

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) = 2017 X 2103

Image Size (Scaled) = 42.58 μ m X 44.40 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) = 275 X 268

Image Size (Scaled) = 5.81 μ m X 5.66 μ m

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 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 -

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')₂ (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

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