BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

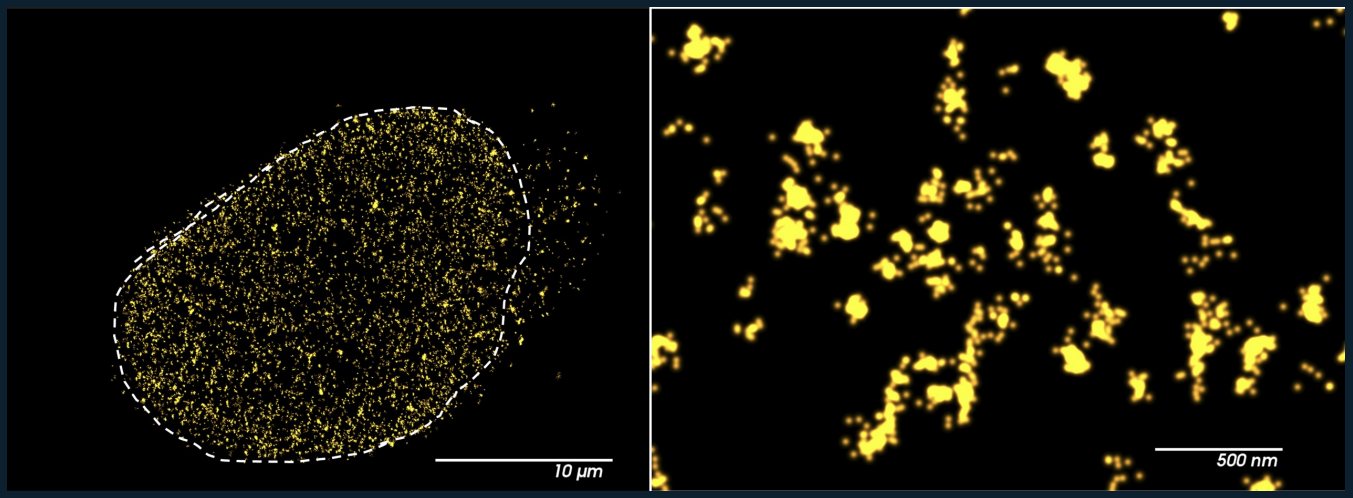
ATM

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human ATM Recombinant Monoclonal

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

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000008 | 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 ATM, 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 PA-RR-000008 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 PA-RR-000008 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; 647	2; 50 Cy5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Widefield SIM — Apotome	405/DAPI; 488; 647	3; 50 Cy5; 38 He eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Confocal	405/DAPI; 488; 647	3; None; 639nm @0.3%; 488nm @0.1%; 405nm @0.2%; 653; 493; 353
Airyscan	405/DAPI; 488; 647; 750	3; None; 639nm @0.5%; 488nm @0.04%; 405nm @0.2%; 653; 493; 353
SIM	405/DAPI; 488; 647	3; 2; 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; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR
SIM²	405/DAPI; 488; 647	3; 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; T2-Axiocam 820-SR; T3-Axiocam 820 #2-SR
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	3; None; 639nm @0.3%; 653; 668

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 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 2; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 472 X 487; Image Size (Pixels): 472 X 487; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705 mono. Timing: Exposure Time: 150ms; 20ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @15%; X-Cite Xylis Lamp Intensity @ 10%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 31.

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; 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: 81 Slices (8.0 µm); Channels: 3; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 1147 X 957; Image Size (Pixels): 1147 X 957; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 mono. Timing: Exposure Time: 100ms; 200 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 15%; X-Cite Xylis Lamp Intensity @ 20.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

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 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 141 Slices (4.20µm); Channels: 3; Scaling (Per Pixel): 0.059µm X 0.059µm x 0.030µm; Image size (Pixels): 1017 X 1017; Image Size (Pixels): 1017 X 1017; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-PMT3; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.33µs; Frame Time: 3.36s. Illumination: Laser Wavelength: 639nm @0.3%; 488nm @0.1%. Optical/scan: Pinhole: 1.00 AU / 66µm; 1.00 AU / 50µm; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.1 (3D, Auto); Filter: 7.2 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, 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 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 142 Slices (1.41µm); Channels: 3; Scaling (Per Pixel): 35.30nm X 35.30nm x 30.00nm; Image size (Pixels): 1248 X 1248; 639 X 756; Image Size (Pixels): 1248 X 1248; 639 X 756; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Airysccan; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.82µs; Frame Time: 6.25s. Illumination: Laser Wavelength: 639nm @0.5%; 488nm @0.04%. Optical/scan: Pinhole: 5.00 AU / 328µm; 5.00 AU / 250µm; Scan Zoom: 2.5; LSM Scan Speed: 7; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 45°, 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 10.0 (3D, Auto); Super Resolution 9.9 (3D, Auto). Structured source metadata values: 63.

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 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647; 750.

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: 278 Slices (8.31µm); 184 Slices (5.49µm) - The Gallery tool was used to reduce the z-stack to provide clarity; Channels: 3; 2; Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm; Image size (Pixels): 5056 X 5056; 1486 X 1775; Image Size (Pixels): 5056 X 5056; 1486 X 1775; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 640nm @2.0%; 488nm @1.0%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; 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: 63.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM, documents platform context as ZEISS Elyra, 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: 209 Slices (8.31µm); 115 Slices (3.42µm) - The Gallery tool was used to reduce the z-stack to provide clarity; Channels: 3; 1; Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm; Image size (Pixels): 3542 X 3672; 1394 X 1246; Image Size (Pixels): 3542 X 3672; 1394 X 1246; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820; Axiocam 820 #2. Timing: Exposure Time: 120ms; 50ms; Frame Time: 1.59s; 676.00ms. Illumination: Laser Wavelength: 640nm @2.0%; 488nm @1.0%; 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 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 64.

VISIBLE OBSERVATION

A preserved presentation image is available for 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: 81.

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 LSM 980. Objective: Plan-Apochromat 63X/1.40 oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.059µm X 0.059µm; Image size (Pixels): 1017 X 1017; Image Size (Pixels): 1017 X 1017; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-PMT3; Detector Type: GaAsp-PMT. Timing: Pixel Time: 0.33µs; Frame Time: 3.36s. Illumination: Laser Wavelength: 639nm @0.3%. Optical/scan: Pinhole: 1.00 AU / 66µm; Scan Zoom: 2.2; LSM Scan Speed: 12; Scan Direction: Bidirectional; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.1 (3D, Auto). Structured source metadata values: 35.

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 LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 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 PA-RR-000008 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

- Antibody and microscopy evidence require orthogonal controls and modality-appropriate interpretation; presentation images alone do not establish analytical specificity.

- Advanced-resolution presentation depends on acquisition, reconstruction or localization quality control before numerical resolution, molecular dimensions or cluster identity can be claimed.

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.30nm X 35.30nm x 30.00nm -> 0.03530 µm X 0.03530 µm X 0.01000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=1248 X 1248; Image Size (Scaled)=44.05 µm X 44.05µm; 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): 21.11nm X 21.11nm X 30.00nm -> 0.02111 µm X 0.02111 µm X 0.03000 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=5056 X 5056; Image Size (Scaled)=106.75µm X 106.75µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 21.11nm X 21.11nm X 30.00nm -> 0.02111 µm X 0.02111 µm X 0.03976 µm. Both numerical magnitude and unit were normalized, then checked against the reported field dimensions; no unit label was changed alone. Supporting evidence: Image Size (Pixels)=3542 X 3672; Image Size (Scaled)=74.78µm X 77.53µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

HIGH CONFIDENCE - SCIENTIST-AUTHORIZED CORRECTION

Cross-tier / treatment concentration: 200 µM; 2µM -> Etoposide = 2 µM. The governing scientist identified every 200 µM occurrence as a technician transcription error. The canonical historical concentration is 2 µM. Supporting evidence: Scientist-authorized correction supplied for the Identifyn historical archive; original technician text remains preserved unchanged. Authority: Dr. Brian T. Bennett. Preservation: Original source text and hashes remain unchanged; only the derivative canonical field is corrected.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["24 hours"], "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"], "Control": ["24 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

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

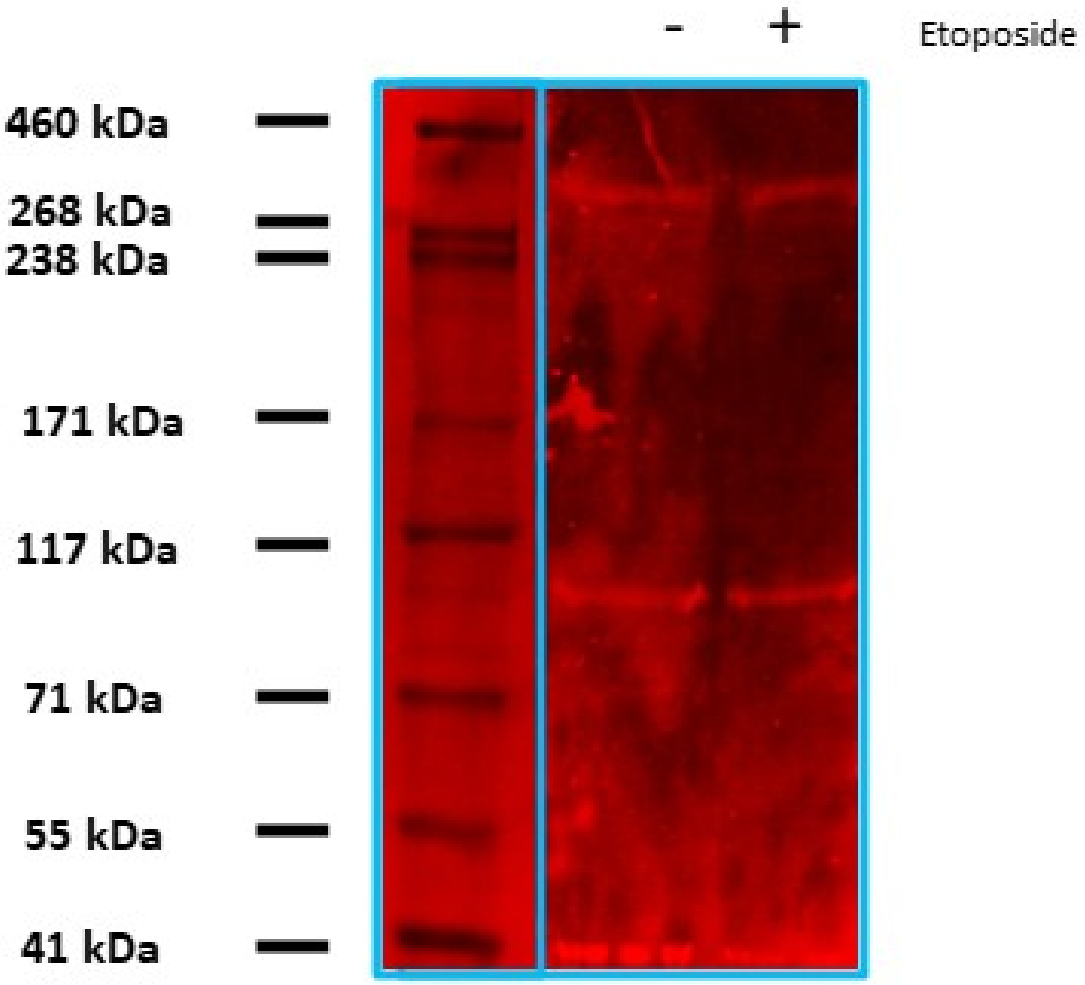
This dossier preserves the complete available evidence progression for PA-RR-000008. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
3	Widefield SIM - Apotome	ZEISS Axio Observer.Z1/7 Apotome	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	PRESERVED
6	SIM	ZEISS Elyra	PRESERVED
7	SIM²	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	K. Small

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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR 000008

Expected Molecular Weight: 350 kDa

HeLa cells were damaged with 200 µM etoposide for 24 hours. Cells were then homogenized using an ultrasonication probe and RIPA lysis buffer, and then centrifuged to pellet the cell debris. The lysate supernatant (100 µg) was mixed with NuPAGE™ LDS Sample Buffer (4X), heated at 80 °C for 10 min, and subjected to electrophoresis using NuPAGE™ Tris-Acetate Mini Protein Gels, 3 to 8%, 1.0 mm, 2D-well in a Mini Gel Tank. Thermo Fisher's HiMark Prestained Protein Standard was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit, PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Rabbit anti-Human ATM Recombinant Monoclonal, IgG for 2 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Rabbit IgG (H + L) was incubated for 1 hour and washed 5 TIMES 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

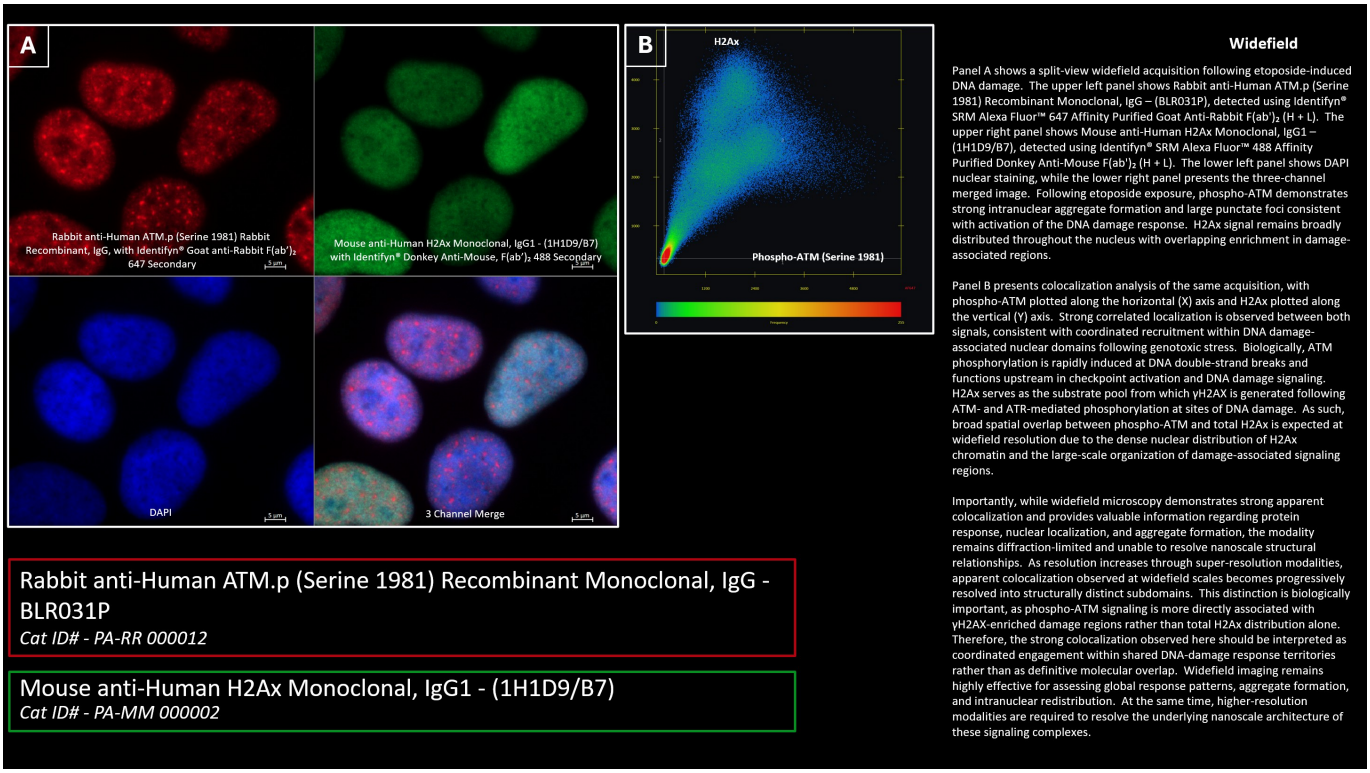
None

Image: K. Small

SOURCE PROVENANCE

Catalog	PA-RR-000008
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV- NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A856638526A47C8A31C25E7C5DF7EF67B6DD9EFF74568F53157A11C2E0E8B840
Method PDF SHA-256	F3B19DCD92FBBD96EE1ED0278C68186FE7DAAA24DB93ED42C6D2AF3498E97102

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - BLR031P
Cat ID# - PA-RR 000012

Mouse anti-Human H2Ax Monoclonal, IgG1 - (1H1D9/B7)
Cat ID# - PA-MM 000002

EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
METADATA VALUES	31

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 705 mono, 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)	0.110 µm X 0.110 µm
Image size (Pixels)	472 X 487
Image Size (Scaled)	51.70 µm X 53.34 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00µm, Y: 0.00µm
ROI Center Offset	X: 0.00µm, 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 @15% | X-Cite Xylis Lamp Intensity @ 10%
Exposure Time	150ms | 20ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705 mono
Camera Adapter	1X
Depth of Focus	1.03µm | 0.72µm
Binning Mode	2,2
Profile Definition	Stroke Thickness 105, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 50.5), Y (Min 43 and Max 1458)

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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:

Single Plane (2D) - Panel A & B

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 472 X 487
Image Size (Scaled) = 51.70 µm X 53.34 µm
Bit Depth = 14 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 = 665 - 715
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @15%
Exposure Time = 150ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
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 @ 10%
Exposure Time = 20ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705 mono
Camera Adapter = 1X
Depth of Focus = 0.72µm
Binning Mode = 2,2

Split View - Panel A

The left panel features Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N), the middle panel shows DAPI nuclear staining, and the right panel displays the merged image of all channels. The Image demonstrates the nuclear localization and brightness of the ATM Rabbit Recombinant antibody.

Profile View Display - Panel B

Profile Definition = Stroke Thickness 105, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 50.5), Y (Min 43 and Max 1458)

The profile display shows the ATM signal across two nuclei, highlighting specificity to the nucleus and relative fluorescent Intensity.

Zeiss Zen Settings - Panel C, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings for the Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N) image acquisition. We demonstrate that an X-Cite LED light input of 15% and a camera exposure time of 150 ms were sufficient to capture the image, further demonstrating excellent localization and high brightness for the Identifyn® Rabbit Recombinant Monoclonal ATM, combined with the Identifyn® 647 secondary antibody.

Image Correction - Panel B

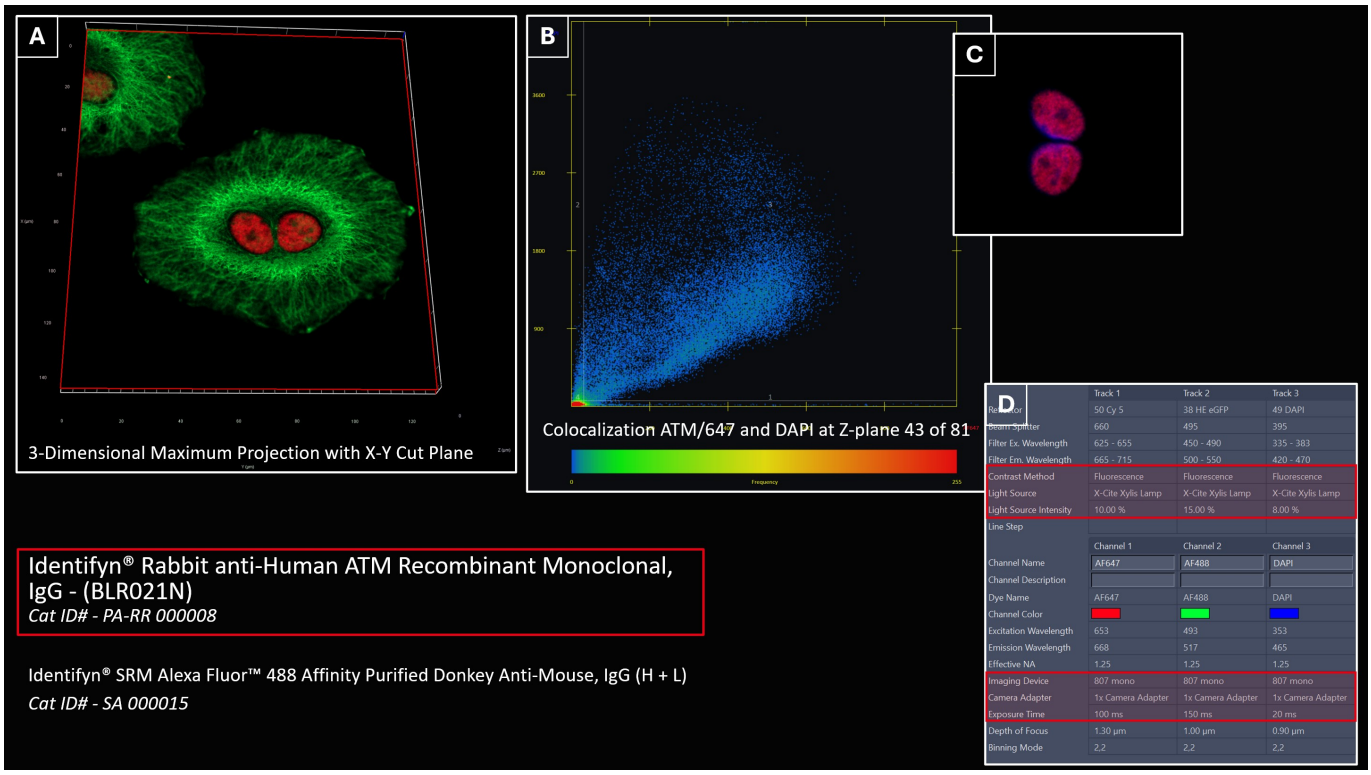
Identifyn® ATM/647 staining was changed from its default red in Zeiss Zen to white to enhance this visualization.

Image by E.F. Simon
Image Analysis and Data Presentation B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
Stage	Widefield
Instrument	ZEISS Axio Imager.M1
Microscope configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705 mono, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	31
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	65821CA6B5C1DEFF66140EC05DF6AB515895E51B3397D7C6DBC91B19CBD62A57
Method PDF SHA-256	2D7AA5F4AE6BC76F7D4170259ACBDA54B556E3CA5743D6A99A049E391816A1FA

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	50

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	81 Slices (8.0 µm)
Channels	3
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels)	1147 X 957
Image Size (Scaled)	163.86 µm X 136.71 µm
Bit Depth	14 Bit
Image Center Position	X: 76.72 mm, Y: 50.18 mm
ROI Center Offset	X: 76.72 µm, Y: 50.18 µm
Reflector	50 Cy5 | 38 He eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @ 15% | X-Cite Xylis Lamp Intensity @ 20.00% | X-Cite Xylis Lamp Intensity @ 8.0%
Exposure Time	100ms | 200 ms | 20 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.25
Imaging Device	Axiocam 807 mono
Camera Adapter	1X
Depth of Focus	1.30µm | 1.00 µm | 0.90µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and ATM signal. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

FIELD	SOURCE-DOCUMENTED VALUE
Position of Clip	9%
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

Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

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

Z Stack - (3D) - Panel A (Maximum projection) and Panel B (Colocalization, z-plane 43 of 81)

Z-Stack = 81 Slices (8.0 µm)

Channels = 3
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image Size (Pixels) = 1147 X 957
Image Size (Scaled) = 163.86 µm X 136.71 µm
Bit Depth = 14 Bit
Image Center Position = X: 76.72 mm, Y: 50.18 mm
ROI Center Offset = X: 76.72 µm, Y: 50.18 µm

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 @ 15%
Exposure Time = 100ms
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

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 @ 20.00%
Exposure Time = 200 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
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 = 20 ms
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.

3D Image Processing - Panel A

3D Maximum Projection = Precise
Appearance = Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel A

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N) to the nuclear compartment.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and ATM signal. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = 9%
Clip Y = -0°
Clip Z =0°

Colocalization View - Panels B and C

Horizontal Axis -DAPI Threshold 32, Range Auto
Vertical Axis - ATM/647, Threshold 70, Range Auto

Colocalization data show that both Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N) and the DAPI nuclear staining are strongly colocalized, with both residing within the nuclear compartment following DNA damage.

Zeiss Zen Settings - Panel D, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings for the Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N) image acquisition, as well as the two other channels collected, 488 and DAPI. We demonstrate that an X-Cite LED light input of 10% and a camera exposure time of 100 ms were sufficient to capture the image, further demonstrating excellent localization and high brightness for the Identifyn® Rabbit Recombinant Monoclonal ATM, combined with the Identifyn® 647 secondary antibody.

We also note that the Identifyn® 488 secondary required a 15% LED input and a 150ms exposure.

Image Correction

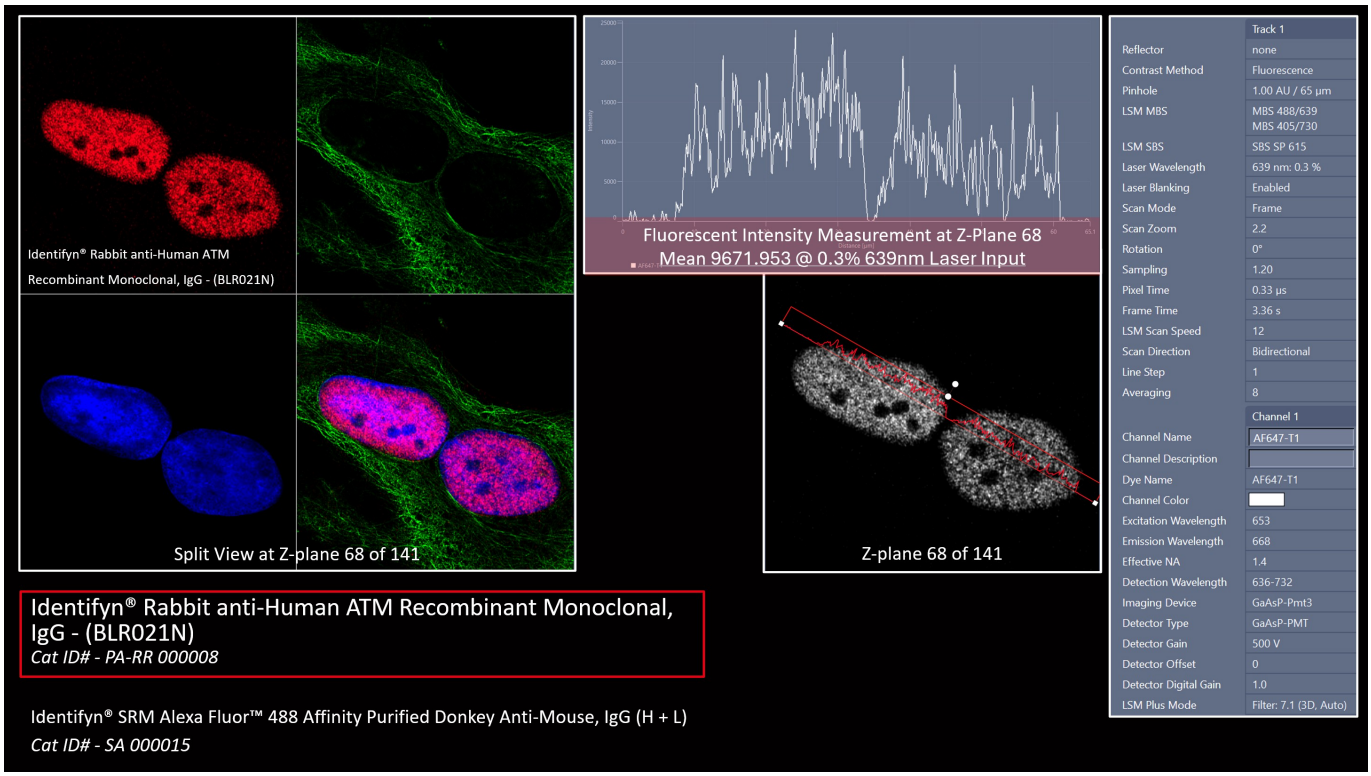
None

Image by E.F. Simon
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	172982C617C1377BD029CE7BF461CCBF4E55BB844E9052BB7A1EDF8B492A8311
Method PDF SHA-256	4DC0DD78DE98E82DE63154C9A13C5FA3B6C952599364E83AAC58E620816A18E3

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	141 Slices (4.20µm)
Channels	3
Scaling (Per Pixel)	0.059µm X 0.059µm x 0.030µm
Image size (Pixels)	1017 X 1017
Image Size (Scaled)	59.81 µm X 59.81 µm
Bit Depth	16 Bit
Image Center Position	X: 88.54 mm, Y: 47.92 mm
Stage Position	X: 88.54 mm, Y: 47.92 mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Pinhole	1.00 AU / 66µm | 1.00 AU / 50µm | 1.00 AU / 43µm
LMS MBS	MBS 488/639 MBS 405/730 | MBS 488/561 MBS 405/730
LMS SBS	SBS SP 615 | Mirror
Laser Wavelength	639nm @0.3% | 488nm @0.1% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.20
Pixel Time	0.33µs
Frame Time	3.36s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	636 - 732 | 489 - 517 | 430 - 470
Imaging Device	GaAsP-PMT3
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.1 (3D, Auto) | Filter: 7.2 (3D, Auto) | Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 65.1), Y (Min 13 and Max 25263)

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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panels A & B (Z-plane 68 of 141)

Z-Stack = 141 Slices (4.20µm)

Channels = 3
Scaling (Per Pixel) = 0.059µm X 0.059µm x 0.030µm
Image size (Pixels) = 1017 X 1017
Image Size (Scaled) = 59.81 µm X 59.81 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.54 mm, Y: 47.92 mm
Stage Position = X: 88.54 mm, Y: 47.92 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 66µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636 - 732
Imaging Device = GaAsP-PMT3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.1 (3D, Auto)

488 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 50µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = Mirror
Laser Wavelength = 488nm @0.1%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 489 - 517
Imaging Device = GaAsP-PMT3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.2 (3D, Auto)

DAPI -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 43µm
LMS MBS = MBS 488/561 MBS 405/730
LMS SBS = Mirror
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 430 - 470
Imaging Device = GaAsP-PMT3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

Split View - Panel A

The upper left features Rabbit anti-Human ATM Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the upper right panel shows Alpha Tubulin monoclonal primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). The lower left panel features DAPI nuclear staining. The lower right panel features the merged image of all channels. This split View was taken at Z-Position 68 of 141.

Profile View Display - Panel B, (Histogram) Panel C (Image Measured)

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 65.1), Y (Min 13 and Max 25263)

The average fluorescent intensity across the two nuclei measured was 9671.953, deploying the 639nm laser at an input power of 0.3%

Zeiss Zen Settings - Panel D, Image Acquisition Settings

A clipped image from Zeiss Zen image acquisition settings for the Rabbit anti-Human ATM Recombinant Monoclonal, IgG (BLR021N) image acquisition, showing all three channels acquired for transparency.

Image Corrections

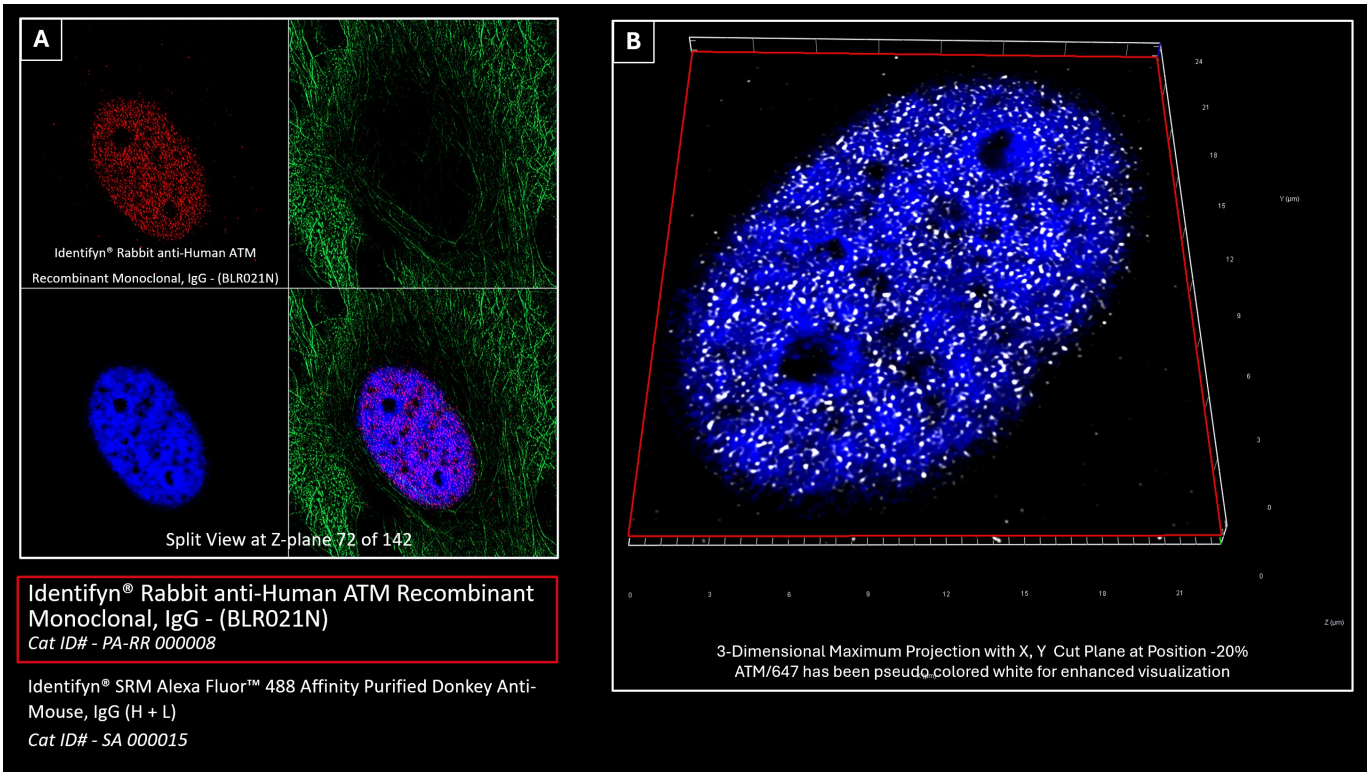
None

Image by J.K. Bennett
Image Analysis and Data Presentation B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6F4BA5271C3809DB0BCB7D1273D3832C1C8F1A9F354B35851AE1615D7CCF6573
Method PDF SHA-256	8AC7C7A3523BF9E7756A0E1717E91FB2C64A520D8E255C94A40A651676D1D617

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



Identifyn® Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR 000008

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647; 750
METADATA VALUES	63

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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Z-Stack	142 Slices (1.41µm)
Channels	3
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.01000 µm
Image size (Pixels)	1248 X 1248 | 639 X 756
Image Size (Scaled)	44.05 µm X 44.05µm | 22.55 µm X 26.68µm
Bit Depth	16 Bit
Image Center Position	X: 88.14 mm, Y: 51.41 mm
Stage Position	X: 88.14 mm, Y: 51.41 mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Pinhole	5.00 AU / 328µm | 5.00 AU / 250µm | 5.00 AU / 214µm
LMS MBS	MBS 488/639 MBS 405/730 | MBS 488/561 MBS 405/730
LMS SBS	SBS SP 640 | SBS SP 550 | SBS SP 505
Laser Wavelength	639nm @0.5% | 488nm @0.04% | 405nm @0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.5
Rotation	0°
Sampling	2.00
Pixel Time	0.82µs
Frame Time	6.25s
LSM Scan Speed	7
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 499 - 548 | 422 - 477
Imaging Device	Airyscan | Airysccan
Detector Type	GaAsp-PMT

FIELD	SOURCE-DOCUMENTED VALUE
Detector Gain	800 V | 750 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	Super Resolution 10.0 (3D, Auto) | Super Resolution 9.9 (3D, Auto) | Super Resolution 7.6 (3D, Auto)
3D Maximum Projection	Precise
Appearance	Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and ATM signal. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.
Position of Clip	20%
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

Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate RAD51 loading onto single-stranded DNA at DSB sites, a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

Z Stack - (3D) - Panel A (Z-plane 72 of 142)

Z-Stack = 142 Slices (1.41µm)

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm x 30.00nm
Image size (Pixels) = 1248 X 1248
Image Size (Scaled) = 44.05 µm X 44.05µm
Bit Depth = 16 Bit
Image Center Position = X: 88.14 mm, Y: 51.41 mm
Stage Position = X: 88.14 mm, Y: 51.41 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panel B

Z-Stack = 142 Slices (1.41µm)

Channels = 3
Scaling (Per Pixel) = 35.30nm X 35.30nm x 30.00nm
Image size (Pixels) = 639 X 756
Image Size (Scaled) = 22.55 µm X 26.68µm
Bit Depth = 16 Bit
Image Center Position = X: 88.14 mm, Y: 51.41 mm
Stage Position = X: 88.14 mm, Y: 51.41 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 328µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 640
Laser Wavelength = 639nm @0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 10.0 (3D, Auto)

488 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 250µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488nm @0.04%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airysccan
Detector Type = GaAsp-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 9.9 (3D, Auto)

DAPI -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 5.00 AU / 214µm
LMS MBS = MBS 488/561 MBS 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.5
Rotation = 0°
Sampling = 2.00
Pixel Time = 0.82µs
Frame Time = 6.25s
LSM Scan Speed = 7
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Detection Wavelength = 422 - 477
Imaging Device = Airyscan
Detector Type = GaAsp-PMT
Detector Gain = 750 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution 7.6 (3D, Auto)

Post Processing - AiryScan 3D Processing

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

Split View - Panel A

The upper left features Rabbit anti-Human ATM Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the upper right panel shows Alpha Tubulin monoclonal primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). The lower left panel features DAPI nuclear staining. The lower right panel features the merged image of all channels. This split View was taken at Z-Position 72 of 142.

3D Image Processing - Panel B

3D Maximum Projection = Precise
Appearance = Threshold 2% all channels, Ramp 0% all channels, Maximum 100% all channels
Background = Black
Light = Brightness 100%, Enabled Tone Mapping
Projection = View Angle 45°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Clipping Image Process - Panel B

The Clipping plane is introduced to demonstrate the specificity of the Rabbit anti-Human ATM Recombinant Monoclonal, IgG - (BLR021N) to the nuclear compartment.

X-Y = Active was selected, and a textured opaque filter was chosen to remove the obstruction from the image-cut plane and highlight the nuclear compartment and ATM signal. A red outline of the clipped perimeter was shown, the Clip Back was selected, and the Clip Front was NOT selected.

Position of Clip = 20%
Clip Y = -0°
Clip Z =0°

Image Corrections

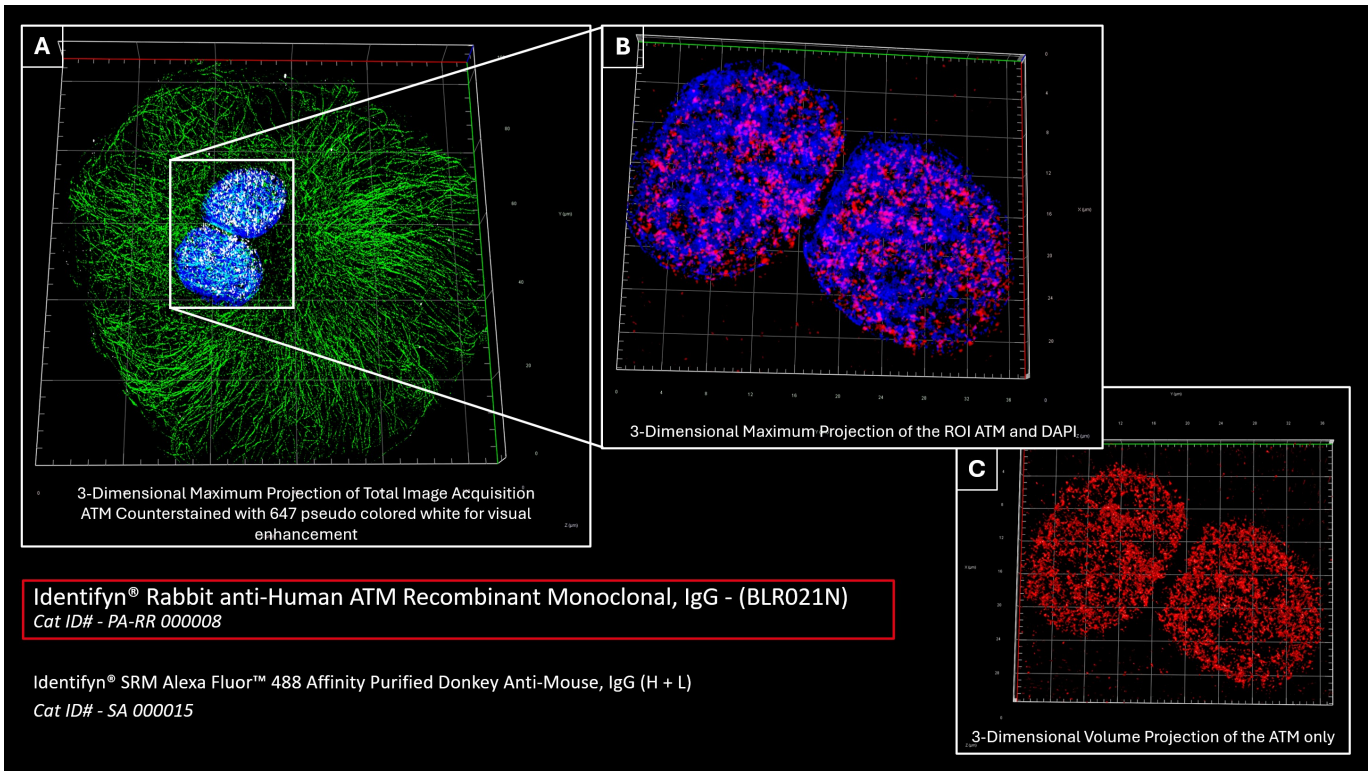
None

Image by J.K. Bennett
Image Analysis and Data Presentation B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
Stage	Airyscan
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	545CA398BE93D46F924FFB02826F5FB1AC1D3C9DA2EB0684C03528E6A8F0DF5A
Method PDF SHA-256	E95CBF81CE168CA50AD291CC90908209233C54666BF400031283361BF4FC8FE8

ORIGINAL IMAGE EVIDENCE / SIM



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

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 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	278 Slices (8.31µm) | 184 Slices (5.49µm) - The Gallery tool was used to reduce the z-stack to provide clarity
Channels	3 | 2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03000 µm
Image Size (Pixels)	5056 X 5056 | 1486 X 1775
Image Size (Scaled)	106.75µm X 106.75µm | 31.37µm X 37.47µm
Bit Depth	16 Bit
Image Center Position	X: 58.60mm, Y: 36.42mm
Stage Position	X: 58.60mm, Y: 36.42mm
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 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525
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 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @2.0% | 488nm @1.0% | 405nm @1.5%
Frame Time	1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Algorithm	SIM

FIELD	SOURCE-DOCUMENTED VALUE
SIM Settings	Standard
Auto Sharpness	On
Sharpness	9.6715 (647), 11.5549 (488), 9.7630 (DAPI)
Fitting	Fast Fit
Normalization	Automatic
3D Maximum Projection	Precise
Appearance	Threshold 2% (647), Threshold 2% (488), Threshold 2% DAPI, Ramp 0% all channels, Maximum 100% all channels | Threshold 3% (647), Threshold 4% DAPI, Ramp 0% all channels, Maximum 100% all channels | Threshold 2% (647), Ramp 98%, Maximum 100%
Background	Black
Light	Brightness 100%, Enabled Tone Mapping | Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting,
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
3D Volume Projection	Precise

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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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) - Panel A, Total Image Acquisition

Z-Stack = 278 Slices (8.31µm)

Channels = 3
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 5056 X 5056
Image Size (Scaled) = 106.75µm X 106.75µm
Bit Depth = 16 Bit
Image Center Position = X: 58.60mm, Y: 36.42mm
Stage Position = X: 58.60mm, Y: 36.42mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels B & C, Region of Interest

Z-Stack = 184 Slices (5.49µm) - The Gallery tool was used to reduce the z-stack to provide clarity

Channels = 2
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 1486 X 1775
Image Size (Scaled) = 31.37µm X 37.47µm
Bit Depth = 16 Bit
Image Center Position = X: 58.60mm, Y: 36.42mm
Stage Position = X: 58.60mm, Y: 36.42mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

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 @2.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm 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 = T2-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 = 488nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm 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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
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 = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @1.5%
Frame Time = 676.00ms
Scan Direction = Unidirectional
Grating 36.5µm grating

Post Processing - SIM Processing

Processing Dimension - 3D
Result Sampling = 4
Channels = 3
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 9.6715 (647), 11.5549 (488), 9.7630 (DAPI)
Fitting = Fast Fit
Normalization = Automatic

3D Image Processing - Panel A

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

3D Image Processing - Panel B

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

Projection = View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

3D Image Processing - Panel C

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

Image Corrections

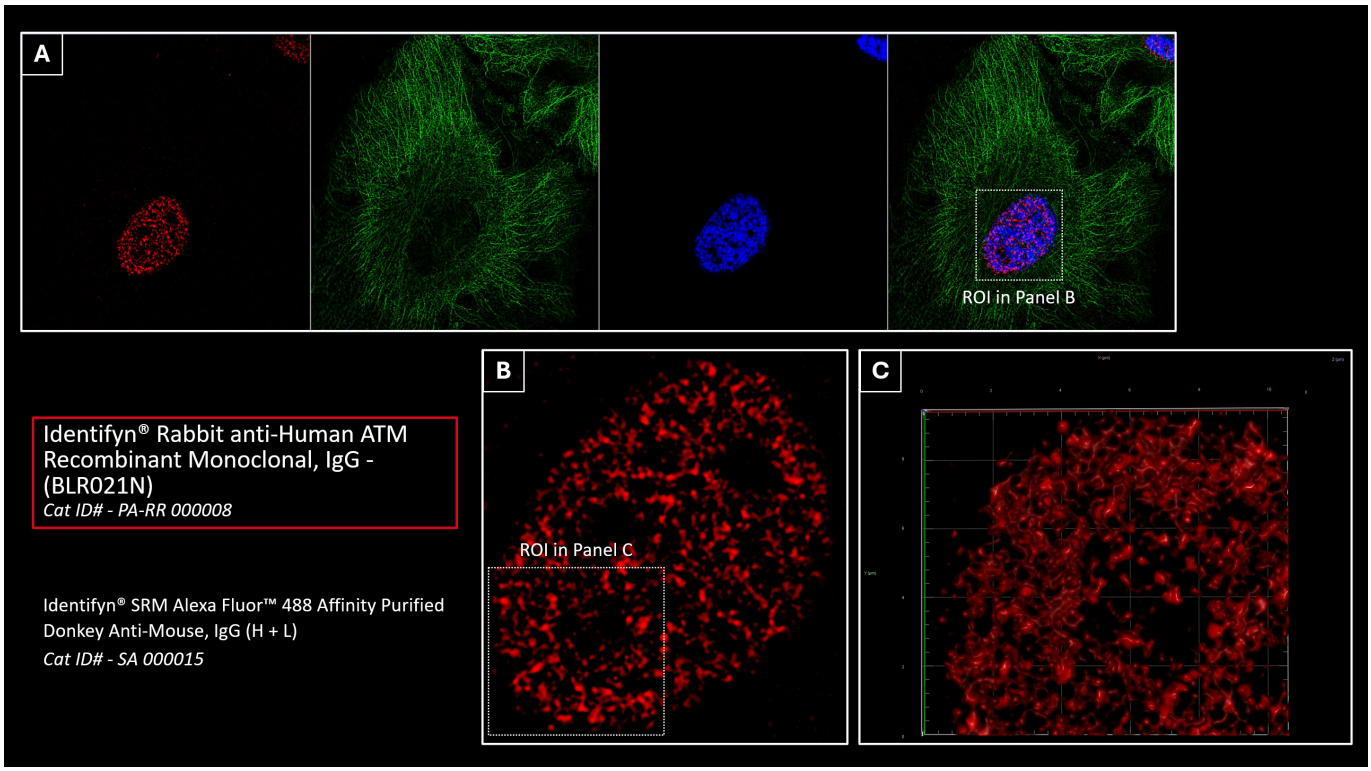
None

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
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	63
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	00F47D924F54DEB13A42728010B4198CFE9FBC852BF4C352D7E0CD44358C5856
Method PDF SHA-256	7A5B9CE0DFCF3423B5B89116030AD9CD661823D3CBE371BA67C19FB52E7D3699

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	64

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	209 Slices (8.31µm) | 115 Slices (3.42µm) - The Gallery tool was used to reduce the z-stack to provide clarity | 115 Slices (3.42µm) -
Channels	3 | 1
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.03976 µm
Image Size (Pixels)	3542 X 3672 | 1394 X 1246 | 501 X 446
Image Size (Scaled)	74.78µm X 77.53µm | 29.43µm X 26.31µm | 10.58µm X 9.42µm
Bit Depth	16 Bit
Image Center Position	X: 58.19mm, Y: 34.08mm
Stage Position	X: 58.19mm, Y: 34.08mm
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 | 405
Channel Name	T1-Axiocam 820 -SR | T2-Axiocam 820-SR | T3-Axiocam 820 #2-SR
Excitation Wavelength	640 | 488 | 405
Emission Wavelength	729 | 525
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 | Axiocam 820 #2
Camera Adapter	1X
Exposure Time	120ms | 50ms
Depth of Focus	1.13µm | 0.81µm | 0.69µm
Binning Mode	2,2
Laser Wavelength	640nm @2.0% | 488nm @1.0% | 405nm @1.5%
Frame Time	1.59s | 676.00ms
Scan Direction	Unidirectional
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 1% (647), Ramp 0%, Maximum 100%
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting,
Projection	View Angle 35°, Scale Z 10%, 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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR-000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000057

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)
Cat ID# - SA 000015

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

The second primary antibody, Alpha Tubulin Monoclonal Antibody (DM1A), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) 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) - Panel A, Total Image Acquisition (2-Dimensional Split View, Z-plane 56)

Z-Stack = 209 Slices (8.31µm)

Channels = 3
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 3542 X 3672
Image Size (Scaled) = 74.78µm X 77.53µm
Bit Depth = 16 Bit
Image Center Position = X: 58.19mm, Y: 34.08mm
Stage Position = X: 58.19mm, Y: 34.08mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels B (2-Dimensional Split view, Z-plane 56, ATM channel only, red)

Z-Stack = 115 Slices (3.42µm) - The Gallery tool was used to reduce the z-stack to provide clarity

Channels = 1
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 1394 X 1246
Image Size (Scaled) = 29.43µm X 26.31µm
Bit Depth = 16 Bit
Image Center Position = X: 58.19mm, Y: 34.08mm
Stage Position = X: 58.19mm, Y: 34.08mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

Z Stack - (3D) - Panels C (3-Dimensional Volume View, ATM channel only, red)

Z-Stack = 115 Slices (3.42µm) -

Channels = 1
Scaling (Per Pixel) = 21.11nm X 21.11nm X 30.00nm
Image Size (Pixels) = 501 X 446
Image Size (Scaled) = 10.58µm X 9.42µm
Bit Depth = 16 Bit
Image Center Position = X: 58.19mm, Y: 34.08mm
Stage Position = X: 58.19mm, Y: 34.08mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

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 @2.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm 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 = T2-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 = 488nm @1.0%
Frame Time = 1.59s
Scan Direction = Unidirectional
Grating 27.5µm 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, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 405
Channel Name = T3-Axiocam 820 #2-SR
Excitation Wavelength = 405
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 = 50ms
Depth of Focus = 0.69µm
Binning Mode = 2,2
Laser Wavelength = 405nm @1.5%
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 = 3
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

Split View - Panel A

The left panel features Rabbit anti-Human ATM Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L), the next panel (second) shows Alpha Tubulin monoclonal primary antibody counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L). The next panel (third) features DAPI nuclear staining. The right panel features the merged image of all channels. This split View was taken at Z-Position 56 of 209 (Panel A) and 56 of 115 (Panel B).

2-Dimensional View of ATM - Panel B

Demonstrates Rabbit anti-Human ATM Recombinant Monoclonal, IgG, counterstained with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) only, at z-plane 56.

3D Image Processing - Panel C

3D Volume Projection = Precise
Appearance = Threshold 1% (647), Ramp 0%, Maximum 100%
Background = Black
Light = Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting,
Enabled Tone Mapping
Projection = View Angle 35°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

Image Corrections

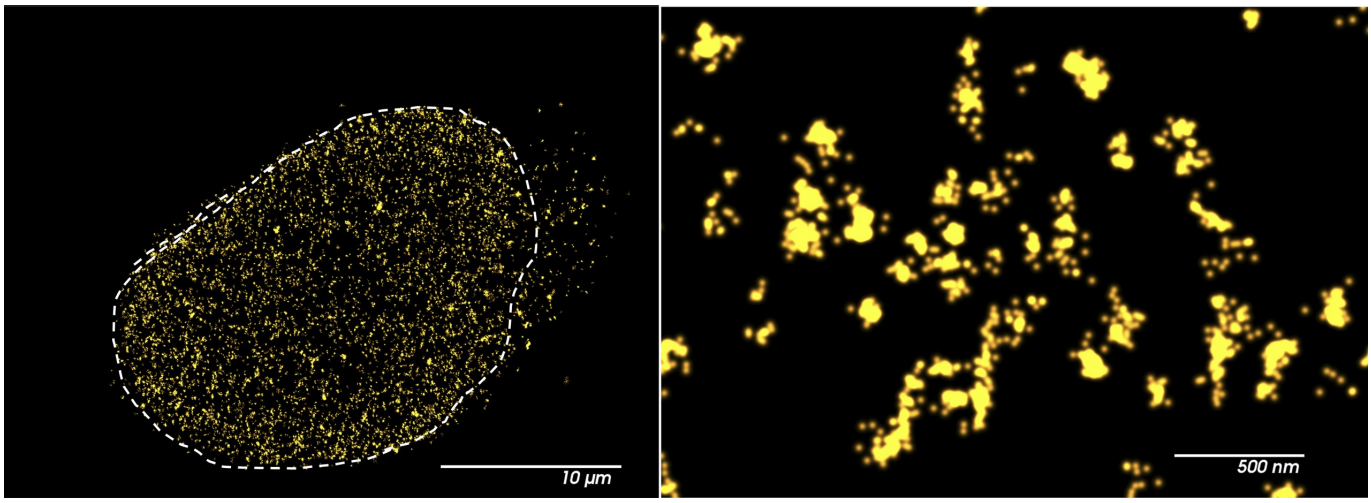
None

Image by P. Raut
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
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	64
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A5F3D7A471FF0D0E4317C9468D752FE66F66C59D0C6BF74D9E5290728E6C0EC6
Method PDF SHA-256	D8D74E787F609B0B3BD5818F4A46DB44E78559A12729CA3232A9AFB519F3F24C

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	81

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	179.1 µm

FIELD	SOURCE-DOCUMENTED VALUE
Widefield 640nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640 nm (640 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100ms
Widefield Laser	"Widefield 640nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50µm is selected
Piezo Record	Begin: 175.5; End: 175.5
SuperRes 640nm	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	7%
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: 5
Sizing Mode	Constant
Particle size	20nm
Color by	Constant
Color Table	Single
Opacity Mode	Constant; Opacity 1: 1.00
Front-to-back Attenuation	Not Selected
Method	Particle (direct)

FIELD	SOURCE-DOCUMENTED VALUE
Time Intervals	5 Intervals
Pixel Size	20nm
Smoothing	8.00
PSF Z offset	-600 to +600
Radial precision	30
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1µm (1000nm)
Maximum Particle Count to Form Cluster	10
Computer Hulls	Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Hull Alpha shape Radius	1000nm
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR 000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown in a µ-Slide 8 Well high Glass-Bottom Ibidi chamber. DNA damage was induced via treatment with 2µM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS, and Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:50. 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)
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 the Sample

Cell(s) or Cellular Structure Localization - The red-bordered box identifies the area of the SuperRes view and defines a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.
Piezo Current: 179.1 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 (640 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: 175.5; End: 175.5

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: 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 640 nm (640 nm Dichroic)
Laser Power: 7%

Expert Configuration Options - The Localization Tab
Beyond the values shown, all other parameters were set to their 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: 5

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: Constant
Particle size: 20nm
Color by: Constant
Color Table: Single
Opacity Mode: Constant; Opacity 1: 1.00
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: -600 to +600
Radial precision: 30
Axial precision: 70
The other parameters were not applied.

Statistical analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

Maximum Particle Distance: 0.1µm (1000nm)
Maximum Particle Count to Form Cluster: 10
Computer Hulls: Selected
Compute Density Isosurfaces: Not Selected
Edit Settings: All Probes
Hull Alpha shape Radius: 1000nm
Compute: Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned: Deselected

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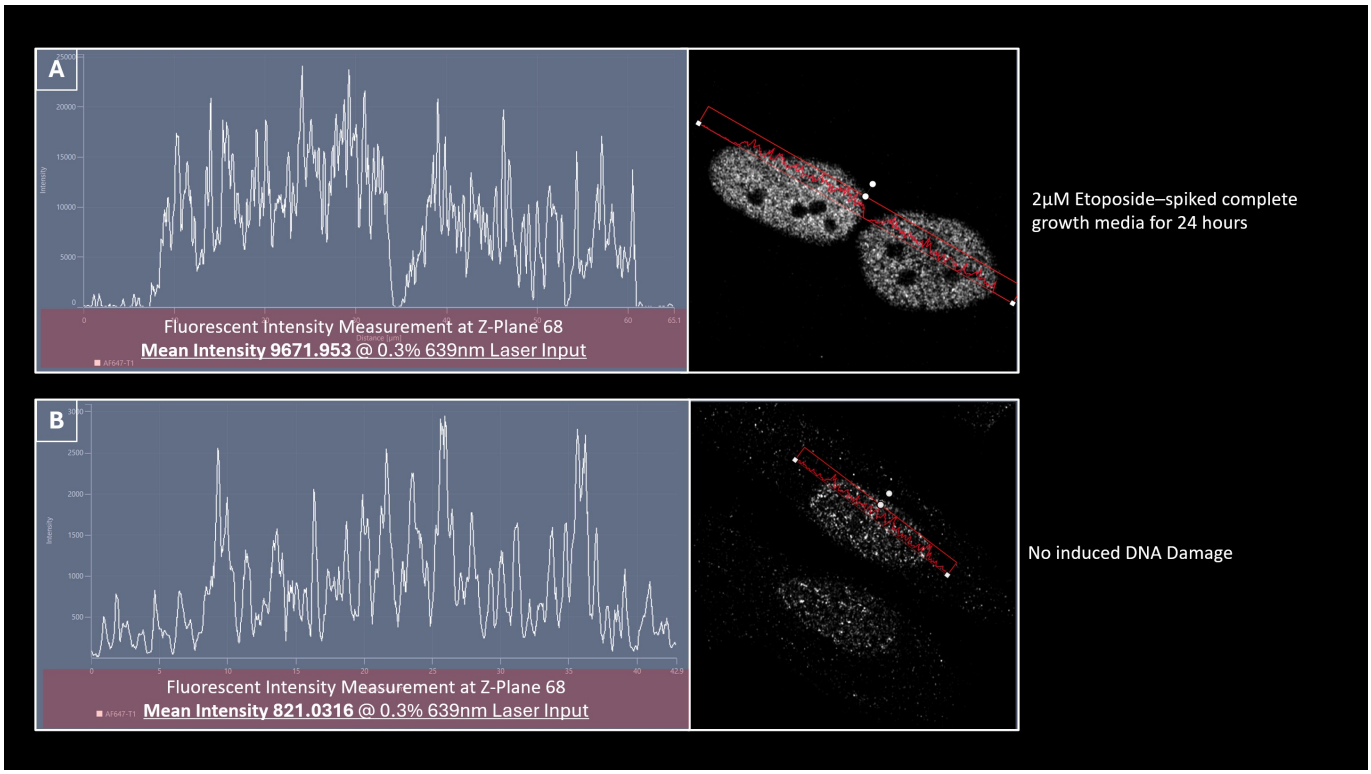
Image by S. Thakur

SOURCE PROVENANCE

Catalog	PA-RR-000008
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	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	6F9E282988AB7D33D7C03E3CCC937B495815DC8355EB3023F315FB7EB2415C2D
Method PDF SHA-256	9D15AE75C5E4ACABEABCFBC288F38E96BB1AADEBFA4CDB45271EDD832B93F2AA

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	35

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 LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63X/1.40 oil DIC M27
Channels	3
Scaling (Per Pixel)	0.059µm X 0.059µm
Image size (Pixels)	1017 X 1017
Image Size (Scaled)	59.81 µm X 59.81 µm
Bit Depth	16 Bit
Image Center Position	X: 88.54 mm, Y: 47.92 mm
Stage Position	X: 88.54 mm, Y: 47.92 mm
ROI Center Offset	X: 0.00µm, Y: 0.00µm
Reflector	None
Pinhole	1.00 AU / 66µm
LMS MBS	MBS 488/639 MBS 405/730
LMS SBS	SBS SP 615
Laser Wavelength	639nm @0.3%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.2
Rotation	0°
Sampling	1.20
Pixel Time	0.33µs
Frame Time	3.36s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	636 - 732
Imaging Device	GaAsP-PMT3
Detector Type	GaAsp-PMT
Detector Gain	500 V
Detector Offset	0

FIELD	SOURCE-DOCUMENTED VALUE
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.1 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 65.1), Y (Min 13 and Max 25263) | Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 42.9), Y (Min 4 and Max 3092)

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 ATM Recombinant Monoclonal, IgG - (BLR021N)
Cat ID# - PA-RR 000008

Identifyn® Secondary Antibodies

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, F(ab')□ (H + L)
Cat ID# - SA 000063

ATM (ataxia-telangiectasia mutated) plays a crucial role in the homologous recombination (HR) pathway, an essential mechanism in the repair of DNA double-strand breaks (DSBs).

ATM is a serine/threonine protein kinase activated in response to DSBs. Once activated, ATM phosphorylates numerous downstream targets, including proteins in DNA repair pathways such as HR. In the context of HR, ATM promotes the repair process by phosphorylating key proteins involved in DNA repair, such as BRCA1 (Breast Cancer 1) and RAD51.

BRCA1 is known to facilitate the loading of RAD51 onto single-stranded DNA at the site of DSBs, which is a crucial step in the HR pathway. ATM-mediated phosphorylation of BRCA1 facilitates the recruitment and stabilization of RAD51 at DNA damage sites, thereby enhancing the HR repair process. ATM also plays a critical role in cell cycle checkpoint control, leading to the initiation of cell cycle checkpoints, which temporarily halt cell cycle progression to allow time for DNA repair. Additionally, ATM activation can trigger the apoptotic cell death pathway, eliminating severely damaged DNA and reducing the propagation of genetic abnormalities.

Overall, ATM serves as a critical guardian of genome integrity, ensuring the maintenance of genomic stability and preventing the accumulation of mutations that could lead to various diseases, including cancer.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2μM 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 ATM Recombinant Monoclonal, IgG- (BLR021N), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® 647 secondary antibody was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI cat#P36962.

Microscope

Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:

2- Dimension Image - Panels A & B (note settings and image sizes were matched for the control to the data from the confocal within this data set)

Channels = 3
Scaling (Per Pixel) = 0.059μm X 0.059μm
Image size (Pixels) = 1017 X 1017

Image Size (Scaled) = 59.81 µm X 59.81 µm
Bit Depth = 16 Bit
Image Center Position = X: 88.54 mm, Y: 47.92 mm
Stage Position = X: 88.54 mm, Y: 47.92 mm
ROI Center Offset = X: 0.00µm, Y: 0.00µm

647 -

Reflector = None
Contrast Method - Fluorescence
Pinhole = 1.00 AU / 66µm
LMS MBS = MBS 488/639 MBS 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @0.3%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.2
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.33µs
Frame Time = 3.36s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging - 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 636 - 732
Imaging Device = GaAsP-PMT3
Detector Type = GaAsp-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.1 (3D, Auto)

Profile View Display - Panel A

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 65.1), Y (Min 13 and Max 25263)

The average fluorescent intensity across the two nuclei measured was 9671.953, deploying the 639nm laser at an input power of 0.3%

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1 Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 42.9), Y (Min 4 and Max 3092)

The average fluorescent intensity across the two nuclei measured was 821.0316, deploying the 639nm laser at an input power of 0.3%

Summary -

We note that when using the same settings in the presence of damage (panel A) and in the absence of induced DNA damage, we observe a ~10-fold decrease in the ATM signal. We also note this is not a modified version of ATM, and in such the increase in total volume of protein as measured by the fluorescent intensity, reveals the actrivation and recruitment of ATM in response to the damage, but does not explicitly show the later version of the ATM response which would be foci aggregates at breaks sites, as this antibody recognizes the antive form. An increase in total protein is expected.;

Image Corrections

None

Image by J.K. Bennett
Image Analysis and Data Presentation B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000008
Stage	Control
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63X/1.40 oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	35
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	84DC04197388472DBA17AD9D70F7612C2D29F5C797C5C32B053EAB8E657CCC86
Method PDF SHA-256	83ECEE3D33F81425007A51F4310220824351AF6A2A38DCA214BEE64BDFF2B801