BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

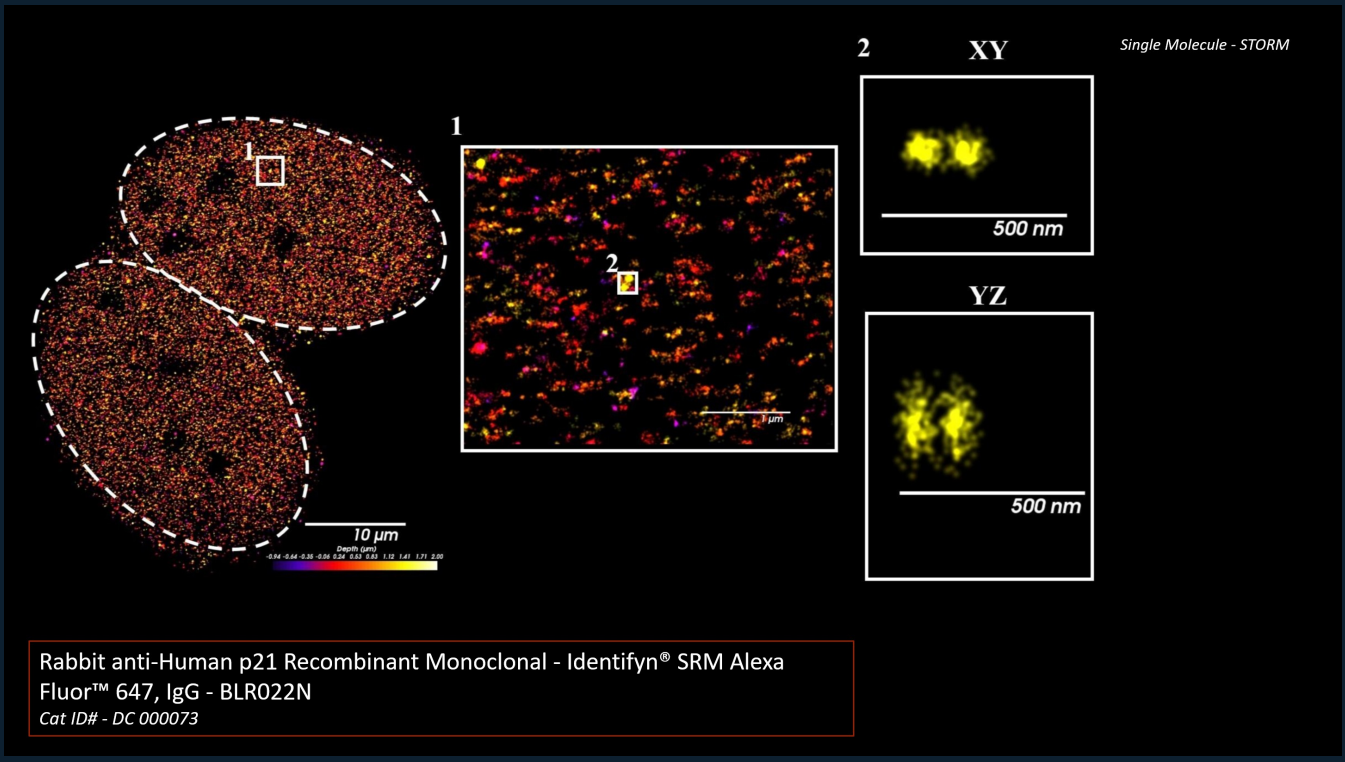
P21

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647

WESTERN BLOT -> WIDEFIELD -> CONFOCAL -> AIRYSCAN -> SINGLE-MOLECULE STORM

5	0	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

DC-000073 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

UNPUBLISHED / PENDING SCIENTIST REVIEW / NO PUBLICATION AUTHORIZED

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
- Single-molecule STORM presentation workflow
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

What source-bounded experimental and microscopy evidence is preserved for P21, and what is the strongest interpretation allowed by the available modalities and controls?

EVIDENCE AVAILABLE

Western blot -> Widefield -> Confocal -> Airyscan -> Single-molecule STORM

BENNETT ASSESSMENT

The preserved DC-000073 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. 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-000073 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. Missing modalities are not represented or inferred.

SETTINGS ASSESSMENT

The record preserves 5 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	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 647	2; 50 Cy 5; 49 DAPI; 625 - 655; 335 - 383; 665 - 715; 420 - 470; 653
Confocal	405/DAPI; 488; 647	1; None; 639 nm @ 2.0%; 653; 668
Airyscan	405/DAPI; 488; 647	1; None; 639 nm @ 2.00%; 405 nm @ 0.3%; 653; 353; 668; 465
Single-molecule STORM	647	Both Channels

MODALITY ASSESSMENT / PART 1 OF 5

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

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

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Western blot, documents platform context as Azure Sapphire FL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 2 OF 5

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): 383 X 362; Image Size (Pixels): 383 X 362; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 350 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; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 5

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: 1; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 830 X 830; Image Size (Pixels): 830 X 830; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.46 µs; Frame Time: 3.15 s. Illumination: Laser Wavelength: 639 nm @ 2.0%. Optical/scan: Pinhole: 1.00 AU / 64 µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 7.8 (3D, Auto). Structured source metadata values: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Confocal, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Optical sectioning and source-bounded 3D presentation do not establish binding, mechanism, or population-level effect.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 5

Airyscan / 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: 68 Slices (2.01 µm); Channels: 1; Scaling (Per Pixel): 35.0 nm X 35.30 nm X 30.00 nm; Image size (Pixels): 1072 X 1094; 229 X 181; Image Size (Pixels): 1072 X 1094; 229 X 181; 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 @ 2.00%; 405 nm @ 0.3%. Optical/scan: Pinhole: 5.00 AU / 328 µm; 5.00 AU / 215 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: SuperResolution 10.0 (3D, Auto); SuperResolution 7.7 (3D, Auto). Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Airyscan, documents platform context as ZEISS LSM 980, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 5

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60X/1.30 silicon oil objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 81.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Single-molecule STORM, documents platform context as Bruker Vutara VXL, and records detected dye/channel descriptors as 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved DC-000073 record contains Western blot; Widefield; Confocal; Airyscan; Single-molecule STORM. 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): 35.0 nm X 35.30 nm X 30.00 nm -> 0.03500 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 1094; Image Size (Scaled)=37.84 µm X 38.61 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.85%.

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

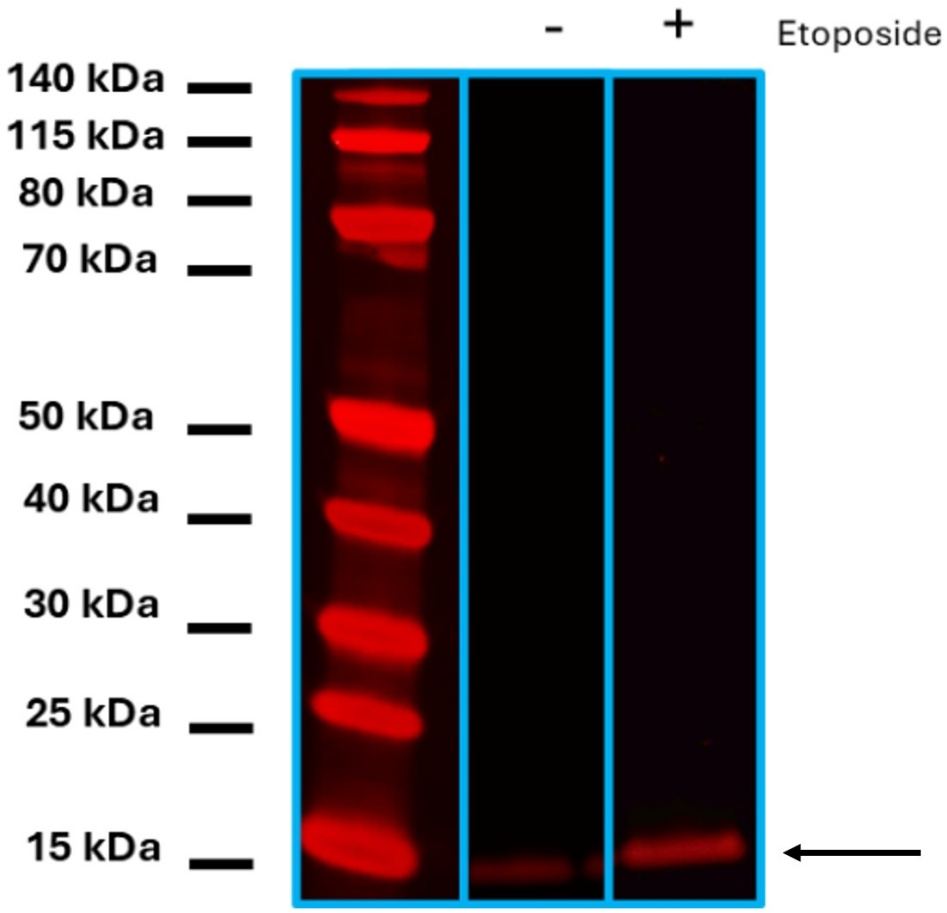
This dossier preserves the complete available evidence progression for DC-000073. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. No separate control record is present in the preserved product record, and none is inferred.

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
8	Single-molecule STORM	Bruker Vutara VXL	PRESERVED

INTERPRETIVE BOUNDARY

Ordering evidence by resolution tier does not make the modalities interchangeable. Each stage retains its acquisition environment and scientific limitations. This pilot organizes the record; it does not synthesize new biological claims.

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	11

STRUCTURED ACQUISITION METADATA / WESTERN BLOT

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane(s)
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane(s)
Detection mode	Fluorescence
Laser	638 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L2
Pixel size	100 µm
Scan Speed	High
Quality	High
Focus	Membrane (0 µm in Z-axis)
Image	K. Small

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N)
Cat ID# - DC 000073

Expected MW: 21 kDa

HeLa cells were treated with 2 µM etoposide for 18 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 using NuPAGE™ 4 to 12% Bis-Tris, 1.0-1.5 mm, Mini Protein Gels in a Mini Gel Tank. Thermo Fisher™ PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using 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 p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) 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(s)

Scan Settings

Detection mode = Fluorescence
Laser = 638 nm
Emission Filter = 676 ± 37 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: K. Small

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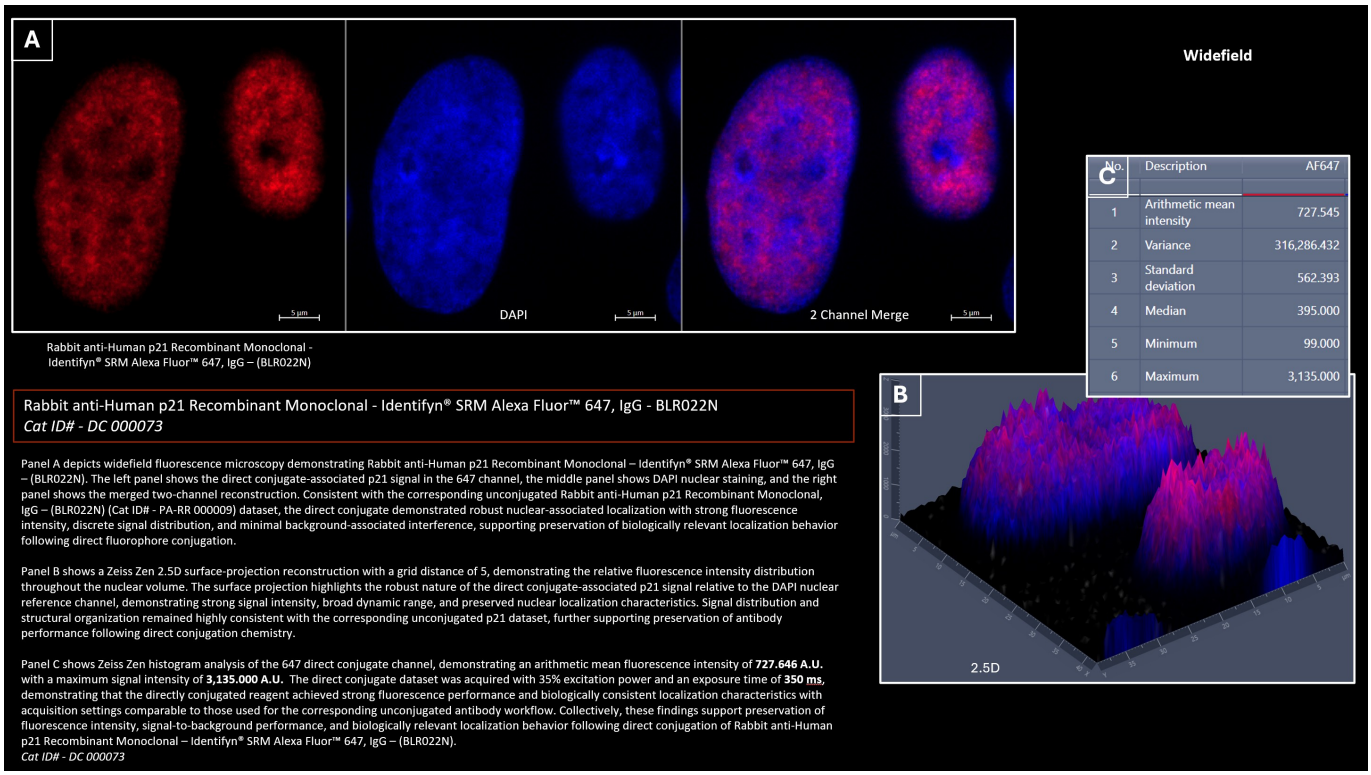
SOURCE PROVENANCE

Catalog	DC-000073
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-NIR and Z-Plane(s)
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	13E34DF4BBF79A8CF237857F36D8F4599E8E7B07659FC582673C8B24C892F38A
Method PDF SHA-256	E9BBA1257EFFCEEB25E8620AD39BFD457A21797BA7BE39BC4EB0FEA31FBDD1FC

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



EVIDENCE TIER	Widefield
INSTRUMENT	ZEISS Axio Imager.M1
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 647
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)	383 X 362
Image Size (Scaled)	41.95 µm X 39.65 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	350 ms | 20 ms
Excitation Wavelength	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1x
Depth of Focus	1.03 µm | 0.72 µm
Binning Mode	2,2
Render Mode	Surface
Grid distance	5
Angle X	54
Angle Y	-53
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 p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG -(BLR022N)
Cat ID# - DC 000073

p21, CDKN1A (cyclin-dependent kinase inhibitor 1A), is a regulatory protein with multiple roles in cell cycle control, DNA damage response, and repair. It is a well-known target of the tumor suppressor protein p53, and its regulation is crucial for cellular responses to DNA damage. The functions of p21 in the context of DNA repair:

Cell Cycle Arrest: p21 is a crucial mediator of cell cycle arrest, particularly in response to DNA damage. It inhibits cyclin-dependent kinases (CDKs), specifically CDK2, CDK4, and CDK6, halting cell cycle progression in the G1 phase. This arrest allows cells time to repair damaged DNA before cell division, reducing the risk of transmitting DNA errors.

Role in DNA Repair: p21, primarily known for its role in cell cycle regulation, can also influence DNA repair mechanisms:

Coordination of Repair Pathways: Inducing cell cycle arrest, p21 provides a stable environment for DNA repair processes. This action helps ensure that repair mechanisms, such as homologous recombination (HR) and nucleotide excision repair (NER), have the time and resources needed to repair DNA damage effectively.

Interacting with DNA Repair Proteins: p21 can interact with various DNA repair proteins, affecting their stability and activity. For example, p21 can bind to proliferating cell nuclear antigen (PCNA), a key component in DNA replication and repair. This interaction can modulate PCNA's activity and help regulate the balance between DNA repair and replication.

Protection from Apoptosis: In some contexts, p21 can protect cells from undergoing apoptosis, providing additional time for DNA repair. This role is complex and context-dependent, as it can also contribute to cellular resistance to specific cancer therapies by prolonging the survival of damaged cells.

Role in Genomic Stability: By facilitating cell cycle arrest and enabling effective repair pathways, p21 helps maintain genomic stability. This role is crucial for preventing mutations and chromosomal aberrations leading to cancer and other genetic diseases.

In summary, p21 plays a multifaceted role in DNA repair by regulating cell cycle progression, coordinating with DNA repair proteins, and providing a stable environment for repair processes. Its ability to interact with components of the cell cycle and the DNA repair machinery makes it a crucial mediator of the cellular response to DNA damage, contributing to genomic integrity and stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced using 2 µM etoposide-spiked complete growth media for 18 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), 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) = 383 X 362
Image Size (Scaled) = 41.95 µm X 39.65 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

647 -

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 = 350 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Imaging Device = Axiocam 705
Camera Adapter = 1x
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
Depth of Focus = 0.72 µm
Binning Mode = 2,2

Split View - Panel A

The left panel shows Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N). The middle panel shows DAPI nuclear counterstaining. The right panel shows the merged image, demonstrating strong nuclear-associated localization and a robust p21 fluorescence signal distribution, supporting effective direct conjugation and the preservation of target-specific localization 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 = 54
Angle Y = -53
Z Scaling = 1.0
Ambient = 50
Specular = 20
Shininess = 50
Light Height = 2.0

ZEN Acquisition Histogram Data - Panel C

Shown is the Zeiss ZEN Histogram analysis for Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), demonstrating an arithmetic mean fluorescence intensity of 727.646 A.U. and a maximum signal intensity of 3,135.000 A.U. in the 647 channel. The direct conjugate dataset was acquired using an X-Cite Xylis Lamp at 30% intensity with an exposure time of 350 ms, supporting robust fluorophore conjugation, strong signal generation, and preservation of biologically consistent localization characteristics under standard widefield fluorescence microscopy acquisition conditions.

Summary

Panel A depicts widefield fluorescence microscopy demonstrating Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N). The left panel shows the direct conjugate-associated p21 signal in the 647 channel, the middle panel shows DAPI nuclear staining, and the right panel shows the merged two-channel reconstruction. Consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) dataset, the direct conjugate demonstrated robust nuclear-associated localization with strong fluorescence intensity, discrete signal distribution, and minimal background-associated interference, supporting preservation of biologically relevant localization behavior following direct fluorophore conjugation.

Panel B shows a Zeiss ZEN 2.5D surface-projection reconstruction with a grid distance of 5, demonstrating the relative fluorescence intensity distribution throughout the nuclear volume. The surface projection highlights the robust nature of the direct conjugate-associated p21 signal relative to the DAPI nuclear reference channel, demonstrating strong signal intensity, a broad dynamic range, and preserved nuclear localization. Signal distribution and structural organization remained highly consistent with the corresponding unconjugated p21 dataset, further supporting preservation of antibody performance following direct conjugation chemistry.

Panel C shows Zeiss ZEN histogram analysis of the 647 direct conjugate channel, demonstrating an arithmetic mean fluorescence intensity of 727.646 A.U. with a maximum signal intensity of 3,135.000 A.U. The direct conjugate dataset was acquired at 30% excitation power and an exposure time of 350 ms, demonstrating that the directly conjugated reagent exhibited strong fluorescence performance and biologically consistent localization characteristics, with acquisition settings comparable to those used for the corresponding unconjugated antibody workflow. Collectively, these findings support preservation of fluorescence intensity, signal-to-background performance, and biologically relevant localization behavior following direct conjugation of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N).

Image Correction

NONE

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

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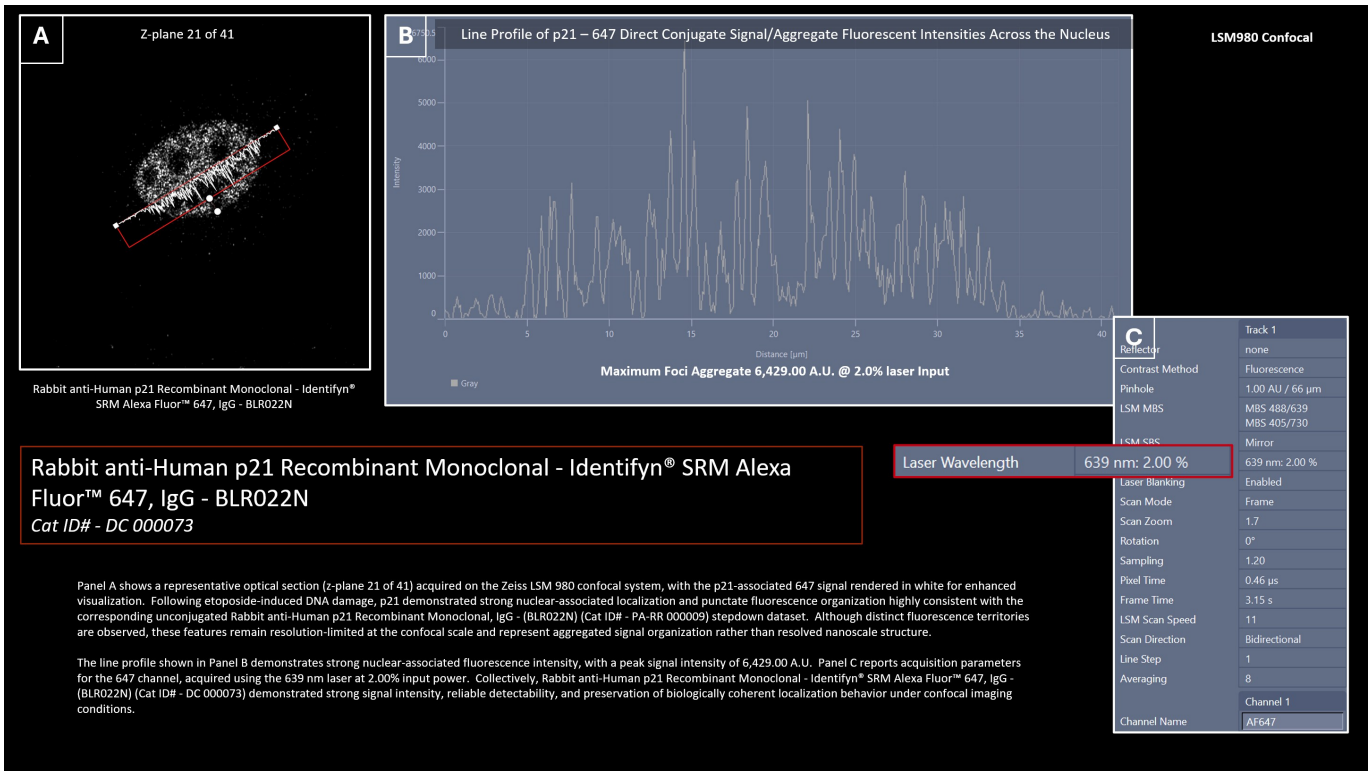
SOURCE PROVENANCE

Catalog	DC-000073
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	39
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	B05CDC3B854E8A204F5609E9006FD6F82A6381B996F075DC5A5758E4E10AB762
Method PDF SHA-256	4F5AF6628992D235A18FAF4D3BDD9FBF22F7BE21CBED29E8649CE30ADE70192E

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	38

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss 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	1
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels)	830 X 830
Image Size (Scaled)	77.00 µm X 77.00 µm
Bit Depth	16 Bit
Image Center Position	X: 86.61 mm, Y: 46.48 mm
Stage Position	X: 86.61 mm, Y: 46.48 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00 AU / 64 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	Mirror
Laser Wavelength	639 nm @ 2.0%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.20
Pixel Time	0.46 µs
Frame Time	3.15 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	635 - 745
Imaging Device	GaAsP-Pmt3

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 7.8 (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 41.0), Y (Min 0 and Max 6750)

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 p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG -(BLR022N)
Cat ID# - DC 000073

p21, CDKN1A (cyclin-dependent kinase inhibitor 1A), is a regulatory protein with multiple roles in cell cycle control, DNA damage response, and repair. It is a well-known target of the tumor suppressor protein p53, and its regulation is crucial for cellular responses to DNA damage. The functions of p21 in the context of DNA repair:

Cell Cycle Arrest: p21 is a crucial mediator of cell cycle arrest, particularly in response to DNA damage. It inhibits cyclin-dependent kinases (CDKs), specifically CDK2, CDK4, and CDK6, halting cell cycle progression in the G1 phase. This arrest allows cells time to repair damaged DNA before cell division, reducing the risk of transmitting DNA errors.

Role in DNA Repair: p21, primarily known for its role in cell cycle regulation, can also influence DNA repair mechanisms:

Coordination of Repair Pathways: Inducing cell cycle arrest, p21 provides a stable environment for DNA repair processes. This action helps ensure that repair mechanisms, such as homologous recombination (HR) and nucleotide excision repair (NER), have the time and resources needed to repair DNA damage effectively.

Interacting with DNA Repair Proteins: p21 can interact with various DNA repair proteins, affecting their stability and activity. For example, p21 can bind to proliferating cell nuclear antigen (PCNA), a key component in DNA replication and repair. This interaction can modulate PCNA's activity and help regulate the balance between DNA repair and replication.

Protection from Apoptosis: In some contexts, p21 can protect cells from undergoing apoptosis, providing additional time for DNA repair. This role is complex and context-dependent, as it can also contribute to cellular resistance to specific cancer therapies by prolonging the survival of damaged cells.

Role in Genomic Stability: By facilitating cell cycle arrest and enabling effective repair pathways, p21 helps maintain genomic stability. This role is crucial for preventing mutations and chromosomal aberrations leading to cancer and other genetic diseases.

In summary, p21 plays a multifaceted role in DNA repair by regulating cell cycle progression, coordinating with DNA repair proteins, and providing a stable environment for repair processes. Its ability to interact with components of the cell cycle and the DNA repair machinery makes it a crucial mediator of the cellular response to DNA damage, contributing to genomic integrity and stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced using 2 µM etoposide-spiked complete growth media for 18 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), 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) - Panel A, 2-Dimensional view at Z-plane 21 of 41, Line Profile Panel B

Z-Stack = 41 Slices (1.20 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image Size (Pixels) = 830 X 830
Image Size (Scaled) = 77.00 µm X 77.00 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.61 mm, Y: 46.48 mm
Stage Position = X: 86.61 mm, Y: 46.48 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00 AU / 64 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = Mirror
Laser Wavelength = 639 nm @ 2.0%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.46 µs
Frame Time = 3.15 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 635 - 745
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.8 (3D, Auto)

2-Dimensional View - Panel A

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) acquired on the Zeiss LSM 980 Confocal microscope at Z-plane 21 of 41 following DNA damage induction. The primary image displays the 647 channel rendered in white to enhance visualization of signal distribution and fluorescence intensity along the selected line profile. The image demonstrates strong nuclear-associated localization, punctate p21-associated fluorescence organization, 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 41.0), Y (Min 0 and Max 6750)

ZEN Acquisition Info Tab - Panel C

Shown is the acquisition of the 647 channel (p21 - 647 direct conjugate) using the 639 nm laser at 2.00%, demonstrating that image acquisition settings do not artificially produce the signal.

Summary

Panel A shows a representative optical section (Z-plane 21 of 41) acquired on the Zeiss LSM 980 confocal system, with the p21-associated 647 signal rendered in white to enhance visualization of fluorescence distribution and structural organization. Following DNA damage induction, p21 demonstrated strong nuclear-associated localization and punctate fluorescence organization highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown dataset. Although distinct fluorescence territories are observed, these features remain resolution-limited at the confocal scale and represent aggregated signal organization rather than resolved nanoscale structure.

The line profile shown in Panel B demonstrates strong nuclear-associated fluorescence, with a peak signal intensity of 6,429.00 A.U., supporting robust fluorescence generation and detection following direct fluorophore conjugation. Panel C reports acquisition parameters for the 647 channel, acquired using the 639 nm laser at 2.00% input power under confocal imaging conditions.

Collectively, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) (Cat ID# - DC 000073) demonstrated strong signal intensity, reliable detectability, and preservation of biologically coherent localization behavior under confocal imaging conditions, further supporting maintenance of antibody functionality and fluorophore performance following direct conjugation.

Image Correction

NONE

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

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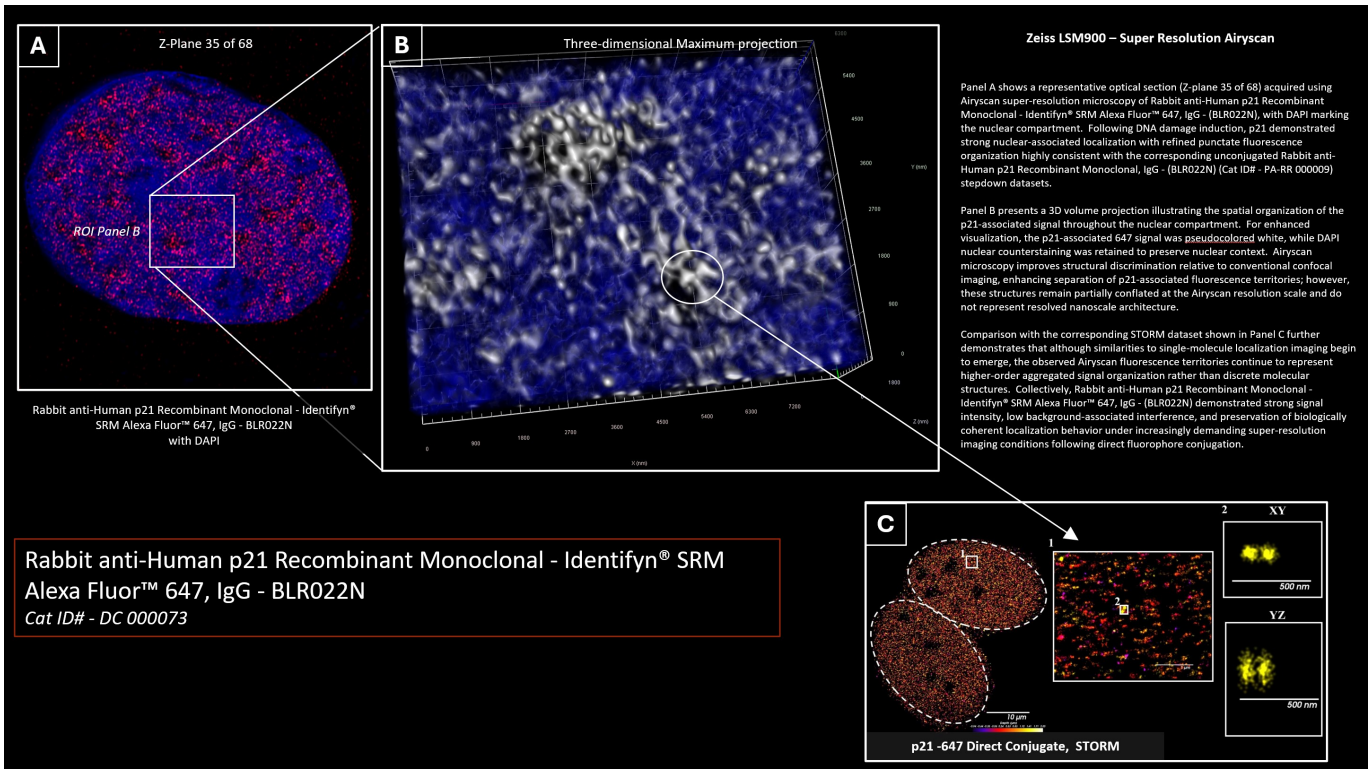
SOURCE PROVENANCE

Catalog	DC-000073
Stage	Confocal

SOURCE PROVENANCE

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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	D2D570365EC86072E0F7CFAD27B266EFC5D06356EC4FE66CA1EEDA8FD68BEDAF
Method PDF SHA-256	41F64222BDF53E3430D992B5CA2865C9C89B7A7D8B58F8FA181221FF16ED0675

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



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

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 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	68 Slices (2.01 µm)
Channels	1
Scaling (Per Pixel)	0.03500 µm X 0.03530 µm X 0.03000 µm
Image Size (Pixels)	1072 X 1094 | 229 X 181
Image Size (Scaled)	37.84 µm X 38.61 µm | 8.08 µm X 6.39 µm
Bit Depth	16 Bit
Image Center Position	X: 86.61 mm, Y: 46.58 mm
Stage Position	X: 86.61 mm, Y: 46.58 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 / 215 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 505
Laser Wavelength	639 nm @ 2.00% | 405 nm @ 0.3%
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	653 | 353
Emission Wavelength	668 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 350 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	850 V | 8000 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 10.0 (3D, Auto) | SuperResolution 7.7 (3D, Auto)
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)

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 p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG -(BLR022N)
Cat ID# - DC 000073

p21, CDKN1A (cyclin-dependent kinase inhibitor 1A), is a regulatory protein with multiple roles in cell cycle control, DNA damage response, and repair. It is a well-known target of the tumor suppressor protein p53, and its regulation is crucial for cellular responses to DNA damage. The functions of p21 in the context of DNA repair:

Cell Cycle Arrest: p21 is a crucial mediator of cell cycle arrest, particularly in response to DNA damage. It inhibits cyclin-dependent kinases (CDKs), specifically CDK2, CDK4, and CDK6, halting cell cycle progression in the G1 phase. This arrest allows cells time to repair damaged DNA before cell division, reducing the risk of transmitting DNA errors.

Role in DNA Repair: p21, primarily known for its role in cell cycle regulation, can also influence DNA repair mechanisms:

Coordination of Repair Pathways: Inducing cell cycle arrest, p21 provides a stable environment for DNA repair processes. This action helps ensure that repair mechanisms, such as homologous recombination (HR) and nucleotide excision repair (NER), have the time and resources needed to repair DNA damage effectively.

Interacting with DNA Repair Proteins: p21 can interact with various DNA repair proteins, affecting their stability and activity. For example, p21 can bind to proliferating cell nuclear antigen (PCNA), a key component in DNA replication and repair. This interaction can modulate PCNA's activity and help regulate the balance between DNA repair and replication.

Protection from Apoptosis: In some contexts, p21 can protect cells from undergoing apoptosis, providing additional time for DNA repair. This role is complex and context-dependent, as it can also contribute to cellular resistance to specific cancer therapies by prolonging the survival of damaged cells.

Role in Genomic Stability: By facilitating cell cycle arrest and enabling effective repair pathways, p21 helps maintain genomic stability. This role is crucial for preventing mutations and chromosomal aberrations leading to cancer and other genetic diseases.

In summary, p21 plays a multifaceted role in DNA repair by regulating cell cycle progression, coordinating with DNA repair proteins, and providing a stable environment for repair processes. Its ability to interact with components of the cell cycle and the DNA repair machinery makes it a crucial mediator of the cellular response to DNA damage, contributing to genomic integrity and stability.

Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced using 2 µM etoposide-spiked complete growth media for 18 hours, followed by fixation in -20 °C methanol and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), 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) - Panel A, 2-Dimensional view at Z-plane 35 of 68

Z-Stack = 68 Slices (2.01 µm)

Channels = 1
Scaling (Per Pixel) = 35.0 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 1072 X 1094
Image Size (Scaled) = 37.84 µm X 38.61 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.61 mm, Y: 46.58 mm
Stage Position = X: 86.61 mm, Y: 46.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Z Stack - (3D) - 3-Dimensional Volume Projection Panel B

Z-Stack = 68 Slices (2.01 µm)

Channels = 1
Scaling (Per Pixel) = 35.0 nm X 35.30 nm X 30.00 nm
Image Size (Pixels) = 229 X 181
Image Size (Scaled) = 8.08 µm X 6.39 µm
Bit Depth = 16 Bit
Image Center Position = X: 86.61 mm, Y: 46.58 mm
Stage Position = X: 86.61 mm, Y: 46.58 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 328 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639 nm @ 2.00%
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 = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 643 - 735
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 850 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00 AU / 215 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405 nm @ 0.3%
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 = 350 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 8000 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 7.7 (3D, Auto)

2-Dimensional View - Panel A

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) acquired on the Zeiss LSM980 Confocal microscope at Z-plane 35 of 68 following DNA damage induction. The primary image displays the 647 channel (red) alongside DAPI nuclear counterstaining to visualize fluorescence distribution, punctate signal organization, and fluorescence intensity within the nuclear compartment. A white boxed region of interest denotes the area selected for further volumetric examination in Panel B. The image demonstrates strong nuclear-associated localization, refined p21-associated fluorescence territories, and robust signal generation following direct fluorophore conjugation.

3D Image Processing - Panel B

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)

STORM

STORM imaging from the same product dataset is shown in Panel C to provide a structural comparison between Airyscan super-resolution microscopy and single-molecule localization imaging. Although Airyscan improves structural discrimination relative to conventional confocal imaging, the observed p21-associated fluorescence territories remain partially conflated at the Airyscan resolution scale. In contrast, STORM imaging further resolves heterogeneous nanoscale fluorescence organization and discrete sub-diffraction-scale structural features that are not fully distinguishable in the Airyscan dataset. Collectively, the STORM comparison further supports preservation of fluorescence integrity, localization fidelity, and compatibility of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) with demanding super-resolution imaging workflows following direct fluorophore conjugation.

Summary

Panel A shows a representative optical section (Z-plane 35 of 68) acquired using Airyscan super-resolution microscopy of Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), with DAPI marking the nuclear compartment. Following DNA damage induction, p21 demonstrated strong nuclear-associated localization with refined punctate fluorescence organization highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown datasets.

Panel B presents a 3D volume projection illustrating the spatial organization of the p21-associated signal throughout the nuclear compartment. For enhanced visualization, the p21-associated 647 signal was pseudocolored white, while DAPI nuclear counterstaining was retained to preserve nuclear context. Airyscan microscopy improves structural discrimination relative to conventional confocal imaging, enhancing separation of p21-associated fluorescence territories; however, these structures remain partially conflated at the Airyscan resolution scale and do not represent resolved nanoscale architecture.

Comparison with the corresponding STORM dataset shown in Panel C further demonstrates that although similarities to single-molecule localization imaging begin to emerge, the observed Airyscan fluorescence territories continue to represent higher-order aggregated signal organization rather than discrete molecular structures. Collectively, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) demonstrated strong signal intensity, low background-associated interference, and preservation of biologically coherent localization behavior under increasingly demanding super-resolution imaging conditions following direct fluorophore conjugation.

Image Correction

NONE

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

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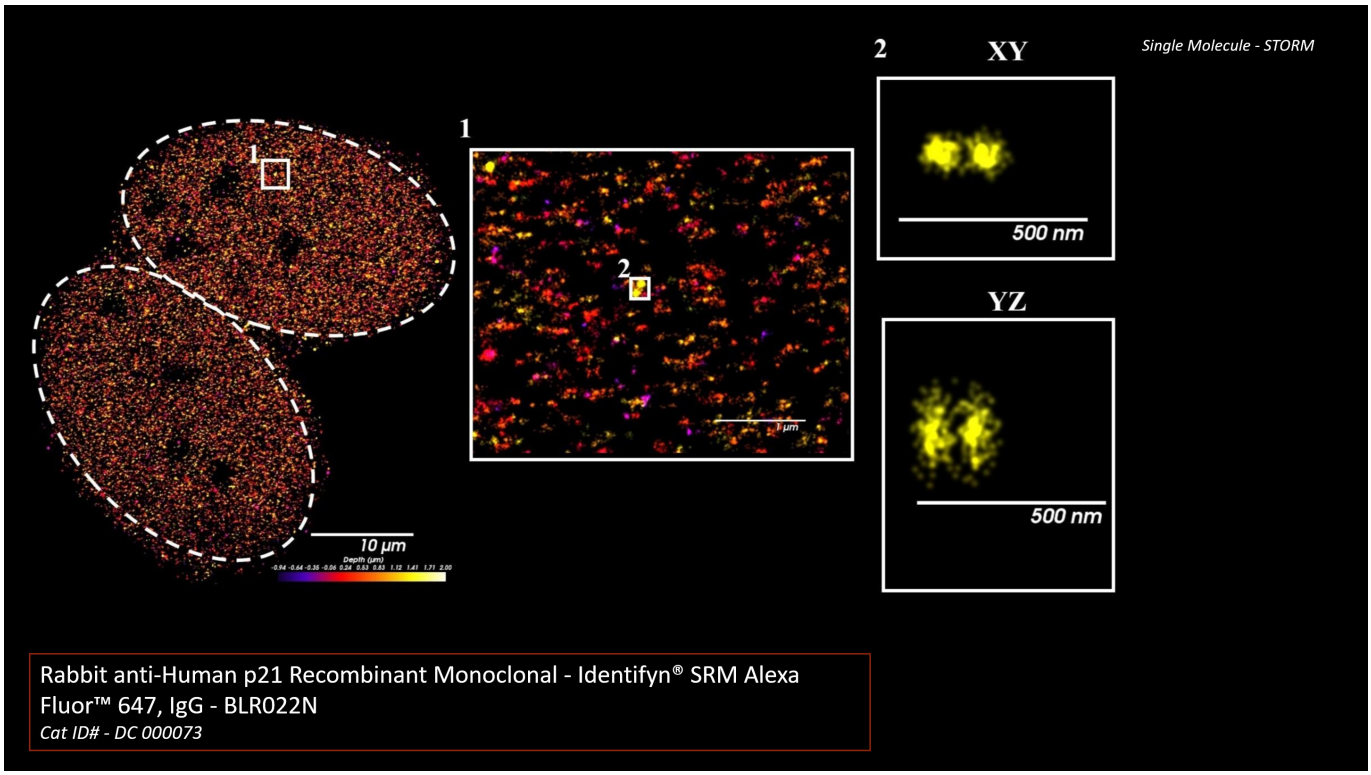
SOURCE PROVENANCE

Catalog	DC-000073
Stage	Airyscan
Instrument	ZEISS LSM 980

SOURCE PROVENANCE

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	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F34C1A778989E3B76A7DD48F3222D8F7ED70270EDBCB9EB66EBB3F1321554331
Method PDF SHA-256	96057E3358A6524A1502B8485B58F2681C306AE0935D9FCDBAD790A909DCD37A

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



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

STRUCTURED ACQUISITION METADATA / SINGLE-MOLECULE STORM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Objective	Olympus 60X/1.30 silicon oil objective
Number of Probes	1
Number of Simultaneous Probes	1
Number of Reference Probes	1
Camera	Both Cameras
Color Channel	Both Channels
Probe Capture Mode	Interleaved
Z -Stack Capture Mode	Interleaved
Multiple Location Capture	Not selected
Post Record Reference	Not Selected
Fluidics	Not Selected
Autofocus Drift Correction	On
Biplane Module	635 nm Dichroic
Objective	Silicon Selected
Stage Insert	Rectangular Selected
Coarse Focus Position	8-Well Chamber Selected
Dichroic	SuperRes
PSF	(Red, Orange)
Heater	Current: 26
Recording Vertical Field of View	100%
Widefield Camera Exposure Time	100 ms | 20 ms
Super Resolution Camera Exposure Time	20 ms
Widefield Camera Binning	1
Per-Probe Exposure Time	Selected
Predefined Probe	First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser	0% Selected
Exposure Time	20 ms Selected
Record Workflow	Selected Options
View Mode	Widefield 200 µm
Capture Mode	Live

FIELD	SOURCE-DOCUMENTED VALUE
Cell(s) or Cellular Structure Localization	The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused. | Positioning of the target Cell or Subsection of the Target Cell is optimized and focused.
Piezo Current	184.2 µm
Widefield 640 nm	0.1X Neutral Density Filter: Selected, @0.2% Laser Input
Reference Probe 1 Laser control 640 nm (635 nm Dichroic)	Not Selected
Widefield camera Exposure Time	100 ms
Widefield Laser	"Widefield 640 nm" was deselected, and "calibrate" under Autofocus was selected.
Current Position	-0.979
Current Intensity	4.5
Calibration Value	-5070 nm/unit
Autofocus Mode	Pre-Capture
SuperRes	50 µm is Selected
Piezo Record	Begin: 184.2; End: 185.4
SuperRes 640nm	0.1X Neutral Density Filter: Selected, @0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	6
Cycles	25
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	25
Cutout Width	16px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1px
Fiducial at Max Accumulation	Not selected
Max Particles Per Frame	No Max
Background SLPE Removal	Not Selected
Cutout/Background Removal	Not Selected
Preview Cutout Positions	Selected
Probe1	BG threshold: 10
Sizing Mode	Radial Precision
Particle size	4 nm
Color by	Depth
Color Table	Single

FIELD	SOURCE-DOCUMENTED VALUE
Opacity Mode	Depth; Opacity 1: 0.35
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	35
Axial precision	70
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Computer Hulls	Not Selected
Compute Density Isosurfaces	Not Selected
Edit Settings	All Probes
Compute	Selected
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned	Deselected

SOURCE METHOD

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

Identifyn Standards for Optical Microscopy Methods™

Identifyn® Primary Antibody

Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG -(BLR022N)
Cat ID# - DC 000073

p21, CDKN1A (cyclin-dependent kinase inhibitor 1A), is a regulatory protein with multiple roles in cell cycle control, DNA damage response, and repair. It is a well-known target of the tumor suppressor protein p53, and its regulation is crucial for cellular responses to DNA damage. The functions of p21 in the context of DNA repair:

Cell Cycle Arrest: p21 is a crucial mediator of cell cycle arrest, particularly in response to DNA damage. It inhibits cyclin-dependent kinases (CDKs), specifically CDK2, CDK4, and CDK6, halting cell cycle progression in the G1 phase. This arrest allows cells time to repair damaged DNA before cell division, reducing the risk of transmitting DNA errors.

Role in DNA Repair: p21, primarily known for its role in cell cycle regulation, can also influence DNA repair mechanisms:

Coordination of Repair Pathways: Inducing cell cycle arrest, p21 provides a stable environment for DNA repair processes. This action helps ensure that repair mechanisms, such as homologous recombination (HR) and nucleotide excision repair (NER), have the time and resources needed to repair DNA damage effectively.

Interacting with DNA Repair Proteins: p21 can interact with various DNA repair proteins, affecting their stability and activity. For example, p21 can bind to proliferating cell nuclear antigen (PCNA), a key component in DNA replication and repair. This interaction can modulate PCNA's activity and help regulate the balance between DNA repair and replication.

Protection from Apoptosis: In some contexts, p21 can protect cells from undergoing apoptosis, providing additional time for DNA repair. This role is complex and context-dependent, as it can also contribute to cellular resistance to specific cancer therapies by prolonging the survival of damaged cells.

Role in Genomic Stability: By facilitating cell cycle arrest and enabling effective repair pathways, p21 helps maintain genomic stability. This role is crucial for preventing mutations and chromosomal aberrations leading to cancer and other genetic diseases.

In summary, p21 plays a multifaceted role in DNA repair by regulating cell cycle progression, coordinating with DNA repair proteins, and providing a stable environment for repair processes. Its ability to interact with components of the cell cycle and the DNA repair machinery makes it a crucial mediator of the cellular response to DNA damage, contributing to genomic integrity and stability.

Method

HeLa cells were grown on µ-Slide 8 Well high Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM Etoposide-spiked complete growth media for 18 hours, followed by -20 °C methanol fixation and blocking with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 5 times in Identifyn®'s proprietary Wash Buffer, followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Microscope

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

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

Number of Probes: 1
Number of Simultaneous Probes: 1
Number of Reference Probes: 1
Camera: Both Cameras
Color Channel: Both Channels
Probe Capture Mode: Interleaved
Z -Stack Capture Mode: Interleaved
Multiple Location Capture: Not selected
Post Record Reference: Not Selected
Fluidics: Not Selected
Autofocus Drift Correction: On

Microscope Configuration

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

Camera Configuration

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

Probe Configuration

Predefined Probe: First probe: 640 nm (635 nm Dichroic); First reference probe: 640 nm (635 nm Dichroic)
Photoactivation/Imaging Laser: 0% Selected
Exposure Time: 20 ms Selected

Experiment Configuration

Record Workflow: Selected Options
View Mode: Widefield 200 µm
Capture Mode: Live

Stage and Piezo Selection

Examination of the Sample

Cell(s) or Cellular Structure Localization: The red-bordered box identifies the area of the SuperRes view and defines the appropriate cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 184.2 µm

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

Advanced Tools Option

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

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

Autofocus - Calibration Values

Current Position: -0.979
Current Intensity: 4.5
Calibration Value: -5070 nm/unit
Autofocus Mode: Pre-Capture

View Mode

SuperRes: 50 µm is Selected

Capture Mode

Live

Stage and Piezo Selection

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

Piezo Record: Begin: 184.2; End: 185.4

Probe 1 Laser control 640 nm (635 nm Dichroic)

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

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

Not Selected

Advanced Tools

Widefield Camera Exposure Time: 20 ms
Default values are maintained unless otherwise noted.

Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 200
Z Positions: 6
Cycles: 25
Time Points: 1
Pause Between Cycles: Not Selected
Automatic Photoactivation Laser Control: Not Selected

Probe 1 Laser control 640 nm (635 nm Dichroic)

Laser Power: 25

Expert Configuration Options - The Localization Tab

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

Localization

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

Record Workflow - Localization Tab

Data Collection

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

Recording Summary

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

Probe1: BG threshold: 10

Region of Interest -

Not selected

Display -

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

Record Workflow - View Tab

Region of Interest

Default values

Visualization Parameters

Point Splatting
Sizing Mode: Radial Precision
Particle size: 4 nm
Color by: Depth
Color Table: Single
Opacity Mode: Depth; Opacity 1: 0.35
Front-to-back Attenuation: Not Selected

Post Acquisition analysis - View Tab

Drift Correction - Computed with the following parameters:

Method: Particle (direct)
Time Intervals: 8 Intervals
Pixel Size: 50 nm
Smoothing: 8.00

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

Radial precision: 35
Axial precision: 70

The other parameters were not applied.

Statistical Analysis - Computed to clean low background signals.

Statistics Dialog - Cluster Analysis

Clustering Algorithm: DBSCAN

Parameters

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

Summary

Collectively, the STORM dataset demonstrates that Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) maintained strong fluorescence performance, localization fidelity, and nanoscale structural signal integrity under demanding single-molecule localization microscopy conditions. Panel 1 demonstrates widespread nuclear-associated p21 fluorescence organization following DNA damage induction, with dense nanoscale fluorescence territories distributed throughout the nuclear compartment. The boxed region of interest is further examined at higher magnification, as shown in Panel 2, which presents orthogonal XY and YZ views demonstrating heterogeneous sub-diffraction fluorescence organization and discrete p21-associated structural territories at the nanoscale.

Importantly, the STORM dataset further confirms that fluorescence territories observed in the corresponding widefield, confocal, and Airyscan datasets represent higher-order aggregated signal organization rather than resolved molecular structures. While similarities to these lower-resolution modalities remain biologically consistent, single-molecule localization imaging further resolves nanoscale structural heterogeneity not distinguishable under diffraction-limited or transitional super-resolution imaging conditions.

Despite the demanding requirements of STORM acquisition workflows, Rabbit anti-Human p21 Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647, IgG - (BLR022N) maintained robust signal intensity, strong signal-to-noise performance, and reproducible nanoscale localization behavior highly consistent with the corresponding unconjugated Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) (Cat ID# - PA-RR 000009) stepdown datasets. Collectively, these findings further support preservation of fluorophore integrity, photoswitching compatibility, and biologically coherent localization behavior following direct fluorophore conjugation under advanced STORM imaging conditions.

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

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SOURCE PROVENANCE

Catalog	DC-000073
Stage	Single-molecule STORM
Instrument	Bruker Vutara VXL
Microscope configuration	Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60X/1.30 silicon oil objective, was used with the following parameters:
Structured source metadata values	81
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	F2463971A6AAE53ECE87C699B9B3D5F5926477C34FF82F5DBF23284E3EE91754
Method PDF SHA-256	5D735ECAA4EC545BDF83E41519D088992A83255FECF2497E6DA0E4A8C09074D0