

PhotoFiTT: A Quantitative Framework for Assessing Phototoxicity in Live-Cell Microscopy Experiments

SYTOX counting

This Fiji macro script automates the process of creating a series of OR-combined images from the frames of a multi-frame image stack, saving each result sequentially. The procedure is as follows:

1. Initialization and Image Duplication:

- The macro starts by obtaining the title of the currently active image and extracting its dimensions, including the number of frames.
- The first frame of the image stack is duplicated to create an initial reference image, named "ref".

2. Loop Through Frames:

- A loop iterates over each frame from the second frame to the last frame in the image stack.
- In each iteration:
 - The current frame is duplicated and renamed "next".
 - The `ImageCalculator` plugin is used to perform a logical OR operation between the "ref" image and the "next" image, creating a new combined image.
 - The "next" image is then closed.
 - The "ref" image is also closed.
 - The resultant combined image is saved with a filename indicating the frame number.
 - The resultant image is renamed "ref" to be used as the reference in the next iteration.

3. Final Cleanup:

- After processing all frames, the final "ref" image is closed.

Detailed Steps in the Script

1. Obtain Image Title and Dimensions:

- `name = getTitle();`
- `getDimensions(width, height, channels, slices, frames);`

2. Duplicate the Initial Reference Image:

- `selectWindow(name);`
- `run("Duplicate...", "duplicate range=1-1");`
- `rename("ref");`

3. Iterate Through Each Frame:

```
java
Copy code
for (t = 1; t < frames; t++) {
    // Duplicate current frame
    selectWindow(name);
    run("Duplicate...", "duplicate range=" + t + "-" + t);
    rename("next");

    // Perform OR operation with the reference image
    imageCalculator("OR create", "ref", "next");
}
```

```

// Close the "next" image
selectWindow("next");
close();

// Close the "ref" image
selectWindow("ref");
close();

// Save the result and rename it to "ref"
saveAs("Tiff", PATH + new_folder_add + image_name + "_" + t +
".tif");
rename("ref");
}

```

4. **Close the Final Reference Image:**

- o selectWindow("ref");
- o close();

This macro is useful for generating a series of images where each image is a logical OR combination of consecutive frames from a multi-frame image stack, which can be useful for various image analysis tasks such as detecting cumulative changes over time.