γH2AX Foci Counting Workflow
Author: Albert Wang
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Summary

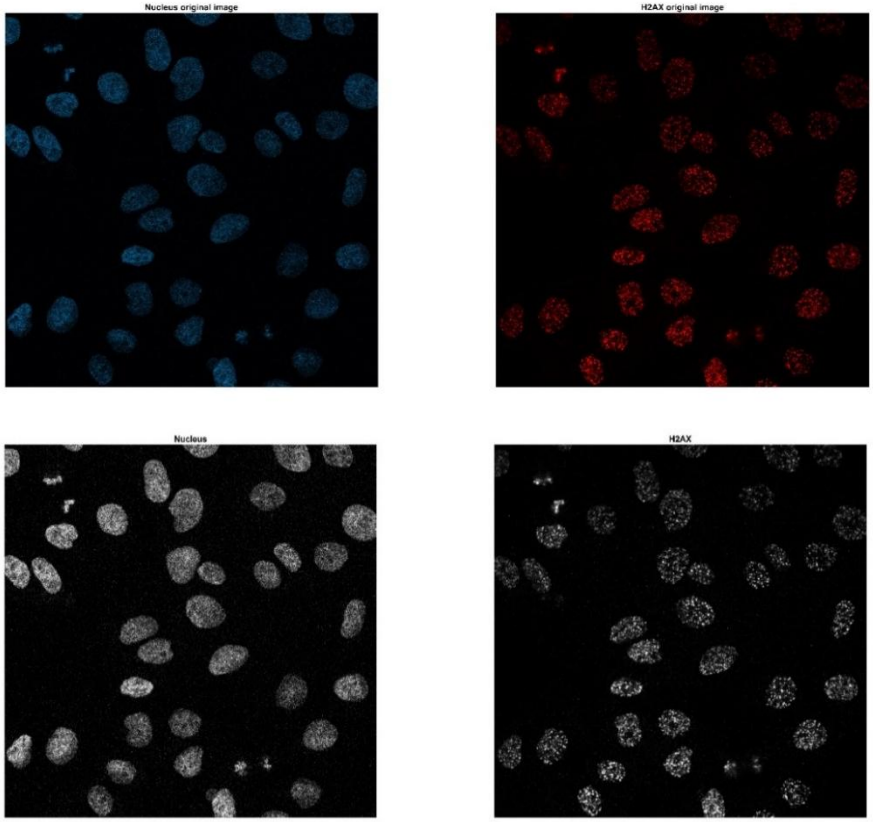
This document describes a semi-automated MATLAB pipeline for quantifying γH2AX foci within individual nuclei. The workflow covers image import, preprocessing, segmentation, foci detection, per-cell metric computation, and results export.

Workflow Steps

MCF10A_CLR_1h_1 is used as example image here

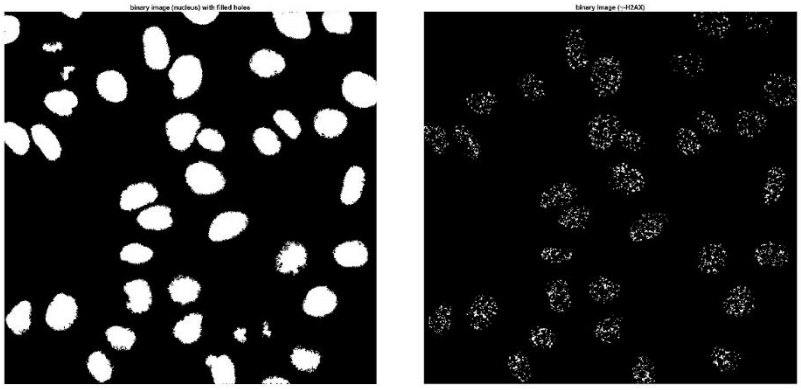
1. Image Import

- Use uigetfile to select two TIFF files: first the nucleus channel, then the γH2AX (foci) channel. Read both images with imread
- Convert each RGB image into a single-channel grayscale intensity map by summing the red, green, and blue channels (I_nucleus, I_foci).



2. Thresholding and Mask Creation

- Prompt the user for:
    - A nucleus threshold value (0–255, used with imbinarize).
    - A foci threshold (0–255 intensity units).
    - Thresholding mode:
        - Dynamic (1): Determines a per-nucleus foci threshold.
        - Universal (2): Uses a single global foci threshold.
- Nucleus mask:
    - Binarize I_nucleus using imbinarize.
    - Clean the binary mask with bwareaopen and imfill.
    - Use bwconncomp to identify connected nuclei.
    - Use regionprops to extract nucleus area and centroid.
    - Discard nuclei smaller than a defined area threshold (e.g., 1,000 pixels) and record remaining indices for downstream analysis.
- Foci mask:
    - Create an initial foci mask by thresholding I_foci.
    - For each retained nucleus:
        - Apply the selected thresholding mode to define foci regions.
        - Remove small specks (< 2 px) with bwareaopen.
        - Aggregate per-nucleus foci masks into a global foci mask.
    - For each discarded (small) nucleus:
        - Remove surrounding foci in a square region (default: 90 px half-width) to eliminate false positives.
- Can also use MATLAB's Color Thresholder App for auto thresholding (https://www.mathworks.com/help/images/image-segmentation-using-the-color-thesholder-app.html)



3. Per-Nucleus Metric Computation

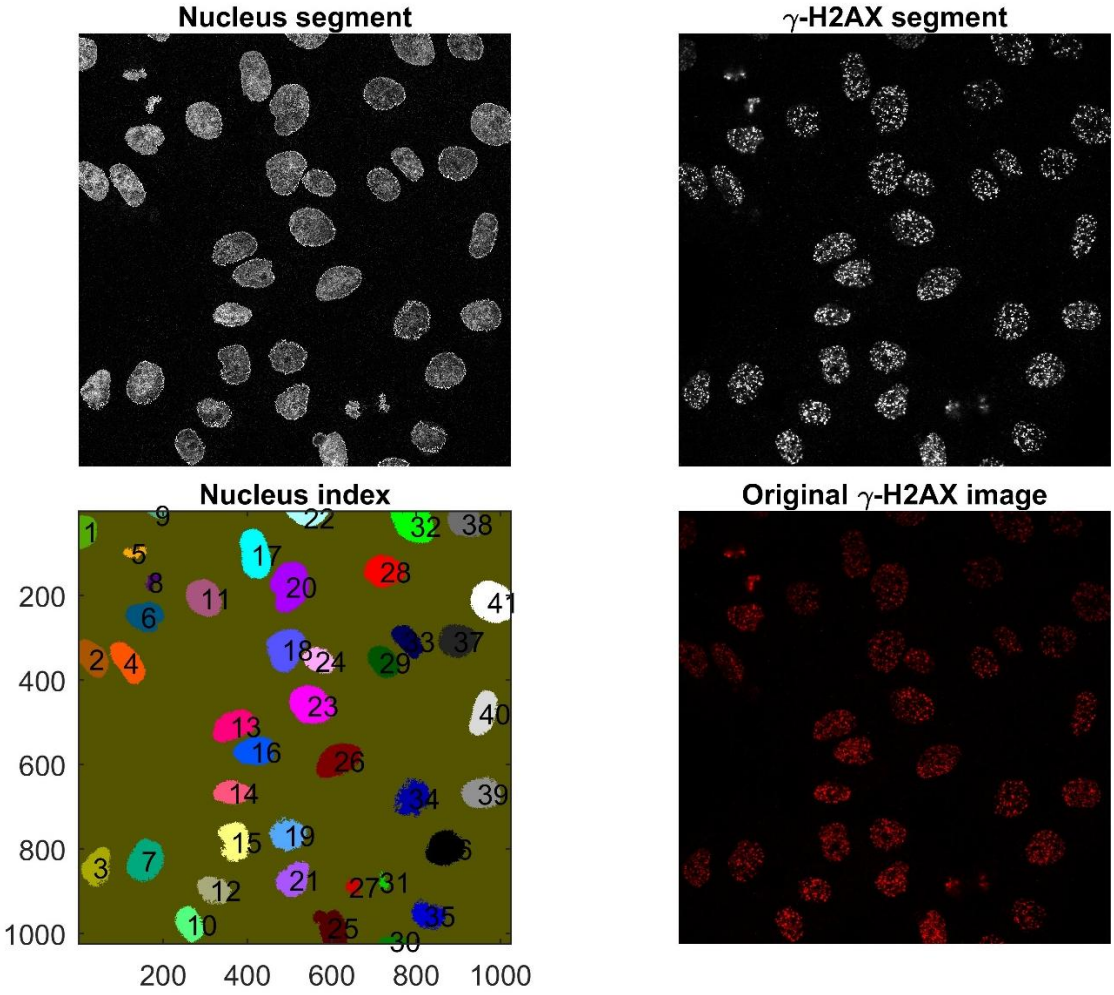
For each retained nucleus:

- Extract the grayscale intensity values from I_foci.

- Compute summary statistics:
    - Mean, median, mode, standard deviation.
- Apply per-nucleus foci thresholding and count foci:
    - Use bwconncomp and regionprops to exclude small objects.
- Store results as a row of:
    - Index, Area, MeanInt, MedianInt, ModeInt, StdInt, FociCount

4. Results Export

- Store all per-nucleus results in a MATLAB table.
- Write the table to an Excel file using writetable.
- Generate a 2×2 segmentation figure including:
    - Nucleus segmentation overlay
    - Foci segmentation overlay
    - Color-coded nucleus index map (with overlaid numbers)
    - Original foci image
- Save the figure as a high-resolution JPEG (print -djpeg -r600).



Note: Cells with their nuclei extremely close to each other (CHLA-20 in particular) would be difficult for the program to distinguish individual nucleus (see example below). Discard these data to avoid skewing the overall trend.