Approach:

Step 1: Read the Image and convert it into double precision.

Step 2: The MATLAB function adapthisteq is used to enhance the contrast between nuclei and background. (will make nuclei more darker and background brighter)

Step 3: Plot the contrast enhanced image.



Fig 1: Contrast enhanced (adapthisteq) Image

Step 4: The MATLAB function graythresh is used to find the global threshold of the image. That will eliminate most of the background. This function is working based on Otsu’s thresholding algorithm. And then binaries the image with the MATLAB function imbinarize using the threshold value.



Fig 2: Threshold binaries image

Step 5: From this step, the morphological operation will start. Complement the threshold Image, so we can highlight the nuclei. As we can see in the Fig 2, some of the portion of the nuclei is not highlighted (see the red circle in Fig 2). So, I am filling that holes with the matlab function imfill. In fig 3, all the holes inside the nuclei is filled (Red circle in Fig 3)



Fig 3: Filling operation on Image 2.

Step 6: following morphological operations, I am using imopen MATLAB function. That will do erosion following by dilation. That will remove all the small unnecessary object from the background. And clean the boundaries. See in Fig 4(Red Circles).



Fig 4: Morphological Opening operation.

Step 7: Flowing operation will eliminate small object in the image that are not the nuclei by using bwareaopen matlab function. Here I have used the function such that it will eliminate all the area which are containing at list 100 pixels.

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Fig 5: Morphological bwareaopen operation.

Step 8: Following operation will do watershading on the detected region of nuclei. For this bwdist matlab function (finding the distance transform binary image) is used followed by watershad function. Then from the distance transform binary Image, whatever value in this matrix has zero value will be discriminating edge between 2 overlapping nuclei, will segment the overlapped nuclei. Fig 6 is the discriminated nuclei Image.



Fig 6A: Edge transform Image

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Fig 6B: detected individual nuclei Image.

Step 9: Following morphological operation is erosion (To make that nuclei smaller), Ultimate erosion Operation with the matlab function bwulterode(to detect this nuclei at 1 point) and dilation (to make that point bigger so it can easily visible). This step is performing for easily counting of the individual nuclei.

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Fig 7: detected individual nuclei.

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Fig 8: Overlaid fig 7 mask with original Image.

Step 10: To count this nuclei, I am using direct matlab function bwlabel. And the count is 265 nuclei.

Step 11: For task 2, we need to find the nuclei which are further than *n* pixels away from the nearest nuclei. For this first I am giving user input to set the distance threshold. And find the pixel distance with the direct matlab function bwdist on the mask. Following to this, whatever the user set the threshold distance, below all the threshold distance value will be zero. And then direct using bwlabel matlab function to find the number of nuclei which are away from threshold distance. Fig 9 is showing us how many nuclei are away from 20 pixels distance. And it is 2 nuclei which are 20 pixels away from the other nuclei.



Fig 9: After giving threshold 20 Image