ProcessND2_RemoveOutlierROIs_FilledColors Macro

User Documentation

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1/21/25

Overview

This macro is designed for use in **Fiji/ImageJ** when analyzing multi-channel ND2 files (e.g., from a microscope). It focuses on **Channel 1** for object detection (such as small circular spots) and **Channel 2** for intensity measurement. A key feature is the *ability to remove outlier ROIs* based on either **area** or **circularity**, highlighting excluded ROIs in red**red** and included ROIs in green**green** before final measurement.

Workflow Summary

1. **Open an ND2 File** with split channels: You should see windows named `C=0`, `C=1`, and `C=2`. The macro automatically closes `C=0`.
2. **Max Projection of C=1:** Converts Channel 1's stack into a single image (`Max_C1`) to isolate bright spots.
3. **Interactive Blur & Threshold:**
   - You specify:
     (a) **Gaussian Blur Sigma** (noise smoothing).
     (b) **Threshold Lower Bound** (exclude background).
   - A temporary `Preview_C1` window shows how the settings look. If unsatisfactory, you can re-enter new values.
4. **Watershed & Analyze Particles:** After confirming the preview, the macro applies blur/threshold to the real `Max_C1`, fills holes, and uses `Watershed` to separate objects. `Analyze Particles` populates the **ROI Manager** with detected ROIs.
5. **Remove Outlier ROIs:**
   - The code measures **Area** and **Circularity** for each ROI.
   - You can toggle filtering by either one:

```
filterByArea = true;
filterByCirc = false;
```

or set both to `true`.

- A "factor" determines how strictly to remove outliers: `factor = 0.5` removes any ROI outside ±50% of the mean area/circularity.
- The macro duplicates `Max_C1` into `Outlier_Preview`, converts it to **RGB**, and *fills* each ROI in color: green**Green** = kept, red**Red** = removed.

6. **Final Intensity Measurement in C=2:** After you confirm removal, outlier ROIs are deleted from the ROI Manager. The macro **Average**-projects Channel 2 (`Avg_C2`) and measures **mean intensity** for only the remaining (kept) ROIs.

Key Parameters & Customization

- `userSigma, userLower`: Set the blur radius and threshold lower bound in the *preview loop*. For 16-bit images, use `userUpper = 65535`; for 8-bit, use `255`.
- `filterByArea` vs. `filterByCirc`:
  - Set `filterByArea = true` if you want to remove ROIs based on area outliers.
  - Set `filterByCirc = true` if you want to remove ROIs based on circularity outliers.
  - Use both at once if needed.
- `factor = 0.5` (default): This means any ROI $< 50\%$ or $> 150\%$ of the average area (or circularity) is discarded. Adjust if you need tighter or looser bounds.
- *Visual Feedback*: The outlier preview image shows red-filled ROIs as removed and green-filled ROIs as kept.

Usage Steps

1. **Open your ND2** in Fiji so `C=0`, `C=1`, `C=2` are visible.
2. **Run the Macro**: In Fiji, go to `File` → `New` → `Script...`, paste the macro code, and click **Run**.
3. **Adjust & Confirm Preview**:
   - Enter `sigma` & `lower` threshold in the dialog.
   - Inspect `Preview_C1`. If unsatisfied, choose "No" to retry with new settings.
4. **Check Outlier Preview**:
   - After particle detection, outlier ROIs are color-filled in `Outlier_Preview` (red vs. green).
   - If satisfied, confirm to remove outliers from the ROI Manager.
5. **Final Measurement**:
   - `C=2` is average-projected to `Avg_C2`.
   - The macro measures only *kept* ROIs' intensities in `Avg_C2`, printing results in the *Results* table.

Tips & Tricks

- If circles are merging too much, lower `sigma`.
- If too many outliers remain, lower `factor` (e.g. `0.3`) for stricter filtering or choose a different threshold.
- For 8-bit images, update all references to `65535` with `255`.