

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')₂ (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 μ M 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')₂ (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 μ m)

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 μ m X 30.09 μ m

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 μ m, Y: 0.00 μ m

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

Z-Stack = 100 Slices (2.97 μ m)

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 μ m X 4.69 μ m

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 μ m, Y: 0.00 μ m

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

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