

IDENTIFYN RESOLUTION STEPDOWN

ATM.P MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN

4

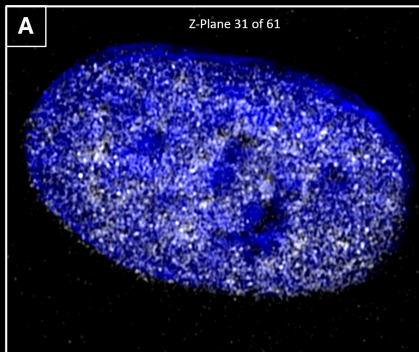
ORDERED EVIDENCE TIERS

0

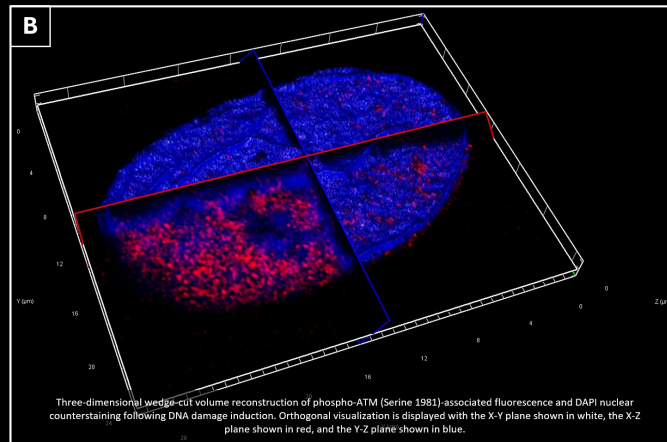
SUPPORTING CONTROL

PENDING

SCIENTIST REVIEW



Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P), pseudo colored white for visual contrast.



Zeiss LSM980 – Super Resolution Airyscan

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG – (BLR031P) acquired at z-plane 31 of 61 following DNA damage induction. The ATM.p-associated 680 signal is displayed in white to enhance visualization of fluorescence distribution and ATM.p-associated foci organization throughout the nuclear compartment, while DAPI nuclear counterstaining is included for structural reference. Strong nuclear-associated localization and discrete 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), as well as the associated widefield and confocal datasets generated within this direct conjugate workflow. Imaging was performed using the 639 nm laser line at only 0.5% laser power, further supporting preservation of fluorescence integrity and robust signal performance following direct conjugation with Identifyn® SRM Alexa Fluor™ 680.

Panel B presents a three-dimensional Airyscan volume reconstruction of phospho-ATM (Serine 1981)-associated fluorescence and DAPI nuclear counterstaining following DNA damage induction. Orthogonal wedge-cut visualization of the X-Y, X-Z, and Y-Z planes was utilized to assess fluorescence distribution throughout the sampled nuclear volume and further evaluate volumetric ATM.p-associated signal organization under super-resolution imaging conditions.

Collectively, the Airyscan dataset further demonstrates that Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG – (BLR031P) maintains strong fluorescence performance, biologically coherent nuclear localization behavior, and compatibility with demanding super-resolution fluorescence microscopy workflows following direct fluorophore conjugation. The preservation of discrete ATM.p-associated foci organization, volumetric signal integrity, and robust fluorescence generation under low excitation conditions further supports the stability and imaging performance of the Identifyn® direct conjugation platform across advanced super-resolution applications.

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P)
Cat ID# - DC 000082

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / AIRYSCAN

DC-000082 | 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-000082 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-000082 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	680	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 680	2; 50 Cy 5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Confocal	405/DAPI; 488; 680	2; None; 639 nm @ 0.4%; 405 nm @ 0.4%; 679; 353; 702; 465
Airyscan	405/DAPI; 488; 680	3; None; 639 nm @ 0.5%; 405 nm @ 0.5%; 679; 353; 702; 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 680.

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): 852 X 735; Image Size (Pixels): 852 X 735; Bit Depth: 14 Bit. Detector/camera: Imaging Device: AxioCam 705. Timing: Exposure Time: 300 ms; 20 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 30.00%; X-Cite Xylis Lamp Intensity @ 10.00%. Optical/scan: Effective NA: 1.4. Structured source metadata values: 39.

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; 680.

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 LSM980 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: 41 Slices (1.20 μm); Channels: 2; Scaling (Per Pixel): 0.059 μm X 0.059 μm X 0.030 μm ; Image size (Pixels): 1121 X 1121; Image Size (Pixels): 1121 X 1121; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.30 μs ; Frame Time: 3.69 s. Illumination: Laser Wavelength: 639 nm @ 0.4%; 405 nm @ 0.4%. Optical/scan: Pinhole: 1.00 AU / 68 μm ; 1.00 AU / 44 μm ; Scan Zoom: 2.0; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 8.1 (3D, Auto); Filter: 7.9 (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; 680.

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 (1.77 μm); Channels: 3; Scaling (Per Pixel): 35.30 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 858 X 704; Image Size (Pixels): 858 X 704; 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: 639 nm @ 0.5%; 405 nm @ 0.5%. Optical/scan: Pinhole: 5.00 AU / 328 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: 10.0 (3D, Auto); Super Resolution: 8.2 (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; 680.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Displayed feature separation remains reconstruction dependent; no unsupported numerical resolution or molecular architecture claim is permitted.

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000082 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)=858 X 704; Image Size (Scaled)=30.28 µm X 24.85 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.02%.

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™ 680

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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



IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

Images, source methods, acquisition settings and provenance follow in the locked
Stepdown order. Scientific wording is preserved; missing modalities are not invented.
No separate control record is present in the preserved product record.

PRODUCT-LEVEL STEPDOWN MAP

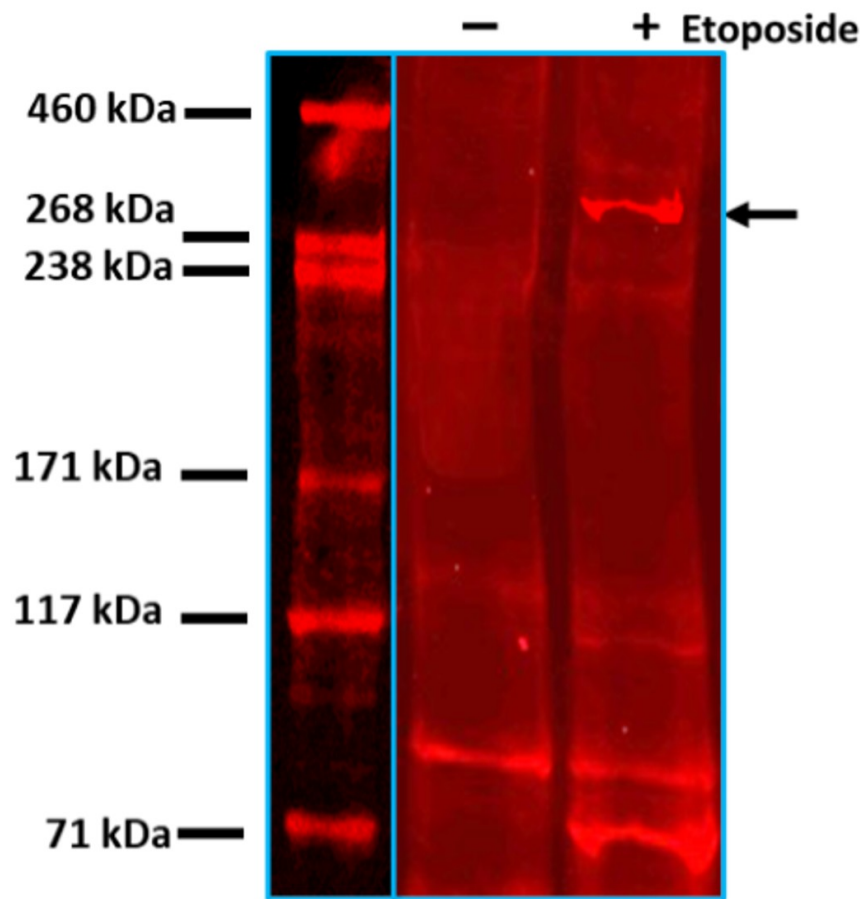
This dossier preserves the complete available evidence progression for DC-000082. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

CANONICAL STEPDOWN	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	680
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	680 nm
Emission Filter	720 ± 24 nm
Detector	PMT
Intensity	L2
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™ 680, IgG - (BLR031P)

Cat ID# - DC 000082

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™ 680, 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 = 680 nm

Emission Filter = 720 ± 24 nm

Detector = PMT

Intensity = L2

Pixel size = 100 µm

Scan Speed = High

Quality = High

Focus = Membrane (0 µm in Z-axis)

Post Processing

NONE

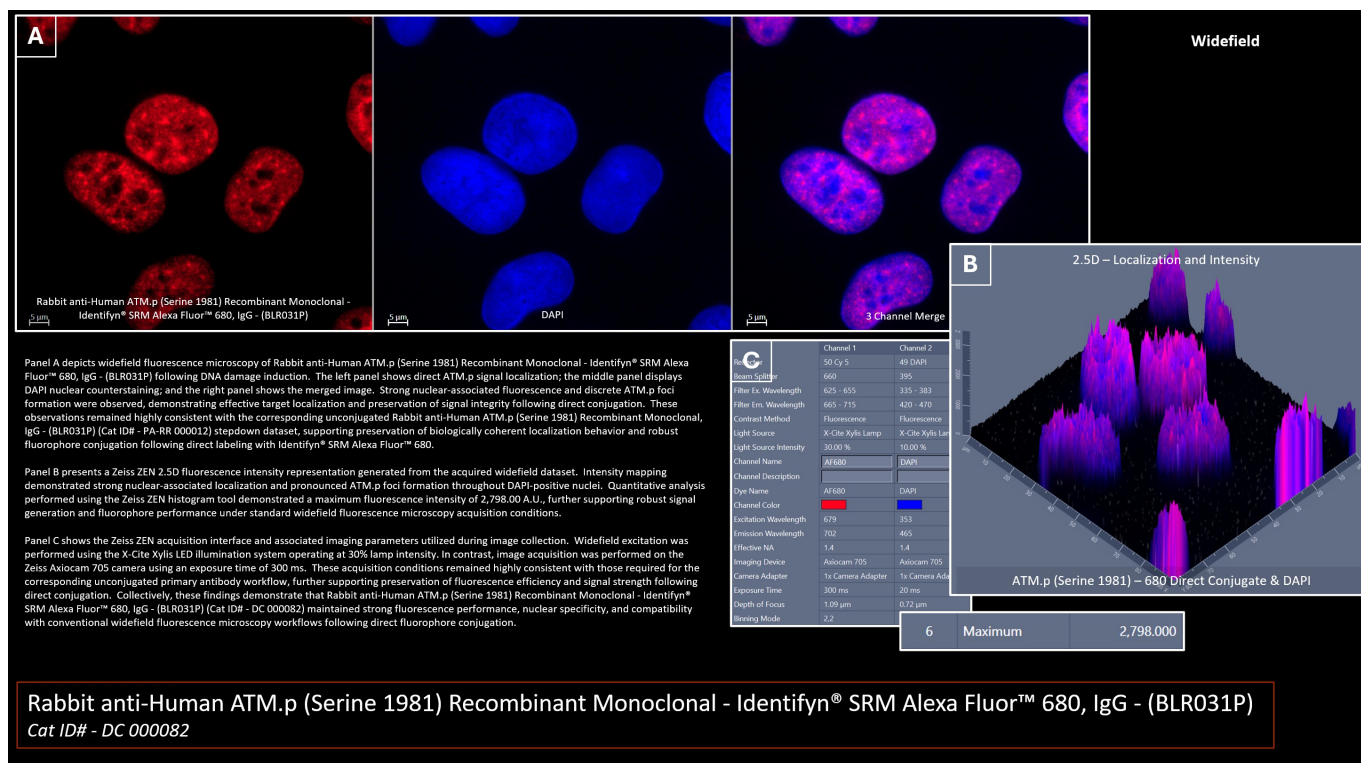
Image: S. Khanal

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SOURCE PROVENANCE

Catalog	DC-000082
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	5E0B0C65FA30CFB9ECE8C9196B1538F50A891562A012E0FB8301DCEBC76BA0DD
Method PDF SHA-256	63C2FBC35D7F48FBC788A1EEB097557E949F4B7FA889B32B096148A204E21596

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 680
METADATA VALUES	39

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)	852 X 735
Image Size (Scaled)	93.91 μm X 82.69 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	50 Cy 5 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 @ 30.00% X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	300 ms 20 ms
Excitation Wavelength	653 353
Emission Wavelength	668 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1x Camera Adapter
Depth of Focus	1.03 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™ 680, IgG - (BLR031P)

Cat ID# - DC 000082

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.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 μ M 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™ 680, 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 μ m X 0.110 μ m

Image Size (Pixels) = 852 X 735

Image Size (Scaled) = 93.91 μ m X 82.69 μ m

Bit Depth = 14 Bit

Image Center Position = X: 0.00 μ m, Y: 0.00 μ m

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

680 -

Reflector = 50 Cy 5
 Beam Splitter = 660
 Filter Excitation Wavelength = 625 - 655
 Filter Emission Wavelength = 665 - 715
 Contrast Method = Fluorescence
 Light Source = X-Cite Xylis Lamp Intensity @ 30.00%
 Exposure Time = 300 ms
 Excitation Wavelength = 653
 Emission Wavelength = 668
 Effective NA = 1.4
 Imaging Device = Axiocam 705
 Camera Adapter = 1x Camera Adapter
 Depth of Focus = 1.03 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 @ 10.00%
 Exposure Time = 20 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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear localization and discrete ATM.p foci formation following DNA damage induction, supporting robust conjugation performance and preservation of target-specific 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™ 680, IgG - (BLR031P) widefield dataset collected using the X-Cite Xylis Lamp and Zeiss Axiocam 705 camera. Imaging was performed using only 30% X-Cite Xylis Lamp intensity with a 300 ms exposure time on the Axiocam 705, demonstrating robust signal generation under standard widefield excitation and collection conditions. The ability to generate a strong nuclear-associated ATM.p signal and to form discrete foci under these moderate acquisition settings supports the robustness of direct conjugation and the preservation of biologically relevant localization characteristics. These findings further demonstrate that fluorescence intensity was achieved without relying on excessive excitation power or prolonged exposure.

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 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) direct conjugate, demonstrating a peak signal intensity of 2,798.000 A.U., supporting robust fluorophore conjugation and strong signal generation under standard widefield acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) following DNA damage induction. The left panel shows direct ATM.p 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 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™ 680.

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 foci formation throughout DAPI-positive nuclei. Quantitative analysis performed using the Zeiss ZEN histogram tool demonstrated a maximum fluorescence intensity of 2,798.00 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 was performed using the X-Cite Xylis LED illumination system operating at 30% lamp intensity, while image acquisition was performed on the Zeiss Axiocam 705 camera using an exposure time of 300 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™ 680, IgG - (BLR031P) (Cat ID# - DC 000082) maintained strong

fluorescence performance, nuclear specificity, and compatibility with conventional widefield fluorescence microscopy workflows following direct fluorophore conjugation.

Image Correction

NONE

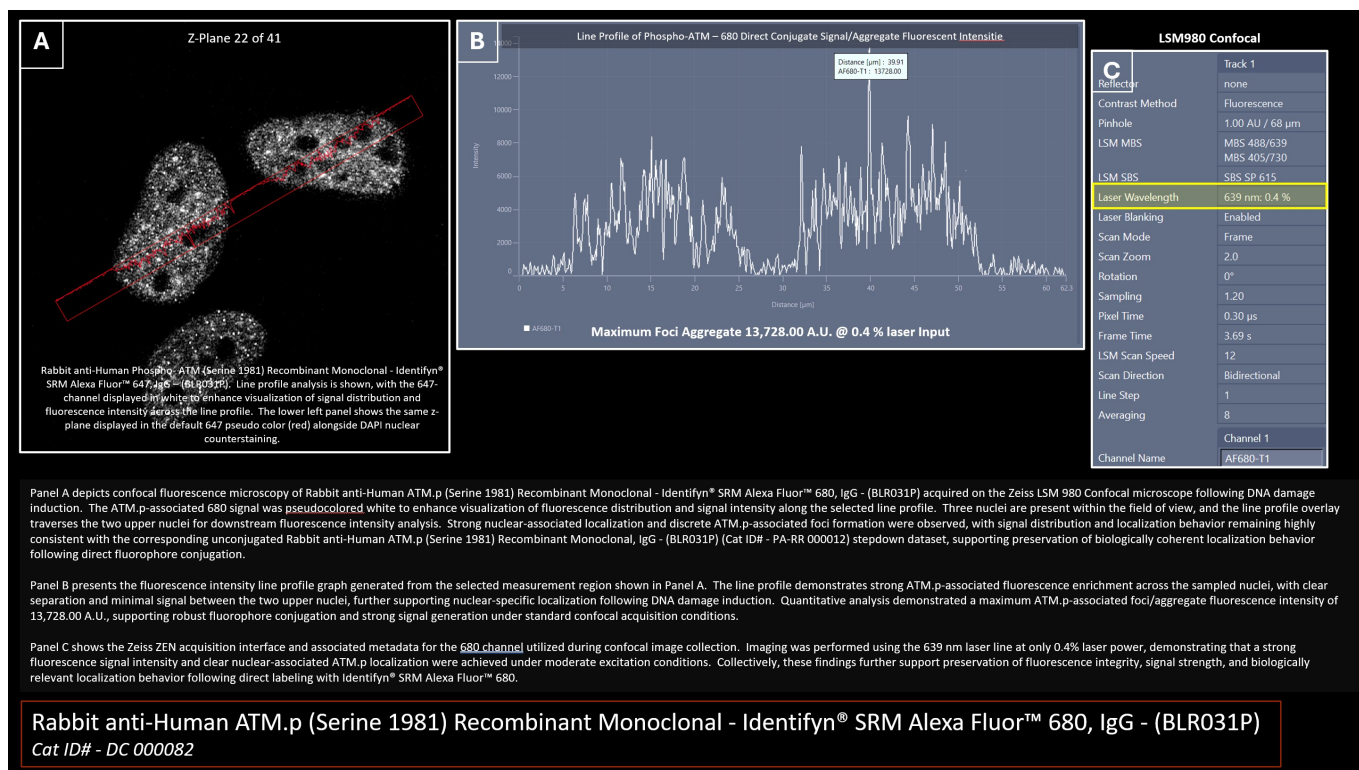
Image by E.F.Simon

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE	
Catalog	DC-000082
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 Xylys Lamp, was used with the following parameters for each dye:
Structured source metadata values	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C73D5A0141AC0CD9A2CCCC60298F42784A54DBEE1A07CA8C6B56B033E682CA58
Method PDF SHA-256	4BEE96DB92BF8A17DA5912BC8236851F6AA1961D0CDCF4BDA9EE630B7BB9837E

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
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 LSM980 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	41 Slices (1.20 μm)
Channels	2
Scaling (Per Pixel)	0.059 μm X 0.059 μm X 0.030 μm
Image Size (Pixels)	1121 X 1121
Image Size (Scaled)	65.93 μm X 65.93 μm
Bit Depth	16 Bit
Image Center Position	X: 85.90 mm, Y: 53.76 mm
Stage Position	X: 85.90 mm, Y: 53.76 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 68 μm 1.00 AU / 44 μm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615 Mirror
Laser Wavelength	639 nm @ 0.4% 405 nm @ 0.4%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	2.0
Rotation	0°
Sampling	1.20
Pixel Time	0.30 μs
Frame Time	3.69 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	679 353
Emission Wavelength	702 465
Effective NA	1.4
Detection Wavelength	674 - 745 435 - 490
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	600 V 500 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.1 (3D, Auto) Filter: 7.9 (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 62.3), Y (Min 0 and Max 14414)

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™ 680, IgG - (BLR031P)

Cat ID# - DC 000082

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.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 μ M 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™ 680, 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 LSM980 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 22 of 41

Z-Stack = 41 Slices (1.20 μ m)

Channels = 2

Scaling (Per Pixel) = 0.059 μ m X 0.059 μ m X 0.030 μ m

Image Size (Pixels) = 1121 X 1121

Image Size (Scaled) = 65.93 μ m X 65.93 μ m

Bit Depth = 16 Bit

Image Center Position = X: 85.90 mm, Y: 53.76 mm

Stage Position = X: 85.90 mm, Y: 53.76 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 68 µm
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = SBS SP 615
 Laser Wavelength = 639 nm @ 0.4%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.30 µs
 Frame Time = 3.69 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Detection Wavelength = 674 - 745
 Imaging Device = GaAsP-Pmt3
 Detector Type = GaAsP-PMT
 Detector Gain = 600 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 LSM Plus Mode = Filter: 8.1 (3D, Auto)

DAPI - Acquired but not Shown in Data Analysis

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 1.00 AU / 44 μ m
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = Mirror
 Laser Wavelength = 405 nm @ 0.4%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 1.20
 Pixel Time = 0.30 μ s
 Frame Time = 3.69 s
 LSM Scan Speed = 11
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 8
 Excitation Wavelength = 353
 Emission Wavelength = 465
 Effective NA = 1.4
 Detection Wavelength = 435 - 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: 7.9 (3D, Auto)

2-Dimensional View - Panel A

Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) acquired on the Zeiss LSM 980 Confocal microscope at Z-plane 22 of 41 following DNA damage induction. The ATM.p-associated 680 signal is rendered in white to enhance visualization of fluorescence distribution and signal intensity along the selected line profile. Three nuclei are present within the field of view, and the line profile overlay traverses the two upper nuclei for downstream fluorescence intensity analysis. Strong nuclear-associated localization and discrete ATM.p-associated foci formation were observed, supporting preservation of biologically coherent localization behavior and robust signal integrity following direct fluorophore 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 62.3), Y (Min 0 and Max 14414)

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 680 channel for Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) using the 639 nm laser at 0.4%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A depicts confocal fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) acquired on the Zeiss LSM 980 Confocal microscope following DNA damage induction. The ATM.p-associated 680 signal was pseudocolored white to enhance visualization of fluorescence distribution and signal intensity along the selected line profile. Three nuclei are present within the field of view, and the line profile overlay traverses the two upper nuclei for downstream fluorescence intensity analysis. Strong nuclear-associated localization and discrete ATM.p-associated foci formation 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 the fluorescence intensity line profile graph generated from the selected measurement region shown in Panel A. The line profile demonstrates strong ATM.p-associated fluorescence enrichment across the sampled nuclei, with clear separation and minimal signal between the two upper nuclei, further supporting nuclear-specific localization following DNA damage induction. Quantitative analysis demonstrated a maximum ATM.p-associated foci/aggregate fluorescence intensity of 13,728.00 A.U., supporting robust fluorophore conjugation and strong signal generation under standard confocal acquisition conditions.

Panel C shows the Zeiss ZEN acquisition interface and associated metadata for the 680 channel utilized during confocal image collection. Imaging was performed using the 639 nm laser line at only 0.4% laser power, demonstrating a strong fluorescence signal intensity and clear nuclear-associated ATM.p localization under moderate excitation conditions. Collectively, these findings further support preservation of fluorescence integrity, signal strength, and biologically relevant localization behavior following direct labeling with Identifyn® SRM Alexa Fluor™ 680.

Image Correction

NONE

Image by J.K. Bennett

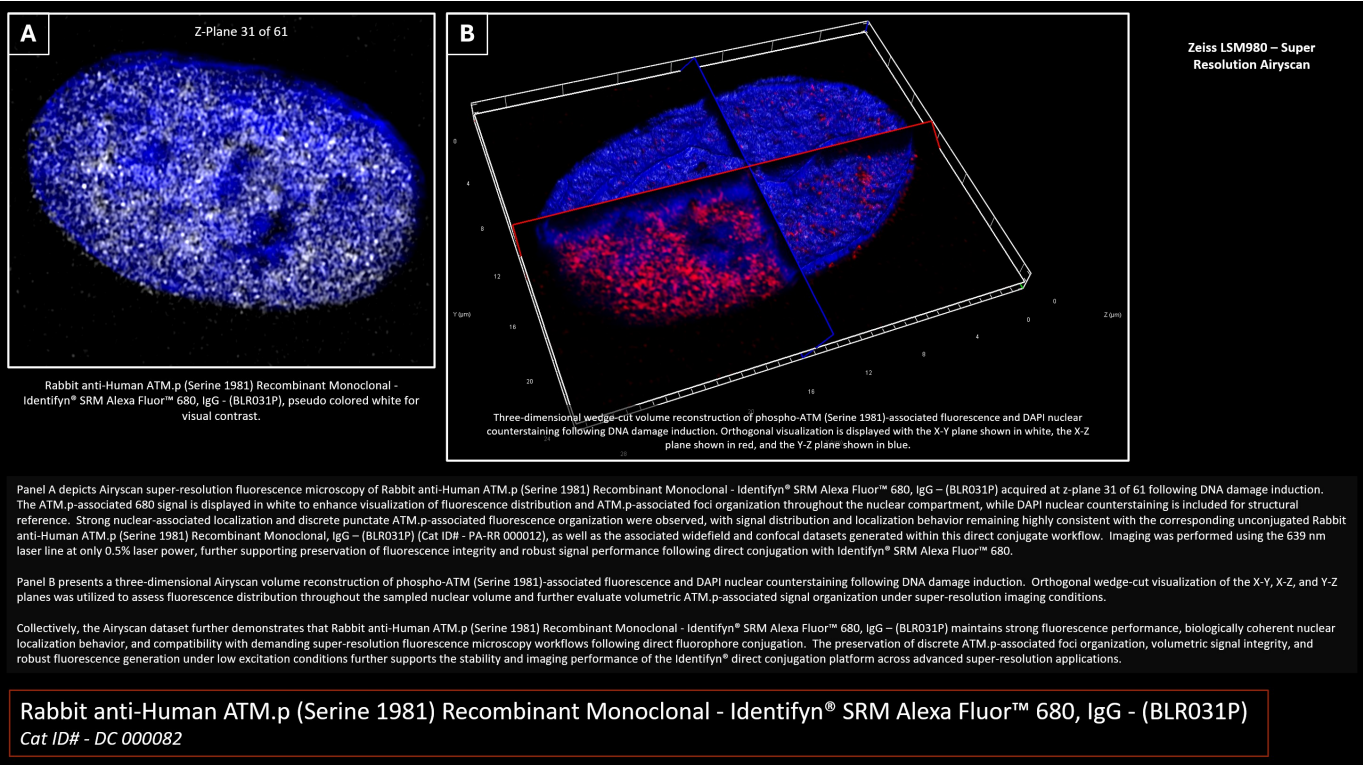
Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000082
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM980 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	205A414DE87C5C3C876CB7CA9C7C640E61B47A6D6FFB06689C8856B27D48D46F
Method PDF SHA-256	87B0EC93B9AFC73DBE95BEBFCF40343D3E1FF9C80072766437F6D3DAE6DCBE9

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 680
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 (1.77 μm)
Channels	3
Scaling (Per Pixel)	0.03530 μm X 0.03530 μm X 0.03000 μm
Image Size (Pixels)	858 X 704
Image Size (Scaled)	30.28 μm X 24.85 μm
Bit Depth	16 Bit
Image Center Position	X: 84.84 mm, Y: 54.69 mm
Stage Position	X: 84.84 mm, Y: 54.69 mm
ROI Center Offset	X: 0.00 μm , Y: 0.00 μm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00 AU / 328 μm 5.00 AU / 214 μm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 SBS SP 505
Laser Wavelength	639 nm @ 0.5% 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	679 353
Emission Wavelength	702 465
Effective NA	1.4
Detection Wavelength	643 - 745 422 - 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: 10.0 (3D, Auto) Super Resolution: 8.2 (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-Y	Activate was selected, textured opaque was chosen, and a white outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Position of Clip	-19% 0%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a red outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	60° -45°
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™ 680, IgG - (BLR031P)

Cat ID# - DC 000082

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.

Method

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 μ M 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™ 680, 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 31 of 61 and Panel B, 3-Dimensional Volume View

Z-Stack = 61 Slices (1.77 μ m)

Channels = 3

Scaling (Per Pixel) = 35.30 nm X 35.30 nm X 30.00 nm

Image Size (Pixels) = 858 X 704

Image Size (Scaled) = 30.28 μ m X 24.85 μ m

Bit Depth = 16 Bit

Image Center Position = X: 84.84 mm, Y: 54.69 mm

Stage Position = X: 84.84 mm, Y: 54.69 mm

ROI Center Offset = X: 0.00 μ m, Y: 0.00 μ m

680 -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 328 μ m
 LMS MBS = MBS 488/639 & 405/730
 LMS SBS = SBS LP 640
 Laser Wavelength = 639 nm @ 0.5%
 Laser Blanking = Enabled
 Scan Mode = Frame
 Scan Zoom = 2.0
 Rotation = 0°
 Sampling = 2.00
 Pixel Time = 1.10 μ s
 Frame Time = 18.77 s
 LSM Scan Speed = 6
 Scan Direction = Bidirectional
 Line Step = 1
 Averaging = 4
 Excitation Wavelength = 679
 Emission Wavelength = 702
 Effective NA = 1.4
 Detection Wavelength = 643 - 745
 Imaging Device = Airyscan
 Detector Type = GaAsP-PMT
 Detector Gain = 850 V
 Detector Offset = 0
 Detector Digital Gain = 1.0
 Airyscan Mode = Super Resolution: 10.0 (3D, Auto)

DAPI -

Reflector = None
 Contrast Method = Fluorescence
 Pinhole = 5.00 AU / 214 μ m
 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 μ s
 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: 8.2 (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™ 680, IgG - (BLR031P) acquired at z-plane 31 of 61 following DNA damage induction. The ATM.p-associated 680 signal is displayed in white to enhance visualization of fluorescence distribution and ATM.p-associated foci organization throughout the nuclear compartment, while DAPI nuclear counterstaining is included for structural reference. Strong nuclear-associated localization and discrete punctate ATM.p fluorescence organization were observed, with signal distribution and localization behavior remaining highly consistent with the corresponding unconjugated, widefield, and confocal datasets generated within this direct conjugate workflow.

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™ 680, IgG - (BLR031P) within the nuclear compartment through a triple-wedge clipping-plane volume cut. Portions of the volumetric dataset were selectively removed across the X-Y, X-Z, and Y-Z planes to facilitate visualization of intranuclear phospho-ATM-associated fluorescence organization throughout the sampled nuclear volume.

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

Position of Clip = -19%

Clip X = 0°

Clip Z = 0°

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

Position of Clip = 0%

Clip Y = 60°

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 = -45°

Clip Z = 0°

Summary

Panel A depicts Airyscan super-resolution fluorescence microscopy of Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) acquired at z-plane 31 of 61 following DNA damage induction. The ATM.p fluorescence organization 680 signal is displayed in white to enhance visualization of fluorescence distribution and ATM.p-associated foci organization throughout the nuclear compartment, while DAPI nuclear counterstaining is included for structural reference. Strong nuclear-associated localization and discrete 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), as well as the associated widefield and confocal datasets generated within this direct conjugate workflow. Imaging was performed using the 639 nm laser line at only 0.5% laser power, further supporting preservation of fluorescence integrity and robust signal performance following direct conjugation with Identifyn® SRM Alexa Fluor™ 680.

Panel B presents a three-dimensional Airyscan volume reconstruction of phospho-ATM (Serine 1981)-associated fluorescence and DAPI nuclear counterstaining following DNA damage induction. Orthogonal wedge-cut visualization of the X-Y, X-Z, and Y-Z planes was utilized to assess fluorescence distribution throughout the sampled nuclear volume and further evaluate volumetric ATM.p-associated signal organization under super-resolution imaging conditions.

Collectively, the Airyscan dataset further demonstrates that Rabbit anti-Human ATM.p (Serine 1981) Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 680, IgG - (BLR031P) maintains strong fluorescence performance, biologically coherent nuclear localization behavior, and compatibility with demanding super-resolution fluorescence microscopy workflows following direct fluorophore conjugation. The preservation of discrete ATM.p-associated foci

organization, volumetric signal integrity, and robust fluorescence generation under low excitation conditions further supports the stability and imaging performance of the Identifyn® direct conjugation platform across advanced super-resolution applications.

Image Correction

NONE

Image by J.K. Bennett

Image Analysis and Data Presentation by B.T. Bennett

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SOURCE PROVENANCE

Catalog	DC-000082
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	069FA4D8801F734E9090F75D4E2B94C96A6E0017784C626D62E0CEEACFF514EE
Method PDF SHA-256	FFEFE623A62709CBC45750BD8EE088D26088DAEDA26AAC3F8E898BBA074E3EED