rnaScope Analysis with FISH Finder

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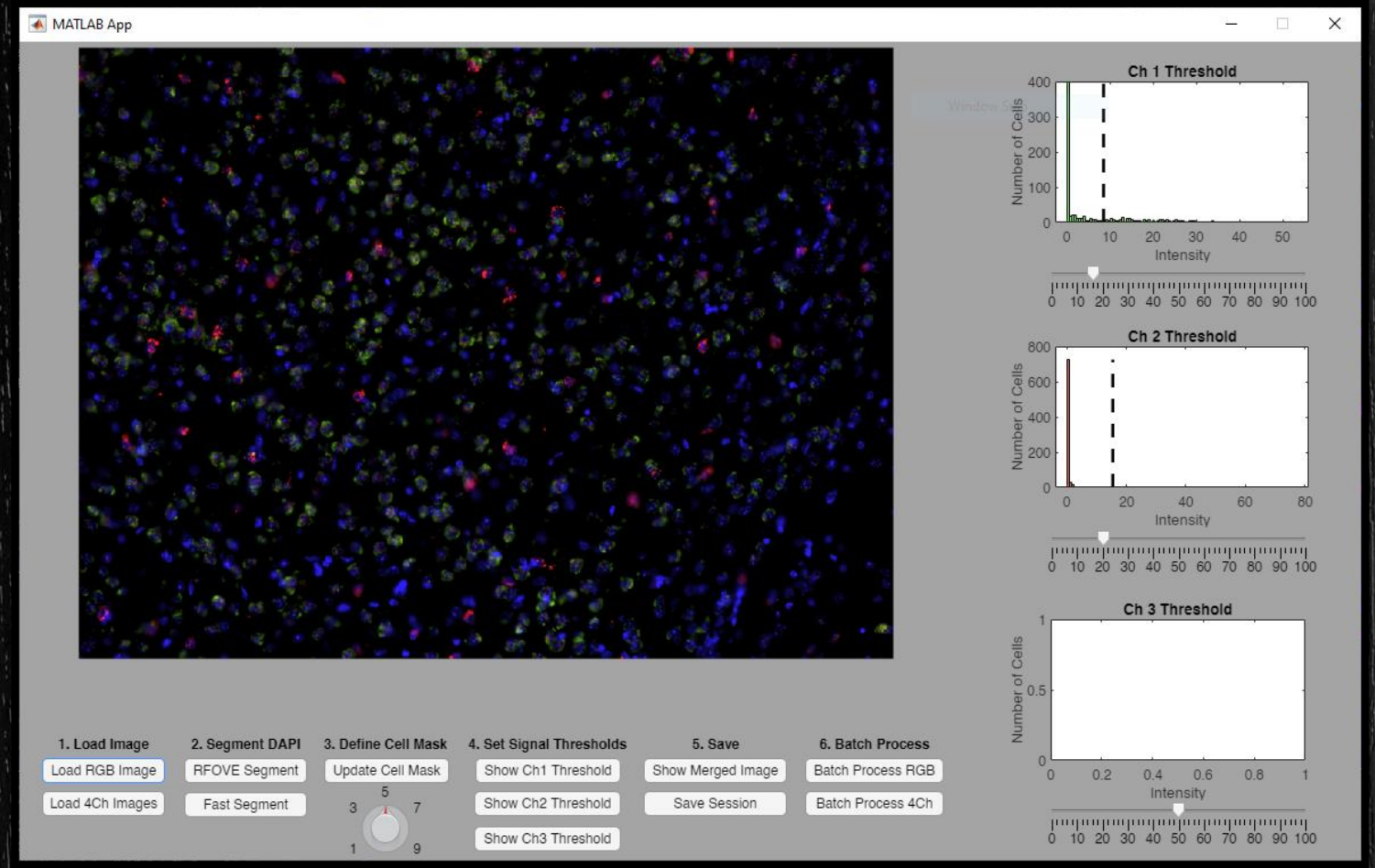


File Naming

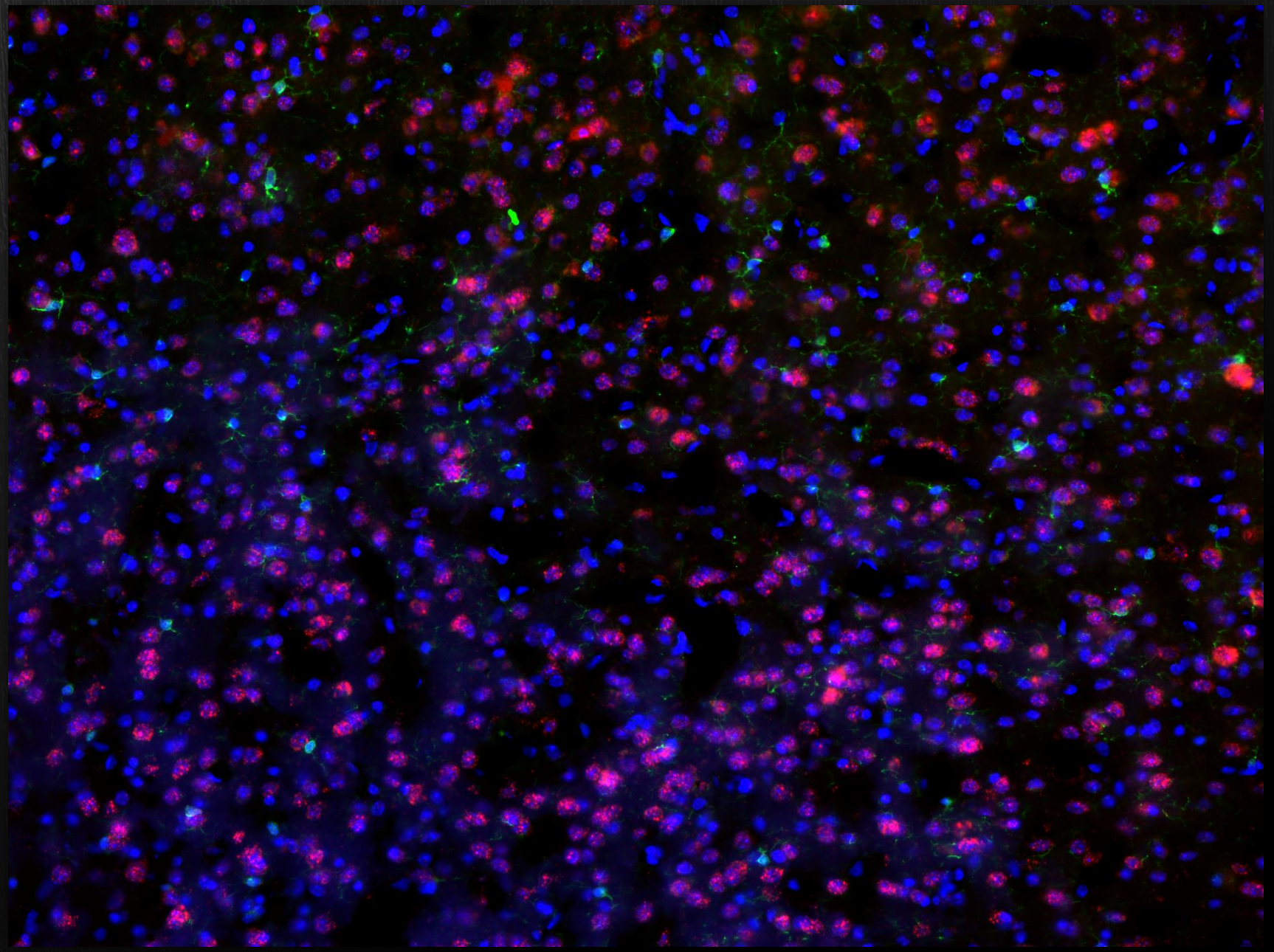
- RGB Images
  - ID.(tif. jpg, png, or bmp)
    - A1.png
    - A2.png
    - Etc.
- 4 Channel Images
  - Project Folder
    - ID Folder – Contains 4 images
      - ID_ch00.tif (DAPI)
      - ID_ch01.tif
      - ID_ch02.tif
      - ID_ch03.tif

Use a Key File

Mouse	Group	Sex	ID
1230	MN	F	A1
1271	MN	M	A2
1439	MS	F	A3
1463	MS	M	A4
1269	SS	F	A5
1274	SS	M	A6
1234	MN	F	B1
1272	MN	M	B2
1440	MS	F	B3
1464	MS	M	B4
1438	SS	F	B5
1275	SS	M	B6
1268	MN	F	C1
1273	MN	M	C2
1441	MS	F	C3
1465	MS	M	C4
1462	SS	F	C5
1466	SS	M	C6

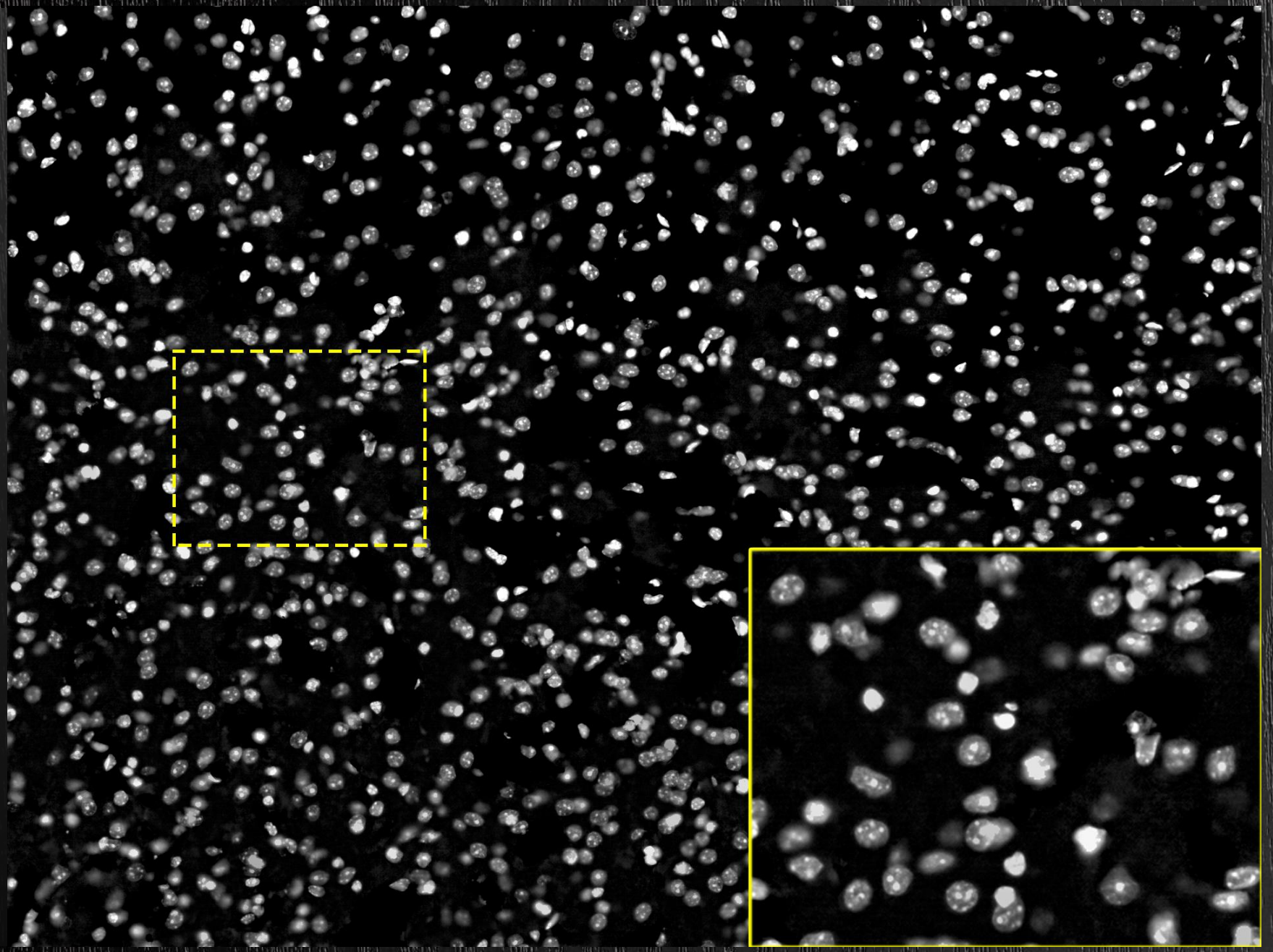
Quality In - Quality Out

- Keyence Example
  - 20x
  - High Resolution – 1920x1440
  - 2D Pinhole optical sectioning



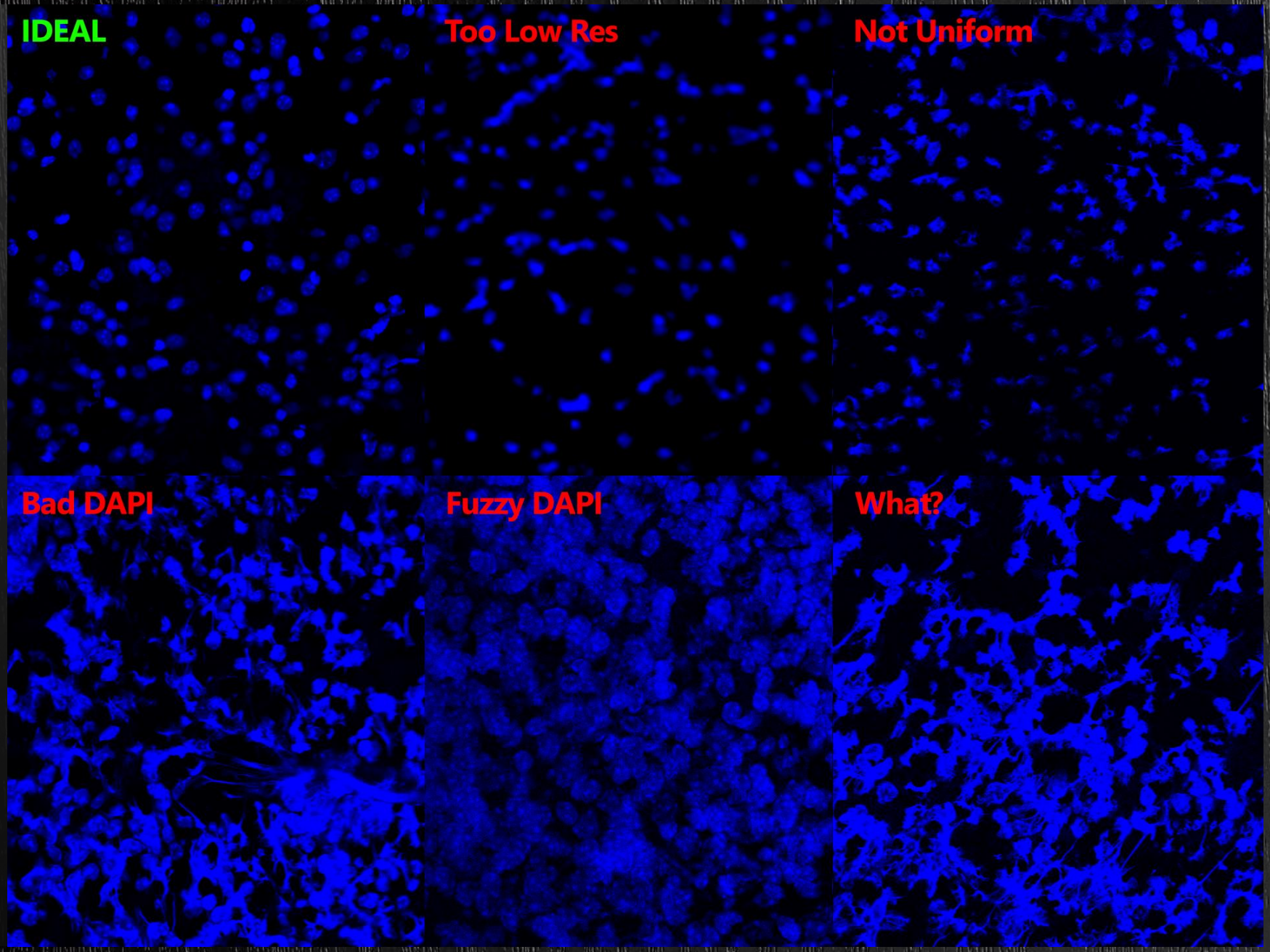
DAPI IS KEY!!!!

- Uniform brightness
- ~20-pixel diameter



Bad DAPI is Bad

- If your DAPI doesn't look like the top left image, something is wrong and automated analysis will suffer.



FISH Finder Pipeline

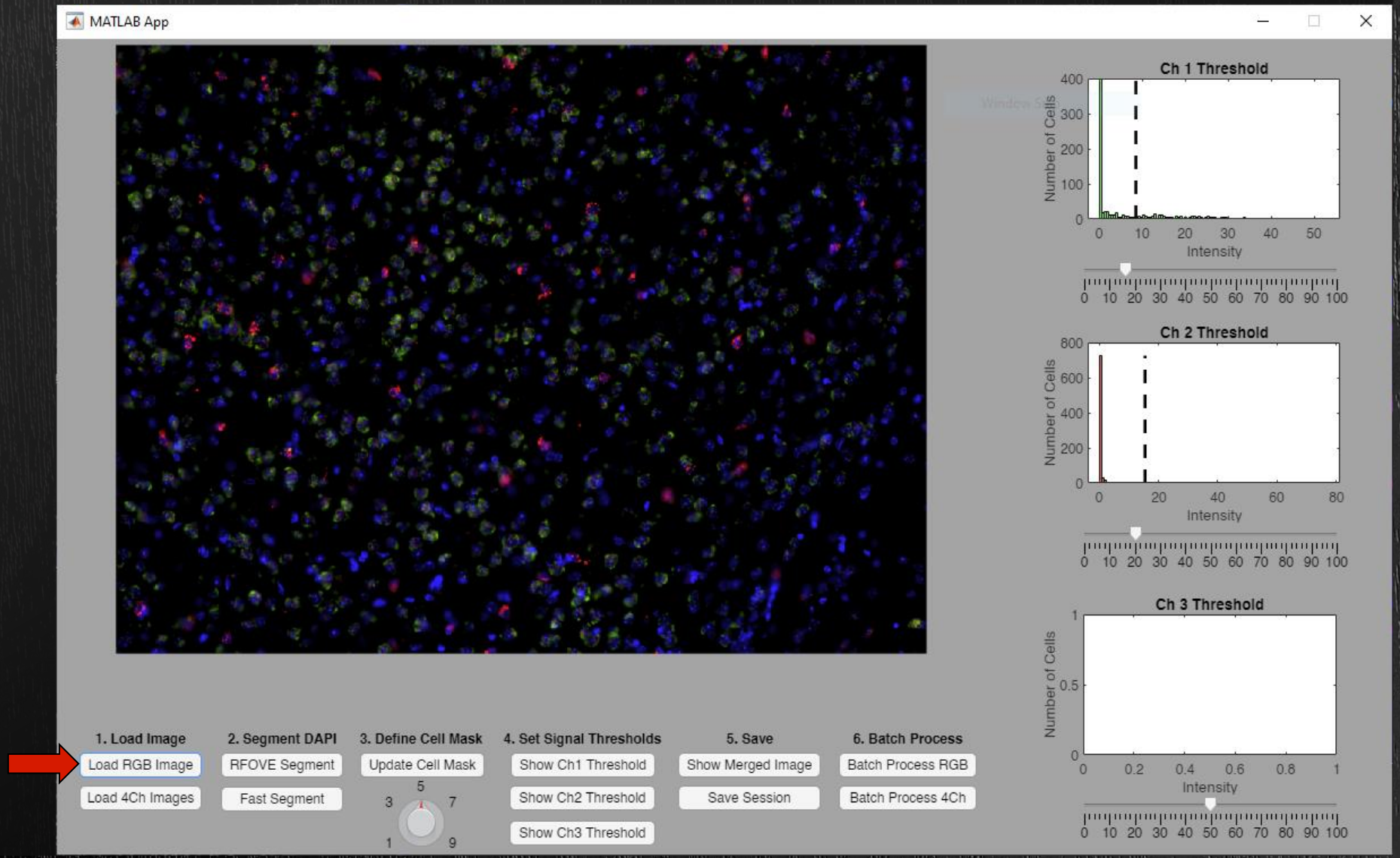
1. Load Representative Image
2. Segment DAPI
3. Define Cell Size
4. Set Signal Channel Thresholds
5. Save Final Image and Settings
6. Batch Process Entire Dataset

Load Representative Image

- Choose a single RGB Image
  - DAPI must be on blue channel

Or

- Choose 4 individual channel images (monochrome or single color)
  - DAPI must be on ch00 or ch0

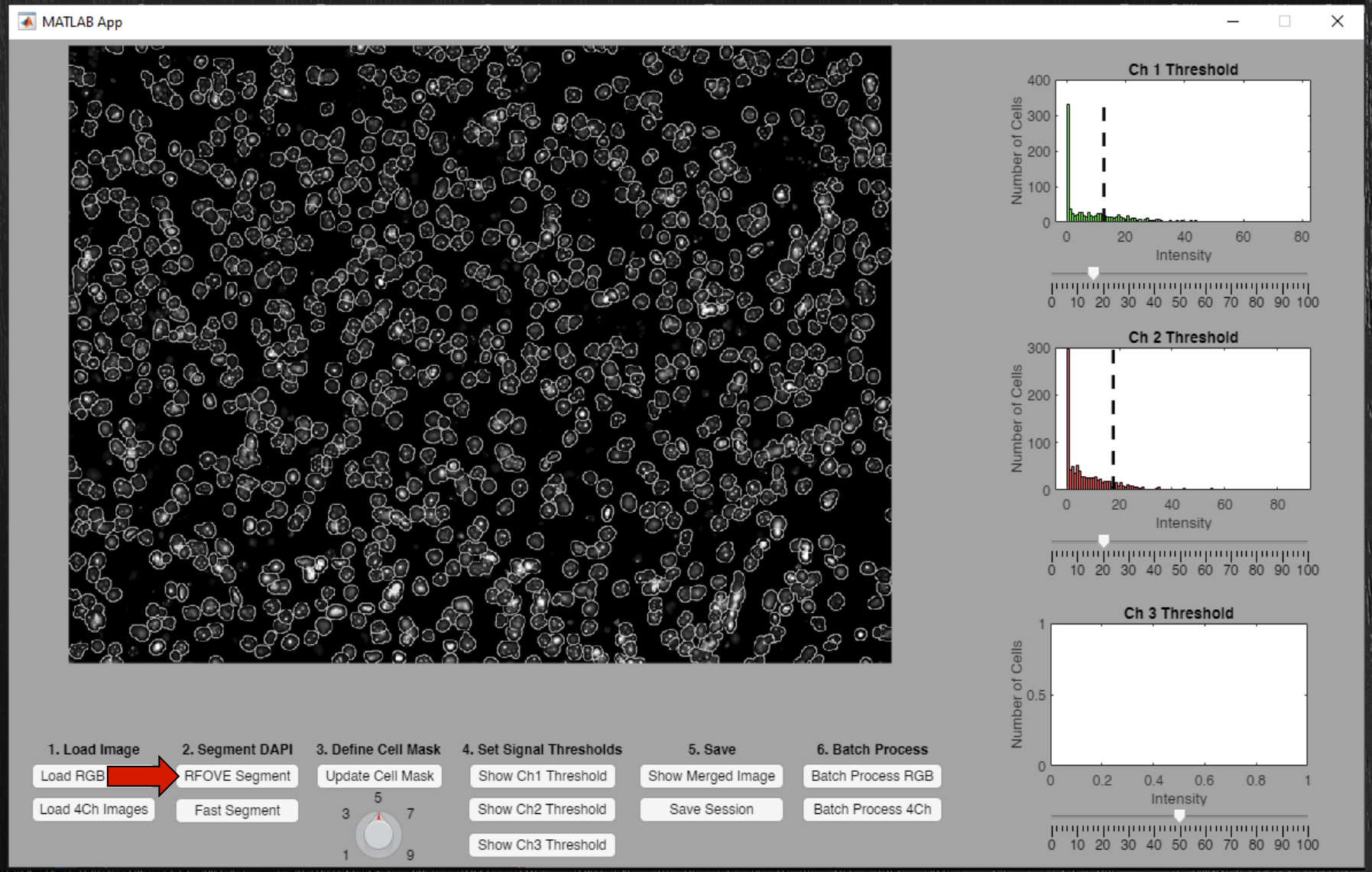


Segment DAPI

- RFOVE Segmentation
  - Very accurate but slow and resource intensive

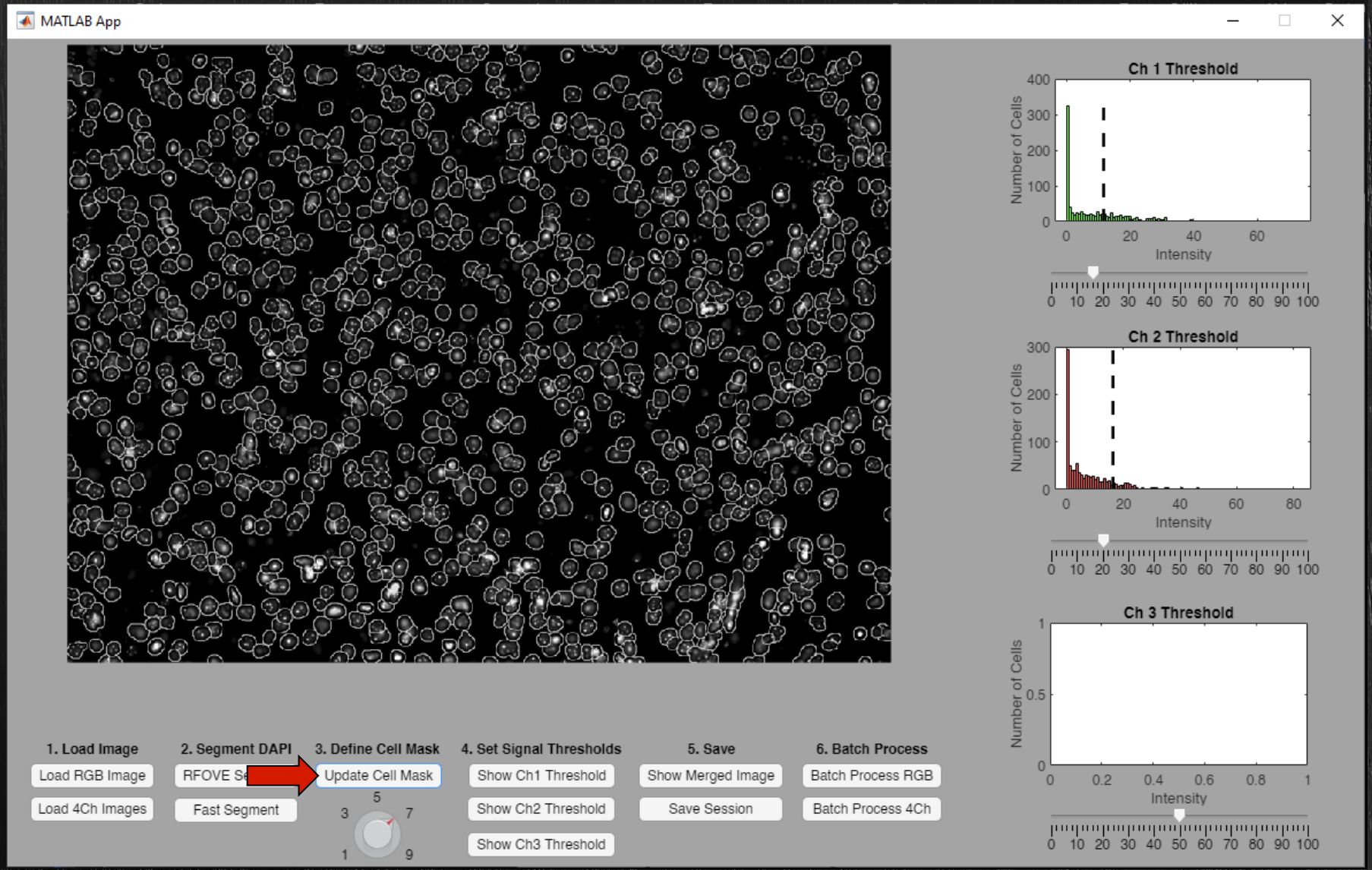
Or

- Fast Segmentation
  - Very fast segmentation but lower accuracy on overlapping nuclei



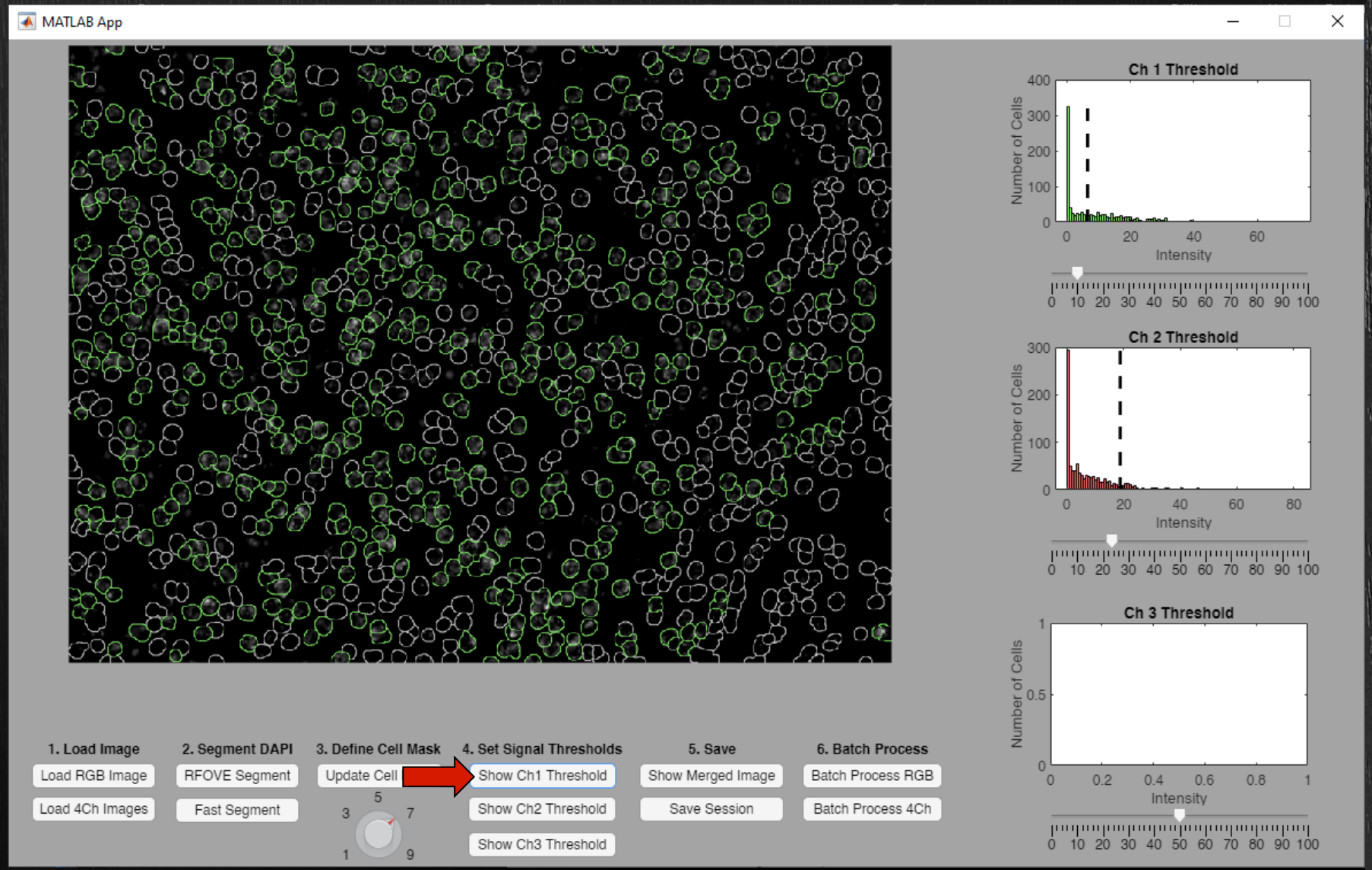
Define Cell Size

- Dilate nuclear mask to define cell size
  - How many pixels should you expand the nucleus to count signal in each cell?



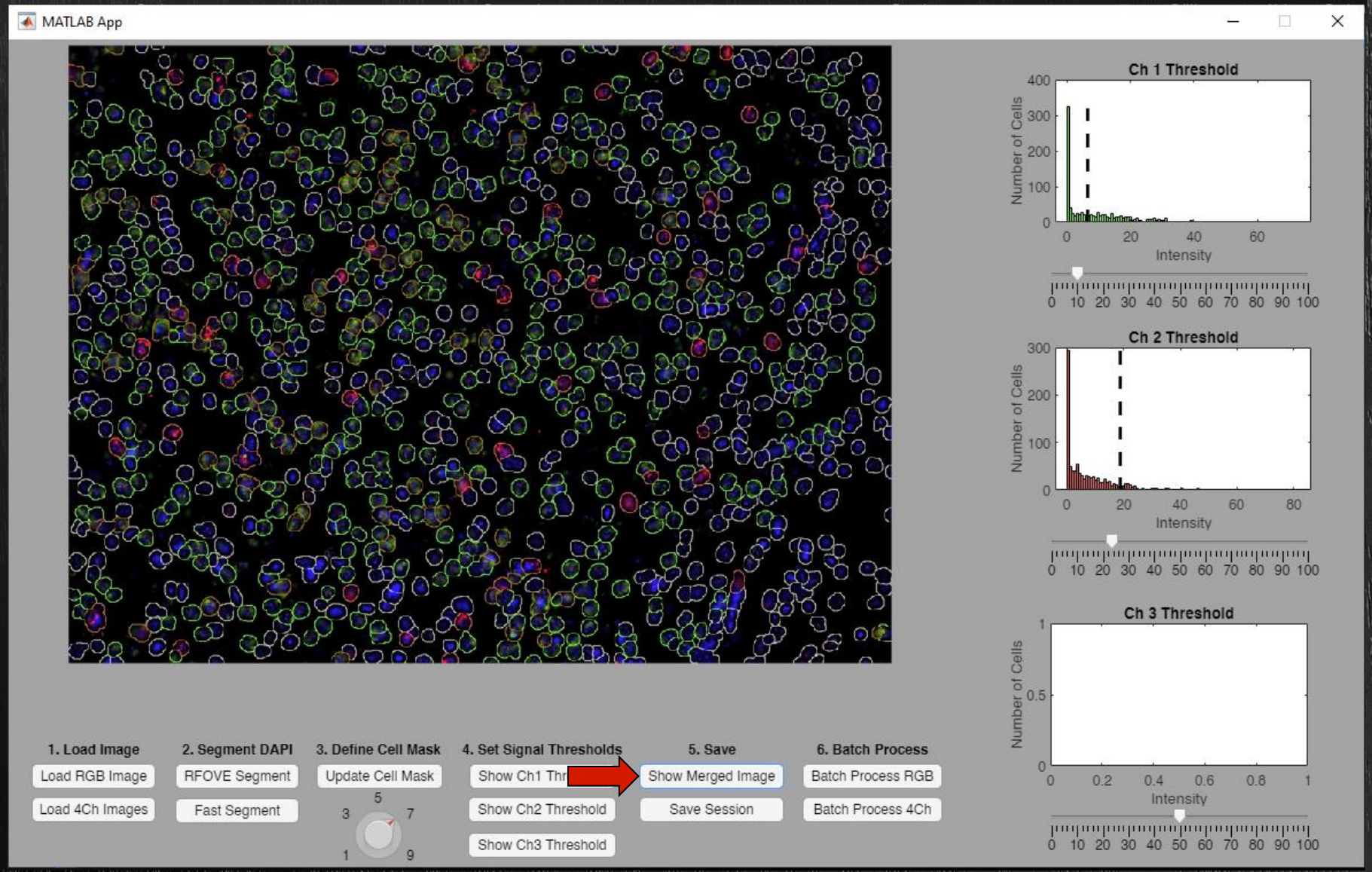
Set Signal Channel Thresholds

- Move Sliders to set thresholds
- Cells on left of the line are “Negative”
- Cells on right of the line are “Positive”
- View each individually



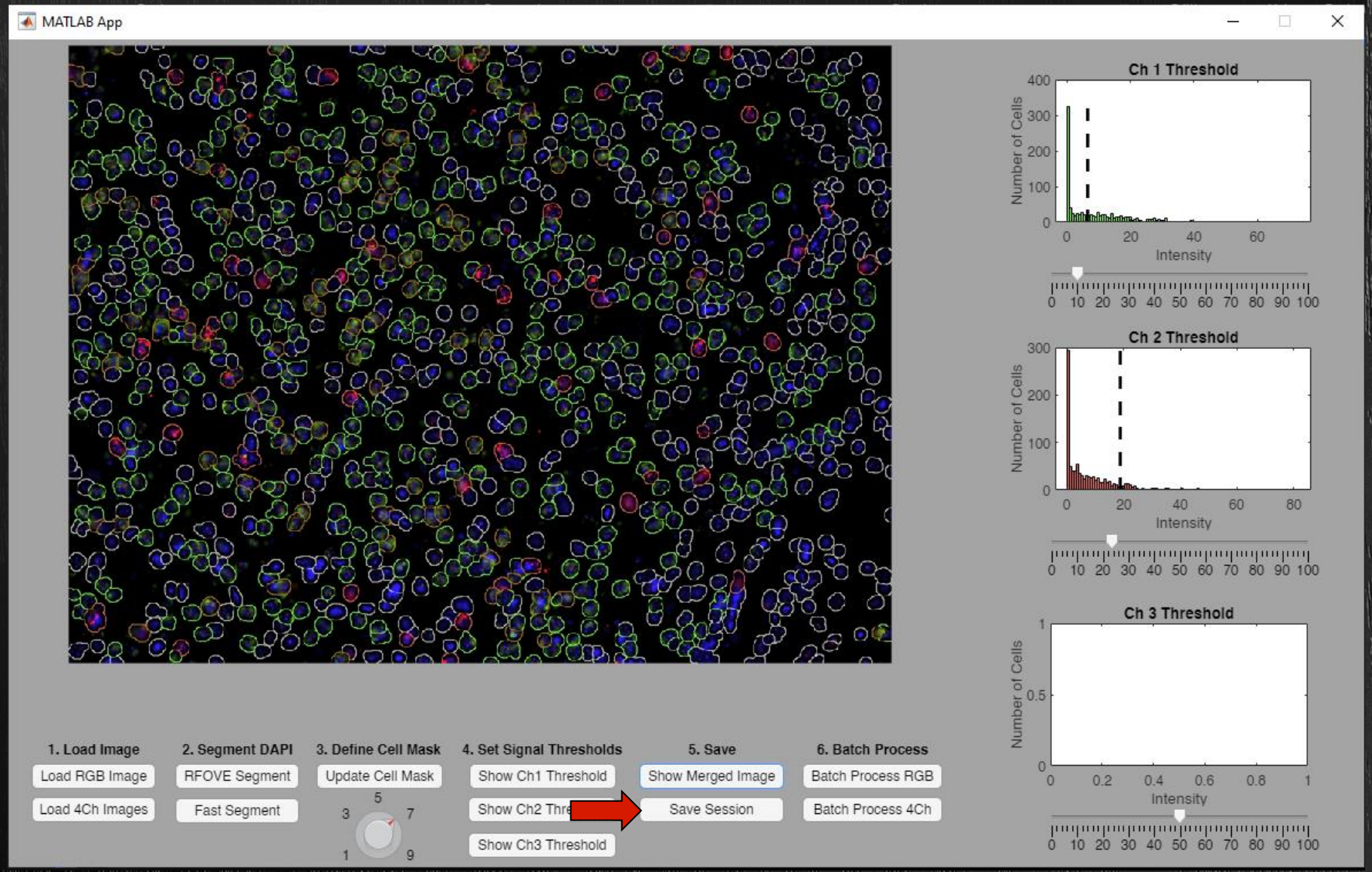
View Composite Image and Verify Thresholds

- Move Sliders to adjust thresholds
- Cells on left of the line are “Negative”
- Cells on right of the line are “Positive”
- Confirm Thresholds visually



Save Final Analysis Settings

- Save the composite image
- Save colocalization statistics for the representative image
- Save settings file for use in batch processing



Batch Process The Entire Dataset

- RGB:
  - Select all the RGB images to analyze with the same settings
- 4 Ch Images:
  - Select parent directory containing 1 folder for each set of 4 images (NO OTHER FOLDERS!)
- Select settings file
- Select directory to save analyses
- Walk away, be patient