BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

IDENTIFYN RESOLUTION STEPDOWN

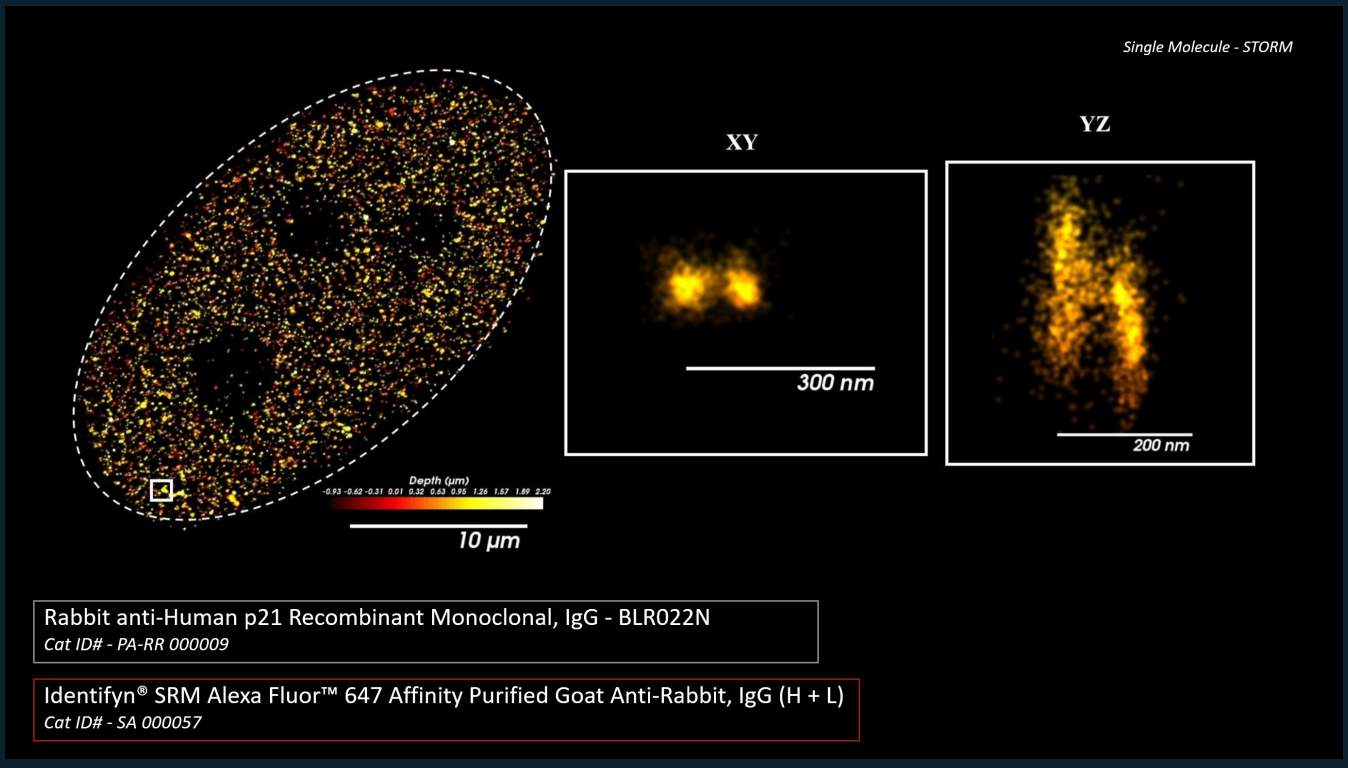
P21

MICROSCOPY EVIDENCE ASSESSMENT

Rabbit anti-Human p21 Recombinant Monoclonal

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

8	1	PENDING
ORDERED EVIDENCE TIERS	SUPPORTING CONTROL	SCIENTIST REVIEW



Rabbit anti-Human p21 Recombinant Monoclonal, IgG - BLR022N
Cat ID# - PA-RR 000009

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

REPRESENTATIVE HIGHEST-AVAILABLE VIEW / SINGLE-MOLECULE STORM

PA-RR-000009 | PRODUCT-LEVEL EVIDENCE DOSSIER

SOURCE-GROUNDED MICROSCOPY + BIOLOGICAL REVIEW / IDENTIFYN EVIDENCE PRESERVED

CRO CAPABILITY DEMONSTRATED

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

- Source-documented sample preparation and antibody/reagent workflow
- Western blot imaging
- Widefield fluorescence microscopy
- Widefield structured illumination / Apotome optical sectioning
- Confocal microscopy
- Airyscan acquisition and reconstruction workflow
- Structured illumination microscopy
- SIM² reconstruction workflow
- Single-molecule STORM presentation workflow
- Preserved control experiment
- Treatment-response experimental workflow
- Z-stack / 3D acquisition
- Multi-channel imaging

SCIENTIFIC QUESTION

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

EVIDENCE AVAILABLE

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

BENNETT ASSESSMENT

The preserved PA-RR-000009 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

BENNETT / MICROSCOPY AND BIOLOGICAL EVIDENCE REVIEW

SOURCE-GROUNDED RETROSPECTIVE REVIEW / PENDING SCIENTIST REVIEW

SOURCE STATE	SOURCE HASH VERIFIED / EVENT RECORDED
BENNETT EVIDENCE REVIEW	COMPLETED / SOURCE-BOUNDED
NATIVE IMAGE REPROCESSING	NOT PERFORMED
LITERATURE SOURCES	INDEPENDENTLY VERIFIED
SCIENTIST REVIEW	PENDING
PUBLICATION	NOT AUTHORIZED

OVERALL CALL / FULL STEPDOWN / SOURCE-BOUNDED REVIEW

The preserved PA-RR-000009 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

SETTINGS ASSESSMENT

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

BIOLOGICAL SUPPORT

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

CONTROL ASSESSMENT

A true preserved control record is present and appears last.

CLAIM CEILING

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

PROCESSING DISCLOSURE

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

REAGENTS AND ACQUIRED CHANNELS

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

EVIDENCE OBJECT	REAGENTS / FLUOROPHORES PRESENT	CHANNELS ACTUALLY ACQUIRED
Western blot	647	Not deterministically stated in the acquisition block
Widefield	405/DAPI; 488; 647	3; 50 Cy 5; 38 eGFP; 49 DAPI; 625 - 655; 450 - 490; 335 - 383; 665 - 715
Widefield SIM — Apotome	405/DAPI; 488; 647	2; 50 Cy 5; 38 HE eGFP; 625 - 655; 450 - 490; 665 - 715; 500 - 550; 653
Confocal	405/DAPI; 488; 647	3; None; 639nm @ 0.10%; 488nm @ 0.10%; 405nm @ 0.2%; 653; 493; 353
Airyscan	405/DAPI; 488; 647	2; None; 639nm @ 0.9%; 488nm @ 0.2%; 405nm @ 0.2%; 653; 493; 353
SIM	405/DAPI; 488; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; 640; 488
SIM²	405/DAPI; 488; 647	2; SR HE LBF 405/488/561/642; 200 - 405, 200 - 488, 200 - 561, 200 - 642; 419 - 470, 503 - 546, 576 - 617, 657 - 800; T1-Axiocam 820-SR; T2-Axiocam 820-SR; 640; 488
Single-molecule STORM	647	Both Channels
Control	405/DAPI; 488; 647	1; None; 639nm @ 0.10%; 653; 668

MODALITY ASSESSMENT / PART 1 OF 9

Western blot / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Azure Sapphire FL. Objective: not explicitly stated. Platform: Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-IR 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 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 9

Widefield / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Imager.M1. Objective: Plan-Apochromat 63x/1.40 Oil M27. Platform: Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:. Acquisition: Channels: 3; Scaling (Per Pixel): 0.110 µm X 0.110 µm; Image size (Pixels): 890 X 683; Image Size (Pixels): 890 X 683; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 705. Timing: Exposure Time: 300 ms; 25 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: 38.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield, documents platform context as ZEISS Axio Imager.M1, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 3 OF 9

Widefield SIM — Apotome / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Axio Observer.Z1/7 Apotome. Objective: EC Plan-Neofluar 63x/1.25 Oil M27. Platform: Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:. Acquisition: Z-Stack: 81 Slices (8.0 µm); Channels: 2; Scaling (Per Pixel): 0.143 µm X 0.143 µm X 0.100 µm; Image size (Pixels): 870 X 826; 284 X 214; Image Size (Pixels): 870 X 826; 284 X 214; Bit Depth: 14 Bit. Detector/camera: Imaging Device: Axiocam 807 Mono. Timing: Exposure Time: 300 ms; 250 ms. Illumination: Light source: X-Cite Xylis Lamp Intensity @ 30.00%; X-Cite Xylis Lamp Intensity @ 25.00%. Optical/scan: Effective NA: 1.25. Processing: Projection: View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 50.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as Widefield SIM — Apotome, documents platform context as ZEISS Axio Observer.Z1/7 Apotome, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 4 OF 9

Confocal / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 201 Slices (6.0 µm); Channels: 3; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; Image size (Pixels): 640 X 681; Image Size (Pixels): 640 X 681; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29 µs; Frame Time: 4.97 s. Illumination: Laser Wavelength: 639nm @ 0.10%; 488nm @ 0.10%. Optical/scan: Pinhole: 1.00AU / 64 µm; 1.00AU / 51 µm; Scan Zoom: 1.7; LSM Scan Speed: 11; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 8.6 (3D, Auto); Filter: 7.7 (3D, Auto). Structured source metadata values: 55.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 5 OF 9

Airyscan / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 141 Slices (4.2 µm); Channels: 2; Scaling (Per Pixel): 35.0 nm X 35.0 nm X 30.0 nm; Image size (Pixels): 720 X 996; 333 X 346; Image Size (Pixels): 720 X 996; 333 X 346; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Airyscan; Detector Type: GaAsP-PMT. Timing: Pixel Time: 1.04 µs; Frame Time: 19.77 s. Illumination: Laser Wavelength: 639nm @ 0.9%; 488nm @ 0.2%. Optical/scan: Pinhole: 5.00AU / 328 µm; 5.00AU / 250 µm; Scan Zoom: 1.9; LSM Scan Speed: 6; Scan Direction: Bidirectional; Averaging: 4; Effective NA: 1.4. Processing: Projection: View Angle 35°, Scale Z 50%, 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 9.3 (3D, Auto). Structured source metadata values: 56.

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 6 OF 9

SIM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 100 Slices (2.97 µm); Channels: 2; Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm; Image size (Pixels): 2017 X 2103; 275 X 268; Image Size (Pixels): 2017 X 2103; 275 X 268; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 640 nm @ 1.5%; 488 nm @ 1.0%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM; Process Sampling: N/A; Result Sampling: 4; Projection: View Angle 35°, Scale Z 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 53.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Reconstruction-dependent structural separation requires native reconstruction QC before numerical resolution or molecular dimensions can be claimed.

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 7 OF 9

SIM² / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS Elyra. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:. Acquisition: Z-Stack: 100 Slices (2.97 µm); Channels: 2; Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm; Image size (Pixels): 1593 X 1425; 231 X 222; Image Size (Pixels): 1593 X 1425; 231 X 222; Bit Depth: 16 Bit. Detector/camera: Imaging Device: Axiocam 820. Timing: Exposure Time: 120 ms; Frame Time: 1.59 s. Illumination: Laser Wavelength: 640 nm @ 1.5%; 488 nm @ 1.0%; Illumination Wavelength: 640; 488. Optical/scan: Scan Direction: Unidirectional; Effective NA: 1.4. Processing: Algorithm: SIM2; Process Sampling: 4; Result Sampling: 4; Projection: View Angle 35°, Scale Z 30%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected); Background: Black. Structured source metadata values: 51.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

The source identifies this object as SIM², documents platform context as ZEISS Elyra, and records detected dye/channel descriptors as 405/DAPI; 488; 647.

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

Further reconstruction-dependent segmentation does not establish molecular dimensions, structure identity, or mechanism without independent support.

CLAIM CEILING

This tier supports a source-bounded statement that the historical SIM² evidence and documented settings are preserved for review; stronger biological or quantitative conclusions require compatible native data, controls and scientist review.

MODALITY ASSESSMENT / PART 8 OF 9

Single-molecule STORM / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: Bruker Vutara VXL. Objective: Olympus 60x/1.30 Silicon Oil Objective. Platform: Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil Objective, was used with the following parameters:. Detector/camera: Camera: Both Cameras. Timing: Exposure Time: 20 ms Selected; Super Resolution Camera Exposure Time: 20 ms; Widefield Camera Exposure Time: 100 ms; 20 ms. Illumination: Photoactivation/Imaging Laser: 0% Selected. Structured source metadata values: 80.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

MODALITY ASSESSMENT / PART 9 OF 9

Control / SOURCE EVIDENCE PRESERVED

DOCUMENTED SETTINGS

Platform: ZEISS LSM 980. Objective: Plan-Apochromat 63x/1.40 Oil DIC M27. Platform: Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:. Acquisition: Z-Stack: 201 Slices (6.0 µm); Channels: 1; Scaling (Per Pixel): 0.059 µm X 0.059 µm X 0.030 µm; 0.059 µm X 0.059 µm; Image size (Pixels): 573 X 656; 1006 X 732; Image Size (Pixels): 573 X 656; 1006 X 732; Bit Depth: 16 Bit. Detector/camera: Imaging Device: GaAsP-Pmt3; Detector Type: GaAsP-PMT. Timing: Pixel Time: 0.29 µs; 0.25 µs; Frame Time: 4.97 s; 10.24 s. Illumination: Laser Wavelength: 639nm @ 0.10%. Optical/scan: Pinhole: 1.00AU / 64 µm; Scan Zoom: 1.7; 1.1; LSM Scan Speed: 11; 9; Scan Direction: Bidirectional; Averaging: 8; Effective NA: 1.4. Processing: LSM Plus Mode: Filter: 8.6 (3D, Auto); Filter: 8.3 (3D, Auto). Structured source metadata values: 54.

VISIBLE OBSERVATION

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

SOURCE-REPORTED RESULT

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

LITERATURE CORRELATION

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

INTERPRETIVE LIMIT

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

CLAIM CEILING

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

CROSS-MODALITY SYNTHESIS

The preserved PA-RR-000009 record contains the full ordered evidence sequence from Western blot through single-molecule STORM. Each modality retains its own acquisition and processing boundary.

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

BENNETT LITERATURE CORRELATION — SOURCES INDEPENDENTLY VERIFIED

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

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

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

REFERENCES

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

RELEASE BOUNDARY

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

SOURCE RECONCILIATION / QA / PROVENANCE

SOURCE STATUS - PRESERVED / AUTO-RECONCILED

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

HIGH CONFIDENCE - AUTO-RECONCILED

Airyscan / Scaling (Per Pixel): 35.0 nm X 35.0 nm X 30.0 nm -> 0.03500 µm X 0.03500 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)=720 X 996; Image Size (Scaled)=25.41 µm X 35.14 µm; PASS - INTERNALLY CONSISTENT. Documented sampling reproduces the reported physical field dimensions. Maximum field-dimension error 0.83%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02111 µm X 0.02111 µm X 0.02970 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)=2017 X 2103; Image Size (Scaled)=42.58 µm X 44.40 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

HIGH CONFIDENCE - AUTO-RECONCILED

SIM² / Scaling (Per Pixel): 0.021 nm X 0.021 nm X 0.030 nm -> 0.02111 µm X 0.02112 µm X 0.02970 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)=1593 X 1425; Image Size (Scaled)=33.63 µm X 30.09 µm; PASS - INTERNALLY CONSISTENT. The source magnitude/unit pair fails by approximately 1,000-fold; independent X and Y field dimensions establish the reconciled sampling. Maximum field-dimension error 0.00%.

UNRESOLVED - PRESERVED AS CONFLICT

Cross-tier / treatment duration: {"Western blot": ["20 hours"], "Widefield": ["24 hours"], "Widefield SIM — Apotome": ["24 hours"], "Confocal": ["24 hours"], "Airyscan": ["24 hours"], "SIM": ["24 hours"], "SIM²": ["24 hours"], "Single-molecule STORM": ["48 hours"], "Control": ["24 hours"]} -> Preserved as modality-specific historical duration values. Genuinely different treatment durations are present across evidence stages, and no source or scientist correction resolves them. Supporting evidence: Only duration values from explicit etoposide-treatment sentences were classified; concentration, wavelength, resolution and pixel-size values were excluded.

BENNETT

AI-POWERED BIOLOGICAL INTELLIGENCE

BENNETT EVIDENCE REVIEW COMPLETE

INTERPRETATION TO EVIDENCE

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

IDENTiFYN®

IDENTIFYN PRESERVED SOURCE EVIDENCE

ORIGINAL RECORD BEGINS

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

PRODUCT-LEVEL STEPDOWN MAP

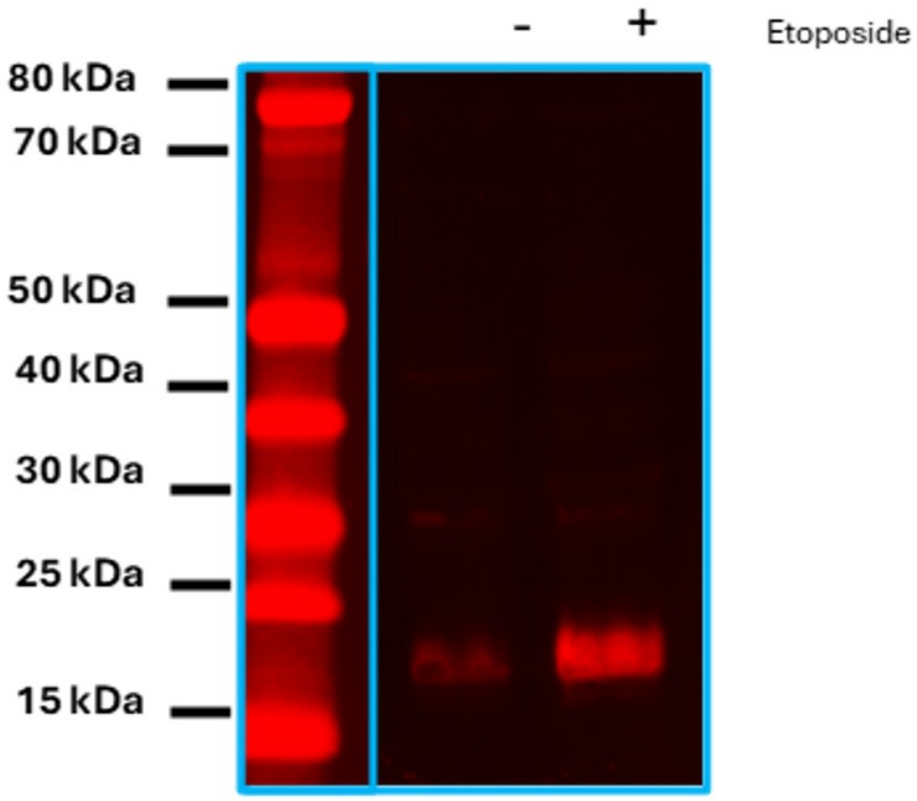
This dossier preserves the complete available evidence progression for PA-RR-000009. Each tier remains tied to its original image, source method, instrument context and cryptographic identity. A preserved control is retained as supporting evidence and appears after every resolution tier.

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

INTERPRETIVE BOUNDARY

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

ORIGINAL IMAGE EVIDENCE / WESTERN BLOT



EVIDENCE TIER	Western blot
INSTRUMENT	Azure Sapphire FL
CELL MODEL	HeLa
DETECTED DYES	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-IR and Z-Plane Imaging.
Scanner	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-IR and Z-Plane Imaging.
Detection mode	Fluorescence
Laser	632 nm
Emission Filter	676 ± 37 nm
Detector	PMT
Intensity	L3
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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Expected MW: 21 kDa

HeLa cells were treated with 2 µM etoposide for 20 hours to induce DNA damage. Cells were homogenized using an ultrasonication probe and RIPA 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's PageRuler™ Prestained Protein Ladder, 10 to 180 kDa, was used as a molecular weight marker.

Post electrophoresis, the proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using the iBlot™ 3 Starter Kit PVDF. The transfer membrane was blocked with Blocker™ Casein for 1 hour, then washed 5 times with PBS. The blocked membrane was incubated with Identifyn® Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N) for 20 hours, followed by five washes in 1X PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated for 1 hour and washed 5 times with 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-IR and Z-Plane Imaging.

Scan Settings

Detection mode = Fluorescence
Laser = 632 nm
Emission Filter = 676 ± 37 nm
Detector = PMT
Intensity = L3
Pixel size = 100 µm
Scan Speed = High
Quality = High
Focus = Membrane (0 µm in Z-axis)

Post Processing

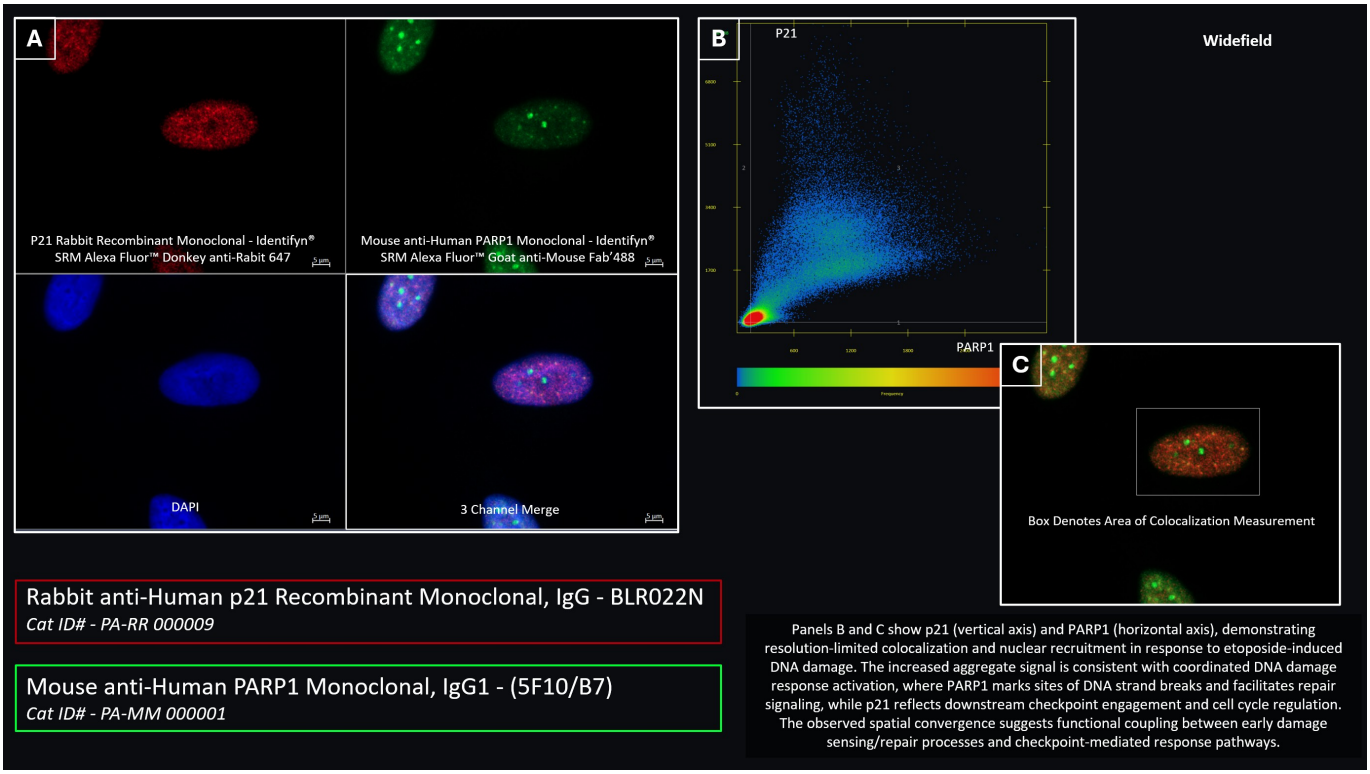
None

Image: K. Small

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	Western blot
Instrument	Azure Sapphire FL
Microscope configuration	Azure Sapphire FL with Customizable Laser and Filter Modules, Enabling UV-IR and Z-Plane Imaging.
Structured source metadata values	11
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	A0768D41080F5CFC45DDB5BC19BE8D0CFC9C60527072E2D40098DF8BD8BB94E1
Method PDF SHA-256	11F46937493B284B4E54479D5CF7E3AC1ECF77D367FEDB2A1543EBBFC6732C43

ORIGINAL IMAGE EVIDENCE / WIDEFIELD



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

STRUCTURED ACQUISITION METADATA / WIDEFIELD

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Imager.M1 Widefield, using a Plan-Apochromat 63x/1.40 Oil M27, Axiocam 705, and X-Cite Xylis Lamp, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil M27
Channels	3
Scaling (Per Pixel)	0.110 µm X 0.110 µm
Image size (Pixels)	890 X 683
Image Size (Scaled)	97.48 µm X 74.80 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 | 38 eGFP | 49 DAPI
Beam Splitter	660 | 495 | 395
Filter Excitation Wavelength	625 - 655 | 450 - 490 | 335 - 383
Filter Emission Wavelength	665 - 715 | 500 - 550 | 420 - 470
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @ 30.00% | X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time	300 ms | 25 ms
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Imaging Device	Axiocam 705
Camera Adapter	1X
Depth of Focus	1.03 µm | 0.80 µm | 0.72 µm
Binning Mode	2,2
Image Measurement	Indicated by White Box

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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

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

Single Plane (2D)

Channels = 3
Scaling (Per Pixel) = 0.110 µm X 0.110 µm
Image size (Pixels) = 890 X 683
Image Size (Scaled) = 97.48 µm X 74.80 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 = 300 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

488 -

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

DAPI -

Reflector = 49 DAPI
Beam Splitter = 395
Filter Excitation Wavelength = 335 - 383
Filter Emission Wavelength = 420 - 470
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 10.00%
Exposure Time = 25 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 top-left panel shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L). The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Colocalization View - Panels B and C

Horizontal Axis - AF647, Threshold 145, Range Auto
Vertical Axis - AF488, Threshold 307, Range Auto

Image Measurement = Indicated by White Box

Summary

Panels B and C show p21 (horizontal axis) and PARP1 (vertical axis), demonstrating resolution-limited colocalization and nuclear recruitment in response to etoposide-induced DNA damage. The increased aggregate signal is consistent with coordinated activation of the DNA damage response, in which PARP1 marks sites of DNA strand breaks and facilitates repair signaling. At the same time, p21 reflects downstream checkpoint engagement and cell cycle regulation. The observed spatial convergence suggests functional coupling between early damage-sensing/repair processes and checkpoint-mediated response pathways.

Image Correction

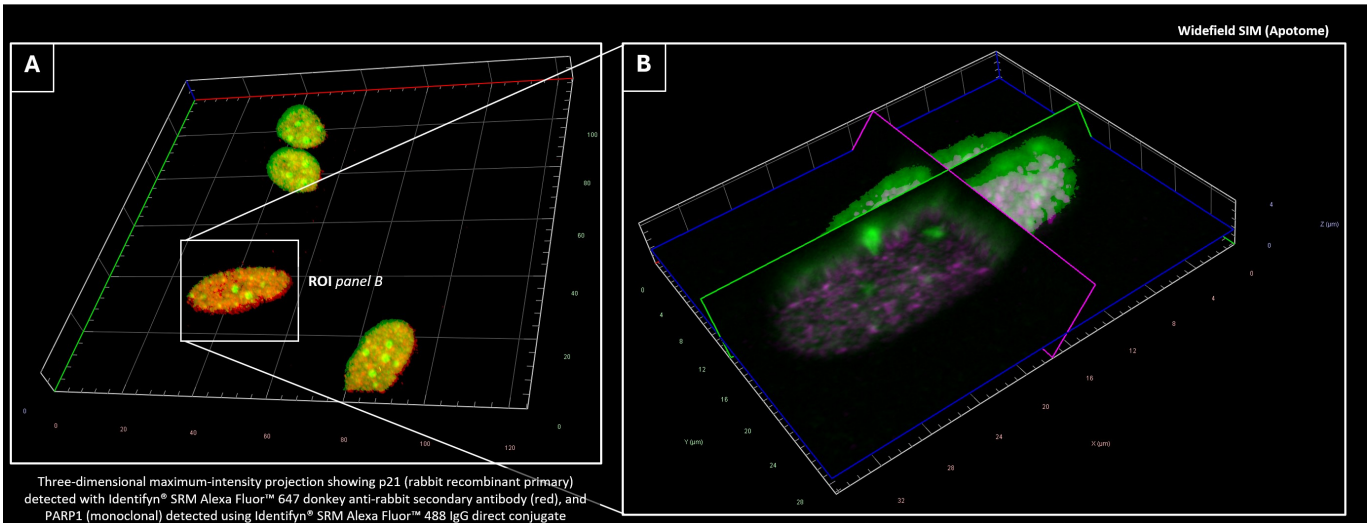
NONE

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

SOURCE PROVENANCE

Catalog	PA-RR-000009
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	38
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	FA6E6A269AFD801D876AF5BF011F9E6F229C0630A81732BF06A5275A478CDB5B
Method PDF SHA-256	361565B60ED1E35203B7868CB2BBD1EDF5117227C63ACBD69BA9467AA442D68B

ORIGINAL IMAGE EVIDENCE / WIDEFIELD SIM - APOTOME



Three-dimensional maximum-intensity projection showing p21 (rabbit recombinant primary) detected with Identifyn® SRM Alexa Fluor™ 647 donkey anti-rabbit secondary antibody (red), and PARP1 (monoclonal) detected using Identifyn® SRM Alexa Fluor™ 488 IgG direct conjugate (green).

Three-dimensional volume-intensity projection (wedge view) with orthogonal plane representation (X–Y, blue; X–Z, green; Y–Z, violet), demonstrating nuclear colocalization of p21 and PARP1 following etoposide-induced DNA damage. The orthogonal reconstructions provide spatial validation of colocalization across all axes, confirming that signal overlap is not due to projection artifacts but reflects true co-distribution within the nuclear volume. The 647 nm (p21) channel was pseudo-colored violet to enhance visual discrimination between channels.

Rabbit anti-Human p21 Recombinant Monoclonal, IgG - BLR022N
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

EVIDENCE TIER	Widefield SIM - Apotome
INSTRUMENT	ZEISS Axio Observer.Z1/7 Apotome
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	50

STRUCTURED ACQUISITION METADATA / WIDEFIELD SIM - APOTOME

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Objective	EC Plan-Neofluar 63x/1.25 Oil M27
Z-Stack	81 Slices (8.0 µm)
Channels	2
Scaling (Per Pixel)	0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels)	870 X 826 | 284 X 214
Image Size (Scaled)	124.29 µm X 118.00 µm | 35.43 µm X 30.57 µm
Bit Depth	14 Bit
Image Center Position	X: 45.68 mm, Y: 50.45 mm
Stage Position	X: 45.68 mm, Y: 50.45 mm
ROI Center Offset	X: 1.14 µm, Y: 0.00 µm
Reflector	50 Cy 5 | 38 HE eGFP
Beam Splitter	660 | 495
Filter Excitation Wavelength	625 - 655 | 450 - 490
Filter Emission Wavelength	665 - 715 | 500 - 550
Contrast Method	Fluorescence
Light source	X-Cite Xylis Lamp Intensity @ 30.00% | X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time	300 ms | 250 ms
Excitation Wavelength	653 | 493
Emission Wavelength	668 | 517
Effective NA	1.25
Imaging Device	Axiocam 807 Mono
Camera Adapter	1X
Depth of Focus	1.30 µm | 1.00 µm
Binning Mode	2,2
Apotome SIM Image Processing	Display Mode "Optical Sectioning" - enabled correction selected.
3D Maximum Projection	Precise
Appearance	Threshold 13% all channels, Ramp 0% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 100%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)
X-Y	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.

FIELD	SOURCE-DOCUMENTED VALUE
Position of Clip	-31% | -13% | -11%
Clip X	0°
Clip Z	0°
X-Z	Activate was selected, textured opaque was chosen, and a green outline of the clipped perimeter was shown; Clip Back was selected, and Clip Front was NOT selected.
Clip Y	30° | -45°
Y-Z	Activate was selected, textured opaque was chosen, and a violet 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 p21 Recombinant Monoclonal, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

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

Z Stack - (3D) - Panel A

Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 870 X 826
Image Size (Scaled) = 124.29 µm X 118.00 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.68 mm, Y: 50.45 mm
Stage Position = X: 45.68 mm, Y: 50.45 mm
ROI Center Offset = X: 1.14 µm, Y: 0.00 µm

Z Stack - (3D) - Panel B, ROI

Z-Stack = 81 Slices (8.0 µm)

Channels = 2
Scaling (Per Pixel) = 0.143 µm X 0.143 µm X 0.100 µm
Image size (Pixels) = 284 X 214
Image Size (Scaled) = 35.43 µm X 30.57 µm
Bit Depth = 14 Bit
Image Center Position = X: 45.68 mm, Y: 50.45 mm
Stage Position = X: 45.68 mm, Y: 50.45 mm
ROI Center Offset = X: 1.14 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 = 300 ms
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.30 µm
Binning Mode = 2,2

488 -

Reflector = 38 HE eGFP
Beam Splitter = 495
Filter Excitation Wavelength = 450 - 490
Filter Emission Wavelength = 500 - 550
Contrast Method = Fluorescence
Light source = X-Cite Xylis Lamp Intensity @ 25.00%
Exposure Time = 250 ms
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.25
Imaging Device = Axiocam 807 Mono
Camera Adapter = 1X
Depth of Focus = 1.00 µm
Binning Mode = 2,2

Post Processing - SIM Processing

Apotome SIM Image Processing = Display Mode "Optical Sectioning" - enabled correction selected. Normalization, Phase Corrector, Fourier Filter, and Deconvolution were NOT used. 3D Image Processing

3D Image Processing - Panel A

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

3D Image Processing - Panel B

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

Clipping Image Process - Panel B

Clipping planes are introduced in Panel B to highlight the Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N) and the Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Within the nuclear compartment, by adjusting or cutting away portions of the image using a triple-wedge cut, removing parts of the image in the X-Y, X-Z, and Y-Z planes.

X-Y = 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 = -31%
Clip X = 0°
Clip Z = 0°

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

Position of Clip = -13%
Clip Y = 30°
Clip Z = 0°

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

Position of Clip = -11%
Clip Y = -45°
Clip Z = 0°

Summary

Panel B presents a wedge-cut, volume-rendered view from a top-down perspective with slight posterior rotation, exposing the region of wedge removal and enabling visualization of signal distribution throughout the z-stack. This orientation provides complementary geometric context and supports spatial colocalization of p21 and PARP1 following etoposide-induced DNA damage. Based on the confined, centrally localized signal morphology-consistent with expected nuclear distribution under these conditions-the observed colocalization likely occurs within the nuclear compartment. The volumetric reconstruction and orthogonal perspective further exclude peripheral or out-of-plane signal contributions, confirming that the observed overlap is not due to projection or boundary artifacts.

Image Correction

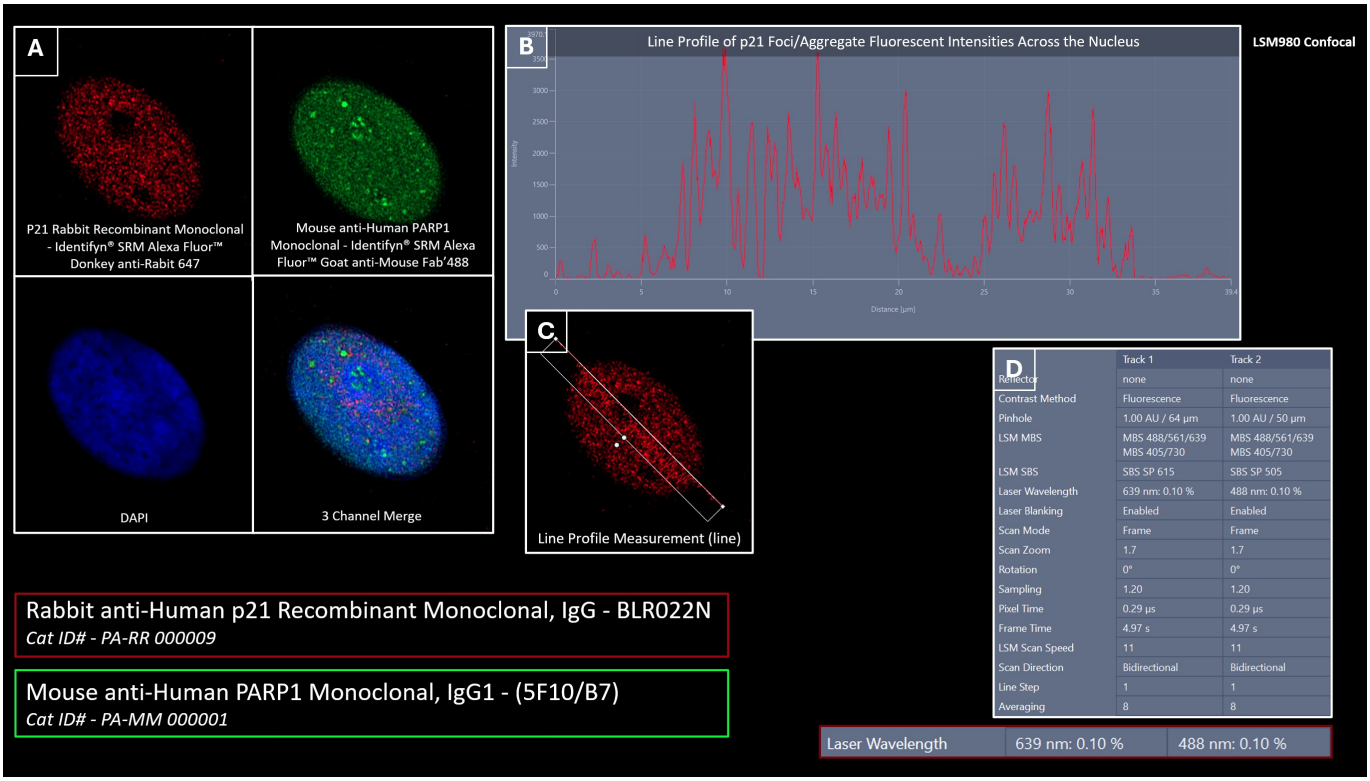
NONE

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

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	Widefield SIM - Apotome
Instrument	ZEISS Axio Observer.Z1/7 Apotome
Microscope configuration	Zeiss Axio Observer.Z1/7 Apotome SIM, using an EC Plan-Neofluar 63x/1.25 Oil M27, Axiocam 807, and X-Cite Xylis Lamp was used with the following parameters for each dye:
Structured source metadata values	50
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	AD131E2A1C16D448565E97DF62BD320E6E4923FC56F25CC82A04BBAF733E0429
Method PDF SHA-256	B1609139104A030CE31518FD15D9201DB89EF07ECA7E6BD62A4AE12DD49AB8C9

ORIGINAL IMAGE EVIDENCE / CONFOCAL



EVIDENCE TIER	Confocal
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	55

STRUCTURED ACQUISITION METADATA / CONFOCAL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	201 Slices (6.0 µm)
Channels	3
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels)	640 X 681
Image Size (Scaled)	37.64 µm X 40.06 µm
Bit Depth	16 Bit
Image Center Position	X: 81.04 mm, Y: 53.39 mm
Stage Position	X: 81.04 mm, Y: 53.39 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 64 µm | 1.00AU / 51 µm | 5.00AU / 235 µm
LMS MBS	MBS 488/639 & 405/730 | MBS 488/561/639 & 405/730
LMS SBS	SBS SP 615 | SBS SP 505
Laser Wavelength	639nm @ 0.10% | 488nm @ 0.10% | 405nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7
Rotation	0°
Sampling	1.20
Pixel Time	0.29 µs
Frame Time	4.97 s
LSM Scan Speed	11
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	645 - 700 | 511 - 545 | 420 - 480, 495 - 499
Imaging Device	GaAsP-Pmt3 | Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	550 V | 500 V | 800 V
Detector Offset	0
Detector Digital Gain	1.0
LSM Plus Mode	Filter: 8.6 (3D, Auto) | Filter: 7.7 (3D, Auto) | Filter: 6.8 (3D, Auto)
Profile Definition	Stroke Thickness 1, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 39.4), Y (Min 0 and Max 3970)

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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss LSM 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) - 2D at Z-Plane 100 of 201, Panels A, B, and C

Z-Stack = 201 Slices (6.0 µm)

Channels = 3
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 640 X 681
Image Size (Scaled) = 37.64 µm X 40.06 µm
Bit Depth = 16 Bit
Image Center Position = X: 81.04 mm, Y: 53.39 mm
Stage Position = X: 81.04 mm, Y: 53.39 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 64 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @ 0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 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 = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.6 (3D, Auto)

488 -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 51 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @ 0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 s
LSM Scan Speed = 11
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 511 - 545
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 500 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 7.7 (3D, Auto)

DAPI -

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 235 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 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 = 420 - 480, 495 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 6.8 (3D, Auto)

Split View - Panel A

The top-left panel shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. The top-right panel shows Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L). The lower-left panel shows DAPI staining, and the bottom-right panel shows the three-channel merged image.

Profile View Display - Panels B & C

Profile Definition = Stroke Thickness 1, Normal View, Grid Distance 1
Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 39.4), Y (Min 0 and Max 3970)

This panel quantifies p21 foci, or resolution-limited aggregates, following etoposide-induced DNA damage. Line profile analysis reveals well-defined intensity peaks and spatial confinement, supporting localized enrichment of p21 at damage-associated regions.

Zen Acquisition Info Tab - Panel D

Shown are the acquisition parameters for the 647 channel (p21) and 488 channel (PARP1), collected using the 639 nm laser at 0.10% power and the 488 nm laser at 0.10% power, respectively. Scan zoom, scan speed, and line-averaging settings are provided to ensure the transparency and reproducibility of line-profile measurements.

Image Correction

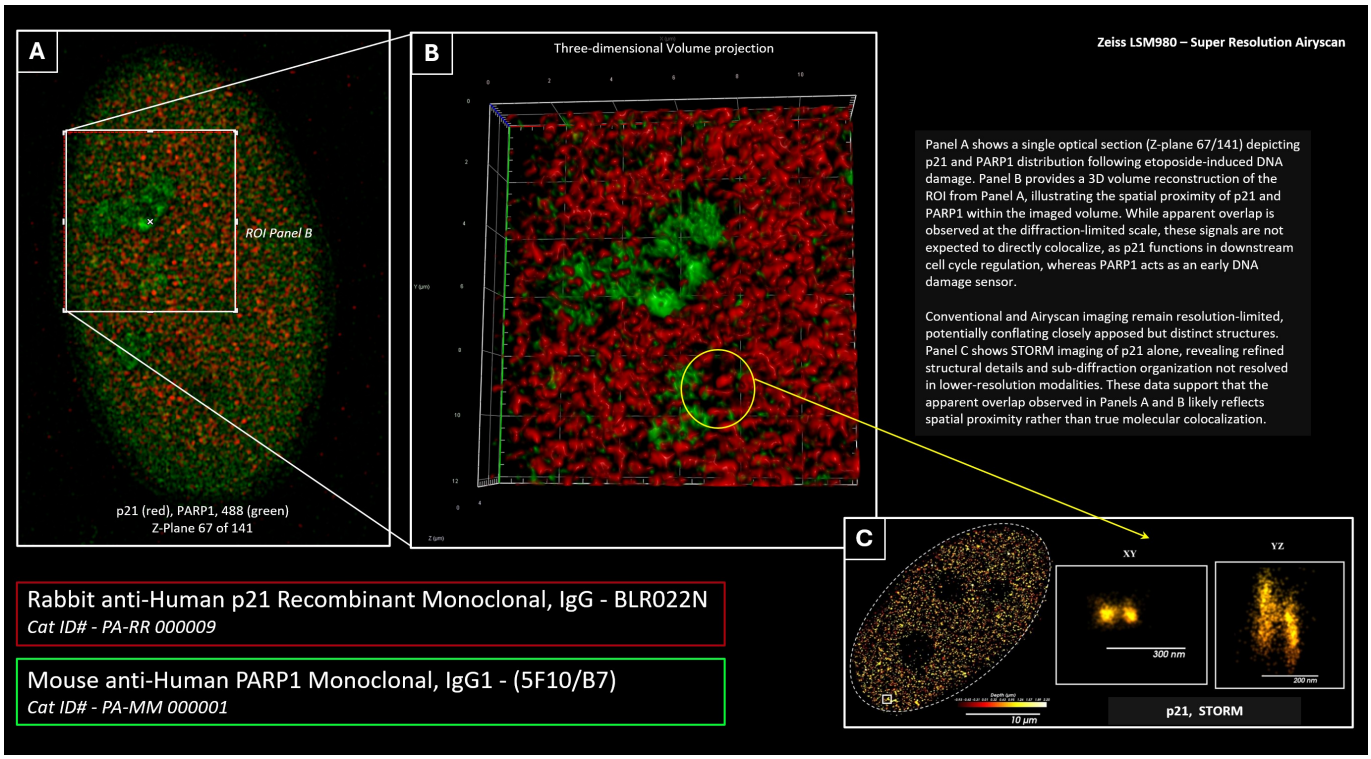
NONE

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

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	Confocal
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	55
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	415E57536793CFF4EEF5FF7F69FC19033C1474ABCD765411C0063489A5315985
Method PDF SHA-256	DCEA711ACC69F2A78E1FF24E20CEBA6B30EEACD52A7D4D28E2790C0E3D49F10A

ORIGINAL IMAGE EVIDENCE / AIRYSCAN



EVIDENCE TIER	Airyscan
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	56

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	141 Slices (4.2 µm)
Channels	2
Scaling (Per Pixel)	0.03500 µm X 0.03500 µm X 0.03000 µm
Image size (Pixels)	720 X 996 | 333 X 346
Image Size (Scaled)	25.41 µm X 35.14 µm | 11.75 µm X 12.21 µm
Bit Depth	16 Bit
Image Center Position	X: 82.17 mm, Y: 53.61 mm
Stage Position	X: 82.17 mm, Y: 53.61 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	5.00AU / 328 µm | 5.00AU / 250 µm | 5.00AU / 235 µm
LMS MBS	MBS 488/561/639 & 405/730
LMS SBS	SBS LP 640 | SBS SP 505
Laser Wavelength	639nm @ 0.9% | 488nm @ 0.2% | 405nm @ 0.2%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.9
Rotation	0°
Sampling	2.00
Pixel Time	1.04 µs
Frame Time	19.77 s
LSM Scan Speed	6
Scan Direction	Bidirectional
Line Step	1
Averaging	4
Excitation Wavelength	653 | 493 | 353
Emission Wavelength	668 | 517 | 465
Effective NA	1.4
Detection Wavelength	643 - 735 | 499 - 548 | 420 - 480, 495 - 499
Imaging Device	Airyscan

FIELD	SOURCE-DOCUMENTED VALUE
Detector Type	GaAsP-PMT
Detector Gain	800 V
Detector Offset	0
Detector Digital Gain	1.0
Airyscan Mode	SuperResolution 10.0 (3D, Auto) | SuperResolution 9.3 (3D, Auto) | SuperResolution 8.6 (3D, Auto)
3D Volume Projection	Precise
Appearance	Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels
Background	Black
Light	Brightness 100%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping
Projection	View Angle 35°, Scale Z 50%, 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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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:

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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.

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Methods

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss LSM 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) - 2D at Z-Plane 67 of 141, Panel A

Z-Stack = 141 Slices (4.2 µm)

Channels = 2
Scaling (Per Pixel) = 35.0 nm X 35.0 nm X 30.0 nm
Image size (Pixels) = 720 X 996
Image Size (Scaled) = 25.41 µm X 35.14 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.17 mm, Y: 53.61 mm
Stage Position = X: 82.17 mm, Y: 53.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 141 Slices (4.2 µm)

Channels = 2
Scaling (Per Pixel) = 35.0 nm X 35.0 nm X 30.0 nm
Image size (Pixels) = 333 X 346
Image Size (Scaled) = 11.75 µm X 12.21 µm
Bit Depth = 16 Bit
Image Center Position = X: 82.17 mm, Y: 53.61 mm
Stage Position = X: 82.17 mm, Y: 53.61 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647
Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 328 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS LP 640
Laser Wavelength = 639nm @ 0.9%
Laser Blanking = Enabled

Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 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 = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 10.0 (3D, Auto)

488

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 250 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 488nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 s
LSM Scan Speed = 6
Scan Direction = Bidirectional
Line Step = 1
Averaging = 4
Excitation Wavelength = 493
Emission Wavelength = 517
Effective NA = 1.4
Detection Wavelength = 499 - 548
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 9.3 (3D, Auto)

DAPI - Not Shown in Data Analysis, but Collected in Image Acquisition

Reflector = None
Contrast Method = Fluorescence
Pinhole = 5.00AU / 235 µm
LMS MBS = MBS 488/561/639 & 405/730
LMS SBS = SBS SP 505
Laser Wavelength = 405nm @ 0.2%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.9
Rotation = 0°
Sampling = 2.00
Pixel Time = 1.04 µs
Frame Time = 19.77 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 = 420 - 480, 495 - 499
Imaging Device = Airyscan
Detector Type = GaAsP-PMT
Detector Gain = 800 V
Detector Offset = 0
Detector Digital Gain = 1.0
Airyscan Mode = SuperResolution 8.6 (3D, Auto)

2-Dimensional View - Panel A

Shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L). A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

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 50%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset to demonstrate the resolution limitations of Airyscan, which are improved relative to conventional confocal imaging, in determining localization and structural details.

Summary

Panel A shows a single optical section (Z-plane 67/141) depicting p21 and PARP1 distribution following etoposide-induced DNA damage. Panel B provides a 3D volume reconstruction of the ROI from Panel A, illustrating the spatial proximity of p21 and PARP1 within the imaged volume. While apparent overlap is observed at the diffraction-limited scale, these signals are not expected to exhibit direct molecular colocalization, as p21 functions in downstream cell-cycle regulation, whereas PARP1 acts as an early DNA-damage sensor.

Conventional and Airyscan imaging remain resolution-limited, potentially conflating closely apposed but distinct structures. Panel C shows STORM imaging of p21 alone, revealing refined structural details and sub-diffraction organization not resolved in lower-resolution modalities. These data support that the apparent overlap observed in Panels A and B likely reflects spatial proximity rather than true molecular colocalization.

Image Correction

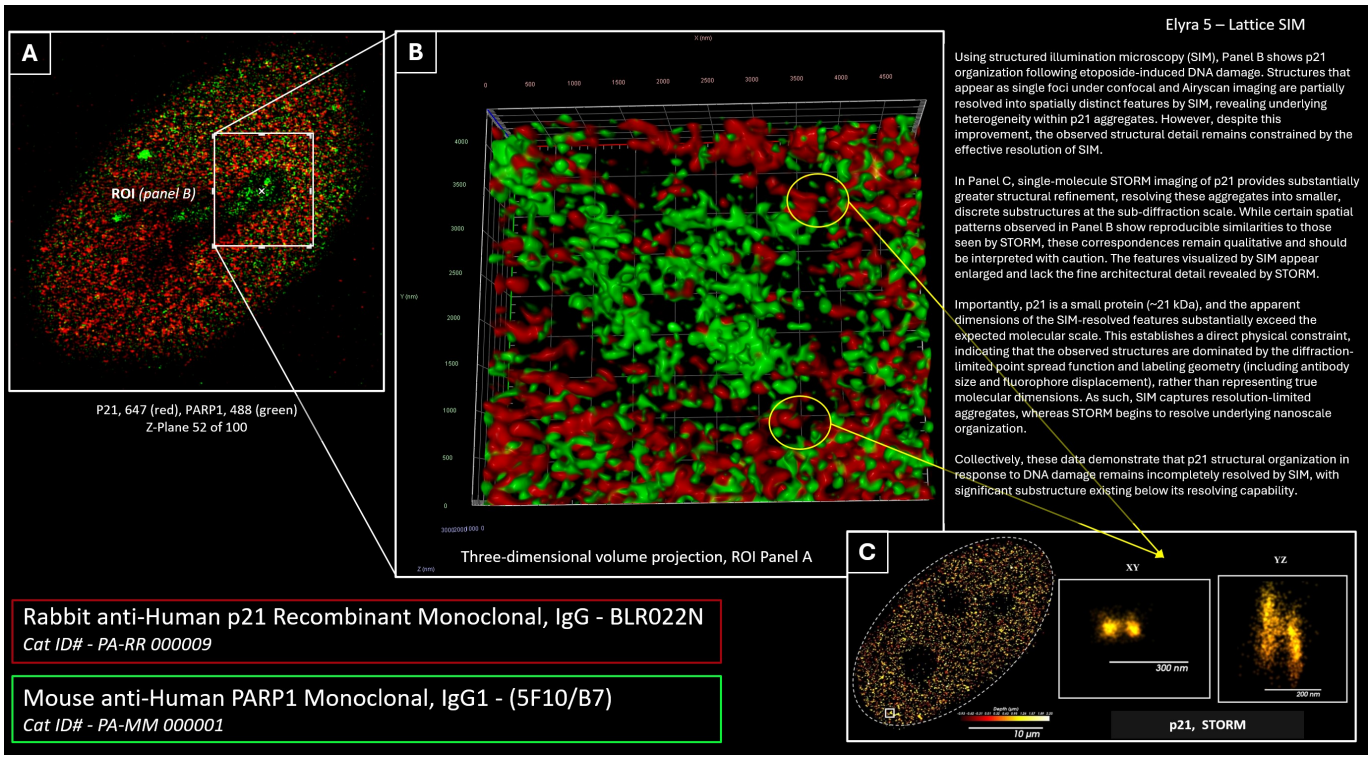
NONE

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

SOURCE PROVENANCE

Catalog	PA-RR-000009
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	56
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	565EF8E4CE8A0780B751788A088E8714E086BCABEEA7BD1AE34E0F740A180D9A
Method PDF SHA-256	CF4D7A824B233CBA996442127A471F2A2598222EF4E4DF7C51BE12BA379471B5

ORIGINAL IMAGE EVIDENCE / SIM



EVIDENCE TIER	SIM
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	53

STRUCTURED ACQUISITION METADATA / SIM

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	100 Slices (2.97 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02111 µm X 0.02970 µm
Image size (Pixels)	2017 X 2103 | 275 X 268
Image Size (Scaled)	42.58 µm X 44.40 µm | 5.81 µm X 5.66 µm
Bit Depth	16 Bit
Image Center Position	X: 59.27 mm, Y: 33.22 mm | X: 69.03 mm, Y: 39.62 mm
Stage Position	X: 59.27 mm, Y: 33.22 mm | X: 69.03 mm, Y: 39.62 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR
Excitation Wavelength	640 | 488
Emission Wavelength	729 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820
Camera Adapter	1x
Exposure Time	120 ms
Depth of Focus	1.13 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 1.5% | 488 nm @ 1.0%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Processing Dimension	3D
Result Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Process Sampling	N/A
Algorithm	SIM
SIM Settings	Standard
Auto Sharpness	On
Sharpness	11.2432 (647), 11.2094 (488)
Fitting	Fast Fit
Normalization	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 80%, 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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

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Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 52 of 100, Panel A

Z-Stack = 100 Slices (2.97 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image size (Pixels) = 2017 X 2103
Image Size (Scaled) = 42.58 µm X 44.40 µm
Bit Depth = 16 Bit
Image Center Position = X: 59.27 mm, Y: 33.22 mm
Stage Position = X: 59.27 mm, Y: 33.22 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 100 Slices (2.97 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image size (Pixels) = 275 X 268
Image Size (Scaled) = 5.81 µm X 5.66 µm
Bit Depth = 16 Bit
Image Center Position = X: 69.03 mm, Y: 39.62 mm
Stage Position = X: 69.03 mm, Y: 39.62 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 1.5%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @ 1.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM Processing

Processing Dimension = 3D
Result Sampling = 4
Process Sampling = N/A
Channels = 2
Algorithm = SIM
SIM Settings = Standard
Auto Sharpness = On
Sharpness = 11.2432 (647), 11.2094 (488)
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View- Panel A

Shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody and Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7) visualized using Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L). A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

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 80%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same product dataset to demonstrate the resolution limitations of Lattice SIM, which are better than Airyscan for determining localization and structural details. Moreover, the signal pattern is consistent with the Airyscan data and is more closely resolution-limited than that of a single-molecule image from STORM.

Summary

Using structured illumination microscopy (SIM), Panel B shows the organization of p21 following etoposide-induced DNA damage. Structures that appear as single foci under confocal and Airyscan imaging are partially resolved into spatially distinct features by SIM, revealing underlying heterogeneity within p21 aggregates. However, despite this improvement, the observed structural detail remains constrained by SIM's effective resolution.

In Panel C, single-molecule STORM imaging of p21 provides substantially greater structural refinement, resolving these aggregates into smaller, discrete substructures at the sub-diffraction scale. While certain spatial patterns observed in Panel B show reproducible similarities to those seen by STORM, these correspondences remain qualitative and should be interpreted with caution. The features visualized by SIM appear enlarged and lack the fine architectural detail revealed by STORM.

Importantly, p21 is a small protein (~21 kDa), and the apparent dimensions of the SIM-resolved features substantially exceed the expected molecular scale. This establishes a direct physical constraint, indicating that the observed structures are dominated by the diffraction-limited point spread function and labeling geometry (including antibody size

and fluorophore displacement), rather than representing true molecular dimensions. As such, SIM captures resolution-limited aggregates, whereas STORM begins to resolve underlying nanoscale organization.

Collectively, these data demonstrate that p21 structural organization in response to DNA damage remains incompletely resolved by SIM, with significant substructure existing below its resolving capability.

Image Correction

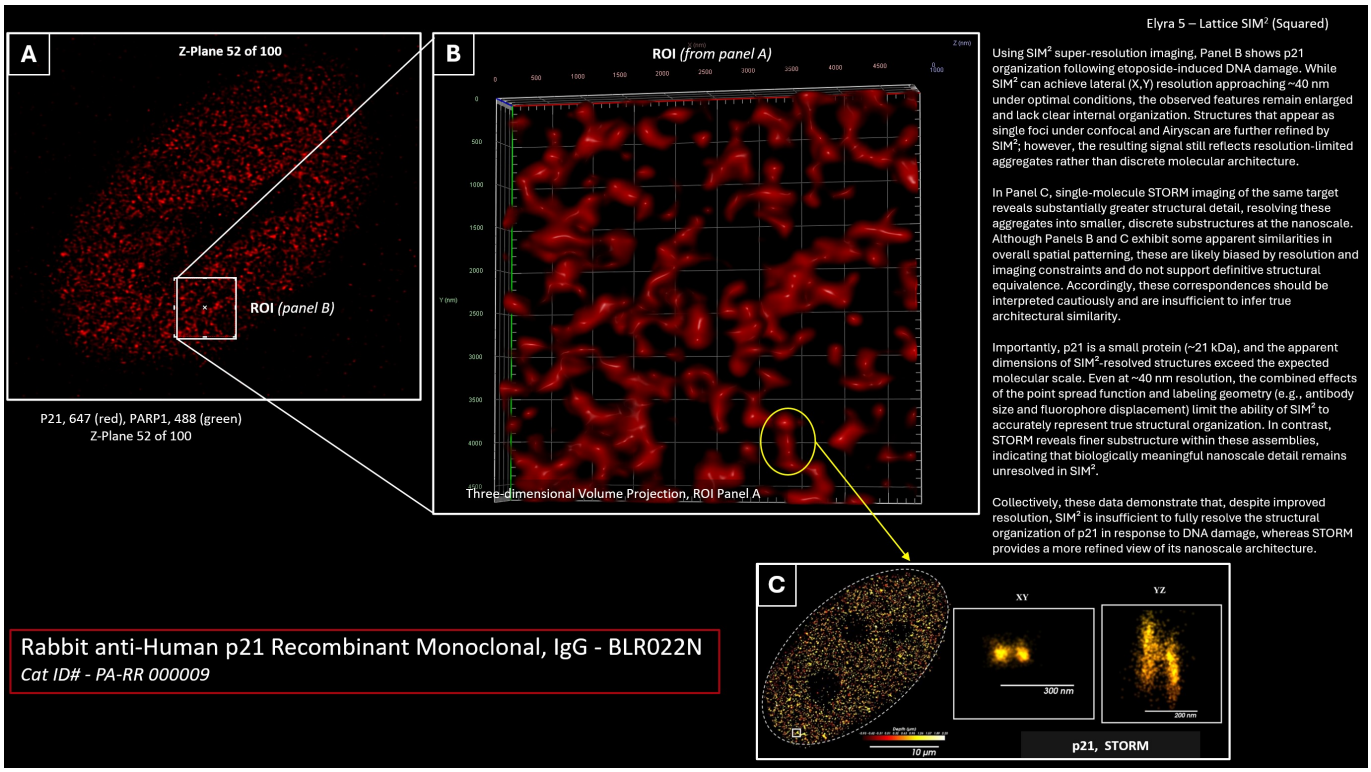
NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	SIM
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	53
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	4C8D3DBF155E38B6D046276B9FA058708DE23A801B57D996FB716FCAF0586067
Method PDF SHA-256	A15D60BA885DEDC44C8ED05D0F14B824993EF9520589488BED3E457AE09A9E4E

ORIGINAL IMAGE EVIDENCE / SIM²



EVIDENCE TIER	SIM²
INSTRUMENT	ZEISS Elyra
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	51

STRUCTURED ACQUISITION METADATA / SIM²

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	100 Slices (2.97 µm)
Channels	2
Scaling (Per Pixel)	0.02111 µm X 0.02112 µm X 0.02970 µm
Image size (Pixels)	1593 X 1425 | 231 X 222
Image Size (Scaled)	33.63 µm X 30.09 µm | 4.88 µm X 4.69 µm
Bit Depth	16 Bit
Image Center Position	X: 59.27 mm, Y: 33.22 mm
Stage Position	X: 59.27 mm, Y: 33.22 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflectors	SR HE LBF 405/488/561/642
Beam Splitter	405, 488, 561, 642
Filter Excitation Wavelength	200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method	Fluorescence
Illumination Wavelength	640 | 488
Channel Name	T1-Axiocam 820-SR | T2-Axiocam 820-SR
Excitation Wavelength	640 | 488
Emission Wavelength	729 | 525
Effective NA	1.4
Detection Wavelength	419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device	Axiocam 820
Camera Adapter	1x
Exposure Time	120 ms
Depth of Focus	1.13 µm | 0.81 µm
Binning Mode	2,2
Laser Wavelength	640 nm @ 1.5% | 488 nm @ 1.0%
Frame Time	1.59 s
Scan Direction	Unidirectional
Grating	36.5 µm grating
Result Sampling	4
Process Sampling	4

FIELD	SOURCE-DOCUMENTED VALUE
Algorithm	SIM2
Input SNR	Low
Regularization Weight	0.0000
Iterations	3
Filter	No Filter
Fitting	Fast Fit
Normalization	Auto
3D Volume Projection	Precise
Appearance	Threshold 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 30%, 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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7)
Cat ID# - PA-MM 000001

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L)
Cat ID# - SA 000017

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 by treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS.

The second primary antibody, Mouse anti-Human PARP1 Monoclonal, IgG1 - (5F10/B7), was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Goat Anti-Mouse, F(ab')□ (H + L) was incubated for 1 hour at room temperature at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI, cat#P36962.

Microscope

Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:

Z Stack - (3D) - 2D at Z-Plane 52 of 100, Panel A

Z-Stack = 100 Slices (2.97 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image size (Pixels) = 1593 X 1425
Image Size (Scaled) = 33.63 µm X 30.09 µm
Bit Depth = 16 Bit
Image Center Position = X: 59.27 mm, Y: 33.22 mm
Stage Position = X: 59.27 mm, Y: 33.22 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

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

Z-Stack = 100 Slices (2.97 µm)

Channels = 2
Scaling (Per Pixel) = 0.021 nm X 0.021 nm X 0.030 nm
Image size (Pixels) = 231 X 222
Image Size (Scaled) = 4.88 µm X 4.69 µm
Bit Depth = 16 Bit
Image Center Position = X: 59.27 mm, Y: 33.22 mm
Stage Position = X: 59.27 mm, Y: 33.22 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 -

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 640
Channel Name = T1-Axiocam 820-SR
Excitation Wavelength = 640
Emission Wavelength = 729
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 120 ms
Depth of Focus = 1.13 µm
Binning Mode = 2,2
Laser Wavelength = 640 nm @ 1.5%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

488 - Collected in Image Acquisition but not Shown in this Analysis

Reflectors = SR HE LBF 405/488/561/642
Beam Splitter = 405, 488, 561, 642
Filter Excitation Wavelength = 200 - 405, 200 - 488, 200 - 561, 200 - 642
Filter Emission Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Contrast Method = Fluorescence
Illumination Wavelength = 488
Channel Name = T2-Axiocam 820-SR
Excitation Wavelength = 488
Emission Wavelength = 525
Effective NA = 1.4
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800
Imaging Device = Axiocam 820
Camera Adapter = 1x
Exposure Time = 120 ms
Depth of Focus = 0.81 µm
Binning Mode = 2,2
Laser Wavelength = 488 nm @ 1.0%
Frame Time = 1.59 s
Scan Direction = Unidirectional
Grating = 36.5 µm grating

Post Processing - SIM2 Processing

Processing Dimension - 3D
Result Sampling = 4
Process Sampling = 4
Channels = 2
Algorithm = SIM2
Input SNR = Low
Regularization Weight = 0.0000
Iterations = 3
Filter = No Filter
Fitting = Fast Fit
Normalization = Auto

2-Dimensional View - Panel A

Shows Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N), visualized using Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) secondary antibody. A Region of Interest is denoted by a white box to be further examined in the 3-Dimensional Volume Projection in Panel B.

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 30%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

STORM - Panel C

Panel C shows STORM imaging from the same dataset to illustrate the resolution characteristics of lattice SIM². While SIM² provides improved lateral resolution relative to both Airyscan and Lattice SIM-enhancing localization and partially refining structural features-the observed signal remains resolution-limited. The overall pattern is consistent with that seen in Airyscan and SIM datasets, but with modestly improved feature separation.

Summary

Using SIM² super-resolution imaging, Panel B shows the organization of p21 following etoposide-induced DNA damage. While SIM² can achieve lateral (X, Y) resolution approaching ~40 nm under optimal conditions, the observed features remain enlarged and lack clear internal organization. Structures that appear as single foci under confocal and Airyscan are further refined by SIM²; however, the resulting signal still reflects resolution-limited aggregates rather than discrete molecular architecture.

In Panel C, single-molecule STORM imaging of the same target reveals substantially greater structural detail, resolving these aggregates into smaller, discrete nanoscale substructures. Although Panels B and C exhibit some apparent similarities in overall spatial patterning, these are likely biased by resolution and imaging constraints and do not support definitive structural equivalence. Accordingly, these correspondences should be interpreted cautiously and are insufficient to infer true architectural similarity.

Importantly, p21 is a small protein (~21 kDa), and the apparent dimensions of SIM²-resolved structures exceed the expected molecular scale. Even at ~40 nm resolution, the combined effects of the point spread function and labeling geometry (e.g., antibody size and fluorophore displacement) limit SIM²'s ability to represent true structural organization

accurately. In contrast, STORM reveals finer substructure within these assemblies, indicating that biologically meaningful nanoscale detail remains unresolved in SIM².

Collectively, these data demonstrate that, despite improved resolution, SIM² is insufficient to fully resolve the structural organization of p21 in response to DNA damage. In contrast, STORM provides a more refined view of its nanoscale architecture.

Image Correction

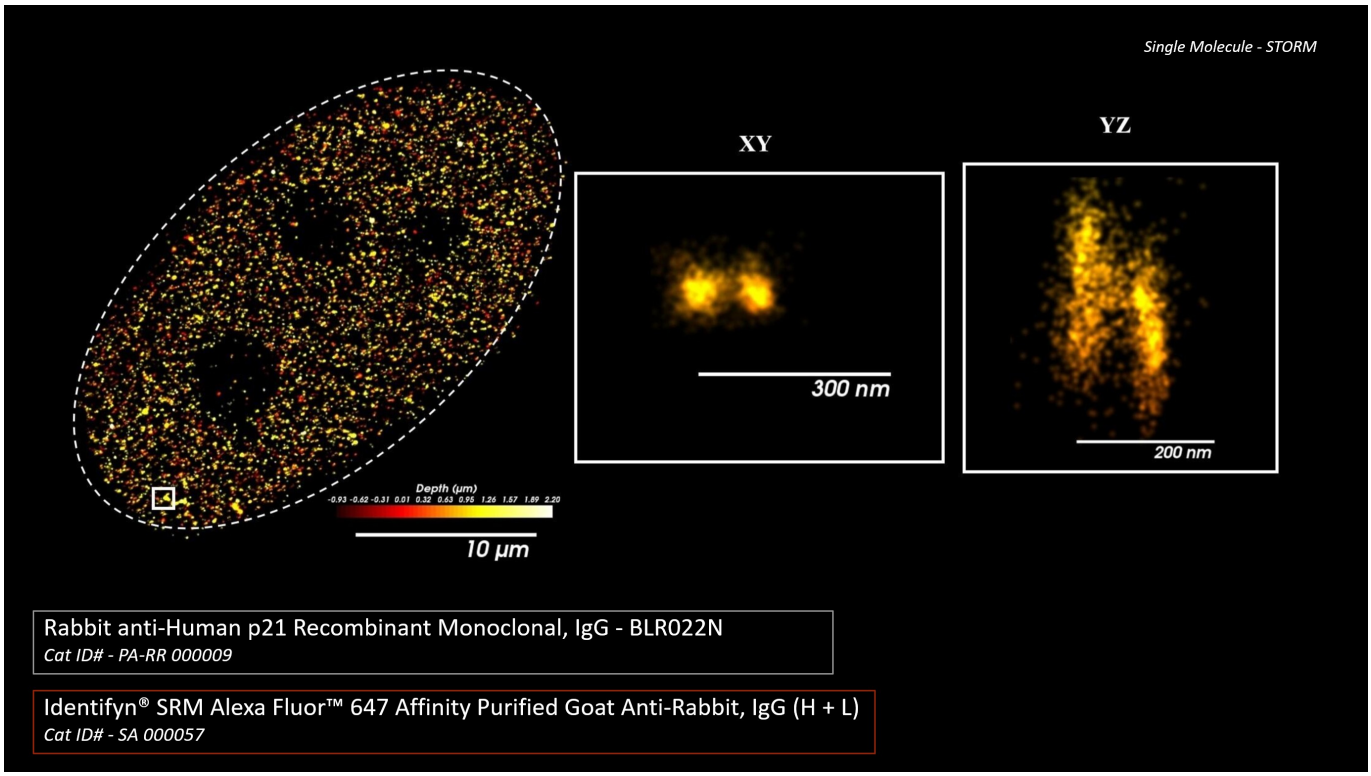
NONE

Image by J. H. Cheong
Image Analysis and Data Presentation by B.T. Bennett

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	SIM²
Instrument	ZEISS Elyra
Microscope configuration	Zeiss Elyra 5 Lattice SIM, AxioObserver.Z1/7 using Plan-Apochromat 63x/1.40 Oil DIC M27, 1.25X Tubelens, was used with the following parameters for each dye:
Structured source metadata values	51
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	E751793DD928292311FDF0F4C8AB5C28E6C4C818BB51858E8C3B253C44F81945
Method PDF SHA-256	D0674989CAB133E38CF6505ABF3BAABD3C5F8DD53382B623A31FE9A97E3C6EFE

ORIGINAL IMAGE EVIDENCE / SINGLE-MOLECULE STORM



EVIDENCE TIER	Single-molecule STORM
INSTRUMENT	Bruker Vutara VXL
CELL MODEL	HeLa
DETECTED DYES	647
METADATA VALUES	80

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	180 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 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: 179; End: 180.4
SuperRes 640 nm	0.1X Neutral Density Filter: Selected, @ 0.02% Laser Input
Record Workflow - Record Tab	Capture Parameters
Frames	Probe 1: 200
Z Positions	7
Cycles	23
Time Points	1
Pause Between Cycles	Not Selected
Automatic Photoactivation Laser Control	Not Selected
Laser Power	30
Cutout Width	16 px
Max Frame Accumulation	1 frame
Max Accumulation Offset	1 px
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: 15
Sizing Mode	Radial Precision
Particle size	4 nm
Color by	Depth
Color Table	Single
Opacity Mode	Depth; Opacity 1: 0.09

FIELD	SOURCE-DOCUMENTED VALUE
Front-to-back Attenuation	Not Selected
Method	Particle (direct)
Time Intervals	8 Intervals
Pixel Size	50 nm
Smoothing	8.00
Radial precision	30
Axial precision	65
Clustering Algorithm	DBSCAN
Maximum Particle Distance	0.1 µm
Maximum Particle Count to Form Cluster	10
Compute 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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Identifyn® Secondary Antibody

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

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 in a µ-Slide 8 Well High Glass Bottom Ibidi chamber. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 48 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, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:100. Cells were washed 3X in Identifyn®'s proprietary Wash Buffer, and Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:100. Cells were washed 5X in Identifyn®'s proprietary Wash Buffer, followed by the addition of Identifyn®'s proprietary STORM Buffer to the sample chamber, enabling image acquisition.

Microscope

Bruker Vutara VXL Biplane Single Molecule Microscope, using an Olympus 60x/1.30 Silicon Oil Objective, was used with the following parameters:

Calibration -

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

https://identifyn.com/storm-calibration

Image Acquisition -

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

Configuration Tab - Capture Configuration

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

Microscope Configuration

Biplane Module: 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: 180 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: 179; End: 180.4

Probe 1 Laser control 640 nm (635 nm Dichroic)

SuperRes 640 nm: 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: 7
Cycles: 23
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: 30

Expert Configuration Options - The Localization Tab

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

Localization

Cutout Width: 16 px
Max Frame Accumulation: 1 frame
Max Accumulation Offset: 1 px
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: 15

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: 0.09
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: 30
Axial precision: 65

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
Compute 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

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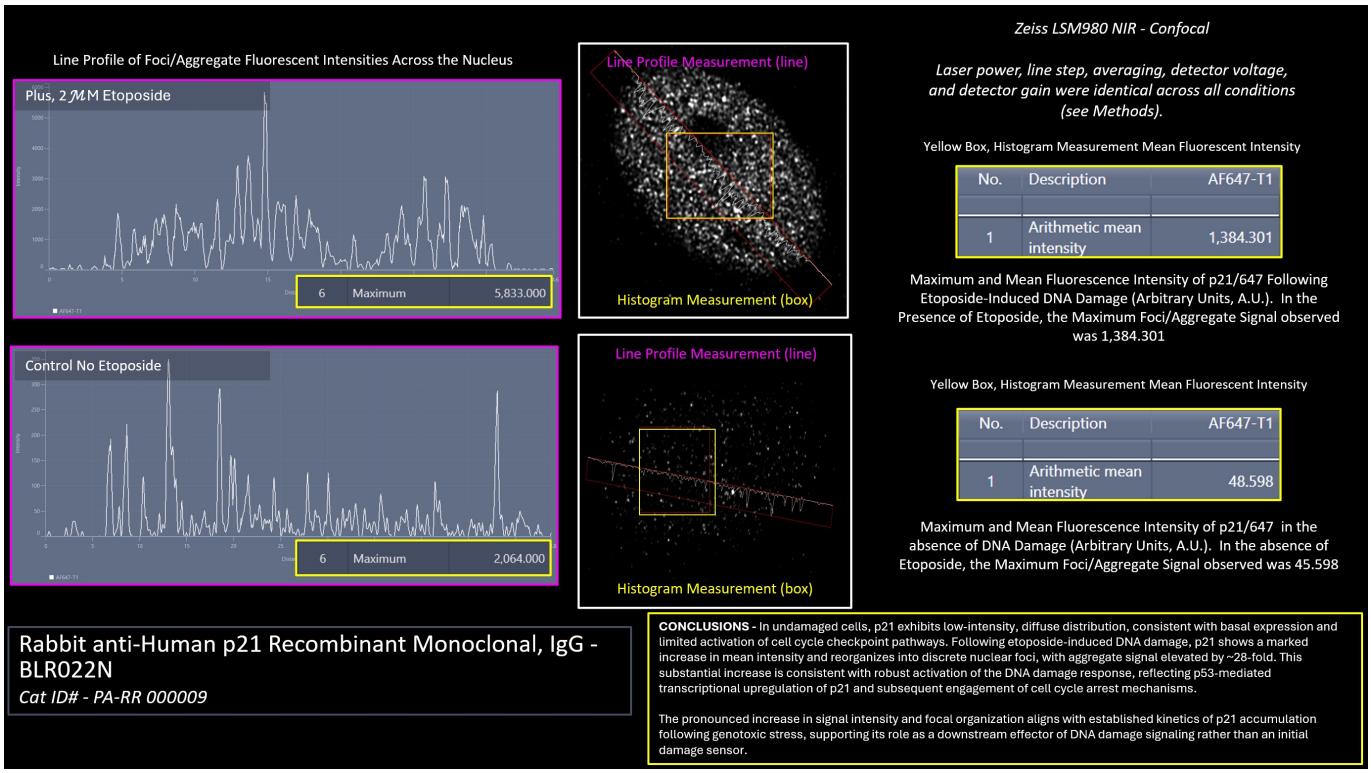
Image by P. Raut

SOURCE PROVENANCE

Catalog	PA-RR-000009
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	80
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT
Cell model	HeLa
Image SHA-256	C465713053260D782A66B5C00D87C1B656FFA4601CBD7D760A0CBCC2D5451B9A
Method PDF SHA-256	92A4A22E2A5CDFB63BBE39000ABA76DF6F383AA5EE3B3FB9B2B163BDB98EFD7A

ORIGINAL IMAGE EVIDENCE / CONTROL



EVIDENCE TIER	Control
INSTRUMENT	ZEISS LSM 980
CELL MODEL	HeLa
DETECTED DYES	405/DAPI; 488; 647
METADATA VALUES	54

STRUCTURED ACQUISITION METADATA / CONTROL

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

FIELD	SOURCE-DOCUMENTED VALUE
Platform / configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Objective	Plan-Apochromat 63x/1.40 Oil DIC M27
Z-Stack	201 Slices (6.0 µm)
Channels	1
Scaling (Per Pixel)	0.059 µm X 0.059 µm X 0.030 µm | 0.059 µm X 0.059 µm
Image size (Pixels)	573 X 656 | 1006 X 732
Image Size (Scaled)	33.70 µm X 35.58 µm | 59.16 µm X 43.05 µm
Bit Depth	16 Bit
Image Center Position	X: 81.04 mm, Y: 53.39 mm | X: 72.53 mm, Y: 51.01 mm
Stage Position	X: 81.04 mm, Y: 53.39 mm | X: 72.53 mm, Y: 51.01 mm
ROI Center Offset	X: 0.00 µm, Y: 0.00 µm
Reflector	None
Contrast Method	Fluorescence
Pinhole	1.00AU / 64 µm
LMS MBS	MBS 488/639 & 405/730
LMS SBS	SBS SP 615
Laser Wavelength	639nm @ 0.10%
Laser Blanking	Enabled
Scan Mode	Frame
Scan Zoom	1.7 | 1.1
Rotation	0°
Sampling	1.20
Pixel Time	0.29 µs | 0.25 µs
Frame Time	4.97 s | 10.24 s
LSM Scan Speed	11 | 9
Scan Direction	Bidirectional
Line Step	1
Averaging	8
Excitation Wavelength	653
Emission Wavelength	668
Effective NA	1.4
Detection Wavelength	645 - 700
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: 8.6 (3D, Auto) | Filter: 8.3 (3D, Auto)
Profile Definition	Stroke Thickness 1.5, Normal View, Grid Distance 1
Profile View	X and Y axes were set to Auto - X (Min 0.0 and Max 32.60), Y (Min 0.0 and Max 6129) | X and Y axes were set to Auto - X (Min 0.0 and Max 53.80), Y (Min 0.0 and Max 366)
Histogram Definition	Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535
Histogram View	X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000) | X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 100000)
Arithmetic Mean intensity	1384.301 | 45.598

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, IgG - (BLR022N)
Cat ID# - PA-RR 000009

Identifyn® Secondary Antibody

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L)
Cat ID# - SA 000060

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

Plus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency. DNA damage was induced via treatment with 2 µM etoposide-spiked complete growth media for 24 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, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Minus Damage -

HeLa cells were grown on acid-etched #1.5H cover glass to ~50% confluency, then fixed in -20°C methanol and blocked with Thermo Fisher Fish Serum Blocker (cat#37527X3). The primary antibody, Rabbit anti-Human p21 Recombinant Monoclonal, IgG - (BLR022N), was incubated for 1 hour at room temperature at a dilution of 1:250. Cells were washed 3X in PBS. Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Donkey Anti-Rabbit, IgG (H + L) was incubated at room temperature for 1 hour at a dilution of 1:1000. Cells were washed 5X in PBS and mounted using ProLong™ Diamond Antifade Mountant with DAPI (cat# P36962).

Microscope

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

Z Stack - (3D) - 2D at Z-Plane 100 of 201, Panel A, from Confocal Data Set

Z-Stack = 201 Slices (6.0 µm)

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm X 0.030 µm
Image size (Pixels) = 573 X 656
Image Size (Scaled) = 33.70 µm X 35.58 µm
Bit Depth = 16 Bit
Image Center Position = X: 81.04 mm, Y: 53.39 mm
Stage Position = X: 81.04 mm, Y: 53.39 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

Single Plane (2D) - Control

Channels = 1
Scaling (Per Pixel) = 0.059 µm X 0.059 µm
Image size (Pixels) = 1006 X 732
Image Size (Scaled) = 59.16 µm X 43.05 µm
Bit Depth = 16 Bit
Image Center Position = X: 72.53 mm, Y: 51.01 mm
Stage Position = X: 72.53 mm, Y: 51.01 mm
ROI Center Offset = X: 0.00 µm, Y: 0.00 µm

647 - Plus Damage, from Confocal Data Set

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 64 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @ 0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.7
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.29 µs
Frame Time = 4.97 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 = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.6 (3D, Auto)

647 - Minus Damage - noting the same laser input power and conditions

Reflector = None
Contrast Method = Fluorescence
Pinhole = 1.00AU / 64 µm
LMS MBS = MBS 488/639 & 405/730
LMS SBS = SBS SP 615
Laser Wavelength = 639nm @ 0.10%
Laser Blanking = Enabled
Scan Mode = Frame
Scan Zoom = 1.1
Rotation = 0°
Sampling = 1.20
Pixel Time = 0.25 µs
Frame Time = 10.24 s
LSM Scan Speed = 9
Scan Direction = Bidirectional
Line Step = 1
Averaging = 8
Excitation Wavelength = 653
Emission Wavelength = 668
Effective NA = 1.4
Detection Wavelength = 645 - 700
Imaging Device = GaAsP-Pmt3
Detector Type = GaAsP-PMT
Detector Gain = 550 V
Detector Offset = 0
Detector Digital Gain = 1.0
LSM Plus Mode = Filter: 8.3 (3D, Auto)

Profile View Display - Plus DNA Damage, Top

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 32.60), Y (Min 0.0 and Max 6129)

This panel measures p21 DNA damage-induced foci/aggregates across an arbitrary line, noting that the foci/aggregate signal reached approximately ~6K A.U. at its maximum.

Measured at the Z-plane 100 of 201.

Profile View Display - Minus DNA Damage, Bottom

Profile Definition = Stroke Thickness 1.5, Normal View, Grid Distance 1

Profile View = X and Y axes were set to Auto - X (Min 0.0 and Max 53.80), Y (Min 0.0 and Max 366)

This panel measures p21 in the absence of DNA damage across an arbitrary line, noting that the foci/aggregate signal reached approximately ~300 A.U. at its maximum.

Histogram Display - Plus DNA Damage, Top

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 5000)

Arithmetic Mean intensity = 1384.301

The histogram was generated from the yellow-boxed region shown to quantify the overall p21 response within a defined nuclear area following DNA-damage induction. This measurement provides additional support for the observed increase in signal relative to the control condition, complementing the line-profile analysis presented alongside it.

Histogram Display - Minus DNA Damage, Bottom

Histogram Definition = Normal, Histo Table set to Frequency, Bin Count 256, Bin Size was 256, Lower Threshold 0, Upper Threshold 65535

Histogram View = X and Y axes were set to Auto - X (Min 0.0 and Max 65535), Y (Min 0 and Max 100000)

Arithmetic Mean intensity = 45.598

The histogram was generated from the yellow-boxed region shown to quantify the overall p21 protein signal within a defined nuclear area in the absence of DNA-damage induction. This measurement provides a baseline comparison for the increased signal observed following DNA-damage induction, complementing the line-profile analysis presented alongside it.

Control Conclusion -

In undamaged cells, p21 exhibits low-intensity, diffuse distribution, consistent with basal expression and limited activation of cell cycle checkpoint pathways. Following etoposide-induced DNA damage, p21 shows a marked increase in mean intensity. It reorganizes into discrete nuclear foci, with an aggregate signal elevated by ~30.4-fold. This substantial increase is consistent with robust activation of the DNA damage response, reflecting p53-mediated transcriptional upregulation of p21 and subsequent engagement of cell cycle arrest mechanisms.

The pronounced increase in signal intensity and focal organization aligns with established kinetics of p21 accumulation following genotoxic stress, supporting its role as a downstream effector of DNA damage signaling rather than an initial damage sensor.

Image Corrections

None

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

SOURCE PROVENANCE

Catalog	PA-RR-000009
Stage	Control
Instrument	ZEISS LSM 980
Microscope configuration	Zeiss LSM 980 Confocal with NIR Detection, Axio Observer.Z1/7, using a Plan-Apochromat 63x/1.40 Oil DIC M27, was used with the following parameters for each dye:
Structured source metadata values	54
Metadata source	PRESERVED_SOURCE_METHOD_PDF_TEXT

SOURCE PROVENANCE

Cell model	HeLa
Image SHA-256	15BDC1B2DFF427ED1E11584A3DF63B9946D1ADFA285376E5F8C08D1A38CF23DA
Method PDF SHA-256	CCC3DC05C31714DF130D8DF3BC6B7206CFAEFC5B1F7A007D3C8A63189F3B36B5