



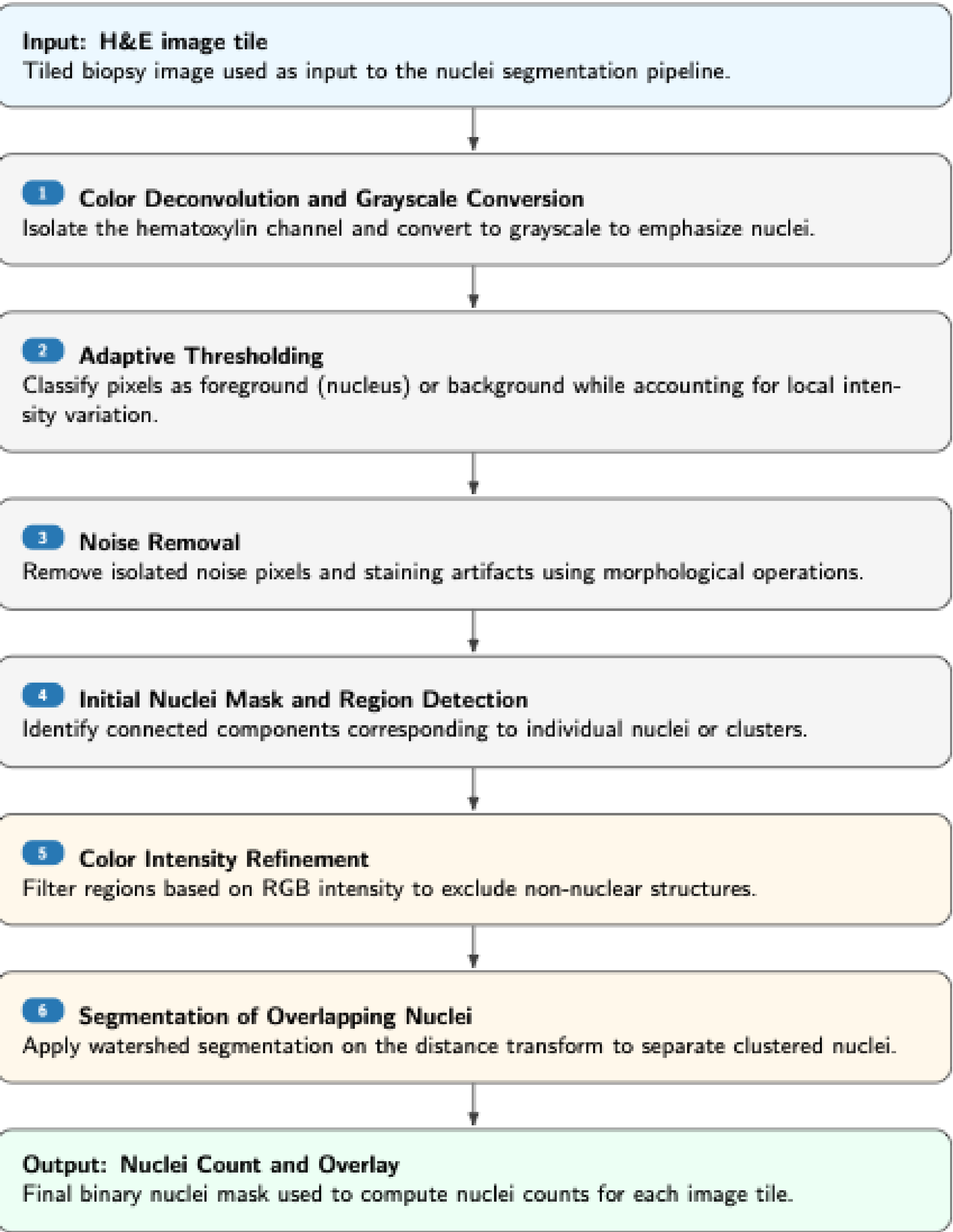
Machine Learning–Based Quantification of Cellular Density for Objective Rejection Monitoring in Facial Vascularized Composite Allotransplantation

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Figure 1. Overview of the automated nuclei segmentation pipeline



Rejection monitoring in facial vascularized composite allotransplantation (fVCA) relies on Banff-graded histopathologic assessment of skin and mucosal biopsies. Although clinically indispensable, this process is subjective and limited by interobserver variability and the scarcity of VCA-experienced pathologists. To advance standardized, multidisciplinary rejection surveillance, we evaluated whether automated quantification of cellular density on routine hematoxylin and eosin (H&E) slides can objectively discriminate rejection from non-rejection in fVCA biopsies.

Methods

Forty-six biopsies (21 skin, 25 mucosa) from nine fVCA recipients (ten transplants) were analyzed. Samples were classified by expert dermatopathologists using the Banff criteria; grade ≥ 2 was considered rejection-positive. Whole-slide images were processed using a custom Python-based nuclei segmentation pipeline incorporating color deconvolution, adaptive thresholding, morphological refinement, and optional watershed separation of clustered nuclei. Cellular density (cells/mm²) was calculated by normalizing automated nuclei counts to tissue area. Group comparisons were performed using one-sided Welch’s t-tests. To validate robustness, analyses were repeated using HoVer-Net, an established deep learning–based nuclei segmentation framework, with identical downstream processing.

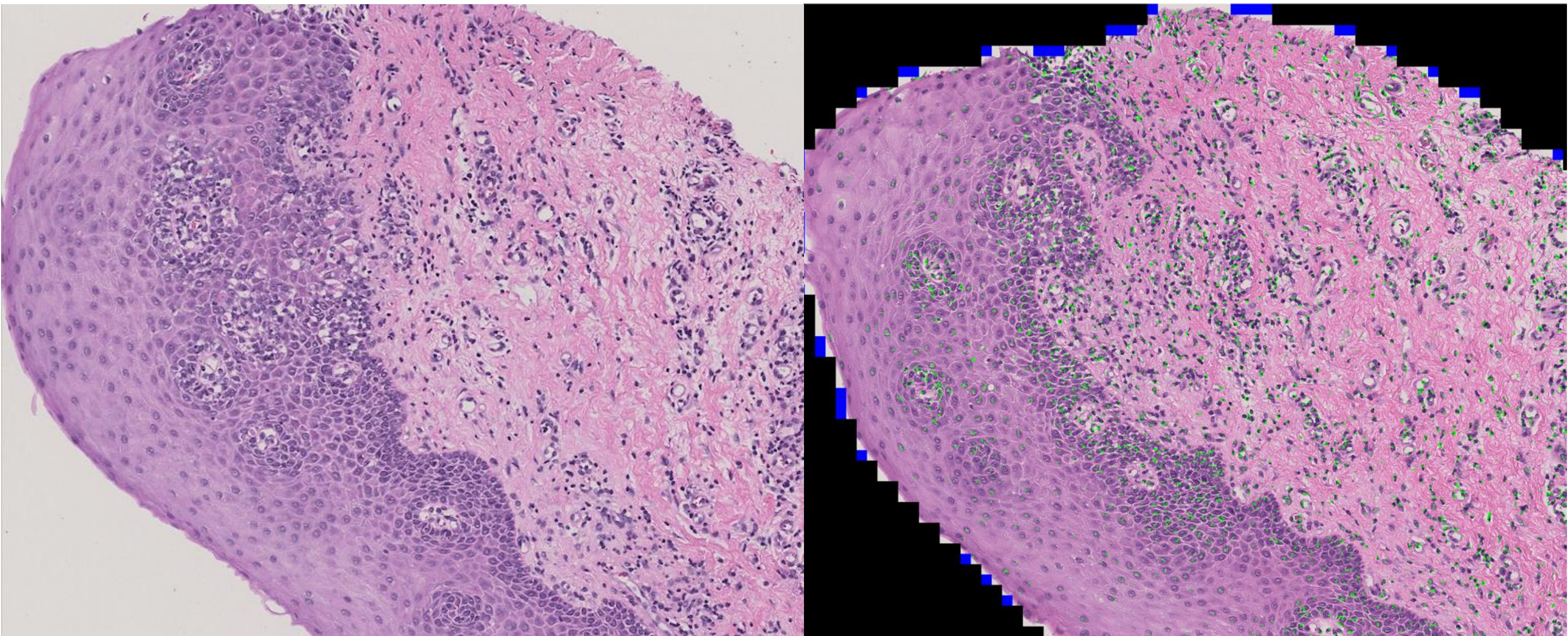


Figure 2. Automated nuclei segmentation and background masking pipeline applied to an H&E-stained mucosal biopsy. Representative hematoxylin and eosin (H&E)–stained whole-slide image of a mucosal biopsy after application of the custom image analysis pipeline.

Results

Sixteen biopsies were rejection-positive and 30 rejection-negative. Using the custom pipeline, cellular density was significantly higher in rejection-positive biopsies compared with controls across all tissues (p=0.014). Stratified analyses demonstrated consistent findings in both skin (p=0.016) and mucosal samples (p=0.026). HoVer-Net–based segmentation reproduced these associations, showing significantly increased cellular density in rejection-positive biopsies (p<0.001), with concordant results in both tissue types. Direct paired comparisons between segmentation methods revealed no statistically significant differences in cellular density estimates, supporting methodological agreement.

Table 1. Summary of Cellular Density Analyses by Segmentation Method and Tissue Type

Analysis Category	Segmentation Method	Tissue Type	N (Biopsies)	p-Value
Primary analysis	Custom pipeline	All samples	46	0.014
Primary analysis	Custom pipeline	Skin	21	0.016
Primary analysis	Custom pipeline	Mucosa	25	0.026
Validation analysis	HoVer-Net	All samples	46	<0.001
Validation analysis	HoVer-Net	Skin	21	0.020
Validation analysis	HoVer-Net	Mucosa	25	0.007

Conclusion

Automated quantification of cellular density provides an objective, reproducible marker of acute rejection in fVCA biopsies. A lightweight, resource-efficient image analysis pipeline yields results concordant with state-of-the-art deep learning methods and may facilitate broader clinical adoption. This work exemplifies a team science approach integrating transplant surgery, dermatopathology, immunology, and computational image analysis to advance standardized, precision diagnostics in facial transplantation.