

**Identifyn Standards for Optical Microscopy Methods™**Identifyn Primary Antibody**Rabbit anti-Human RAD50 Polyclonal, IgG***Cat ID# - PA-RP 000007*Identifyn® Secondary Antibodies**Identifyn®SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L)***Cat ID# - SA 000057***Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L)***Cat ID# - SA 000015*

Rad50 recognizes and processes double-strand breaks during DNA repair. It is a component of the MRN complex (Mre11-Rad50-Nbs1), which is crucial for identifying and processing DNA double-strand breaks. Rad50 also provides tethering and bridging of DNA ends. In its homodimeric state, Rad50 contains an ATPase domain at its ends, facilitating the alignment of broken DNA strands. Rad50 is also associated with telomere maintenance. Dysregulation or mutations in Rad50 or the MRN complex can lead to genomic instability, increased cancer susceptibility, and other genetic disorders.

**Method**

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 RAD50 Polyclonal, IgG, was incubated for 1 hour at room temperature at a 1:250 dilution. Cells were washed 3X in PBS. 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 1:1000. Cells were washed 3 times in PBS.

The second primary antibody, [Alpha Tubulin Monoclonal Antibody \(DM1A\)](#), 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 Donkey Anti-Mouse, IgG (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:

**Single Plane (2D) - Z-plane 32 of 63 total - Panel A**

Channels = 3 - (488 and DAPI not shown)

Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm

Image Size (Pixels) = 1220 X x 1049

Image Size (Scaled) = 25.76 µm X 22.15 µm

Bit Depth = 16 Bit

Image Center Position = X: 61.21 mm, Y: 36.03 mm

Stage Position = X: 61.21 mm, Y: 36.03 mm  
ROI Center Offset = X: 0.00  $\mu$ m, Y: 0.00  $\mu$ m

**Z Stack - (3D) - Panel B****Z-Stack = Slices 63 (1.86  $\mu$ m)**

Channels = 3 - (488 and DAPI not shown)  
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm  
Image Size (Pixels) = 318 X x 286  
Image Size (Scaled) = 6.71  $\mu$ m X 6.04  $\mu$ m  
Bit Depth = 16 Bit  
Image Center Position = X: 61.21 mm, Y: 36.03 mm  
Stage Position = X: 61.21 mm, Y: 36.03 mm  
ROI Center Offset = X: 0.00  $\mu$ m, Y: 0.00  $\mu$ m

**Z Stack - (3D) - Panel C****Z-Stack = Slices 63 (1.86  $\mu$ m)**

Channels = 3 - (488 and DAPI not shown)  
Scaling (Per Pixel) = 21.11 nm X 21.11 nm X 30.00 nm  
Image Size (Pixels) = 118 X x 120  
Image Size (Scaled) = 2.49  $\mu$ m X 2.53  $\mu$ m  
Bit Depth = 16 Bit  
Image Center Position = X: 61.21 mm, Y: 36.03 mm  
Stage Position = X: 61.21 mm, Y: 36.03 mm  
ROI Center Offset = X: 0.00  $\mu$ m, Y: 0.00  $\mu$ 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 = 100 ms  
Depth of Focus = 1.13  $\mu$ m  
Binning Mode = 2,2  
Laser Wavelength = 640 nm @1.00%  
Frame Time = 1.33 s  
Scan Direction = Unidirectional  
Grating = 27.5  $\mu$ m 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 = 100 ms  
Depth of Focus = 0.81  $\mu$ m  
Binning Mode = 2,2  
Laser Wavelength = 488 nm @1.00%  
Frame Time = 1.33 s  
Scan Direction = Unidirectional  
Grating = 27.5  $\mu$ m grating

**DAPI -**

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 = 405  
Channel Name = T3-Axiocam 820 #2-SR  
Excitation Wavelength = 405  
Emission Wavelength = 445  
Effective NA = 1.4  
Detection Wavelength = 419 - 470, 503 - 546, 576 - 617, 657 - 800  
Imaging Device = Axiocam 820 #2  
Camera Adapter = 1X  
Exposure Time = 50 ms  
Depth of Focus = 0.69  $\mu$ m  
Binning Mode = 2,2  
Laser Wavelength = 405 nm @2.00%  
Frame Time = 676.00 ms  
Scan Direction = Unidirectional  
Grating = 36.5  $\mu$ m 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**

Panel features Rabbit anti-Human RAD50 Polyclonal, IgG with Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L).

**Not Shown but Aquired** - Mouse Monoclonal Alpha Tubulin primary, counterstained with Identifyn® SRM Alexa Fluor™ 488 Affinity Purified Donkey Anti-Mouse, IgG (H + L) and DAPI,

Image was taken from Z-stack Acquisition at Z-plane 32 of 63.

### **3D Image Processing - Panel B**

3D Maximum Projection = Precise  
Appearance = Threshold 1% all channels, Ramp 0% all channels, Maximum 100% all channels  
Background = Black  
Light = Brightness 100%, 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)

### **3D Image Processing - Panel C**

3D Volume Projection = Precise  
Appearance = Threshold 2% all channels, Ramp 98% all channels, Maximum 100% all channels  
Background = Black  
Light = Brightness 120%, Azimuth 80°, Elongation -130°, Enabled Directional Lighting, Enabled Tone Mapping  
Projection = View Angle 50°, Scale Z 10%, Reverse Z-Axis NOT Selected, Stereo Anaglyph NOT Selected, Camera Separation 5% (deselected), Parallax Shift 0% (deselected)

### **Panel E - STORM**

#### **Summary -**

Super-resolution lattice structured illumination microscopy squared (SIM<sup>2</sup>) was used to characterize the intranuclear distribution and organization of RAD50 following etoposide-induced DNA damage within the Identifyn® Standards for Optical Microscopy Methods™ step-down framework. HeLa cells were labeled with a rabbit anti-human RAD50 polyclonal IgG, detected with Identifyn® SRM Alexa Fluor™ 647 affinity-purified secondary antibodies, along with  $\alpha$ -tubulin and DAPI (not shown in the data set). Across all SIM<sup>2</sup> views, the RAD50 signal was strictly confined to the nuclear compartment, consistent with

its established role as a core component of the MRE11–RAD50–NBS1 (MRN) complex, which is involved in DNA double-strand break (DSB) detection and signaling.

Two-dimensional SIM<sup>2</sup> optical sections (Panel A) acquired from a 63-slice Z-stack reveal punctate and aggregated intranuclear RAD50 distributions with markedly increased spatial definition relative to Airyscan and Lattice SIM imaging. Panel B presents a maximum three-dimensional projection, illustrating the full nuclear distribution and continuity of RAD50 signal across the entire Z-volume. Three-dimensional volume reconstructions and higher-magnification region-of-interest analyses (Panel C) further resolve damage-associated RAD50 assemblies throughout the nuclear space. At SIM<sup>2</sup> lateral resolution (~40–60 nm in the X–Y plane), these signal patterns exhibit reproducible organizational features that closely approximate those observed by single-molecule localization microscopy.

Biologically, RAD50 functions as a structural and mechanochemical component of the MRN complex, mediating DNA end tethering, stabilizing broken DNA ends, and initiating ATM-dependent damage signaling. While SIM<sup>2</sup> does not resolve all molecular substructures detectable by STORM, the substantially improved resolution reduces signal convolution and enables more precise separation of adjacent RAD50 assemblies within damage-associated foci. Direct comparison with STORM imaging from the same dataset indicates that some SIM<sup>2</sup>-resolved features remain composites of multiple RAD50 sub-assemblies, entirely separable only at the single-molecule level.

Collectively, these data position SIM<sup>2</sup> as a critical structural-resolution stage within the step-down workflow, bridging enrichment-level localization (confocal/Airyscan) and definitive molecular resolution (STORM). By resolving RAD50 organization beyond diffraction-limited and conventional SIM modalities, SIM<sup>2</sup> provides essential structural context for interpreting MRN complex engagement and early DNA damage sensing following genotoxic stress.

### Image Corrections

Identifyn® SRM Alexa Fluor™ 647 Affinity Purified Goat Anti-Rabbit, IgG (H + L) was pseudo-colored in Zen Software from red, the default color, to white to provide visual contrast in Panels B and C.

----

Image by P. Raut

Image Analysis and Data Presentation by B.T. Bennett

© Copyright 2026 GMD12 LLC.