This method is designed to analyze tissue images, focusing on identifying and quantifying purple-stained regions.

The process begins with generating an epithelium mask to isolate tissue regions using contour detection.

The input image is preprocessed through grayscale conversion, blurring, and thresholding to create a binary mask.

Morphological operations help refine this mask by smoothing edges and removing noise. Contours outlining the epithelium are then extracted and drawn on the original image, creating a

clear visual distinction of the tissue region. The next step, superpixel segmentation, uses color and texture similarity

to divide the image into smaller segments. Each segment is analyzed for purple concentration based on defined HSV color thresholds,

allowing a more consistent analysis across lighting variations. Regions with high staining concentration retain their original

color, moderate concentrations are marked in white, and low concentrations in black, emphasizing tissue areas by staining intensity.

Instruction Manual for Use:

1. To use this code, start by organizing your images and ensuring that they are named

and stored in a directory that matches the image path specified in the code. Also make sure they are in .tif format.

2. Make sure OpenCV, NumPy, skimage, and Matplotlib are installed.

3. Run the code.

4. Adjust parameters like `num\_segments` for superpixel count and

`lower\_purple`/`upper\_purple` to refine HSV color thresholds as needed.

The code will output segmented images with regions classified by purple concentration,

with the ability to visualize and quantify these staining patterns.

Limitations:

While this method effectively highlights stained regions, it has some limitations.

It relies on specific threshold and morphological settings, which may need adjustment for different images.

Variations in lighting or stain intensity can also affect results, requiring color threshold adjustments.

Additionally, the code is not fully developed for handling outside regions and struggles with overlapping

tissue areas where superpixels may blend inside and outside regions. Expanding these capabilities would

improve its effectiveness across a wider range of tissue samples.