BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

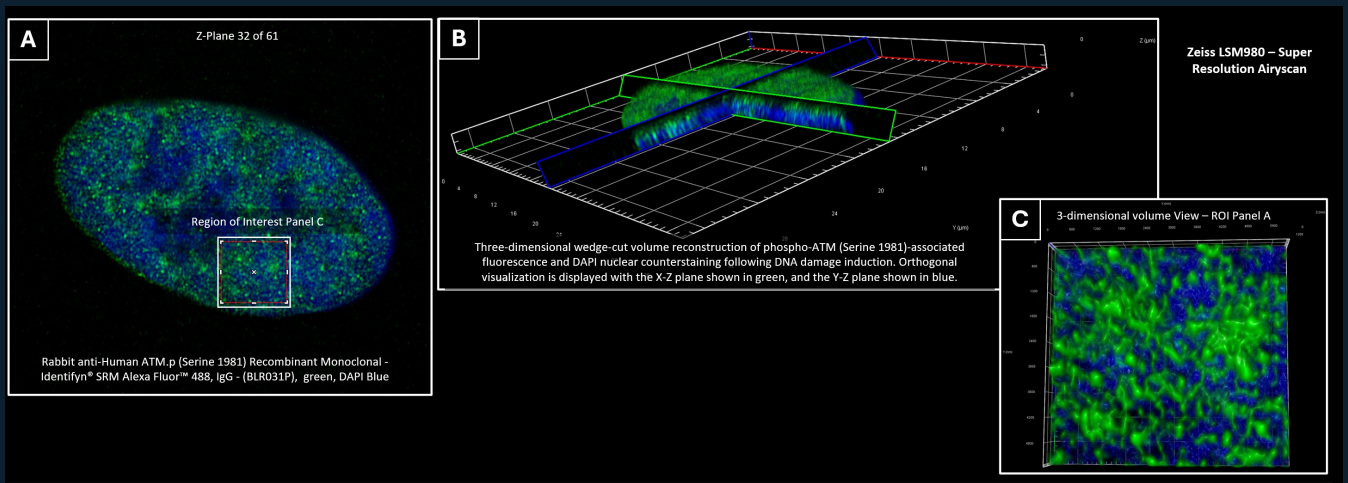
ATM.P

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN

4	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired at z-plane 32 of 61 following DNA damage induction. The phospho-ATM-associated 488 signal is displayed in green, while DAPI nuclear counterstaining is displayed in blue. A white boxed region of interest identifies a subnuclear area selected for downstream volumetric analysis in Panel C. Strong nuclear-associated localization and punctate ATM.p-associated fluorescence organization were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - (BLR031P) (Cat ID# - PA-RR 000012) stepdown dataset, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents a three-dimensional wedge-cut Airyscan volume reconstruction demonstrating the organization of intranuclear phospho-ATM-associated fluorescence following DNA damage induction. Orthogonal wedge-cut visualization is displayed with the X-Z plane shown in green and the X-Y plane shown in red to facilitate assessment of volumetric fluorescence distribution and intranuclear signal intensity throughout the sampled nuclear volume. The reconstruction further demonstrates preservation of volumetric ATM.p-associated fluorescence organization under super-resolution imaging conditions following direct conjugation.

Panel C presents a three-dimensional Airyscan volume projection of the region of interest identified in Panel A, displaying both the ATM.p-associated 488 signal and DAPI nuclear counterstaining throughout the sampled volumetric dataset. The super-resolution morphology, fluorescence intensity, and intranuclear ATM.p-associated signal organization remained highly consistent with the corresponding unconjugated ATM.p dataset, further supporting preservation of fluorescence integrity, biologically coherent localization behavior, and robust super-resolution imaging performance following direct labeling with Identifyn® SRM Alexa Fluor™ 488.

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / AIRYSCAN

DC-000078 | 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
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for ATM.p, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Confocal -> Airyscan

BENNETT ASSESSMENT

The preserved DC-000078 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

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 / PARTIAL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved DC-000078 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 4 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

No separate control record is preserved; none is inferred from method or template language.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	488	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488	2; 38 eGFP; 49 DAPI; 450 - 490; 335 - 383; 500 - 550; 420 - 470; 493
Confocal	405/DAPI; 488	2; None; 488 nm @ 0.9%; 405 nm @ 0.4%; 493; 353; 517; 465
Airyscan	405/DAPI; 488	2; None; 488 nm @ 1.0%; 405 nm @ 0.5%; 493; 353; 517; 465

MODALITY ASSESSMENT / PART 1 OF 4

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 Imaging.. 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 4

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, 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): 578 X 498; Image Size (Pixels): 578 X 498; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 30 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 30.00%; X-Cite Xylis Lamp Intensity @ 15.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 40.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 4

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: 81 Slices (4.5 µm); Channels: 2; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 1248 X 1248; Image Size (Pixels): 1248 X 1248; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.27 µs; Frame Time: 4.10 s. Illumination: Laser Wavelength: 488 nm @ 0.9%; 405 nm @ 0.4%. Optical/scan: Pinhole: 1.00 AU / 51 µm; 1.00 AU / 44 µm; Scan Zoom: 1.8; LSM Scan Speed: 12; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.2 (3D, Auto); Filter: 6.4 (3D, Auto). Structured source metadata values: 46.

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.

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 4 OF 4

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: 61 Slices (1.80 µm); Channels: 2; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1072 X 951; 164 X 152; Image Size (Pixels): 1072 X 951; 164 X 152; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.10 µs; Frame Time: 18.77 s. Illumination: Laser Wavelength: 488 nm @ 1.0%; 405 nm @ 0.5%. Optical/scan: Pinhole: 5.00 AU / 250 µm; 5.00 AU / 214 µm; Scan Zoom: 2.0; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black; AiryScan Mode: Super Resolution: 9.0 (3D, Auto); Super Resolution: 8.6 (3D, Auto). Structured source metadata values: 58.

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.

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.

CROSS-MODALITY SYNTHESIS

The preserved DC-000078 record contains Western blot; Widefield; Confocal; Airyscan. Missing modalities are not represented or inferred.

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): 35.30 nm X 35.30 nm X 30.00 nm -> 0.03530 µm X 0.03530 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)=1072 X 951; Image Size (Scaled)=37.84 µm X 33.57 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - SOURCE-DERIVED METADATA CANONICALIZATION

Product identity / phosphosite: Blank structured canonical field -> Serine 1981. The phosphorylation site is explicitly stated in the preserved source product identity. Supporting evidence: Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488

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. No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

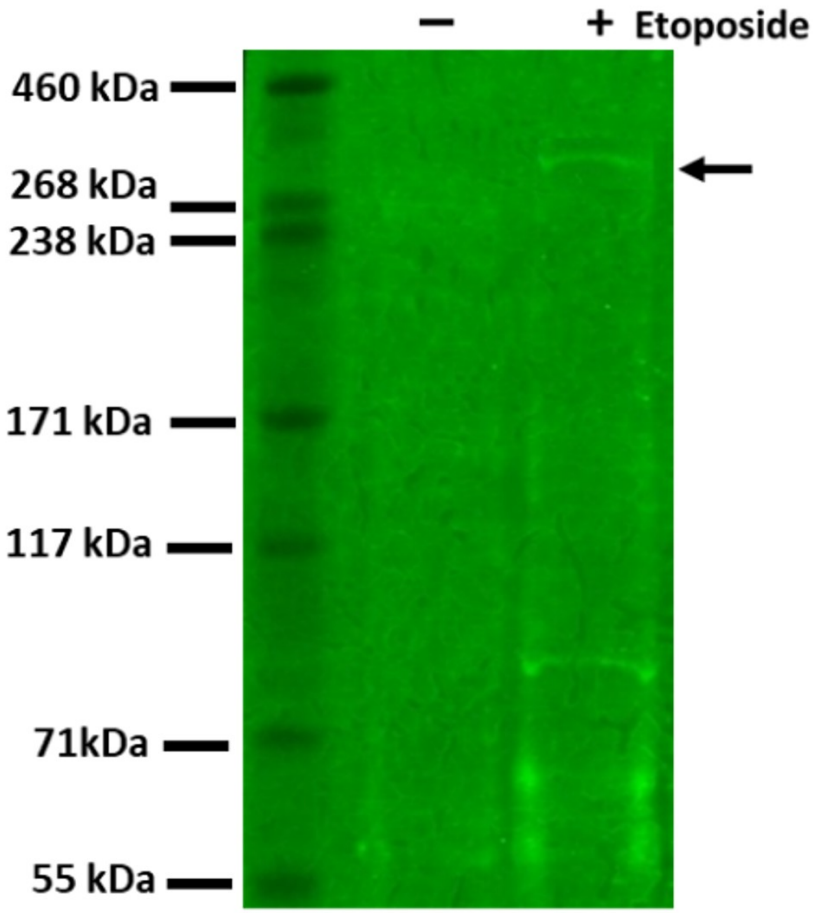
This dossier preserves the complete available evidence progression for DC-000078. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN TIER	EVIDENCE STAGE	INSTRUMENT	STATUS
1	Western blot	Azure Sapphire FL	PRESERVED
2	Widefield	ZEISS Axio Imager.M1	PRESERVED
4	Confocal	ZEISS LSM 980	PRESERVED
5	Airyscan	ZEISS LSM 980	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 Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	488 nm
Emission Filter	513 ± 17 nm
Detector	PMT
Intensity	L3
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	S. Khanal

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.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

Expected MW: 350 kDa

HeLa cells were treated with 2 µM etoposide for 12 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and a nuclear lysis buffer, 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 on NuPAGE™ Tris-Acetate Mini Protein Gels (3-8%, 1.0 mm) in a Mini Gel Tank. Thermo Fisher™ HiMark™ Pre-stained 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 1X PBS. The blocked membrane was incubated with Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) for 20 hours, followed by five washes in 1X 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 Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 488 nm
Emission Filter = 513 ± 17 nm
Detector = PMT
Intensity = L3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

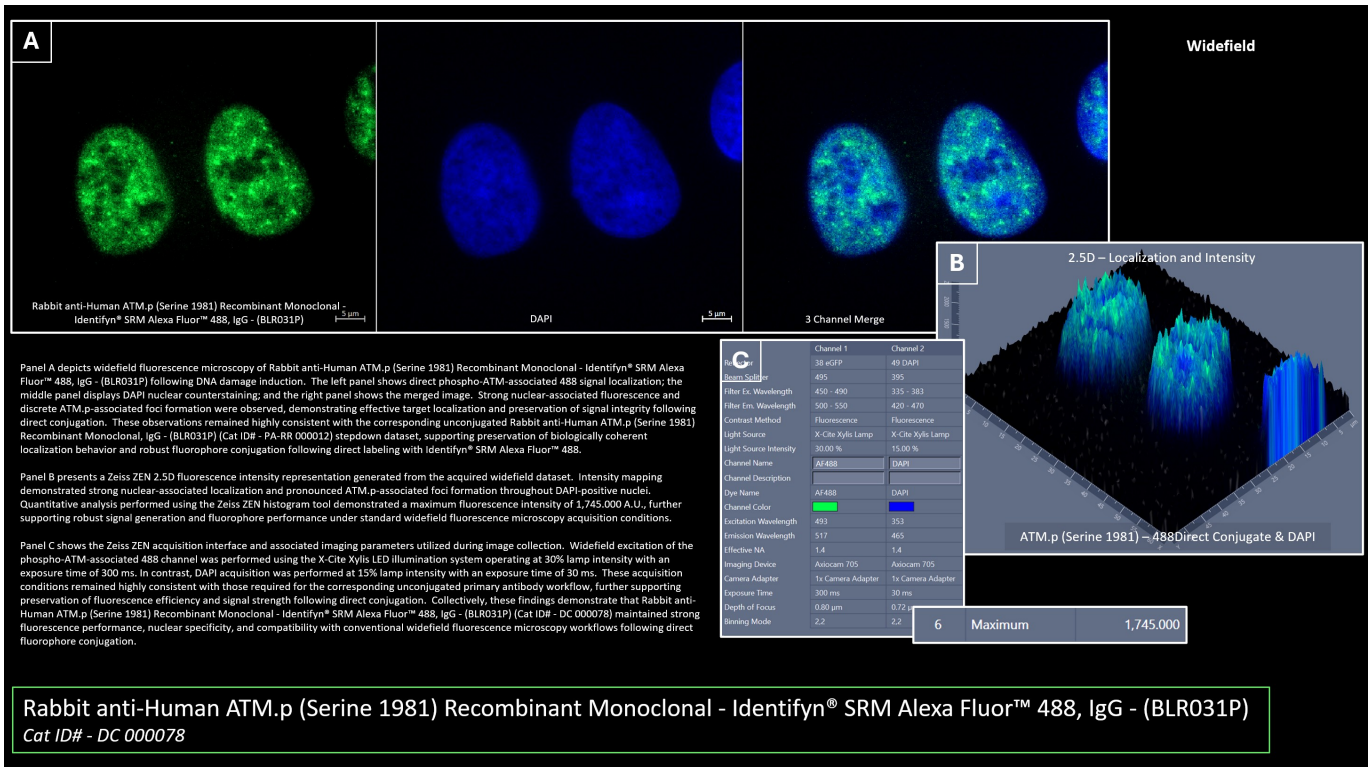
NONE

Image: S. Khanal

SOURCE PROVENANCE

Catalog	DC-000078
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	DAD06E7534BBAFFEF64BB5BF570A3A26BDAF6861C29C8E108F50F928E80F03B6
Method PDF SHA-256	7CB5429D48DCB5D726BF60C40FB4CC247EA4C9F728AD43248CC074DB436E7572

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	40

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, 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)	578 X 498
Image Size (Scaled)	63.30 µm X 54.54 µm
Bit Depth	14 Bit
Image Center Position	X: 0.00 µm, Y: 0.00 µm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	38 eGFP | 49 DAPI
Beam Splitter	495 | 395
Filter Excitation Wavelength	450 - 490 | 335 - 383
Filter Emission Wavelength	500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light Source	X-Cite Xylis Lamp Intensity @ 30.00% | X-Cite Xylis Lamp Intensity @ 15.00%
Exposure Time	300 ms | 30 ms
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X | 1x Camera Adapter
Depth of Focus	0.80 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	45
Angle Y	-45
Z Scaling	1.0
Ambient	50
Specular	20
Shininess	50
Light Height	2.0

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.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

ATM (ataxia-telangiectasia mutated) is a crucial serine/threonine kinase that plays a central role in the DNA damage response (DDR), particularly in response to DNA double-strand breaks (DSBs). ATM's activation and subsequent phosphorylation cascades are essential for detecting DNA damage and initiating repair processes. Here's a detailed overview of how ATM is activated, its targets, and its role in the DDR:

Activation of ATM
In response to DNA damage, particularly DSBs, ATM is activated through a series of events:

Detection of DNA Breaks: ATM can sense DNA breaks by associating with the MRN complex (MRE11, RAD50, and NBS1). This complex recognizes DSBs, leading to ATM's recruitment to the damaged site.

Autophosphorylation: Upon detection of DNA damage, ATM undergoes autophosphorylation at serine 1981 (S1981), leading to its activation. This phosphorylation event is critical for ATM's kinase activity and its ability to phosphorylate downstream targets.

Dimer-to-Monomer Transition: In its inactive form, ATM is a dimer. It transitions to an active monomeric state upon activation, allowing it to interact with and phosphorylate various downstream proteins.

Phosphorylation Targets of ATM

Once activated, ATM phosphorylates many proteins involved in the DNA damage response, cell cycle regulation, and apoptosis. Here are some critical targets of ATM:

CHK2: ATM phosphorylates CHK2, a cell cycle checkpoint control kinase. This phosphorylation helps to halt the cell cycle, allowing time for DNA repair.

p53: ATM phosphorylates p53, activating this tumor suppressor, leading to cell cycle arrest or apoptosis if the damage is irreparable.

H2AX: ATM phosphorylates histone H2AX, forming γ-H2AX, a marker of DNA damage. This modification helps signal the presence of DSBs and recruit DNA repair machinery.

BRCA1: ATM's phosphorylation of BRCA1 is critical for homologous recombination, a key DNA repair pathway for DSBs.

NBS1: As part of the MRN complex, NBS1 phosphorylation by ATM enhances the complex's DNA repair functions.

53BP1: ATM phosphorylation of 53BP1 helps recruit this protein to sites of DSBs, guiding repair pathways like non-homologous end joining (NHEJ).

Role of ATM Phosphorylation in DNA Repair

ATM-mediated phosphorylation plays a central role in coordinating the DNA damage response:

Signal Propagation: Phosphorylation cascades initiated by ATM amplify the signal for DNA damage, ensuring an efficient response.

Recruitment of Repair Machinery: Phosphorylation of specific proteins helps recruit DNA repair complexes to the sites of DNA damage.

Cell Cycle Regulation: ATM's activation can lead to cell cycle checkpoints, preventing cells from dividing with damaged DNA.

Apoptosis: If the DNA damage is too severe to repair, ATM's activation can lead to apoptosis, preventing the propagation of damaged cells

ATM's phosphorylation in response to DNA damage is crucial for detecting DNA double-strand breaks and coordinating the DNA damage response. By phosphorylating a range of critical proteins, ATM orchestrates a complex network of signaling events that facilitate DNA repair, cell cycle regulation, and apoptosis, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

Microscope

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

Single Plane (2D)

Channels = 2
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image Size (Pixels) = 578 X 498
Image Size (Scaled) = 63.30 µm X 54.54 µm
Bit Depth = 14 Bit
Image Center Position = X: 0.00 µm, Y: 0.00 µm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = 38 eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light Source = X-Cite Xylis Lamp Intensity @ 30.00%
Exposure Time = 300 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1X
Depth of Focus = 0.80 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 @ 15.00%
Exposure Time = 30 ms
Excitation Wavelength = 353
Emission Wavelength = 465
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x Camera Adapter
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The left panel shows Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear localization and discrete ATM.p-associated foci formation following DNA damage induction, supporting robust conjugation performance and preservation of target-specific fluorescence signal under widefield fluorescence microscopy conditions.

2.5D Image Processing - Panel B

2.5D Display

Render Mode = Surface
Grid distance = 5
Palette and Show Faces at Side are both Selected

2.5D Display Options

Angle X = 45
Angle Y = -45
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition interface for the Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) widefield dataset collected using the X-Cite Xylis Lamp and Zeiss Axiocam 705 camera. Imaging of the phospho-ATM-associated 488 channel was performed using 30% X-Cite Xylis Lamp intensity with a 300 ms exposure time on the Axiocam 705, while DAPI acquisition was performed using 15% lamp intensity with a 30 ms exposure time. These acquisition conditions demonstrated robust fluorescence signal generation and strong nuclear-associated ATM.p localization under standard widefield excitation and collection settings. The ability to generate discrete ATM.p-associated foci and strong target-specific fluorescence signal under these moderate acquisition parameters further supports the robustness of direct conjugation and preservation of biologically coherent localization behavior following labeling with Identifyn® SRM Alexa Fluor™ 488.

ZEN Acquisition Histogram Data - Panel C

Shown is the maximum fluorescence intensity measurement obtained from the Zeiss ZEN Histogram tool for the Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) direct conjugate, demonstrating a peak fluorescence intensity of 1,745.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield fluorescence microscopy acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) following DNA damage induction. The left panel shows direct phospho-ATM-associated 488 signal localization; the middle panel displays DAPI nuclear counterstaining; and the right panel shows the merged image. Strong nuclear-associated fluorescence and discrete ATM.p-associated foci formation were observed, demonstrating effective target localization and preservation of signal integrity following direct conjugation. These observations remained highly consistent with the corresponding unconjugated Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - (BLR031P) (Cat ID# - PA-RR 000012) stepdown dataset, supporting preservation of biologically coherent localization behavior and robust fluorophore conjugation following direct labeling with Identifyn® SRM Alexa Fluor™ 488.

Panel B presents a Zeiss ZEN 2.5D fluorescence intensity representation generated from the acquired widefield dataset. Intensity mapping demonstrated strong nuclear-associated localization and pronounced ATM.p-associated foci formation throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 1,745.000 A.U., further supporting robust signal generation and fluorophore performance under standard widefield fluorescence microscopy acquisition conditions.

Panel C shows the Zeiss ZEN acquisition interface and associated imaging parameters utilized during image collection. Widefield excitation of the phospho-ATM-associated 488 channel was performed using the X-Cite Xylis LED illumination system operating at 30% lamp intensity with an exposure time of 300 ms, while DAPI acquisition was performed at 15% lamp intensity with an exposure time of 30 ms. These acquisition conditions remained highly consistent with those required for the corresponding unconjugated primary antibody workflow, further supporting

preservation of fluorescence efficiency and signal strength following direct conjugation. Collectively, these findings demonstrate that Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) (Cat ID# - DC 000078) maintained strong fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

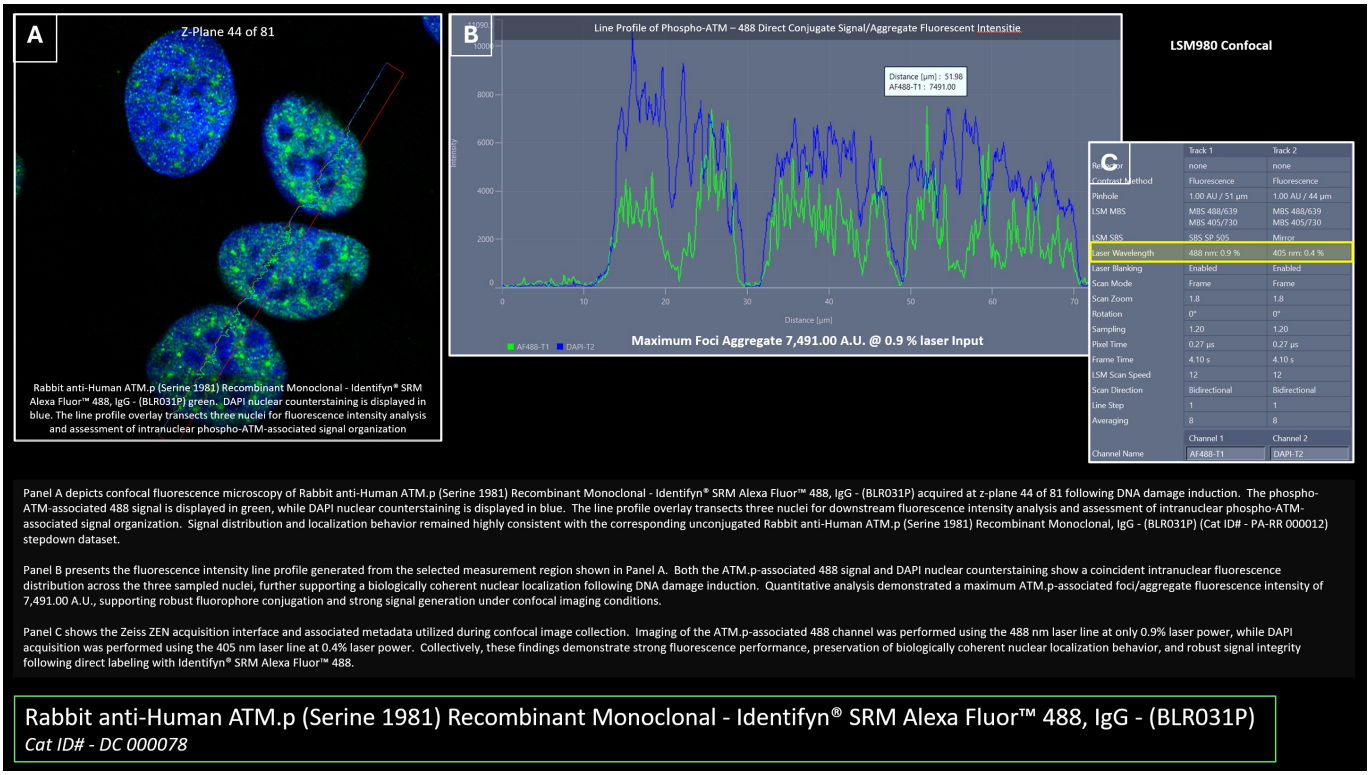
NONE

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

SOURCE PROVENANCE

Catalog	DC-000078
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, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Structured source metadata values	40
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F08304793EE92D9FE60EF8FAAFD0F2B41FF0E7F31E97E17514BD7BA9824D0069
Method PDF SHA-256	EF97607B15671D19F7FB4431D9C22C3E51F9C15CD7BAAC5A301C8B068E653AEE

ORIGINAL IMAGE EVIDENCE / CONFOCAL



Panel A depicts confocal fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired at z-plane 44 of 81 following DNA damage induction. The phospho-ATM-associated 488 signal is displayed in green, while DAPI nuclear counterstaining is displayed in blue. The line profile overlay transects three nuclei for downstream fluorescence intensity analysis and assessment of intranuclear phospho-ATM-associated signal organization. Signal distribution and localization behavior remained highly consistent with the corresponding unconjugated Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - (BLR031P) (Cat ID# - PA-RR 000012) stepdown dataset.

Panel B presents the fluorescence intensity line profile generated from the selected measurement region shown in Panel A. Both the ATM.p-associated 488 signal and DAPI nuclear counterstaining show a coincident intranuclear fluorescence distribution across the three sampled nuclei, further supporting a biologically coherent nuclear localization following DNA damage induction. Quantitative analysis demonstrated a maximum ATM.p-associated foci/aggregate fluorescence intensity of 7,491.00 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal imaging conditions.

Panel C shows the Zeiss ZEN acquisition interface and associated metadata utilized during confocal image collection. Imaging of the ATM.p-associated 488 channel was performed using the 488 nm laser line at only 0.9% laser power, while DAPI acquisition was performed using the 405 nm laser line at 0.4% laser power. Collectively, these findings demonstrate strong fluorescence performance, preservation of biologically coherent nuclear localization behavior, and robust signal integrity following direct labeling with Identifyn® SRM Alexa Fluor™ 488.

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	46

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	81 Slices (4.5 µm)
Channels	2
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	1248 X 1248
Image Size (Scaled)	73.41 µm X 73.41 µm
Bit Depth	16 Bit
Image Center Position	X: 92.13 mm, Y: 53.62 mm
Stage Position	X: 92.13 mm, Y: 53.62 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 51 µm | 1.00 AU / 44 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 505 | Mirror
Laser Wavelength	488 nm @ 0.9% | 405 nm @ 0.4%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.8
Rotation	0°
Sampling	1.20
Pixel Time	0.27 µs
Frame Time	4.10 s
LSM Scan Speed	12
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	511 - 550 | 430 - 490
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.2 (3D, Auto) | Filter: 6.4 (3D, Auto)
Profile Definition	Stroke Thickness 1.0, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 75.0), Y (Min 0 and Max 11090)

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.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

ATM (ataxia-telangiectasia mutated) is a crucial serine/threonine kinase that plays a central role in the DNA damage response (DDR), particularly in response to DNA double-strand breaks (DSBs). ATM's activation and subsequent phosphorylation cascades are essential for detecting DNA damage and initiating repair processes. Here's a detailed overview of how ATM is activated, its targets, and its role in the DDR:

Activation of ATM
In response to DNA damage, particularly DSBs, ATM is activated through a series of events:

Detection of DNA Breaks: ATM can sense DNA breaks by associating with the MRN complex (MRE11, RAD50, and NBS1). This complex recognizes DSBs, leading to ATM's recruitment to the damaged site.

Autophosphorylation: Upon detection of DNA damage, ATM undergoes autophosphorylation at serine 1981 (S1981), leading to its activation. This phosphorylation event is critical for ATM's kinase activity and its ability to phosphorylate downstream targets.

Dimer-to-Monomer Transition: In its inactive form, ATM is a dimer. It transitions to an active monomeric state upon activation, allowing it to interact with and phosphorylate various downstream proteins.

Phosphorylation Targets of ATM

Once activated, ATM phosphorylates many proteins involved in the DNA damage response, cell cycle regulation, and apoptosis. Here are some critical targets of ATM:

CHK2: ATM phosphorylates CHK2, a cell cycle checkpoint control kinase. This phosphorylation helps to halt the cell cycle, allowing time for DNA repair.

p53: ATM phosphorylates p53, activating this tumor suppressor, leading to cell cycle arrest or apoptosis if the damage is irreparable.

H2AX: ATM phosphorylates histone H2AX, forming γ-H2AX, a marker of DNA damage. This modification helps signal the presence of DSBs and recruit DNA repair machinery.

BRCA1: ATM's phosphorylation of BRCA1 is critical for homologous recombination, a key DNA repair pathway for DSBs.

NBS1: As part of the MRN complex, NBS1 phosphorylation by ATM enhances the complex's DNA repair functions.

53BP1: ATM phosphorylation of 53BP1 helps recruit this protein to sites of DSBs, guiding repair pathways like non-homologous end joining (NHEJ).

Role of ATM Phosphorylation in DNA Repair

ATM-mediated phosphorylation plays a central role in coordinating the DNA damage response:

Signal Propagation: Phosphorylation cascades initiated by ATM amplify the signal for DNA damage, ensuring an efficient response.

Recruitment of Repair Machinery: Phosphorylation of specific proteins helps recruit DNA repair complexes to the sites of DNA damage.

Cell Cycle Regulation: ATM's activation can lead to cell cycle checkpoints, preventing cells from dividing with damaged DNA.

Apoptosis: If the DNA damage is too severe to repair, ATM's activation can lead to apoptosis, preventing the propagation of damaged cells

ATM's phosphorylation in response to DNA damage is crucial for detecting DNA double-strand breaks and coordinating the DNA damage response. By phosphorylating a range of critical proteins, ATM orchestrates a complex network of signaling events that facilitate DNA repair, cell cycle regulation, and apoptosis, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

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 and B, Z-plane 44 of 81

Z-Stack = 81 Slices (4.5 µm)

Channels = 2
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 1248 X 1248
Image Size (Scaled) = 73.41 µm X 73.41 µm
Bit Depth = 16 Bit
Image Center Position = X: 92.13 mm, Y: 53.62 mm
Stage Position = X: 92.13 mm, Y: 53.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 51 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488 nm @ 0.9%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27 µs
Frame Time = 4.10 s
LSM Scan Speed = 12
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 550
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 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 / 44 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 405 nm @ 0.4%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.8
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.27 µs
Frame Time = 4.10 s
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 - 490
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.4 (3D, Auto)

2-Dimensional View - Panel A

Panel A depicts Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired on the Zeiss LSM 980 Confocal microscope at z-plane 44 of 81 following DNA damage induction. The primary image displays the phospho-ATM-associated 488 signal in green alongside DAPI nuclear counterstaining displayed in blue. A line profile overlay transects three nuclei for downstream fluorescence intensity analysis, demonstrating strong nuclear-associated localization, discrete ATM.p-associated foci formation, and robust signal generation following direct conjugation.

Profile View Display - Panel B

Profile Definition = Stroke Thickness 1.0, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 75.0), Y (Min 0 and Max 11090)

ZEN Acquisition Info Tab - Panel C

Shown are the acquisition settings for the phospho-ATM-associated 488 direct conjugate and DAPI channels. Imaging of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) was performed using the 488 nm laser line at 0.9% laser power, while DAPI acquisition was performed using the 405 nm laser line at 0.4%, demonstrating that the observed fluorescence signal and nuclear-associated localization were not artificially generated through excessive excitation conditions.

Summary

Panel A depicts confocal fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired at z-plane 44 of 81 following DNA damage induction. The phospho-ATM-associated 488 signal is displayed in green, while DAPI nuclear counterstaining is displayed in blue. The line profile overlay transects three nuclei for downstream fluorescence intensity analysis and assessment of intranuclear phospho-ATM-associated signal organization. Signal distribution and localization behavior remained highly consistent with the corresponding unconjugated Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - (BLR031P) (Cat ID# - PA-RR 000012) stepdown dataset.

Panel B presents the fluorescence intensity line profile generated from the selected measurement region shown in Panel A. Both the ATM.p-associated 488 signal and DAPI nuclear counterstaining show a coincident intranuclear fluorescence distribution across the three sampled nuclei, further supporting a biologically coherent nuclear localization following DNA damage induction. Quantitative analysis demonstrated a maximum ATM.p-associated foci/aggregate fluorescence intensity of 7,491.00 A.U., supporting robust fluorophore conjugation and strong signal generation under confocal imaging conditions.

Panel C shows the Zeiss ZEN acquisition interface and associated metadata utilized during confocal image collection. Imaging of the ATM.p-associated 488 channel was performed using the 488 nm laser line at only 0.9% laser power, while DAPI acquisition was performed using the 405 nm laser line at 0.4% laser power. Collectively, these findings demonstrate strong fluorescence performance, preservation of biologically coherent nuclear localization behavior, and robust signal integrity following direct labeling with Identifyn® SRM Alexa Fluor™ 488.

Image Correction

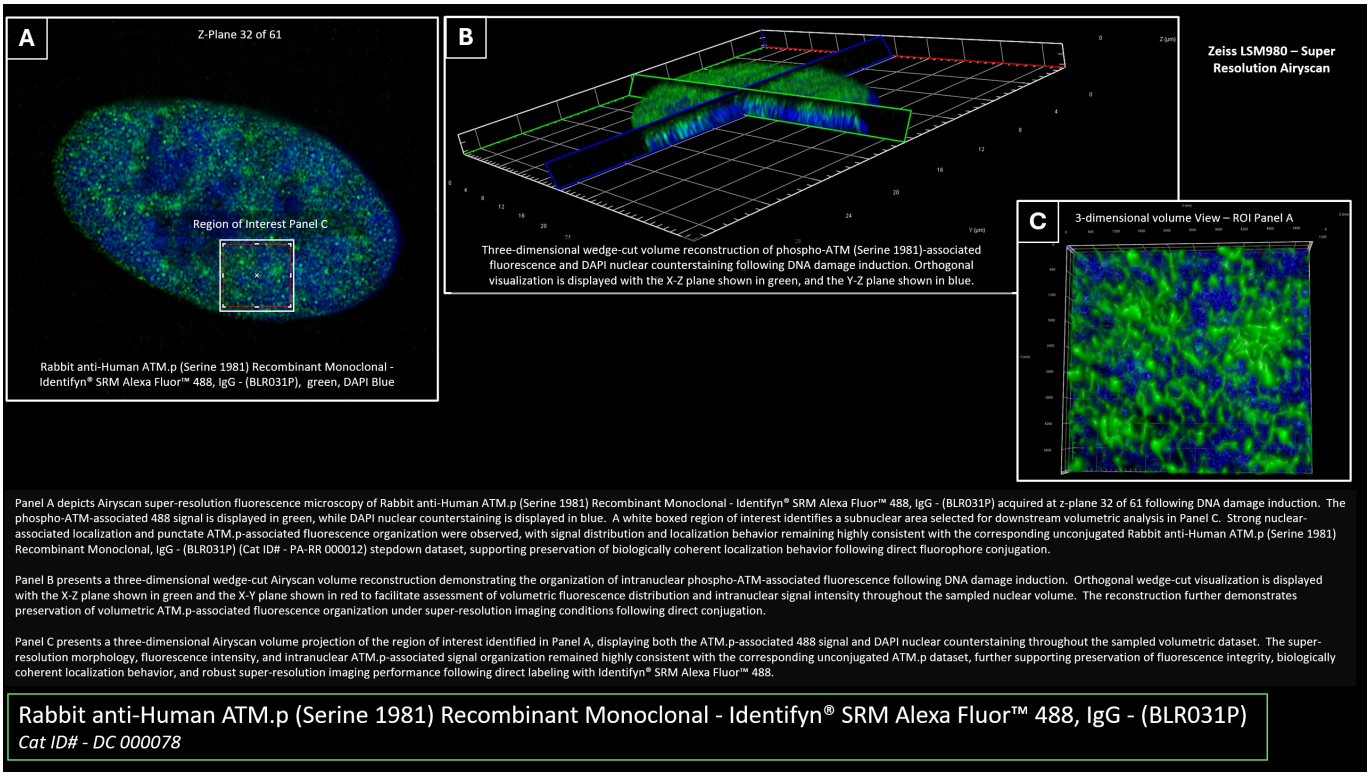
NONE

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

SOURCE PROVENANCE

Catalog	DC-000078
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	46
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	5D407663168BA347CF94AB92DBB495701F4821FAA51DD0E4B19035BC2A52386C
Method PDF SHA-256	1E4B02F154F4EF913208AC7DC49CF6517065AC4720FF389734A8E8AB4DA5102B

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488
METADATA VALUES	58

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	61 Slices (1.80 µm)
Channels	2
Scaling (Per Pixel)	0.03530 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1072 X 951 | 164 X 152
Image Size (Scaled)	37.84 µm X 33.57 µm | 5.79 µm X 5.36 µm
Bit Depth	16 Bit
Image Center Position	X: 92.58 mm, Y: 54.29 mm
Stage Position	X: 92.58 mm, Y: 54.29 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 250 µm | 5.00 AU / 214 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 550 | SBS SP 505
Laser Wavelength	488 nm @ 1.0% | 405 nm @ 0.5%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	2.00
Pixel Time	1.10 µs
Frame Time	18.77 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	493 | 353
Emission Wavelength	517 | 465
Effective NA	1.4
Detection Wavelength	499 - 548 | 422 - 477
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	Super Resolution: 9.0 (3D, Auto) | Super Resolution: 8.6 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-3% | 0%
Clip Y	-30° | 30°
Clip Z	0°
Y-Z	Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P)
Cat ID# - DC 000078

ATM (ataxia-telangiectasia mutated) is a crucial serine/threonine kinase that plays a central role in the DNA damage response (DDR), particularly in response to DNA double-strand breaks (DSBs). ATM's activation and subsequent phosphorylation cascades are essential for detecting DNA damage and initiating repair processes. Here's a detailed overview of how ATM is activated, its targets, and its role in the DDR:

Activation of ATM
In response to DNA damage, particularly DSBs, ATM is activated through a series of events:

Detection of DNA Breaks: ATM can sense DNA breaks by associating with the MRN complex (MRE11, RAD50, and NBS1). This complex recognizes DSBs, leading to ATM's recruitment to the damaged site.

Autophosphorylation: Upon detection of DNA damage, ATM undergoes autophosphorylation at serine 1981 (S1981), leading to its activation. This phosphorylation event is critical for ATM's kinase activity and its ability to phosphorylate downstream targets.

Dimer-to-Monomer Transition: In its inactive form, ATM is a dimer. It transitions to an active monomeric state upon activation, allowing it to interact with and phosphorylate various downstream proteins.

Phosphorylation Targets of ATM

Once activated, ATM phosphorylates many proteins involved in the DNA damage response, cell cycle regulation, and apoptosis. Here are some critical targets of ATM:

CHK2: ATM phosphorylates CHK2, a cell cycle checkpoint control kinase. This phosphorylation helps to halt the cell cycle, allowing time for DNA repair.

p53: ATM phosphorylates p53, activating this tumor suppressor, leading to cell cycle arrest or apoptosis if the damage is irreparable.

H2AX: ATM phosphorylates histone H2AX, forming γ-H2AX, a marker of DNA damage. This modification helps signal the presence of DSBs and recruit DNA repair machinery.

BRCA1: ATM's phosphorylation of BRCA1 is critical for homologous recombination, a key DNA repair pathway for DSBs.

NBS1: As part of the MRN complex, NBS1 phosphorylation by ATM enhances the complex's DNA repair functions.

53BP1: ATM phosphorylation of 53BP1 helps recruit this protein to sites of DSBs, guiding repair pathways like non-homologous end joining (NHEJ).

Role of ATM Phosphorylation in DNA Repair

ATM-mediated phosphorylation plays a central role in coordinating the DNA damage response:

Signal Propagation: Phosphorylation cascades initiated by ATM amplify the signal for DNA damage, ensuring an efficient response.

Recruitment of Repair Machinery: Phosphorylation of specific proteins helps recruit DNA repair complexes to the sites of DNA damage.

Cell Cycle Regulation: ATM's activation can lead to cell cycle checkpoints, preventing cells from dividing with damaged DNA.

Apoptosis: If the DNA damage is too severe to repair, ATM's activation can lead to apoptosis, preventing the propagation of damaged cells

ATM's phosphorylation in response to DNA damage is crucial for detecting DNA double-strand breaks and coordinating the DNA damage response. By phosphorylating a range of critical proteins, ATM orchestrates a complex network of signaling events that facilitate DNA repair, cell cycle regulation, and apoptosis, thereby maintaining genomic stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 12 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 5 times in 1X PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Control Data - Primary Unconjugated Antibody

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 32 of 61 & Panel B, 3-Dimensional Volume View

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1072 X 951
Image Size (Scaled) = 37.84 µm X 33.57 µm
Bit Depth = 16 Bit
Image Center Position = X: 92.58 mm, Y: 54.29 mm
Stage Position = X: 92.58 mm, Y: 54.29 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3-Dimensional Volume View, Panel C

Z-Stack = 61 Slices (1.80 µm)

Channels = 2
Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 164 X 152
Image Size (Scaled) = 5.79 µm X 5.36 µm
Bit Depth = 16 Bit
Image Center Position = X: 92.58 mm, Y: 54.29 mm
Stage Position = X: 92.58 mm, Y: 54.29 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 250 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 550
Laser Wavelength = 488 nm @ 1.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 9.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 214 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.5%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 2.0
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.10 µs
Frame Time = 18.77 s
LSM Scan Speed = 6
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 = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = Super Resolution: 8.6 (3D, Auto)

2-Dimensional View - Panel A

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired at z-plane 32 of 61 following DNA damage induction. The phospho-ATM-associated 488 signal is displayed in green alongside DAPI nuclear counterstaining displayed in blue, demonstrating strong nuclear localization, punctate ATM.p-associated fluorescence organization, and robust signal integrity, consistent with the corresponding unconjugated, widefield, and confocal datasets within this direct conjugate workflow. A white boxed region of interest identifies a subnuclear region selected for downstream volumetric analysis in Panel C.

3D Image Processing - Panel B

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

Clipping Image Processing - Panel B

Clipping planes are introduced in Panel B to highlight Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) within the nuclear compartment through orthogonal wedge-cut visualization. Portions of the volumetric dataset were selectively removed across the X-Z and Y-Z planes to facilitate assessment of intranuclear phospho-ATM-associated fluorescence distribution and volumetric signal organization.

X-Z = Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = -3%
Clip Y = -30°
Clip Z = 0°

Y-Z = Activate was selected, textured opaque was chosen, and a blue outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.

Position of Clip = 0%
Clip Y = 30°
Clip Z = 0°

3D Image Processing - Panel C

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

Summary

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 488, IgG - (BLR031P) acquired at z-plane 32 of 61 following DNA damage induction. The phospho-ATM-associated 488 signal is displayed in green, while DAPI nuclear counterstaining is displayed in blue. A white boxed region of interest identifies a subnuclear area selected for downstream volumetric analysis in Panel C. Strong nuclear-associated localization and punctate ATM.p-associated fluorescence organization were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal, IgG - (BLR031P) (Cat ID# - PA-RR 000012) stepdown dataset, supporting preservation of biologically coherent localization behavior following direct fluorophore conjugation.

Panel B presents a three-dimensional wedge-cut Airyscan volume reconstruction demonstrating the organization of intranuclear phospho-ATM-associated fluorescence following DNA damage induction. Orthogonal wedge-cut visualization is displayed with the X-Z plane shown in green and the Y-Z plane shown in blue to facilitate assessment of volumetric fluorescence distribution and intranuclear signal intensity throughout the sampled nuclear volume. The reconstruction further demonstrates preservation of volumetric ATM.p-associated fluorescence organization under super-resolution imaging conditions following direct conjugation.

Panel C presents a three-dimensional Airyscan volume projection of the region of interest identified in Panel A, displaying both the ATM.p-associated 488 signal and DAPI nuclear counterstaining throughout the sampled volumetric dataset. The super-resolution morphology, fluorescence intensity, and intranuclear ATM.p-associated signal

organization remained highly consistent with the corresponding unconjugated ATM.p dataset, further supporting preservation of fluorescence integrity, biologically coherent localization behavior, and robust super-resolution imaging performance following direct labeling with Identifyn® SRM Alexa Fluor™ 488.

Image Correction

NONE

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

SOURCE PROVENANCE

Catalog	DC-000078
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	58
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	9036CBD86B81900AC83C7D0119BBCD3FEE2466C070646410E122657557D4E137
Method PDF SHA-256	EE0FBA62DD3DBA60BCA1C32125D386EFC2721DE58A2B43D60B805476E703A960