IAS Imaging Analysis Report

Fluorescence Microscopy RGB Composite Image: Automated Extraction of Cells, Nuclei, Fluorescence Intensity, and Morphological Features

Analysis target: 1000 x 1000 px, 8-bit RGB composite image. Because the scale bar, pixel size, exposure conditions, and original single-channel images were not provided, all quantitative values are reported in pixel units and relative intensity units.

1. Executive Summary

In this image, 402 nuclear candidates derived from the blue channel were detected, and 376 estimated cell objects were extracted using the nuclei as seeds. Forty-three candidates touched the image border, while 333 were interior objects. The area fractions were approximately 46.1% for the cell/fluorescence-positive region, 65.6% for the green-positive region, 25.6% for the red-positive region, and 6.1% for the blue nuclear region.

Morphologically, the field contains an adherent, well-spread cell population with strong green filamentous signals, and clear variation in cell size and fluorescence intensity. Nuclei are generally oval, and widespread nuclear loss or pronounced nuclear fragmentation is not conspicuous. However, because the analysis was performed from an RGB composite image and neither original channel images nor microscopy metadata were available, additional data are required for rigorous quantification, cell-type identification, and viability assessment.

2. Sample Recognition and Analysis Scope

The input was automatically recognized as an RGB composite fluorescence cell image. The analysis application was set to Sample QC, and the evaluation was limited to nuclear candidates, estimated cell regions, channel-specific positive regions, and fluorescence-intensity variability in a single 2D field of view.

Measurement summary: The image size was 1000 x 1000 px. A total of 402 nuclear candidates and 376 nucleus-seeded estimated cell objects were detected. Of these, 43 candidates touched the image border and 333 were interior objects.

Positive-area summary: The cell/fluorescence-positive region occupied 46.1% of the image, the green-positive region 65.6%, the red-positive region 25.6%, and the blue nuclear region 6.1%. Median estimated cell area [IQR] was 1013 px^2 [635-1607], and median nuclear area [IQR] was 132 px^2 [109-164].

3. Measurement Items and Parameters

For this report, the measurement items that could be extracted automatically from the analysis target, together with the preprocessing, thresholding, and per-cell separation conditions used for each measurement, were fixed as shown on the following page.

3. Measurement Items and Parameters

Measurement item	Value	Unit	Source
Analysis image size	1000 x 1000	px	Automatic input-image recognition
Nuclear candidates	402	objects	Blue/DAPI-like channel
Estimated cell objects	376	objects	Cell-contour estimation
Objects touching image border	43	objects	Boundary QC
Interior objects	333	objects	Boundary QC
Cell/fluorescence-positive area fraction	46.1%	% area	Foreground mask
Green-positive area fraction	65.6%	% area	Green-channel mask
Red-positive area fraction	25.6%	% area	Red-channel mask
Nuclear area fraction	6.1%	% area	Blue-channel mask
Estimated cell area, median [IQR]	1013 px^2 [635-1607]	px^2	Object measurement table
Nuclear area, median [IQR]	132 px^2 [109-164]	px^2	Nuclear label map
Nuclear-to-estimated-cell area ratio, median [IQR]	0.127 [0.089-0.208]	ratio	Paired nuclear/cell labels
Mean green intensity, median [IQR]	90.9 a.u. [80.2-105.9]	relative intensity	Green channel
Mean red intensity, median [IQR]	93.6 a.u. [77.0-109.2]	relative intensity	Red channel
Green/red mean-intensity ratio, median [IQR]	0.995 [0.858-1.174]	ratio	Green/red object means
Red-Green Pearson correlation	0.5308	r	Cell-foreground pixels
PCA explained variance	PC1=47.98%, PC2=14.42%, PC3=12.48%	%	Object-level numeric features

Measurement parameter	Setting	Purpose
Input image	1000 x 1000 px; RGB composite; relative 8-bit intensity	Fix the analysis target and unit system
Analysis resize	analysis=1000 x 1000 px; resize_scale=1.0; max_side_px=1000	Define the measurement coordinate system
Channel normalization	method=per-channel percentile normalization to uint8; low_percentile=0.5; high_percentile=99.5	Channel-specific quantification from the RGB composite image
Nuclear-candidate extraction	method=raw_blue_channel_otsu; threshold_uint8=45.0; blur_sigma=1.0 px; open_kernel=3 px; close_kernel=5 px; min_area=20 px^2; max_area=12000 px^2	Extract Blue/DAPI-like nuclear regions
Cell/foreground-region extraction	method=raw_rgb_max_triangle_minus_background_margin; threshold_uint8=29.0; open_kernel=3 px; close_kernel=7 px	Generate the foreground mask for cytoplasm/cell-body candidates
Red-marker extraction	method=raw_red_channel_otsu; threshold_uint8=51.0; open_kernel=3 px	Quantify red-positive regions
Per-cell object separation	method=nuclear_seeded_ai_ready_marker_controlled_watershed; watershed_compactness=0.01; max_radius=31 px; min_cell_area=85.8 px^2; max_cell_area=3696.0 px^2	Recognize cell contours and convert them into individual objects

4. Figure Evidence

The following panels present channel separation, nuclear/estimated-cell segmentation, per-cell crops, positive-area measurements, intensity and area distributions, linear and nonlinear latent structure, cluster validity, feature contributions, outlier QC, and spatial phenotype correspondence.

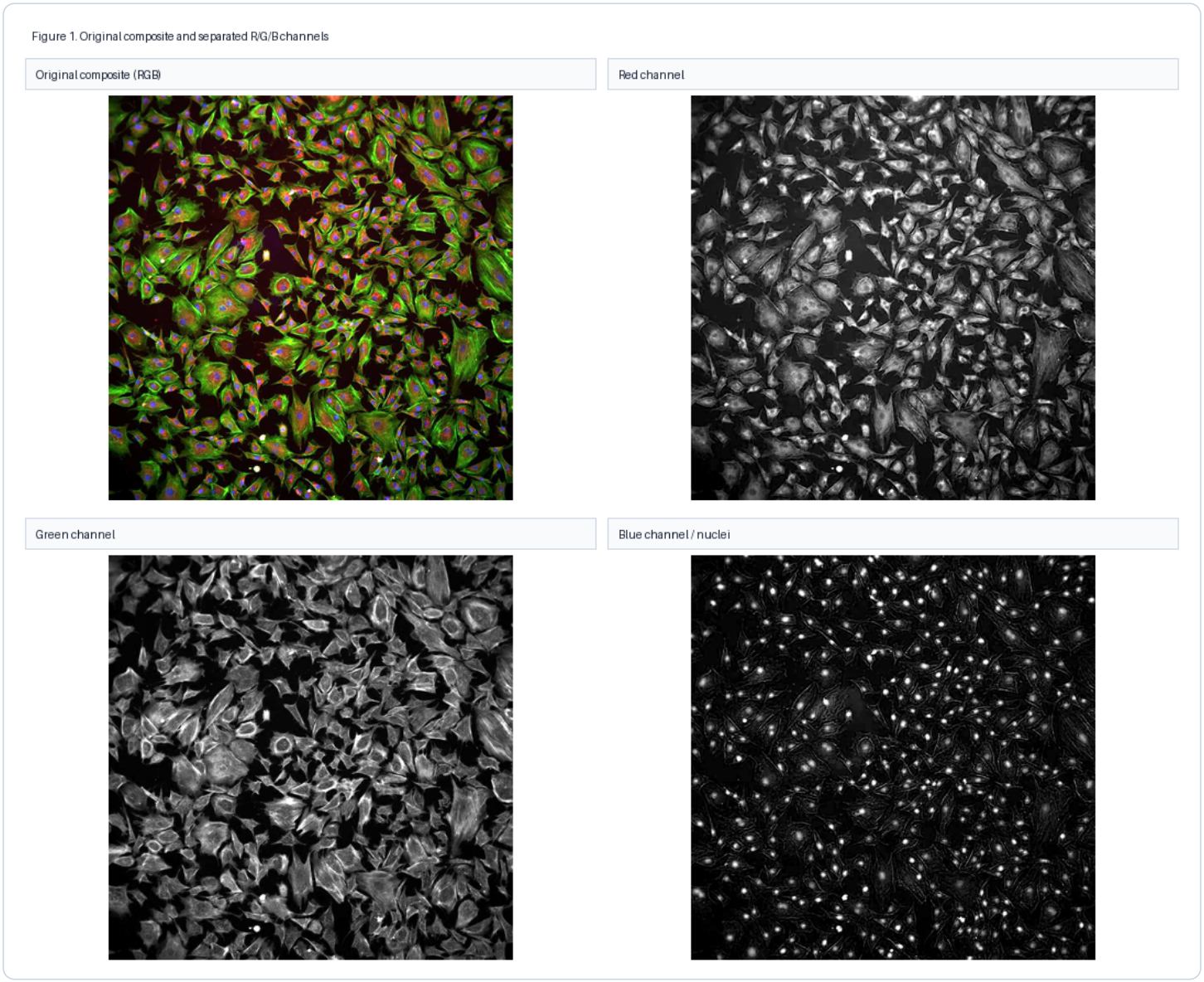

Figure 1. RGB composite image and separated R/G/B channels.

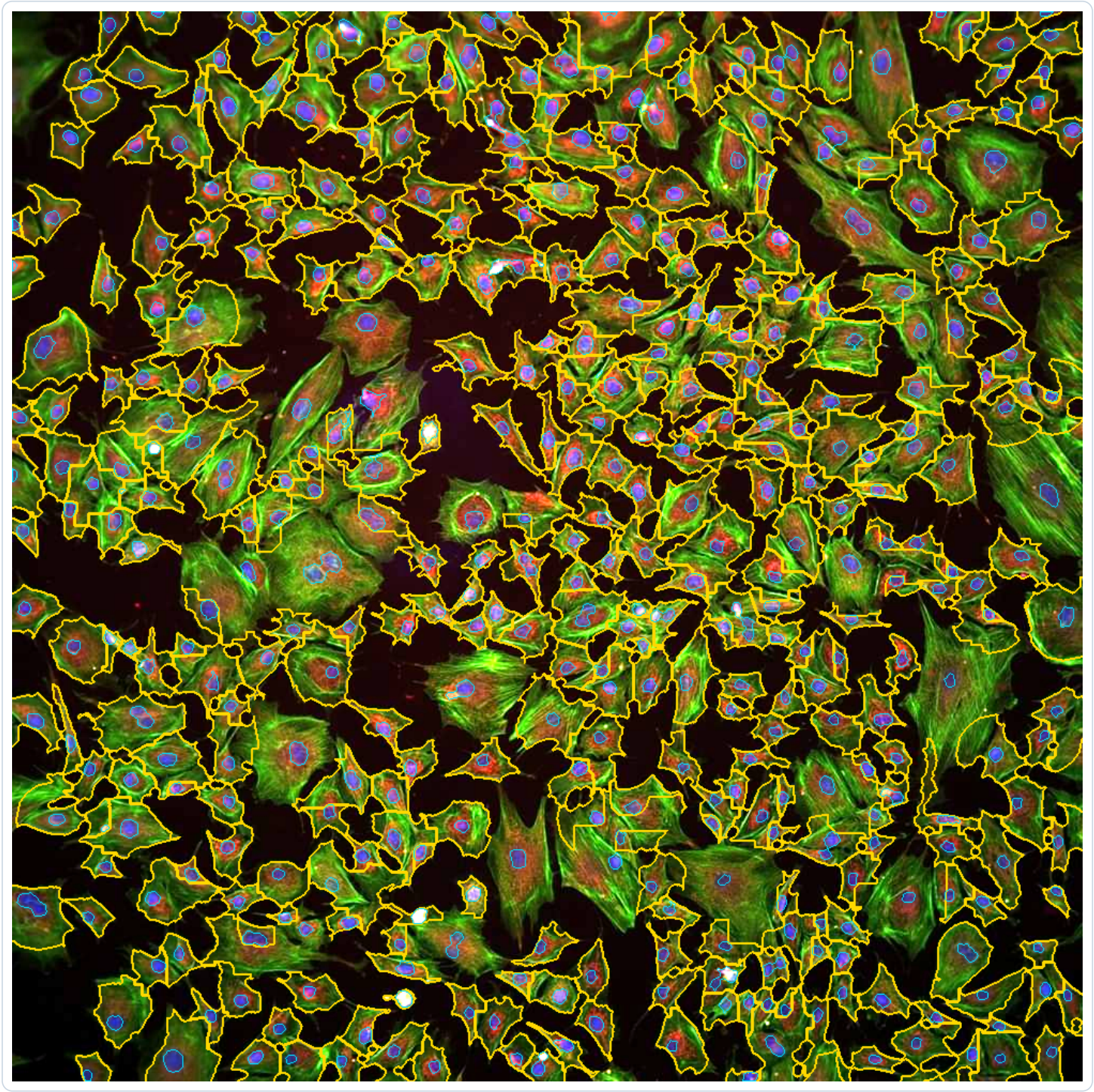

Figure 2. Overlay of estimated cell boundaries (yellow) and nuclear boundaries (cyan).

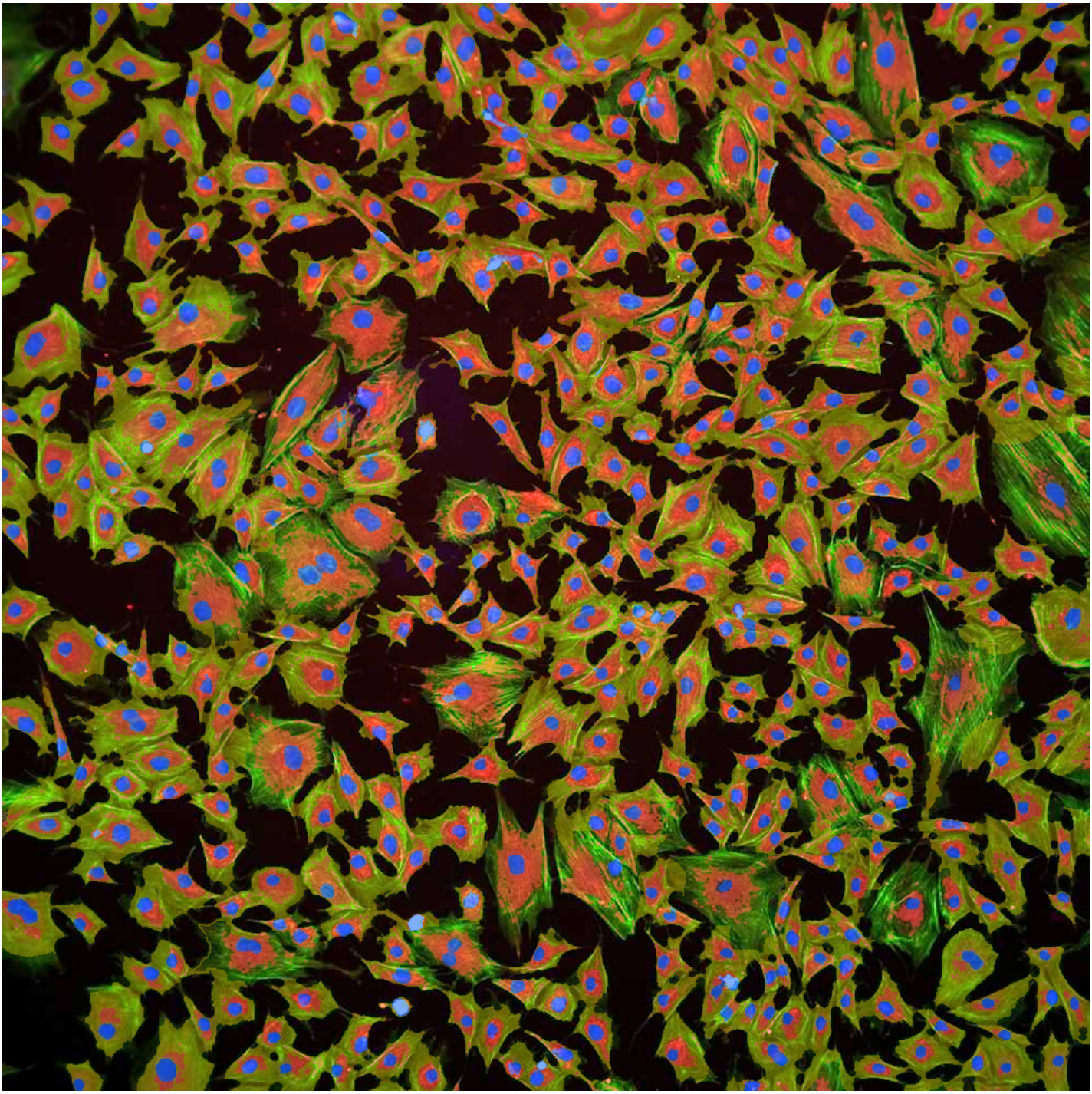

Figure 3. Cell/fluorescence-positive region mask.

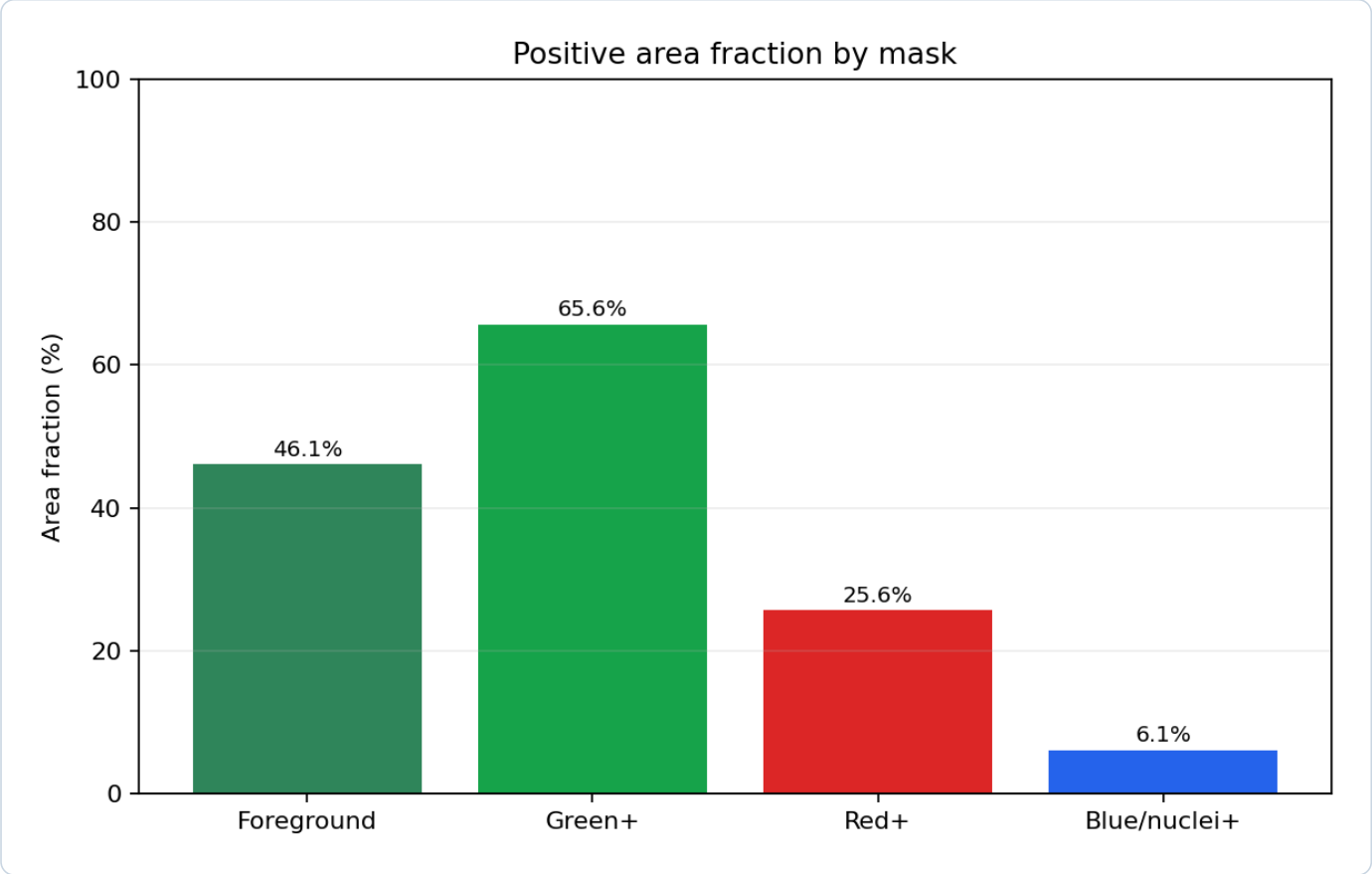

Figure 4. Positive area fraction by channel.

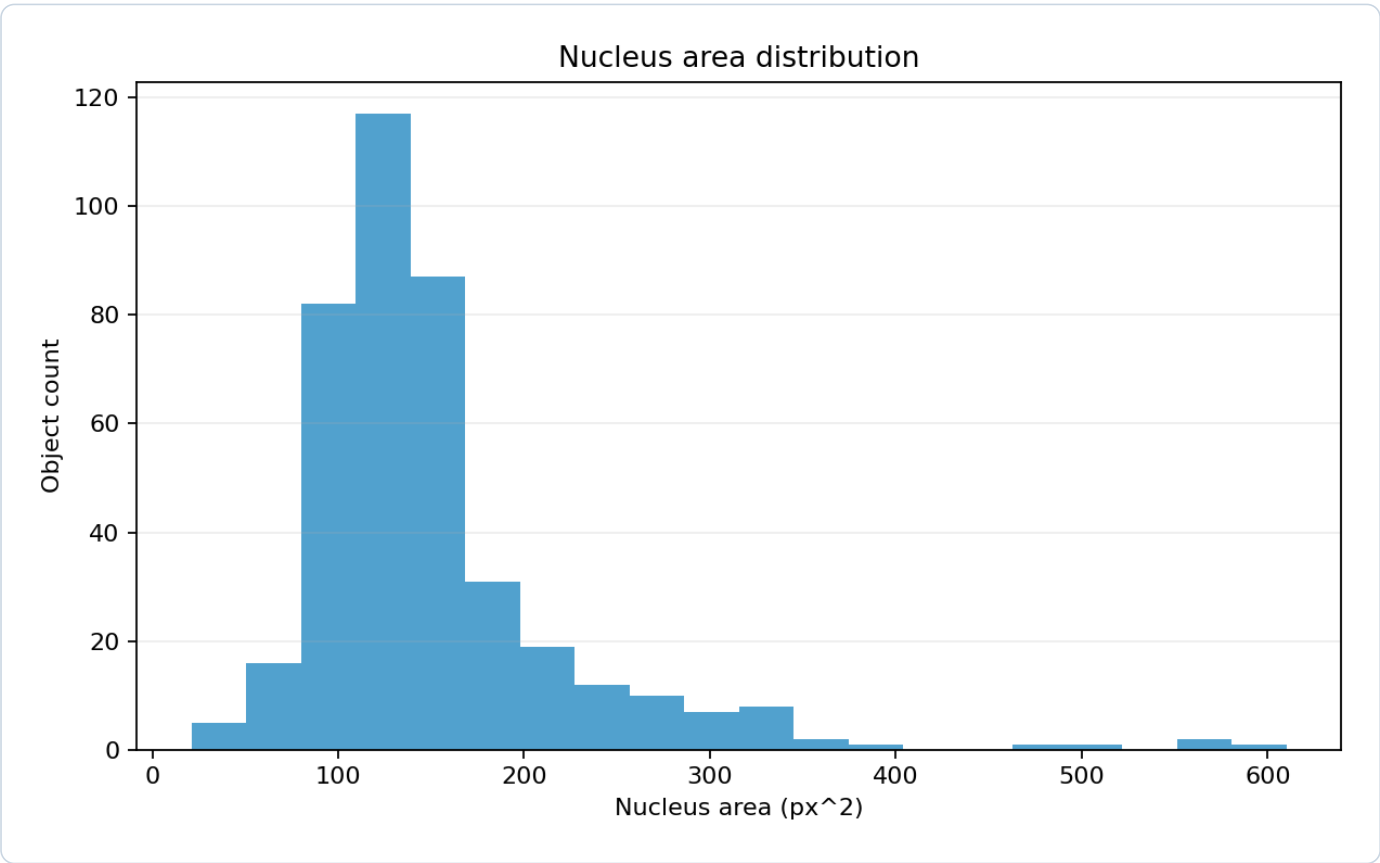

Figure 5. Nuclear area distribution.

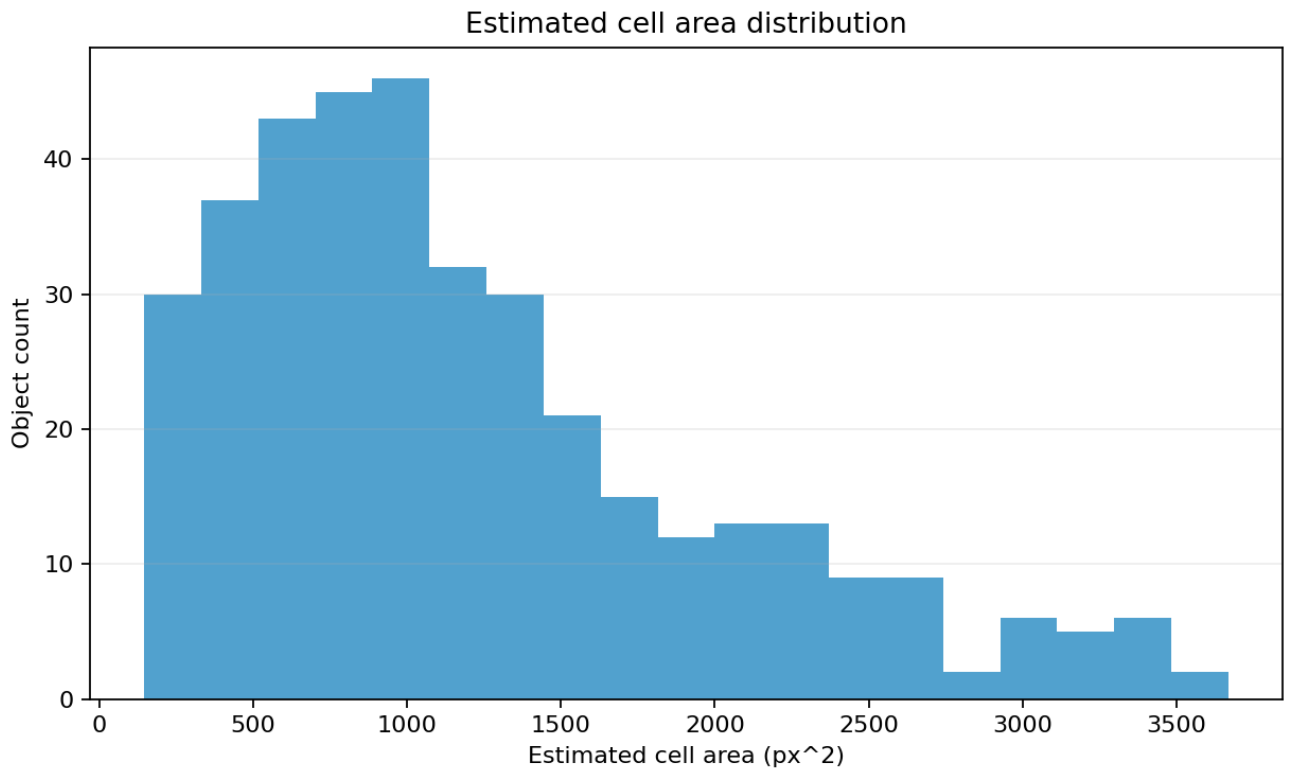

Figure 6. Estimated cell area distribution.

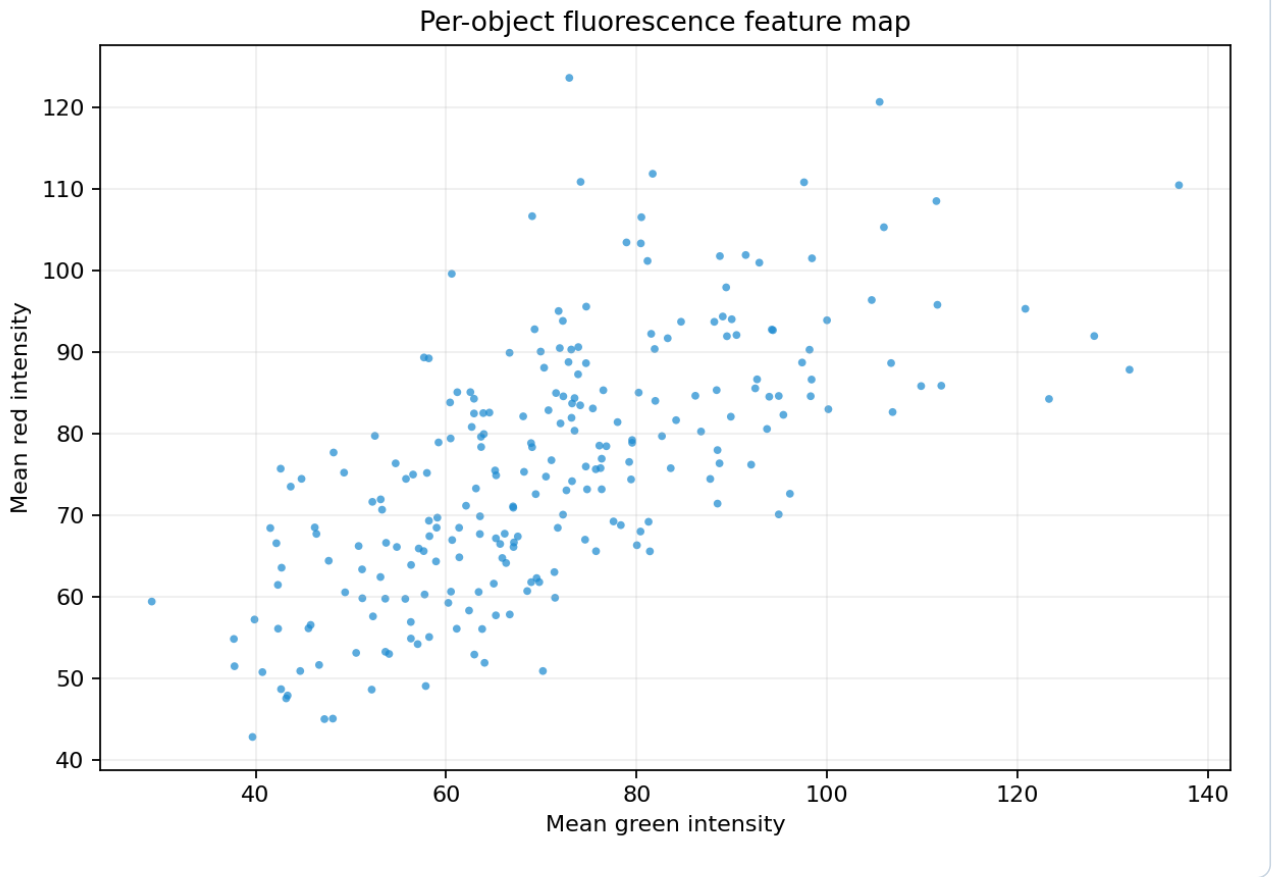

Figure 7. Per-object Red-Green fluorescence feature map.

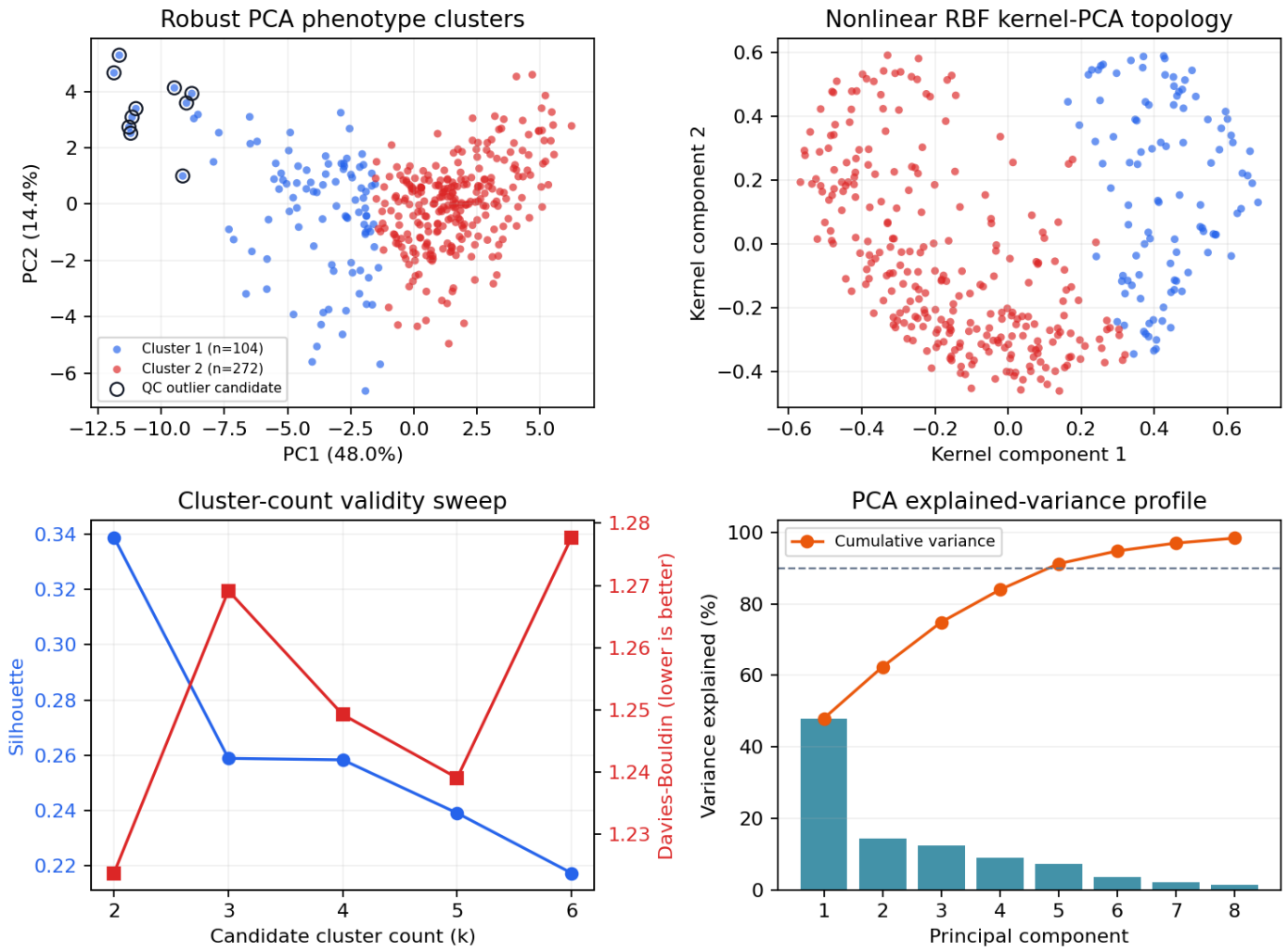

Figure 8. Latent structure, nonlinear embedding, and cluster validity.

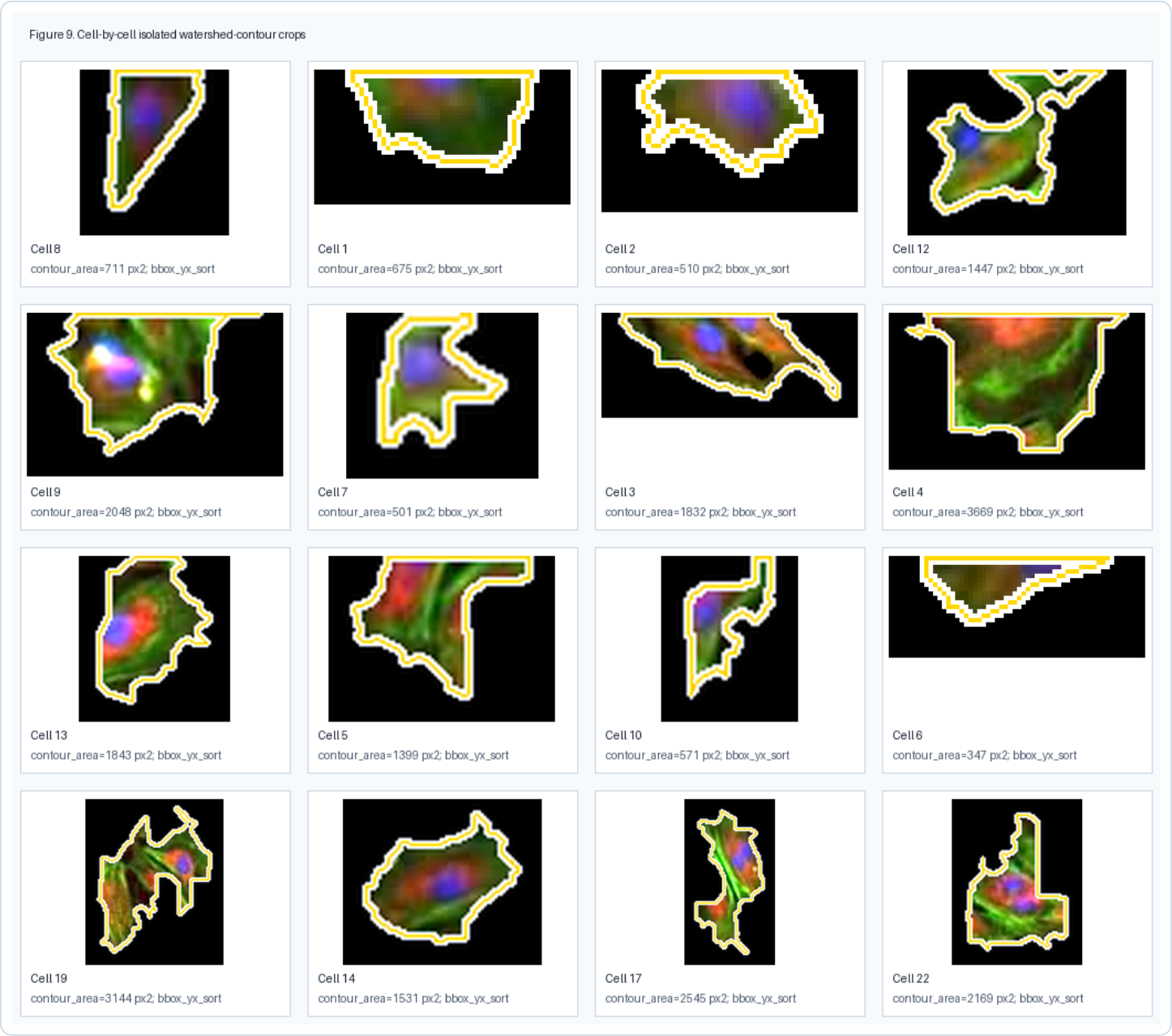

Figure 9. Per-cell crops with estimated boundaries.

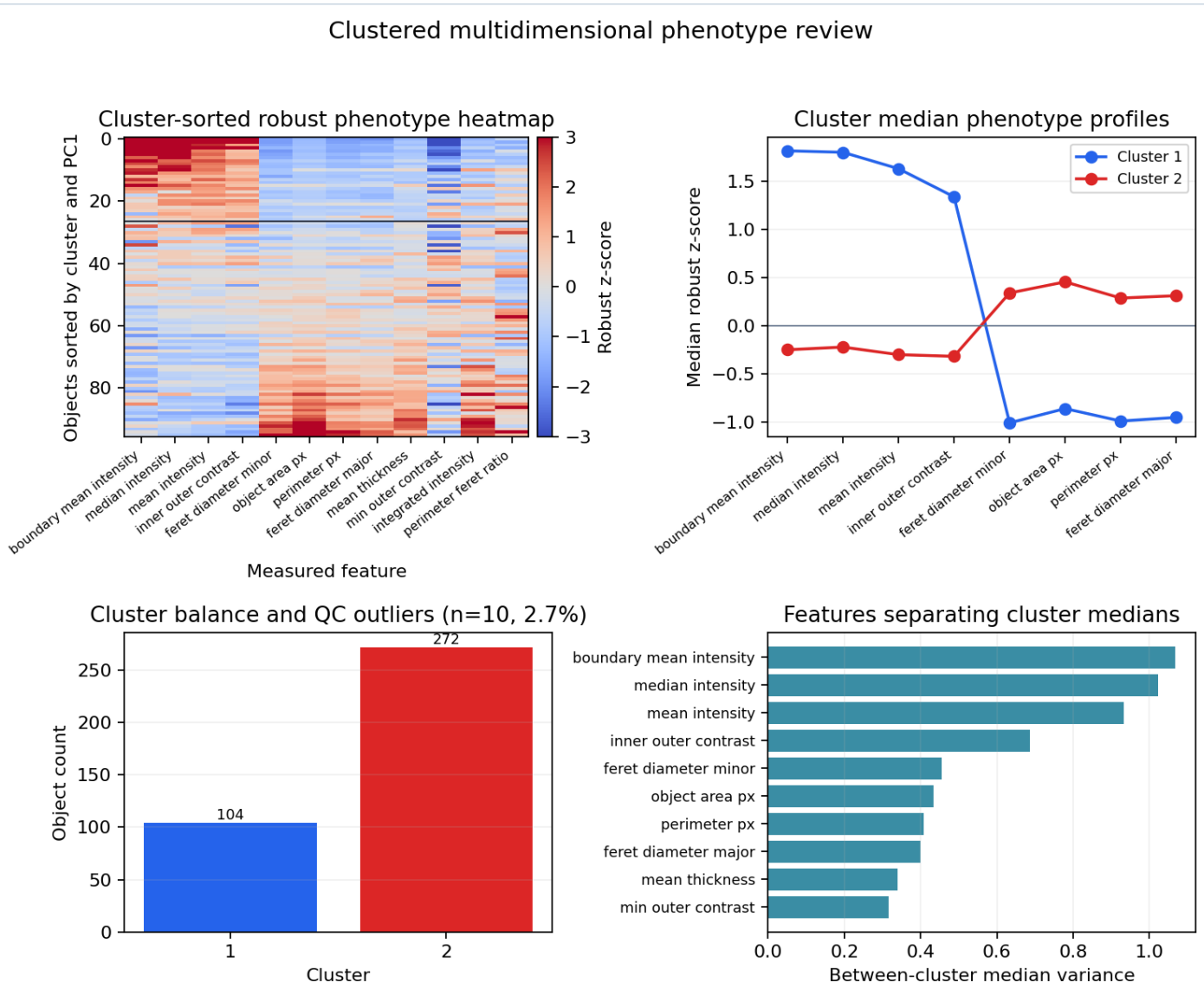

Figure 10. Multidimensional phenotype review by cluster.

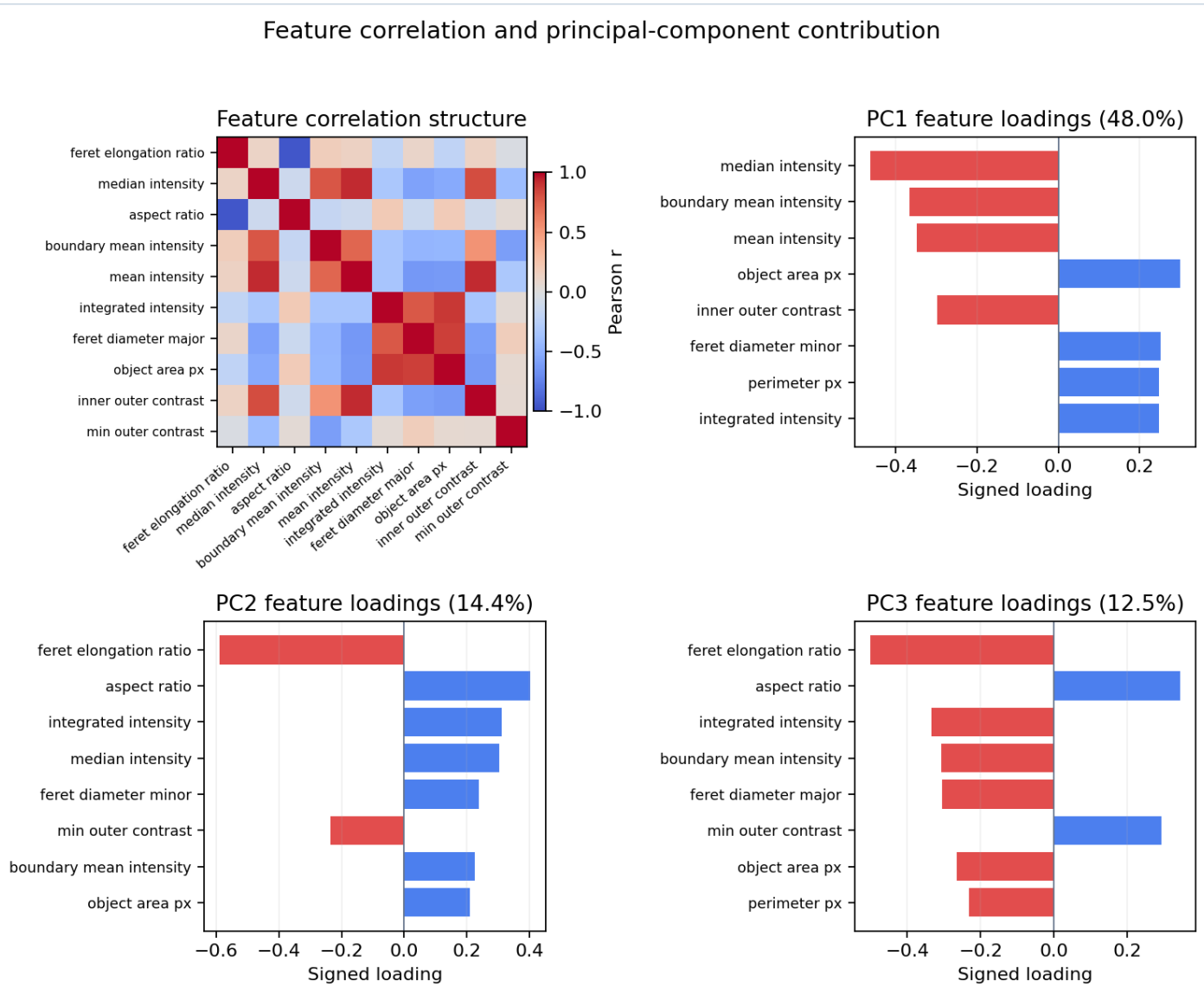

Figure 11. Feature correlations and principal-component contributions.

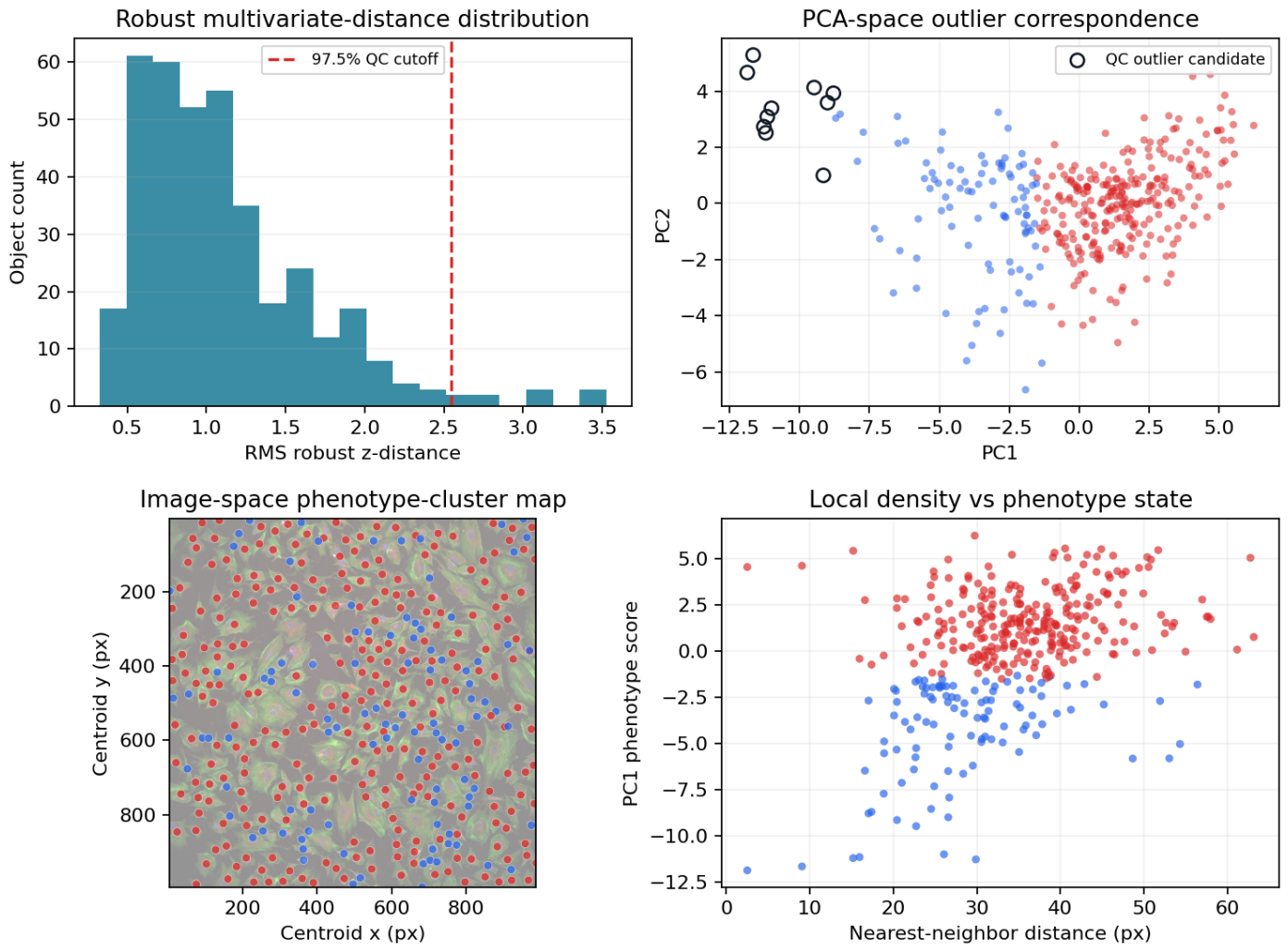

Figure 12. Outlier QC and spatial phenotype correspondence.

5. Scientific Draft

The green channel was positive over a broad area, with strong filamentous and fibrous structures observed throughout the cytoplasm and toward the cell periphery. This pattern suggests well-developed cytoskeletal/fibrous structures in adherent cells. However, because the stain identity was not provided, the signal cannot be assigned definitively to a specific marker.

Some objects showed relatively strong red-channel accumulation in the cytoplasm and perinuclear region, and this signal did not completely coincide with the green signal. The Red-Green scatter plot contained both green-dominant and red-dominant cells, indicating heterogeneity in cell state or staining intensity.

Blue-channel nuclear detection suggested a relatively high cell density within the field of view. At the same time, because cell-cell contact, cells truncated at the border, and bright small particles/debris candidates were present, fully automated nuclear and cell counts may require correction ranging from several percent to the mid-teens.

Multidimensional phenotype analysis used robust scaling of 16 features and selected k=2, with PC1=47.98%, PC2=14.42%, PC3=12.48%, silhouette=0.339, Davies-Bouldin=1.224, Calinski-Harabasz=187.4, feature-subset stability ARI=0.695, and 10 outlier-QC candidates. The clusters should be interpreted not as biological cell types, but as an exploratory QC layer for image-derived phenotypes.

6. Quality Control

- Image-analysis parameters: Blue Otsu threshold=45; foreground triangle threshold=29; green threshold=15; red threshold=51.
- Multidimensional features: Red-Green scatter, area histograms, robust PCA (PC1=47.98%, PC2=14.42%, PC3=12.48%), RBF kernel-PCA, cluster-validity sweep, clustered phenotype heatmap, PC1-PC3 loadings, robust-distance outlier QC, and an image-space cluster map.
- Multidimensional QC results: Sixteen features were robustly scaled and k=2 was selected. silhouette=0.339, Davies-Bouldin=1.224, Calinski-Harabasz=187.4, feature-subset stability ARI=0.695, and 10 outlier-QC candidates were obtained.
- Cluster labels and outlier flags are exploratory image-QC candidates and do not directly indicate cell type, pathology, diagnosis, or drug efficacy. Interpret them in conjunction with control groups, replicates, acquisition conditions, and marker information.
- Because the analysis was performed on an RGB composite PNG, the original 16-bit/12-bit single-channel images, exposure time, gain, flat-field correction, and scale information could not be incorporated.
- Precise cell boundaries are estimates generated by nucleus-seeded watershed; touching cells, overlapping cells, and thin protrusions may be over-segmented or under-segmented.
- To raise quantification to a contract-measurement level, use original channel images, a scale bar or pixel size, negative and positive controls, identical exposure settings, multiple fields of view, and manually reviewed training data.
- For HCA applications, add a well/field/replicate hierarchy, per-field QC, Z-stack or time-lapse information, well-level statistical aggregation, and outlier flags.

7. Output Files

In addition to this report, the output package included an object-level measurement CSV, a multidimensional-coordinate/cluster/outlier-trace CSV, a multidimensional-analysis summary JSON, and the figure panels as PNG files.