AR Nuclear Translocation Quantification Workflow

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Summary

This document outlines a semiautomated MATLAB workflow for quantifying androgen receptor (AR) nuclear translocation using fluorescence microscopy images. The workflow includes grayscale conversion, threshold-based segmentation of cytoplasm and nucleus regions, AR signal quantification within each compartment, nucleus-to-cytoplasm (NC) ratio calculation, and optional visualization export.

AR is typically located in the cytoplasm in its inactive state and translocates to the nucleus upon binding to androgens, where it functions as a transcription factor. Quantifying AR nuclear translocation is essential for assessing androgen receptor activation, as its movement from the cytoplasm to the nucleus is a key step in regulating gene expression involved in development, differentiation, and disease progression, particularly in prostate cancer.

This script reads cytoplasm, nucleus, and AR channel TIFF images selected by the user, converts each RGB image into a summed grayscale intensity map, creates binary masks for the cytoplasm and nucleus regions via user-defined thresholding, computes the AR signal sum and mean intensity in each region, calculates both raw and intensity-normalized nucleus-to-cytoplasm (NC) ratios, outlines and visualizes the segmented regions on original images, counts nuclei while excluding small objects based on area, and exports the AR segmentation figure with overlays to a PNG file.

Example usage:

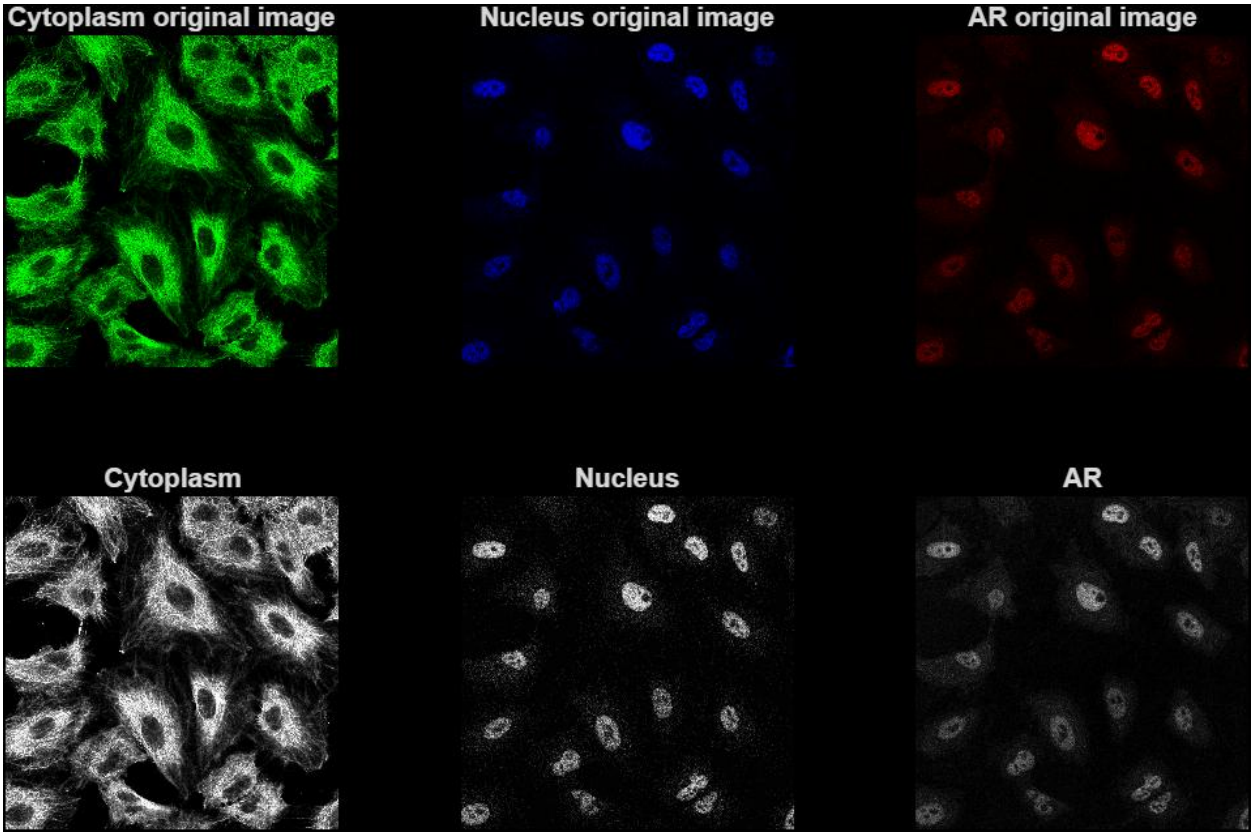
https://doi.org/10.1016/j.gendis.2023.07.001

Wang, A. R., Baschnagel, A. M., Ni, Z., Brennan, S. R., Newton, H. K., Buehler, D., . . . Iyer, G. (2024). Network analyses: Inhibition of androgen receptor signaling reduces inflammation in the lung through AR-MAF-IL6 signaling axes. Genes & Diseases, 11(3), 101072.

Workflow Steps

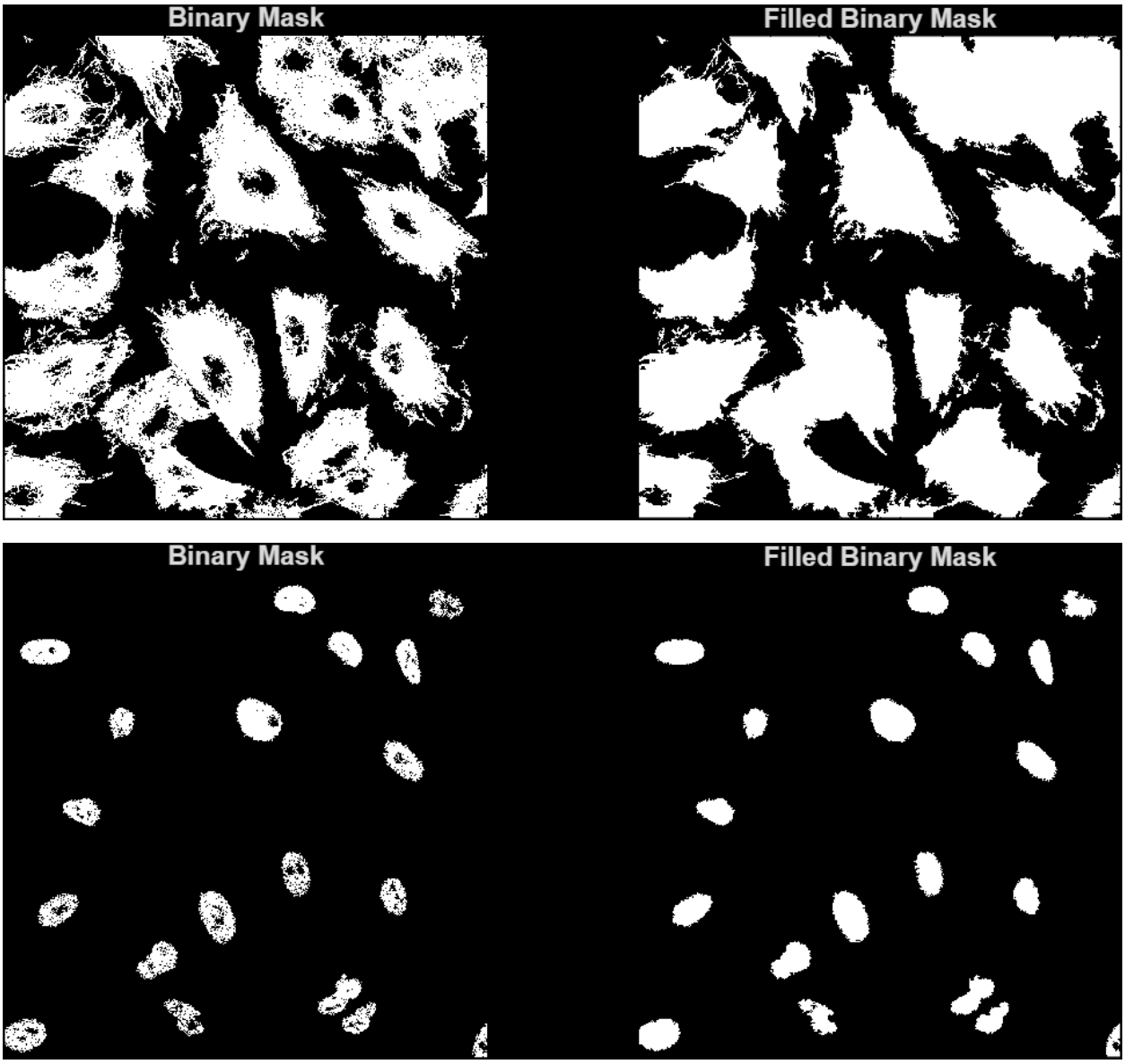
1. Image Import and Conversion

- Use uigetfile to select three TIFF files: one each for the cytoplasm, AR, and nucleus channels.
- Read each image using imread.
- Convert RGB images to grayscale by summing the red, green, and blue channels.



2. Thresholding and Mask Generation

- Prompt the user to input threshold values for:
  - Cytoplasm mask (range: 0–1)
  - Nucleus mask (range: 0–1)
- For each channel:
  - Apply imbinarize with the specified threshold.
  - Clean the binary mask using bwareaopen and imfill.
  - Store the linear indices of mask pixels for downstream signal quantification.



3. Segmentation Visualization

- Use bwperim to outline binary mask boundaries for the cytoplasm and nucleus.
- Overlay outlines on grayscale cytoplasm, nucleus, and AR images to visualize segmentation.
- Display side-by-side panels for:
    - Cytoplasm segmentation
    - Nucleus segmentation
    - AR overlay (cytoplasm)
    - AR overlay (nucleus)

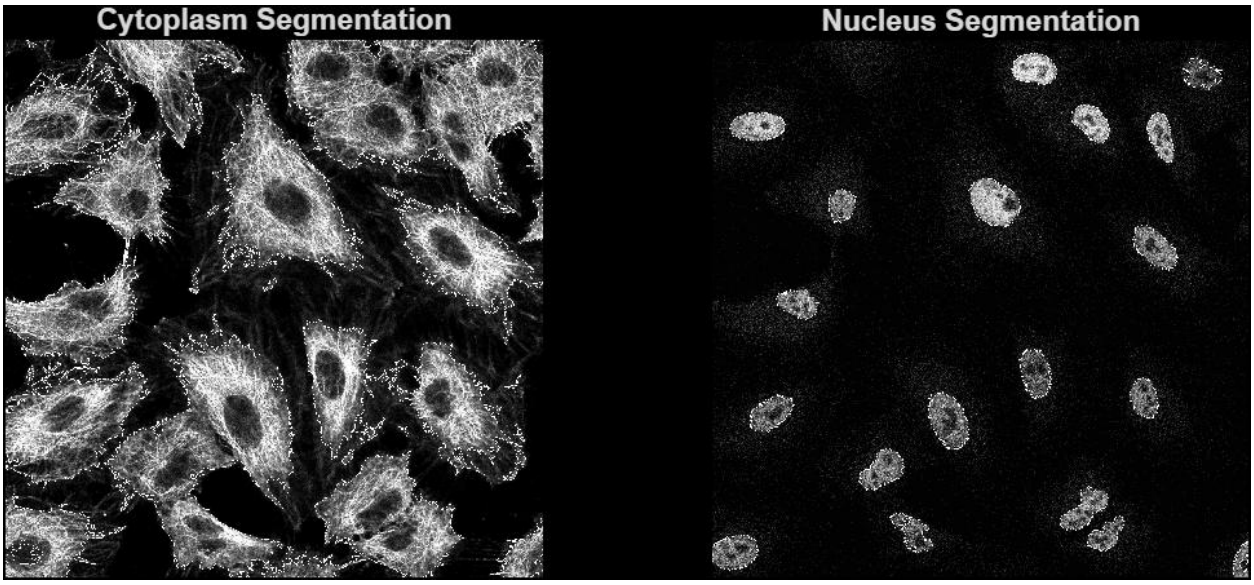
4. AR Signal Quantification

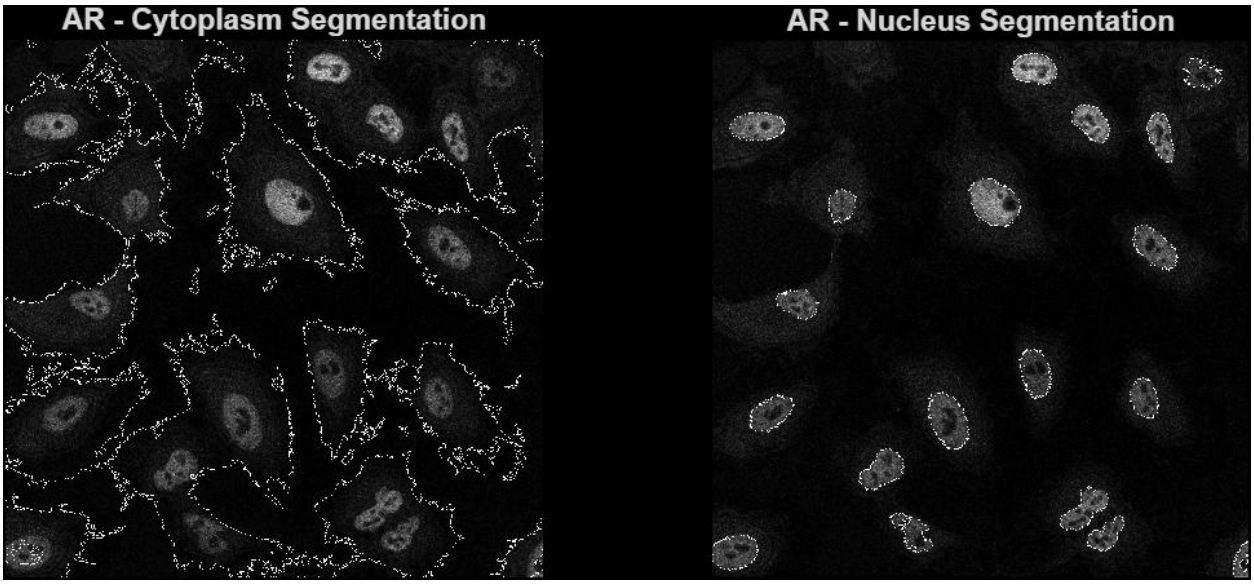
- Use the binary masks to extract AR intensity values:
    - ARsignal_cyto = AR intensity in cytoplasm mask
    - ARsignal_nuc = AR intensity in nucleus mask
- Compute the total AR signal within each compartment by summing the respective pixel values.
- Calculate the nucleus-to-cytoplasm ratio (NCratio):
    - NCratio = ARsum_nuc / ARsum_cyto

5. Integrated Density and Normalized NC Ratio

- Calculate the number of pixels (area) for each region.
- Normalize AR intensity by region size to obtain mean intensity:
    - int_nuc = ARsum_nuc / area_nuc
    - int_cyto = ARsum_cyto / area_cyto
- Calculate the normalized NC ratio:
    - NCratio_int = int_nuc / int_cyto

Use the masks to determine the sum of AR signal within cytoplasm and nucleus





6. Nucleus Counting (Optional)

- Use bwconncomp and regionprops to count connected nuclei in the nucleus mask.
- Exclude small nuclei (e.g., <300 pixels).
- Report the number of valid nuclei.

7. Result Display and Export

- Display the NC ratio results
- Save the final segmentation figure as a high-resolution .png file using saveas.

Notes

- This workflow assumes images are acquired with consistent exposure and channel alignment.
- The accuracy of NC ratio measurement depends on proper threshold selection and mask quality.
- For statistical analysis, repeat the workflow across multiple images or fields of view and aggregate NC ratios.