

IDENTIFYN RESOLUTION STEPDOWN

SECONDARY ANTIBODY MICROSCOPY EVIDENCE ASSESSMENT

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

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

8

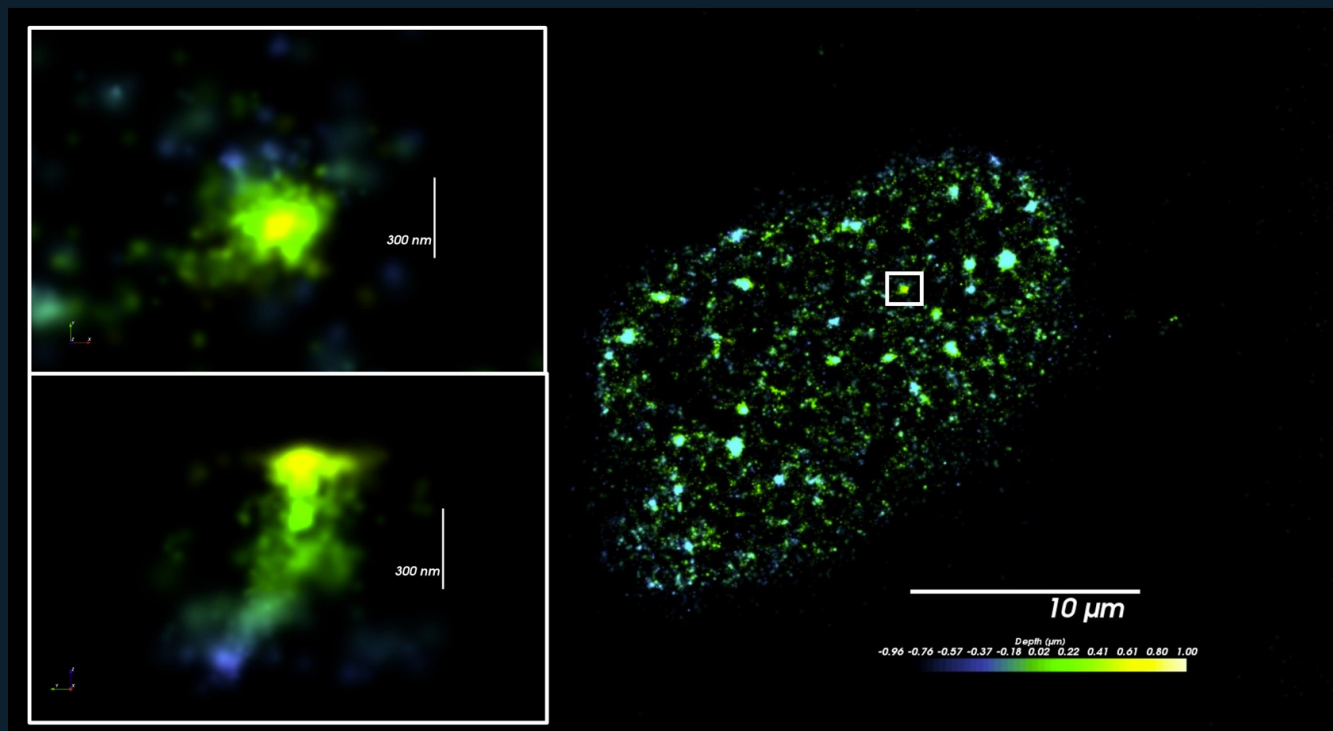
ORDERED EVIDENCE TIERS

1

SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

SA-000016 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

Identifyn performed the experimental and imaging work documented below. BENNETT converts that operating history into a governed, source-bounded scientific record.

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- 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™ 488 Affinity Purified Donkey Anti-Human, IgG (H + L), and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Widefield SIM — Apotome -> Confocal -> Airyscan -> SIM -> SIM² -> Single-molecule STORM -> Control

BENNETT ASSESSMENT

The preserved SA-000016 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-000016 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 38 HE eGFP; 50 Cy5; 49 DAPI; 450 - 490; 625 - 655; 335-383; 500 - 550
Widefield SIM — Apotome	405/DAPI; 488; 594	2; 38 He eGFP; 45 Texas Red; 450 - 490; 540 - 580; 500 - 550; 593 - 668; 493
Confocal	405/DAPI; 488; 647	2; None; 488nm @0.6%; 640nm @0.5%; 493; 653; 517; 668
Airyscan	405/DAPI; 488	2; None; 488nm @0.3%; 405nm @1.5%; 493; 353; 517; 465
SIM	405/DAPI; 488; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T2-Axiocam 820 #2 -SR; T2-Axiocam 820 #2-SR; 488; 405
SIM ²	405/DAPI; 488; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 647 - 800; T2-Axiocam 820 #2 -SR; T2-Axiocam 820 #2-SR; 488; 405
Single-molecule STORM	488	Both Channels
Control	405/DAPI; 488	2; 38 HE eGFP; 49 DAPI; 450 - 490; 335-383; 500 -550; 420-470; 493

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 3; Scaling (Per Pixel): 0.110µm X 0.110µm; Image size (Pixels): 477 X 556; Image Size (Pixels): 477 X 556; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam MR R3. Timing: Exposure Time: 150ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @8.0%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 38.

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: Z-Stack: 23 Slices (4.4µm); Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm X 0.200µm; Image size (Pixels): 961 X 816; Image Size (Pixels): 961 X 816; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807. Timing: Exposure Time: 150ms; 100ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @20%; X-Cite Xylis Lamp Intensity @15%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 50°, Scale Z 59%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 594.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 900. Objective: with a Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 27 Slices (1.82µm); Channels: 2; Scaling (Per Pixel): 70.57nm X 70.57nm X 70.00nm; Image size (Pixels): 1437 X 1437; Image Size (Pixels): 1437 X 1437; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; GaAsP-PMT 2; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.54µs; Frame Time: 5.23s. Illumination: Laser Wavelength: 488nm @0.6%; 640nm @0.5%. Optical/scan: Pinhole: 1.00AU / 43µm; 1.00AU / 57µm; Scan Zoom: 1.0; LSM Scan Speed: 8; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: Projection: View Angle 37°, Scale Z 196%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 45.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 900, and records detected dye/channel descriptors as 405/DAPI; 488; 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 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:. Acquisition: Z-Stack: 38 Slices (1.85µm); Channels: 2; Scaling (Per Pixel): 35.29nm X 35.29nm X 50nm; Image size (Pixels): 944 X 858; Image Size (Pixels): 944 X 858; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.59µs; Frame Time: 4.44s. Illumination: Laser Wavelength: 488nm @0.3%; 405nm @1.5%. Optical/scan: Pinhole: 5.00 AU / 219µm; 5.00 AU / 181µm; Scan Zoom: 1.6; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 2; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution 8.3 (3D, Auto); Super Resolution 7.8 (3D, Auto). Structured source metadata values: 46.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

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: 54 Slices (1.06µm); Channels: 2; Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm; Image size (Pixels): 2116 X 1635; Image Size (Pixels): 2116 X 1635; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 125ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 488nm @3.00%; 405nm @6.0%; Illumination Wavelength: 488; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: NA; Result Sampling: 4; Projection: View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 57.

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

SIM² / 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: 64 Slices (1.26µm); Channels: 2; Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm; Image size (Pixels): 864 X 666; Image Size (Pixels): 864 X 666; Bit Depth: 16 Bit. Detector/camera: Imaging Device: AxioCam 820 #2. Timing: Exposure Time: 125ms; 50ms; Frame Time: 1.33s; 676.00ms. Illumination: Laser Wavelength: 488nm @3.00%; 405nm @6.0%; Illumination Wavelength: 488; 405. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 58.

VISIBLE OBSERVATION

A preserved presentation image is available for SIM². BENNETT records the visible source presentation without creating a new native measurement.

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, 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 SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

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

Structured source metadata values: 71.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63X/1.25 oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, AxioCam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye: Acquisition: Channels: 2; Scaling (Per Pixel): 0.143µm X 0.143µm; Image size (Pixels): 1100 X 1095; Image Size (Pixels): 1100 X 1095; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 807 mono. Timing: Exposure Time: 200ms; 30ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @25%; X-Cite Xylis Lamp Intensity @10%. Optical/scan: Effective NA: 1.25. Structured source metadata values: 30.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved SA-000016 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Confocal / Scaling (Per Pixel): 70.57nm X 70.57nm X 70.00nm -> 0.07057 μ m X 0.07057 μ m X 0.07000 μ 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)=1437 X 1437; Image Size (Scaled)=101.41 μ m X 101.41 μ 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): 35.29nm X 35.29nm X 50nm -> 0.03529 μ m X 0.03529 μ m X 0.05000 μ 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)=944 X 858; Image Size (Scaled)=33.31 μ m X 30.28 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.01%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm -> 0.01320 μ m X 0.01320 μ m X 0.02000 μ 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)=2116 X 1635; Image Size (Scaled)=27.92 μ m X 21.57 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.06%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 13.20nm X 13.20nm X 20.00nm -> 0.01320 μ m X 0.01320 μ m X 0.02000 μ 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)=864 X 666; Image Size (Scaled)=11.40 μ m X 8.79 μ m; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.04%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["6 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

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

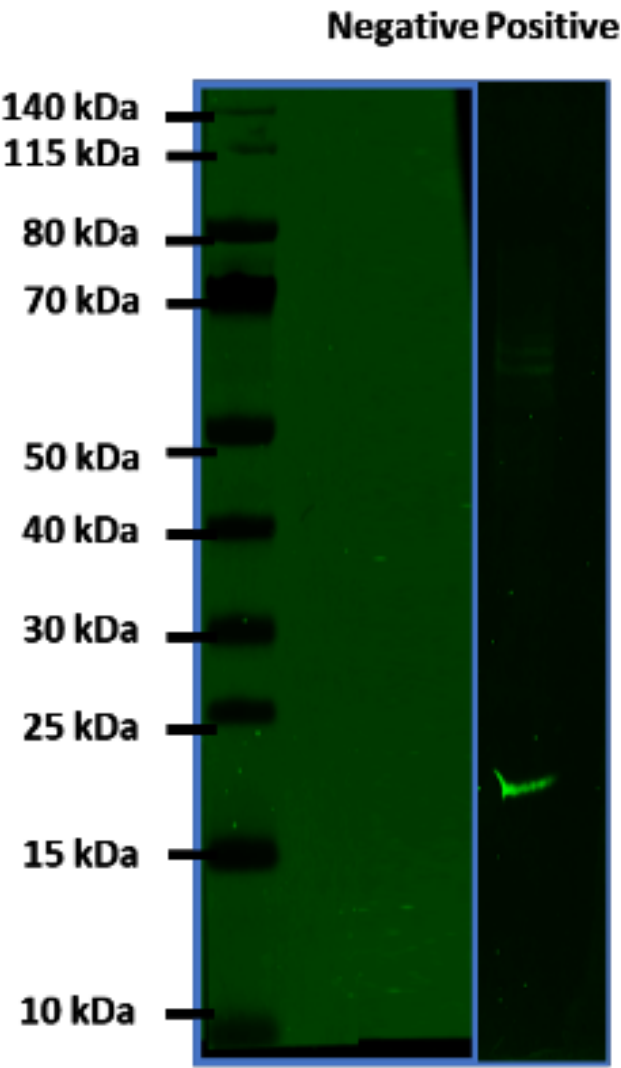
This dossier preserves the complete available evidence progression for SA-000016. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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 ²	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	488
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	488nm
Emission Filter	513±17nm
Detector	PMT
Intensity	2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	Identifyn® LLC

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

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

Cat ID# - SA 000016

Etoposide-treated (2 µM) 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 Identifyn® Rabbit Anti-Human γH2AX chimeric antibody (Human Fc) for 2 hours, followed by 5X PBS washes. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey anti-Human IgG was incubated for 1 hour, followed by washing 5X with PBS. The membrane was dried for 10 minutes at room temperature before imaging.

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

Scan Settings

Detection mode = Fluorescence

Laser = 488nm

Emission Filter = 513±17nm

Detector = PMT

Intensity = 2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

None

Image: Identifyn® LLC

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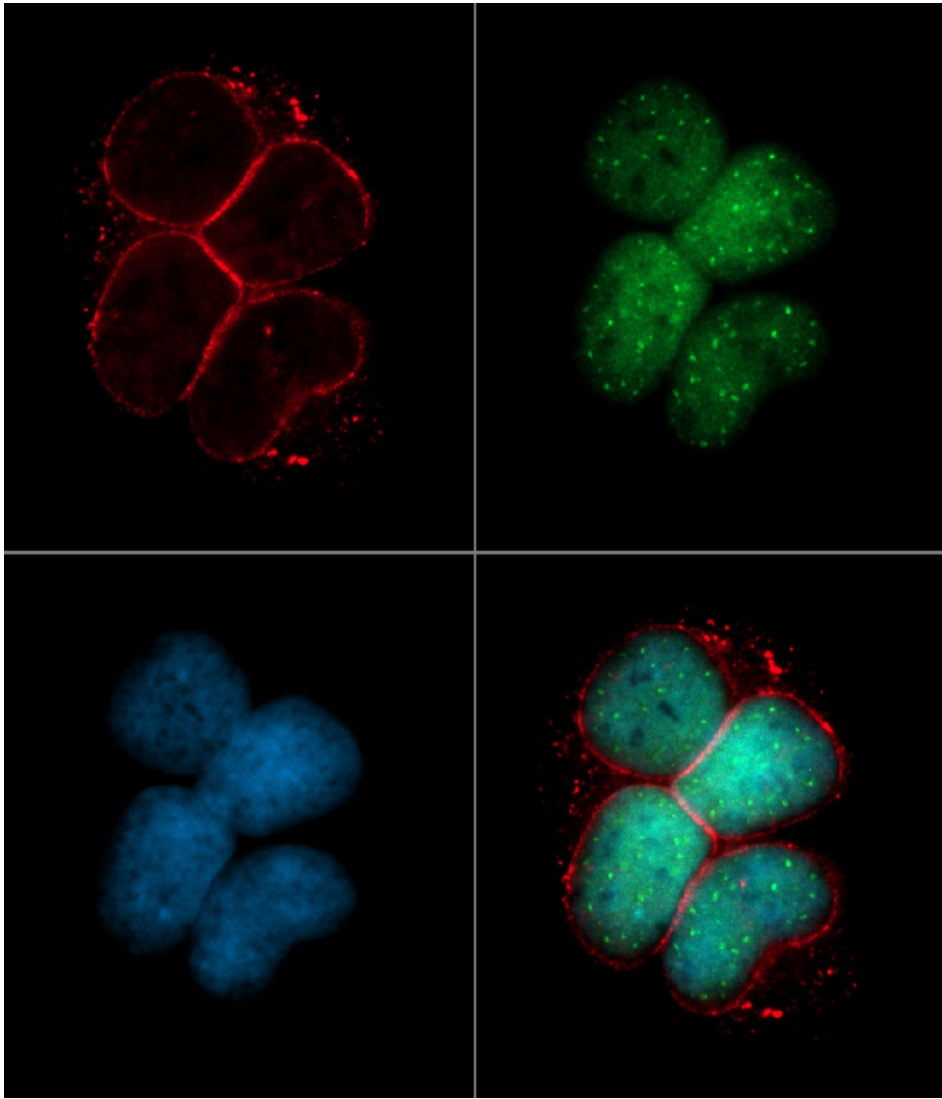
SOURCE PROVENANCE

Catalog	SA-000016
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	C0D580AEB384457E23B57647C4AAD82205A358412F9E8072DF1EB0AA71C54ABD
Method PDF SHA-256	8EB36B5EBBC153D0BBBAB15FBF87E1EFBBA4F0ABF65BFE91B9599B09BD80706A

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110µm X 0.110µm
Image size (Pixels)	477 X 556
Image Size (Scaled)	52.24µm X 60.90µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	38 HE eGFP 50 Cy5 49 DAPI
Beam Splitter	495 660 395
Filter Excitation Wavelength	450 - 490 625 - 655 335-383
Filter Emission Wavelength	500 - 550 665 - 715 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @8.0%
Exposure Time	150ms 100ms 40ms
Excitation Wavelength	493 653 353
Emission Wavelength	517 668 465
Effective NA	1.4
Imaging Device	Axiocam MR R3
Camera Adapter	1X
Depth of Focus	0.80µm 1.03µm 0.72µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human, IgG (H + L)
Cat ID# - SA 000016

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000056

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, 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.

The second primary antibody followed this, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam MR R3, and X-Cite Xylis Lamp was used with the following parameters for each dye:

Single Plane (2D)

Channels = 3

Scaling (Per Pixel) = 0.110µm X 0.110µm

Image size (Pixels) = 477 X 556

Image Size (Scaled) = 52.24µm X 60.90µm

Bit Depth = 14 Bit

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

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

488 -

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 - 550

Contrast Method = Fluorescence

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

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 = 2,2

647-

Reflector = 50 Cy5

Beam Splitter = 660

Filter Excitation Wavelength = 625 - 655

Filter Emission Wavelength = 665 - 715

Contrast Method = Fluorescence

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

Exposure Time = 100ms

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 = 2,2

DAPI -

Reflector = 49 DAPI
 Beam Splitter = 395
 Filter Excitation Wavelength = 335-383
 Filter Emission Wavelength = 420-470
 Contrast Method = Fluorescence
 Light source = X-Cite Xylis Lamp Intensity @8.0%
 Exposure Time = 40ms
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA =1.4
 Imaging Device = AxioCam MR R3
 Camera Adapter = 1X
 Depth of Focus = 0.72µm
 Binning Mode = 2,2

Post Processing

None

Image Correction

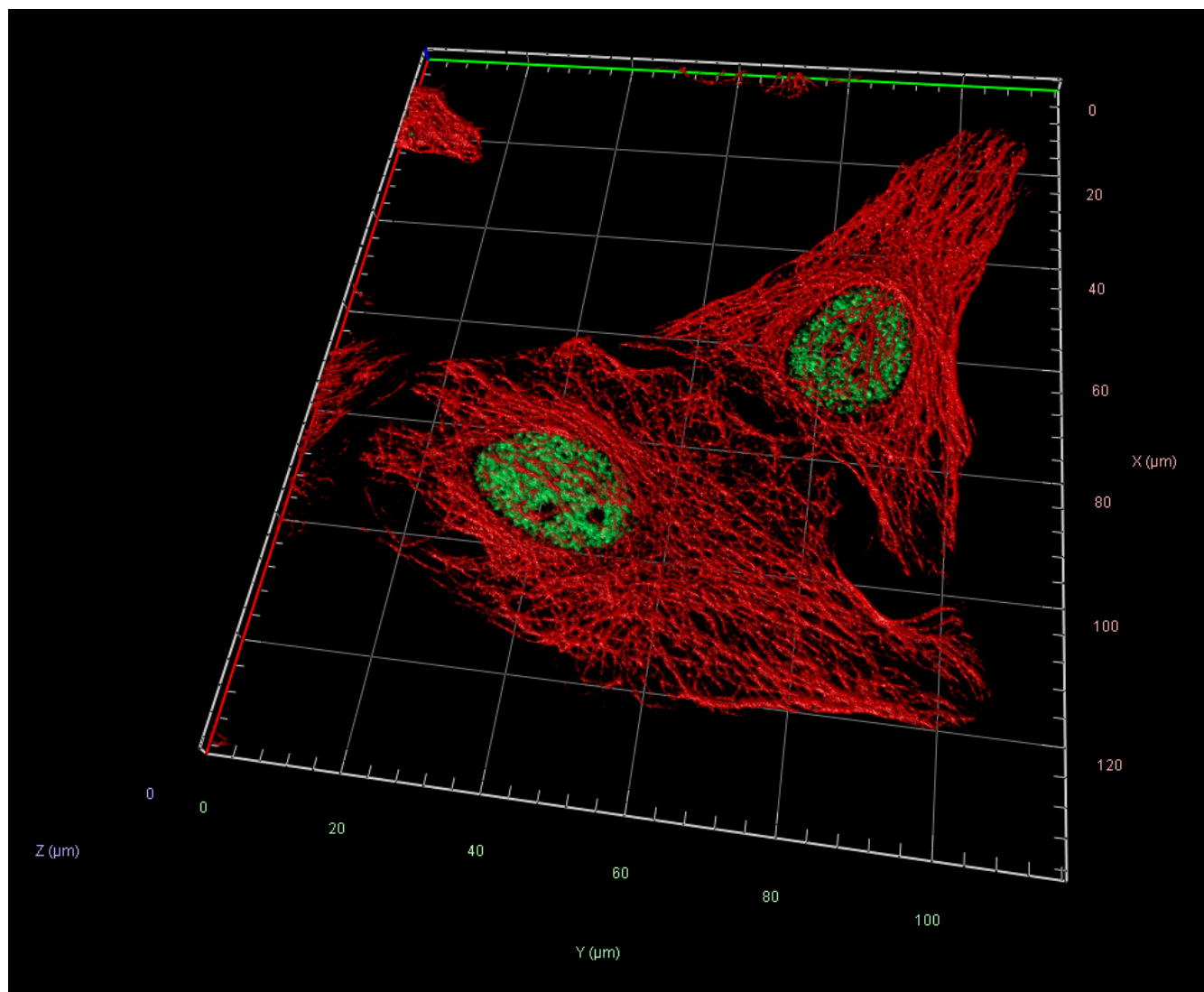
None

Image by K. Shivanna

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SOURCE PROVENANCE	
Catalog	SA-000016
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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	8A1ACB1E8B44B59AE7E23C06C37ABE5C767B4D14DC15F8CA3D1F111C62F9A6FD
Method PDF SHA-256	FAA2DE97A9DD9F4B41C56300CC085F04CD2E1C3A82302529209079AF693145EC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 594
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Z-Stack	23 Slices (4.4µm)
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm X 0.200µm
Image Size (Pixels)	961 X 816
Image Size (Scaled)	137.29µm X 116.57µm
Bit Depth	14 Bit
Image Center Position	X: 77.44mm, Y: 50.30mm
Stage Position	X: 77.44mm, Y: 50.30mm
ROI Center Offset	X: 1.14, Y: 0.00µm
Reflector	38 He eGFP 45 Texas Red
Beam Splitter	495 585
Filter Excitation Wavelength	450 - 490 540 - 580
Filter Emission Wavelength	500 - 550 593 - 668
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @20% X-Cite Xylis Lamp Intensity @15%
Exposure Time	150ms 100ms
Excitation Wavelength	493 590
Emission Wavelength	517 618
Effective NA	1.25
Imaging Device	Axiocam 807
Camera Adapter	1X
Depth of Focus	1.00µm 1.20µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Volume Projection	Precise
Appearance	Threshold 13% (488), Threshold 13% (594), Ramp 50% (488), Ramp 50% (594), Maximum 100% all channels
Background	Black
Light	Brightness 153%, Azimuth 46°, Elongation -143°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 50°, Scale Z 59%, 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

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Identifyn® SRM Alexa Fluor™ 594 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000045

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, 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.

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

Z Stack - (3D)

Z-Stack = 23 Slices (4.4µm)

Channels = 2
Scaling (Per Pixel) = 0.143µm X 0.143µm X 0.200µm
Image Size (Pixels) = 961 X 816
Image Size (Scaled) = 137.29µm X 116.57µm
Bit Depth = 14 Bit
Image Center Position = X: 77.44mm, Y: 50.30mm
Stage Position = X: 77.44mm, Y: 50.30mm
ROI Center Offset = X: 1.14, Y: 0.00µm

488 -

Reflector = 38 He eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @20%
Exposure Time = 150ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 1.00µm
Binning Mode = 2,2

594 -

Reflector = 45 Texas Red
Beam Splitter = 585
Filter Excitation Wavelength = 540 - 580
Filter Emission Wavelength = 593 - 668
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 100ms
Excitation Wavelength = 590
Emission Wavelength = 618
Effective NA = 1.25
Imaging Device = AxioCam 807
Camera Adapter = 1X
Depth of Focus = 1.20µm
Binning Mode = 2,2

Post Processing - SIM Processing

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

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

3D Image Processing

3D Volume Projection = Precise

Appearance = Threshold 13% (488), Threshold 13% (594), Ramp 50% (488), Ramp 50% (594), Maximum 100% all channels

Background = Black

Light = Brightness 153%, Azimuth 46°, Elongation -143°, Enabled Directional Lighting, Enabled Tone Mapping

Projection = View Angle 50°, Scale Z 59%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

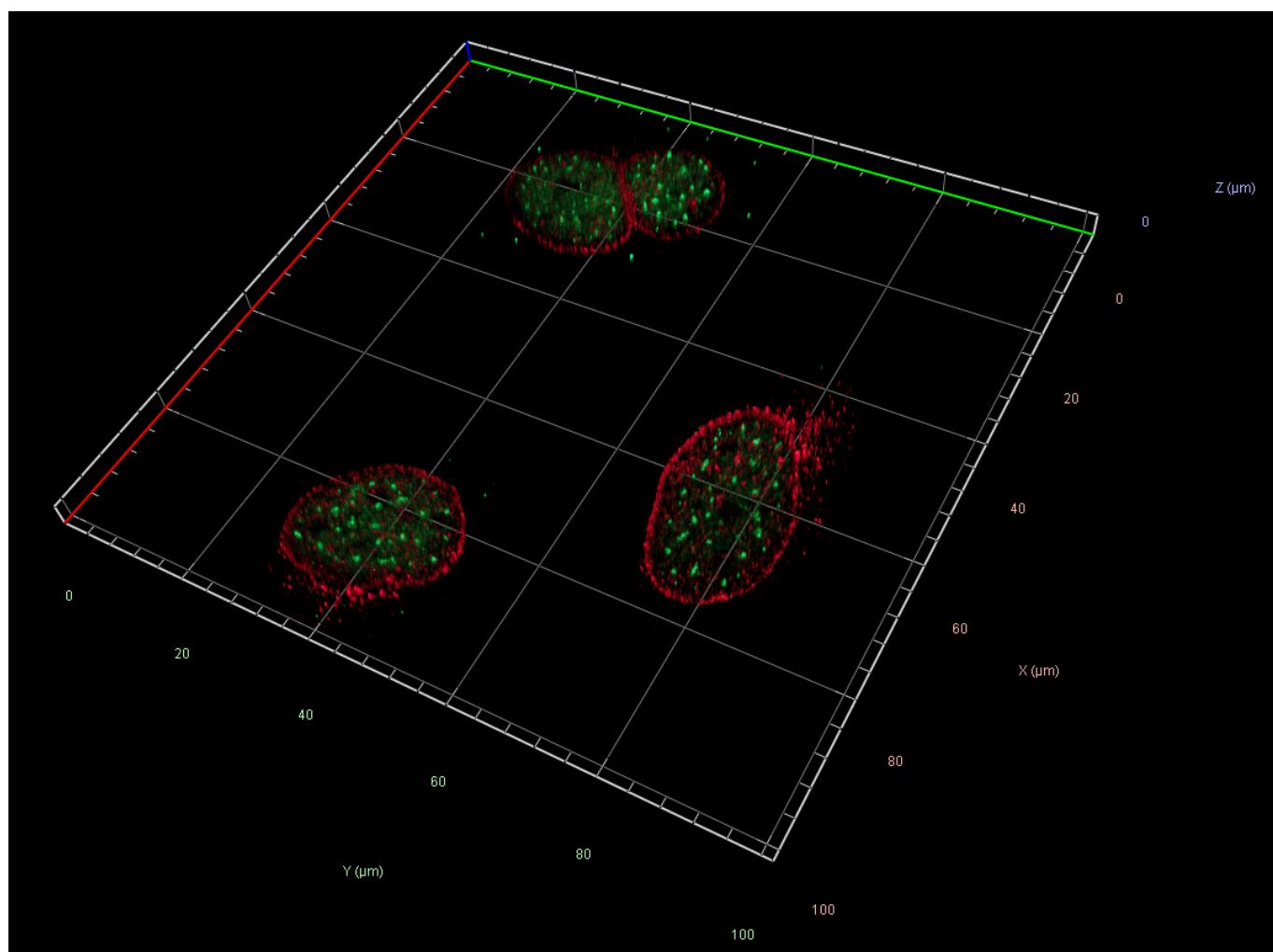
Image by E.F. Simon

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SOURCE PROVENANCE

Catalog	SA-000016
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6EC0D65639B80A44026E1AD5CB84EDC0A635B2A250537B6A9CBE9D566ECA432B
Method PDF SHA-256	C15FCF0C3C23950D245654FB2ED9D157BA0E972A12F8AFBE424DCBB0EF8DD2B2

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 900
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	45

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	27 Slices (1.82µm)
Channels	2
Scaling (Per Pixel)	0.07057 µm X 0.07057 µm X 0.07000 µm
Image Size (Pixels)	1437 X 1437
Image Size (Scaled)	101.41µm X 101.41µm
Bit Depth	16 Bit
Image Center Position	X: 78.45mm, Y: 48.71mm
Stage Position	X: 78.45mm, Y: 48.71mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 43µm 1.00AU / 57µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @0.6% 640nm @0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.0
Rotation	0°
Sampling	1.00
Pixel Time	0.54µs
Frame Time	5.23s
LSM Scan Speed	8
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	493 653
Emission Wavelength	517 668
Effective NA	1.4
Detection Wavelength	478 - 517 656 - 700
Imaging Device	Airyscan GaAsp-PMT 2
Detector Type	GaAsp-PMT
Detector Gain	700 V 650 V

FIELD	SOURCE-DOCUMENTED VALUE
Detector Offset	0
Detector Digital Gain	1.0
3D Volume Projection	Precise
Appearance	Threshold 2% (488), Threshold 2% (647), Ramp 98% (488), Ramp 98% (647), Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 81°, elongation -30°, Enabled Directional Light and Tone Mapping
Projection	View Angle 37°, Scale Z 196%, 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

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Mouse, IgG (H + L), (Subclasses 1+2a+2b+3), Fcy
Fragment Specific
Cat ID# - SA 000056

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, 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.

The second primary antibody followed this, Nuclear Pore Complex Monoclonal Antibody (39C7), 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 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 = 27 Slices (1.82µm)

Channels = 2

Scaling (Per Pixel) = 70.57nm X 70.57nm X 70.00nm

Image Size (Pixels) = 1437 X 1437

Image Size (Scaled) = 101.41µm X 101.41µm

Bit Depth = 16 Bit

Image Center Position = X: 78.45mm, Y: 48.71mm

Stage Position = X: 78.45mm, Y: 48.71mm

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

488 -

Reflector = None

Contrast Method = Fluorescence

Pinhole = 1.00AU / 43µm

LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)

Laser Wavelength = 488nm @0.6%

Laser Blanking = Enabled

Scan Mode = Frame

Scan Zoom = 1.0

Rotation = 0°

Sampling = 1.00

Pixel Time = 0.54µs

Frame Time = 5.23s

LSM Scan Speed = 8

Scan Direction = Bidirectional

Line Step = 1

Averaging - 4

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.4

Detection Wavelength = 478 - 517

Imaging Device = Airyscan

Detector Type = GaAsP-PMT

Detector Gain = 700 V

Detector Offset = 0

Detector Digital Gain = 1.0

647 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00AU / 57µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 640nm @0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.0
 Rotation = 0°
 Sampling = 1.00
 Pixel Time = 0.54µs
 Frame Time = 5.23s
 LSM Scan Speed = 8
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging - 4
 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

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 2% (488), Threshold 2% (647), Ramp 98% (488), Ramp 98% (647), Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 81°, elongation -30°, Enabled Directional Light and Tone Mapping
 Projection = View Angle 37°, Scale Z 196%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

None

Image by E.F. Simon

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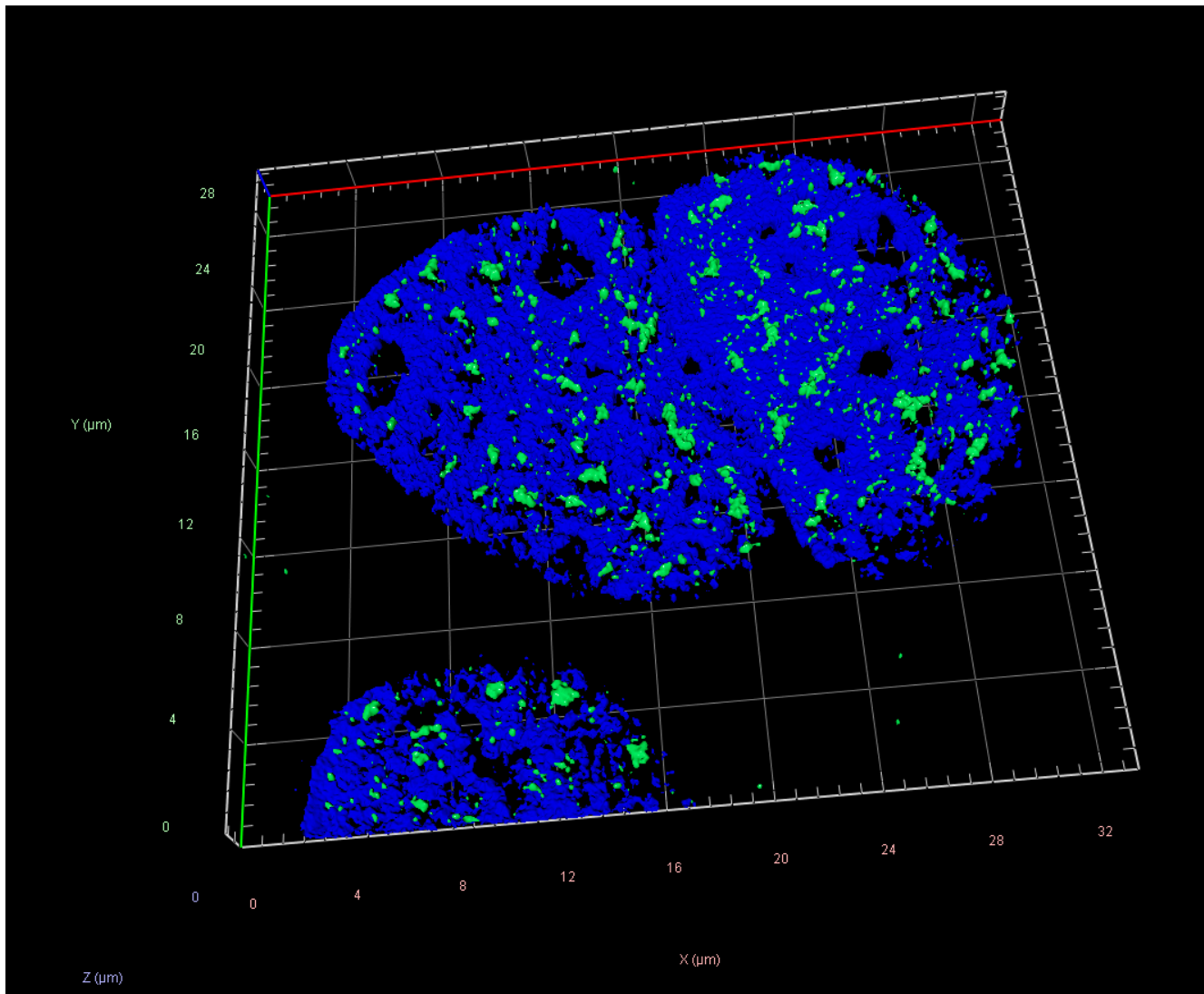
SOURCE PROVENANCE

Catalog	SA-000016
Stage	Confocal

SOURCE PROVENANCE

Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 Confocal, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	45
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	41A6979F7354513115580F981DC82C1B0D5464EDD65A5CC7F2F0C23BED9E8BAA
Method PDF SHA-256	4E348E5EB5B7BE2000CED50234ADF426282868DAF20FCBCD6110307CA67C9073

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



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

STRUCTURED ACQUISITION METADATA / AIRYSCAN

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Objective	with a Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	38 Slices (1.85µm)
Channels	2
Scaling (Per Pixel)	0.03529 µm X 0.03529 µm X 0.05000 µm
Image Size (Pixels)	944 X 858
Image Size (Scaled)	33.31µm X 30.28µm
Bit Depth	16 Bit
Image Center Position	X: 73.26mm, Y: 45.60mm
Stage Position	X: 73.26mm, Y: 45.60mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 219µm 5.00 AU / 181µm
LMS MBS	MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength	488nm @0.3% 405nm @1.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.6
Rotation	0°
Sampling	2.00
Pixel Time	0.59µs
Frame Time	4.44s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	2
Excitation Wavelength	493 353
Emission Wavelength	517 465
Effective NA	1.4
Detection Wavelength	480-560 400-460
Imaging Device	Airyscan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	850 V
Detector Offset	0
Detector Digital Gain	1.0
AiryScan Mode	Super Resolution 8.3 (3D, Auto) Super Resolution 7.8 (3D, Auto)
3D Surface Projection	Precise
Appearance	Threshold 20% (488), Threshold 20% (405), Ambient Light 0% all channels, Specular Light 0% all channels, Shininess 30% all channels
Background	Black
Light	Azimuth 80°, Elongation -130°, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Z Stack - (3D)

Z-Stack = 38 Slices (1.85 μ m)

Channels = 2
Scaling (Per Pixel) = 35.29nm X 35.29nm X 50nm
Image Size (Pixels) = 944 X 858
Image Size (Scaled) = 33.31 μ m X 30.28 μ m
Bit Depth = 16 Bit
Image Center Position = X: 73.26mm, Y: 45.60mm
Stage Position = X: 73.26mm, Y: 45.60mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 219µm
LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
Laser Wavelength = 488nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.6
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.59µs
Frame Time = 4.44s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 2
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 480-560
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
AiryScan Mode = Super Resolution 8.3 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 181µm
 LMS MBS = MBS 405 + 488 + 561 + 640(T10/R90)
 Laser Wavelength = 405nm @1.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 1.6
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 0.59µs
 Frame Time = 4.44s
 LSM Scan Speed = 7
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 2
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 400-460
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 AiryScan Mode = Super Resolution 7.8 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

3D Image Processing

3D Surface Projection = Precise
 Appearance = Threshold 20% (488), Threshold 20% (405), Ambient Light 0% all channels, Specular Light 0% all channels, Shininess 30% all channels
 Background = Black
 Light = Azimuth 80°, Elongation -130°, Enabled Tone Mapping
 Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections -

None

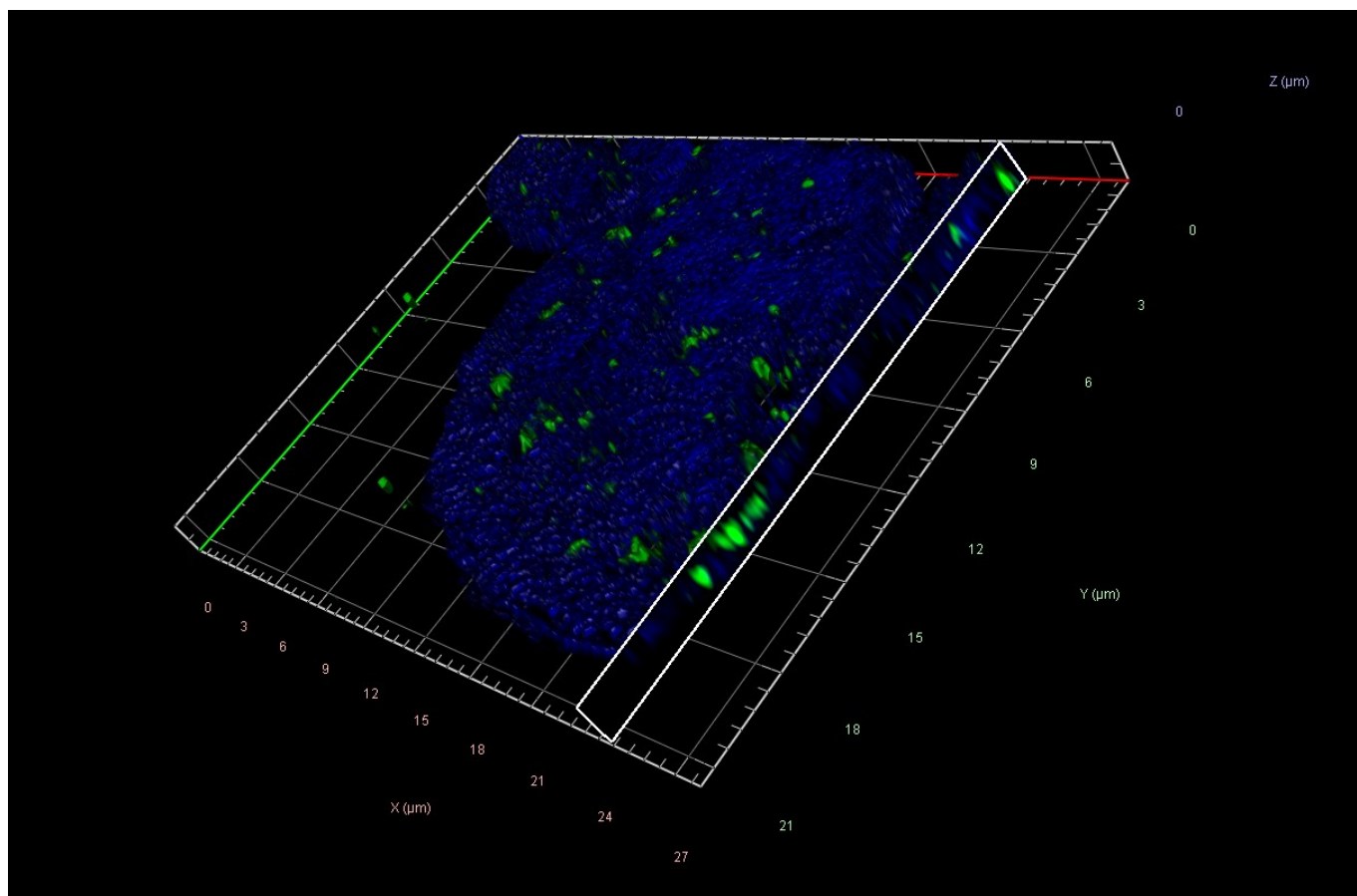
Image by P. Raut

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SOURCE PROVENANCE

Catalog	SA-000016
Stage	Airyscan
Instrument	ZEISS LSM 900
Microscope configuration	Zeiss LSM 900 AiryScan, Axio Observer.Z1/7, using with a Plan-Apochromat 63X/1.40 oil DIC M27 was used with the following parameters for each dye:
Structured source metadata values	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	CA5F16E77D2C677E006A56FE1C7EF12B51E61151F9241C22E57E18874B6A5A0C
Method PDF SHA-256	07ACB6A6823722AF99B4F0535548471E689FF6127679E77D429BA1A84BE69D0C

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	57

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	54 Slices (1.06µm)
Channels	2
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.02000 µm
Image Size (Pixels)	2116 X 1635
Image Size (Scaled)	27.92µm X 21.57µm
Bit Depth	16 Bit
Image Center Position	X: 5.49mm, Y: -1.07mm
Stage Position	X: 5.49mm, Y: -1.07mm
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, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 405
Channel Name	T2-Axiocam 820 #2 -SR T2-Axiocam 820 #2-SR
Excitation Wavelength	488 405
Emission Wavelength	525 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	125ms 50ms
Depth of Focus	0.81µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @3.00% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Grating	36.5µm grating
Result Sampling	4
Process Sampling	NA

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.0186 (488), 8.4959 (DAPI)
Fitting	Fast Fit
Histogram	Scale to Min/Max
Baseline	Cut
3D Volume Projection	Precise
Appearance	Threshold 5% (488), Threshold 5% (DAPI), Ramp 95% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	The Clipping plane is introduced to demonstrate signal specificity and localization of Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG to the nuclear compartment and specificity of the Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L) to the primary antibody.
Y - Z	Activate and textured opaque View (blue) was selected to highlight the clipped plane and demonstrate the localization of primary/secondary antibody localization. A white Outline of the clipped perimeter was shown. Clip Back was Selected, and Clip Front was NOT selected.
Position of Clip	7%
Clip Y	-0°
Clip Z	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 Primary Antibody

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 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 = 54 Slices (1.06 μ m)

Channels = 2
Scaling (Per Pixel) = 13.20nm X 13.20nm X 20.00nm
Image Size (Pixels) = 2116 X 1635
Image Size (Scaled) = 27.92 μ m X 21.57 μ m
Bit Depth = 16 Bit
Image Center Position = X: 5.49mm, Y: -1.07mm
Stage Position = X: 5.49mm, Y: -1.07mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

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, 647 - 800

Contrast Method = Fluorescence

Illumination Wavelength = 488

Channel Name = T2-Axiocam 820 #2 -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 #2

Camera Adapter = 1X

Exposure Time = 125ms

Depth of Focus = 0.81µm

Binning Mode = 2,2

Laser Wavelength = 488nm @3.00%

Frame Time = 1.33s

Scan Direction = Unidirectional

Grating = 36.5µm grating

DAPI -

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, 647 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating = 36.5µm grating
 Post Processing - SIM Processing
 Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = NA
 Channels = 2
 Algorithm = SIM
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 9.0186 (488), 8.4959 (DAPI)
 Fitting = Fast Fit
 Histogram = Scale to Min/Max
 Baseline = Cut

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 5% (488), Threshold 5% (DAPI), Ramp 95% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Light and Tone Mapping
 Projection = View Angle 35°, Scale Z 134%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = The Clipping plane is introduced to demonstrate signal specificity and localization of IdentifyN® Rabbit anti-Human γH2Ax Recombinant Monoclonal, Human Fc, IgG to the nuclear compartment and specificity of the IdentifyN® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L) to the primary antibody.

Y - Z = Activate and textured opaque View (blue) was selected to highlight the clipped plane and demonstrate the localization of primary/secondary antibody localization. A white Outline of the clipped perimeter was shown. Clip Back was Selected, and Clip Front was NOT selected.

Position of Clip = 7%

Clip Y = -0°

Clip Z = 0°

Image Corrections

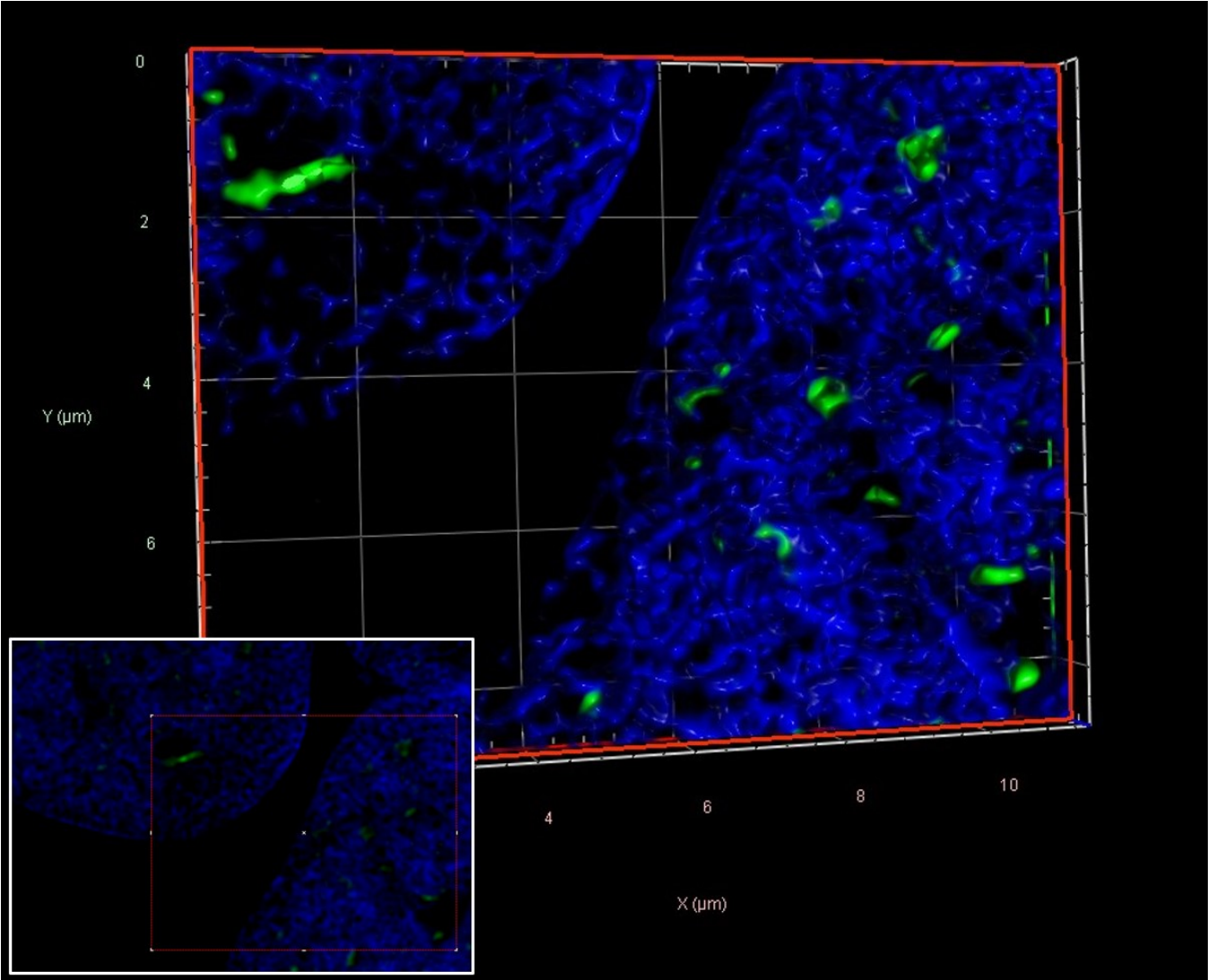
None

Image by P. Raut

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SOURCE PROVENANCE	
Catalog	SA-000016
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	57
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	03EE2AB76FB0CC66AD5E28B0B6D0C9772018F2293AF949195F25BD73296D23D0
Method PDF SHA-256	E864DEEA361B2D78DF492511E9B44F5C6B946FD57117F4DB7EB104FD103BFB11

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM ²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	58

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 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	64 Slices (1.26µm)
Channels	2
Scaling (Per Pixel)	0.01320 µm X 0.01320 µm X 0.02000 µm
Image Size (Pixels)	864 X 666
Image Size (Scaled)	11.40µm X 8.79µm
Bit Depth	16 Bit
Image Center Position	X: 6.30mm, Y: -1.30mm
Stage Position	X: 6.30mm, Y: -1.30mm
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, 647 - 800
Contrast Method	Fluorescence
Illumination Wavelength	488 405
Channel Name	T2-Axiocam 820 #2 -SR T2-Axiocam 820 #2-SR
Excitation Wavelength	488 405
Emission Wavelength	525 445
Effective NA	1.4
Detection Wavelength	419-470, 503-546, 576-617, 657-800 419-470, 503 - 546, 576 - 617, 657-800
Imaging Device	Axiocam 820 #2
Camera Adapter	1X
Exposure Time	125ms 50ms
Depth of Focus	0.81µm 0.69µm
Binning Mode	2,2
Laser Wavelength	488nm @3.00% 405nm @6.0%
Frame Time	1.33s 676.00ms
Scan Direction	Unidirectional
Grating	36.5µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
SIM Settings	Standard
Auto Sharpness	On
Sharpness	8.3541 (488), 7.7383 (DAPI)
Order Combination	Wiener Filter
Regularization	None
Regularization Weight	0.0000
Iterations	3
3D Volume Projection	Precise
Appearance	Threshold 4% (488), Threshold 4% (DAPI), Ramp 96% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 135%, Azimuth 60°, Elongation -26°, Enabled Directional Light and Tone Mapping
Projection	View Angle 35°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
Clipping Image Process	The Clipping plane is introduced to demonstrate signal specificity and localization of Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG to the nuclear compartment and specificity of the Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L) to the primary antibody.
X - Y	Activate. Invisible View (clear) was selected to highlight the clipped plane and demonstrate the primary/secondary antibody localization's localization. An orange Outline of the clipped perimeter was shown. Clip Front was Selected, and Clip Back was NOT selected.
Position of Clip	-7%
Clip X	-0°
Clip Y	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 Primary Antibody

Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG
Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L)
Cat ID# - SA 000016

Gamma H2AX (γ 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, γ H2AX is rapidly phosphorylated explicitly at the serine 139 residue (referred to as Ser139 phosphorylation or γ -phosphorylation) near the site of the break. This phosphorylation event forms γ 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 μ 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 γ H2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS, and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Elyra 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 = 64 Slices (1.26 μ m)

Channels = 2
Scaling (Per Pixel) = 13.20nm X 13.20nm X 20.00nm
Image Size (Pixels) = 864 X 666
Image Size (Scaled) = 11.40 μ m X 8.79 μ m
Bit Depth = 16 Bit
Image Center Position = X: 6.30mm, Y: -1.30mm
Stage Position = X: 6.30mm, Y: -1.30mm
ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

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, 647 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 488
 Channel Name = T2-Axiocam 820 #2 -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 #2
 Camera Adapter = 1X
 Exposure Time = 125ms
 Depth of Focus = 0.81µm
 Binning Mode = 2,2
 Laser Wavelength = 488nm @3.00%
 Frame Time = 1.33s
 Scan Direction = Unidirectional
 Grating = 36.5µm grating

DAPI -

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, 647 - 800
 Contrast Method = Fluorescence
 Illumination Wavelength = 405
 Channel Name = T2-Axiocam 820 #2-SR
 Excitation Wavelength = 405
 Emission Wavelength = 445
 Effective NA = 1.4
 Detection Wavelength = 419-470, 503 - 546, 576 - 617, 657-800
 Imaging Device = Axiocam 820 #2
 Camera Adapter = 1X
 Exposure Time = 50ms
 Depth of Focus = 0.69µm
 Binning Mode = 2,2
 Laser Wavelength = 405nm @6.0%
 Frame Time = 676.00ms
 Scan Direction = Unidirectional
 Grating = 36.5µm grating

Post Processing - SIM2 Processing
 Processing Dimension - 3D
 Result Sampling = 4
 Process Sampling = 4
 Channels = 2

Algorithm = SIM2
 SIM Settings = Standard
 Auto Sharpness = On
 Sharpness = 8.3541 (488), 7.7383 (DAPI)
 Order Combination = Wiener Filter
 Regularization = None
 Regularization Weight = 0.0000
 Iterations = 3

3D Image Processing

3D Volume Projection = Precise
 Appearance = Threshold 4% (488), Threshold 4% (DAPI), Ramp 96% all channels, Maximum 100% all channels
 Background = Black
 Light = Brightness 135%, Azimuth 60°, Elongation -26°, Enabled Directional Light and Tone Mapping
 Projection = View Angle 35°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process = The Clipping plane is introduced to demonstrate signal specificity and localization of Identifyn® Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG to the nuclear compartment and specificity of the Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Human IgG (H + L) to the primary antibody.

X - Y = Activate. Invisible View (clear) was selected to highlight the clipped plane and demonstrate the primary/secondary antibody localization's localization. An orange Outline of the clipped perimeter was shown. Clip Front was Selected, and Clip Back was NOT selected.

Position of Clip = -7%

Clip X = -0°

Clip Y = 0°

Image Corrections

The lower left-hand corner shows the 2-dimensional representation of the entire image and the Region of Interest (ROI) that was selected.

Image by P. Raut

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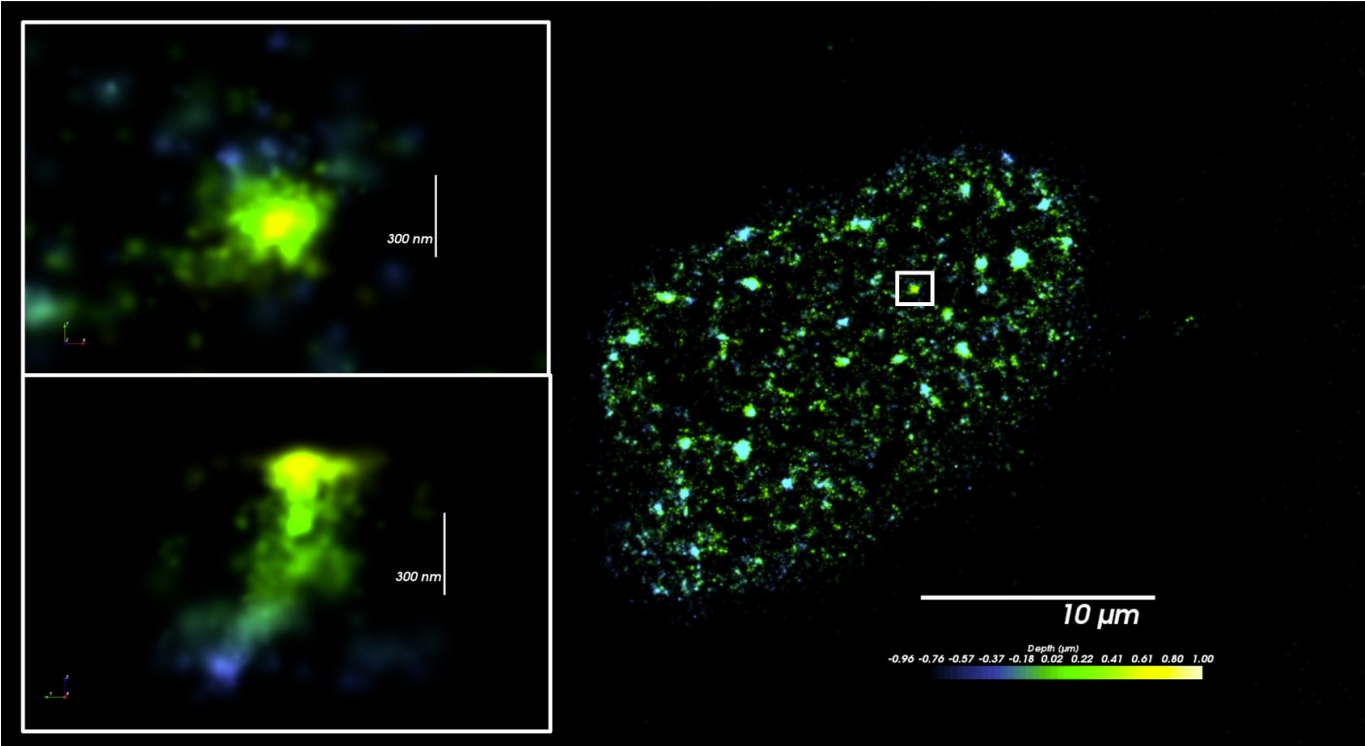
SOURCE PROVENANCE

Catalog	SA-000016
Stage	SIM²
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	58

SOURCE PROVENANCE

Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	123A4A900FC375A96427F4D6118AF9D8681543E0B05A6927385478A5A0D7868A
Method PDF SHA-256	86B79D842DFA87BE795C9448A952495BBF54C2D7CBAB4E90B282F04B44C5A1C6

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



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

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Course Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange, Green)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100ms 20ms
Super Resolution Camera Exposure Time	20ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 488 nm (635 nm Dichroic); First reference probe: 488488 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	181 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 488nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control (488 nm (635 nm Dichroic))	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 488nm" was deselected, and "calibrate" under Autofocus was selected.
Current Intensity	4.5
Calibration Value	-5084 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is Selected
Piezo Record	Begin: 180; End: 180
SuperRes 488nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 20000
Z Positions	1 using Steps: 0.2µm (200nm)
Cycles	1
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	10%
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 7
Sizing Mode	Radial Precision
Particle size	4nm
Color by	Constant
Color Table	Single
Opacity Mode	Constant; Opacity 1: 2
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	5 Intervals

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

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn Primary Antibody

Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG

Cat ID# - PA-RR 000004

Identifyn® Secondary Antibodies

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

Cat ID# - SA 000016

Hela cells were grown on μ -Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 μ M Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. The primary antibody, Rabbit anti-Human γ H2Ax Recombinant Monoclonal, Human Fc, IgG, was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn's™ proprietary Wash Buffer and Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyn's™ proprietary Wash Buffer followed by the addition of Identifyn's™ proprietary STORM Buffer to the sample chamber enabling image acquisition.

Microscope

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

Calibration -

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

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

Image Acquisition -

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

Configuration Tab - Capture Configuration

Number of Probes: 1

Number of Simultaneous Probes: 1

Number of Reference Probes: 1

Camera: Both Cameras

Color Channel: Both Channels

Probe Capture Mode: Interleaved

Z -Stack Capture Mode: Interleaved

Multiple Location Capture: Not selected

Post Record Reference: Not Selected

Fluidics: Not Selected

Autofocus Drift Correction: On

Microscope configuration

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

Camera Configuration

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

Probe Configuration

Predefined Probe: First probe: 488 nm (635 nm Dichroic); First reference probe: 488488 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: 181 µm

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

Widefield 488nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input
 Reference Probe 1 Laser control (488 nm (635 nm Dichroic): Not Selected

Advanced Tools Option

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

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

Autofocus - Calibration Values

Current Position" -0.979

Current Intensity: 4.5

Calibration Value: -5084 nm/unit

Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50µm is Selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 180; End: 180

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

SuperRes 488nm: 0.1X Neutral Density Filter: Selected, @0.02% Laser Input

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

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20ms

Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 20000

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

Cycles: 1

Time Points: 1

Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

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

Laser Power: 10%

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 7

Region of Interest -

Not selected

Display

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
 Sizing Mode: Radial Precision
 Particle size: 4nm
 Color by: Constant
 Color Table: Single
 Opacity Mode: Constant; Opacity 1: 2
 Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

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

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

PSF Z offset: -450 to +450

Radial precision: 35

Axial precision: 65

The other parameters were not applied.

Statistical analysis - N/A

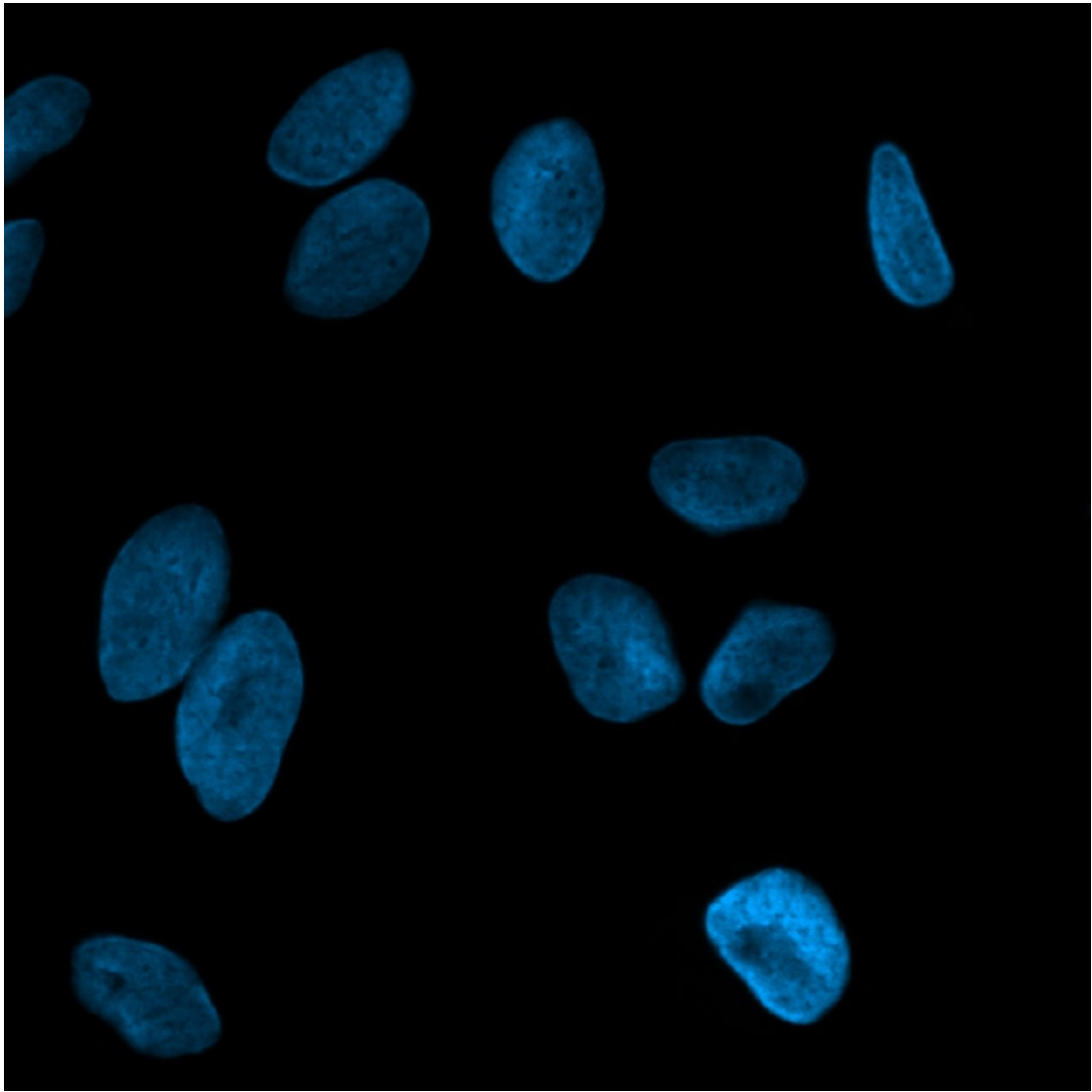
Image by S. Thakur

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SOURCE PROVENANCE

Catalog	SA-000016
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	18C4E8AE4322C4D919204AC7978DABC58AE82C931EA4D12B78269BE947D8BB63
Method PDF SHA-256	41EBE8AA421B59B101AA486EBC258EBE16861B883BAEF7373D468F40E8D1FB28

ORIGINAL IMAGE EVIDENCE / CONTROL



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

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63X/1.25 oil M27
Channels	2
Scaling (Per Pixel)	0.143µm X 0.143µm
Image Size (Pixels)	1100 X 1095
Image Size (Scaled)	157.14µm X 156.43µm
Bit Depth	14 Bit
Image Center Position	X: 46.77mm, Y: 49.24mm
ROI Center Offset	X: 46.77µm, Y: 49.24µm
Reflector	38 HE eGFP 49 DAPI
Beam Splitter	495 395
Filter Excitation Wavelength	450 - 490 335-383
Filter Emission Wavelength	500 -550 420-470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @25% X-Cite Xylis Lamp Intensity @10%
Exposure Time	200ms 30ms
Excitation Wavelength	493 353
Emission Wavelength	517 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.0µm 0.90µm
Binning Mode	2,2

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

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

Cat ID# - SA 000016

Hela Cells were grown on acid-etched #1.5H cover glass to ~50% confluency, followed by -20°C methanol fixation and blocked with Thermo Fisher Fish Serum Blocker cat#37527X3. In the absence of a primary antibody, the Identifyn® 488 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 2

Scaling (Per Pixel) = 0.143µm X 0.143µm

Image Size (Pixels) = 1100 X 1095

Image Size (Scaled) = 157.14µm X 156.43µm

Bit Depth = 14 Bit

Image Center Position = X: 46.77mm, Y: 49.24mm

ROI Center Offset = X: 46.77µm, Y: 49.24µm

488 -

Reflector = 38 HE eGFP

Beam Splitter = 495

Filter Excitation Wavelength = 450 - 490

Filter Emission Wavelength = 500 -550

Contrast Method = Fluorescence

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

Exposure Time = 200ms

Excitation Wavelength = 493

Emission Wavelength = 517

Effective NA = 1.25

Imaging Device = Axiocam 807 mono

Camera Adapter = 1X

Depth of Focus = 1.0µm

Binning Mode = 2,2

DAPI -

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

Post Processing -

None

Image Corrections

None

Control Summary -

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

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

Image by J.K. Bennett

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SOURCE PROVENANCE

Catalog	SA-000016
Stage	Control
Instrument	ZEISS Axio Imager.M1; ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63X/1.25 oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	30
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa

SOURCE PROVENANCE

Image SHA-256	E98204BF1D878B562DD170DDA2EFDC50276D530B0F037B6382788A2B8D324208
Method PDF SHA-256	A0C04CB389C055AC953179139297B3629F985F5BCE17F0C318B8AC5AA06D0D2D