

**Identifyn Standards for Optical Microscopy Methods™**Identifyn Primary Antibody**Rabbit anti-Human  $\gamma$ H2AX Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate***Cat ID# - DC 000020*

Gamma H2AX ( $\gamma$ H2AX) is a variant of the histone H2A protein that plays a crucial role in the cellular response to DNA double-strand breaks (DSBs). Its primary function is as a marker of DNA damage, particularly in the context of DSBs. When DSBs occur in chromatin,  $\gamma$ H2AX is rapidly explicitly phosphorylated at the serine 139 residue (referred to as Ser139 phosphorylation or  $\gamma$ -phosphorylation) near the site of the break. This phosphorylation event forms  $\gamma$ H2AX foci, visualized via immunofluorescence microscopy techniques as an aggregate of fluorescently labeled protein at the site of breaks.

**Method**

Hela cells were grown on a  $\mu$ -Slide 8 Well high Glass Bottom [Ibidi chamber](#). DNA damage was induced via treatment with 2 $\mu$ M Etoposide-spiked complete growth media for 6 hours, followed by -20°C methanol fixation and blocked with [Thermo Fisher Fish Serum Blocker cat#37527X3](#). The primary antibody, Rabbit anti-Human  $\gamma$ H2AX Recombinant Monoclonal - Identifyn® SRM Alexa Fluor™ 647 - Direct Conjugate, was incubated for 1 hour at room temperature 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.

[Control Data - Primary Unconjugated Antibody](#)

**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: 635nm Dichroic  
Objective: Silicon Selected  
Stage Insert: Rectangular Selected  
Course 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: 100ms  
Super Resolution Camera Exposure Time: 20ms  
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: 20ms Selected

### Experiment Configuration

Record Workflow: Selected Options  
View Mode: Widefield 200µm  
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 a suitable cell(s) or cellular structure. The cell(s) or structures are optimally focused.

Piezo Current: 181.0 µm

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

Widefield 640nm: 0.1X Neutral Density Filter: Selected, @0.2% Laser Input

Reference Probe 1 Laser control (640 nm (640 nm Dichroic): Not Selected

### Advanced Tools Option

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

Widefield Laser: "Widefield 640nm" 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: 50µm 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 optimally focused.

Piezo Record: Begin: 180; End: 180.8

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

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

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

Not Selected

### Advanced Tools

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

### Record Workflow - Record Tab: Capture Parameters

Frames: Probe 1: 300  
Z Positions: 4 using Steps: 0.2µm (200nm)  
Cycles: 20  
Time Points: 1  
Pause Between Cycles: Not Selected

Automatic Photoactivation Laser Control: Not Selected

### **Probe 1 Laser control (640 nm (640 nm Dichroic)**

Laser Power: 30%

### **Expert Configuration Options - The Localization Tab**

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

#### **Localization**

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

#### **Record Workflow - Localization Tab**

#### **Data Collection**

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

#### **Recording Summary**

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

Probe1: BG threshold: 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: 4nm  
Color by: Constant  
Color Table: Single  
Opacity Mode: Constant; Opacity 1: 03  
Front-to-back Attenuation: Not Selected

### Post Acquisition analysis - View Tab

**Drift Correction** - Computed with the following parameters:

Method: Particle (direct)  
Time Intervals: 7 Intervals  
Pixel Size: 50nm  
Smoothing: 8.00

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

PSF Z offset: -600 to +600  
Radial precision: 25  
Axial precision: 60

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: 0.07 $\mu$ m (700nm)  
Maximum Particle Count to Form Cluster: 20  
Computer Hulls: Selected  
Compute Density Isosurfaces: Not Selected  
Edit Settings: All Probes  
Hull Alpha shape Radius: 600nm  
Compute: Selected  
Following Cluster Analysis Computation, under the Results Tab, ID Unassigned:  
Deselected

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Image by S. Thakur

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