

KeepIsolatedROIs Plugin – User Guide

Purpose

The **KeepIsolatedROIs** plugin for ImageJ/Fiji identifies and retains only those Regions of Interest (ROIs) that have **no other ROIs within a specified distance** (in microns). This is especially useful in biological imaging where isolated features (e.g., single cells, nuclei, particles) need to be analyzed without interference from neighbors.

Features

- Works on **boundary pixel distances** (not just centroids).
 - Input distance threshold in **microns**.
 - Removes all ROIs that have at least one neighbor within the specified threshold.
 - Usable as both GUI plugin and via macro script.
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Installation

If using precompiled JAR:

1. Place Keep_IsolatedROIs.jar into:

bash

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Fiji.app/plugins/

2. Restart Fiji.

If compiling yourself:

1. Place Keep_IsolatedROIs_.java in a folder.
2. Open terminal/command prompt and run:

bash

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```
javac --release 8 -cp "C:\softwares\fiji-win64\Fiji.app\jars\*" -d bin Keep_IsolatedROIs_.java  
jar cf Keep_IsolatedROIs.jar -C bin .
```

3. Copy Keep_IsolatedROIs.jar into the plugins folder and restart Fiji.
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How to Use

From GUI:

1. Load your image and add ROIs using the ROI Manager.
2. Go to **Plugins > Keep Isolated ROIs**.
3. Enter the **distance threshold in microns** (e.g., 15).
4. Click OK. All non-isolated ROIs will be removed.

From Macro:

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```
run("Keep Isolated ROIs", "threshold=15");
```

You can batch this across images for automation.

How It Works

1. The plugin gets image calibration data (pixel width/height).
 2. For each ROI, it checks all **boundary pixels** against every other ROI's boundary.
 3. Calculates **Euclidean distance** between boundary pixels (converted to microns).
 4. If any two ROIs have boundary points closer than the threshold, they are considered **neighbors**.
 5. Only **non-neighboring ROIs are kept**.
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Requirements

- Fiji/ImageJ (with Java 8 runtime)
 - Java Development Kit (JDK) 8 or higher (for compilation)
 - javac compiler (use --release 8 if using JDK > 8)
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Troubleshooting

Issue	Solution
UnsupportedClassVersionError	Use javac --release 8 to compile
Plugin not showing	Restart Fiji after placing .jar in plugins/
Duplicate plugin error	Remove duplicate .class or .jar versions

Output

- Only isolated ROIs are kept in ROI Manager.
- The rest are deleted.
- Can be exported or analyzed further.