Color-Based Segmentation Using K-Means Clustering

Image Processing Toolbox™ offers a variety of techniques for image segmentation. When working with color images, color-based segmentation is a common way to identify objects.

This example shows how to segment colors automatically using the L*a*b* color space and K-means clustering in Statistics and Machine Learning Toolbox™.

Step 1: Reading the Image

Suppose you have a file `hestain.png`, which is an image of tissue stained with hemotoxylin and eosin (H&E). This staining method helps pathologists distinguish different tissue types. Get started by reading this image into MATLAB®.

```
he = imread('hestain.png');
imshow(he), title('H&E image');
text(size(he,2),size(he,1)+15,...
      'Image courtesy of Alan Partin, Johns Hopkins University', ...
      'FontSize',7,'HorizontalAlignment','right');
```

H&E image

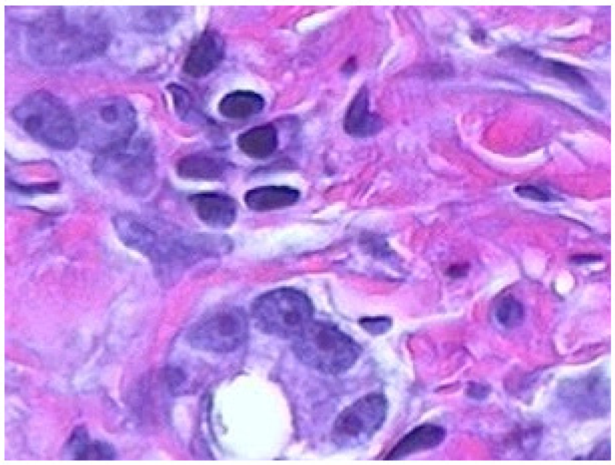


Image courtesy of Alan Partin, Johns Hopkins University

Step 2: Converting the Image from the RGB Color Space to the L*a*b* Color Space

How many colors do you see in the image if you ignore variations in brightness? There are three colors: white, blue, and pink. Notice how easily you can visually distinguish these colors from one another. The L*a*b* color space (also known as CIELAB or CIE L*a*b*) enables you to quantify these visual differences.

The L*a*b* color space is derived from the CIE XYZ tristimulus values. The L*a*b* space consists of a luminosity layer L*, chromaticity layer a* indicating where color falls along the red-green axis, and chromaticity layer b* indicating where the color falls along the blue-yellow axis. All of the color information is in the a* and b* layers. You can measure the difference between two colors using the Euclidean distance metric.

Using the functions `makecform` and `applycform`, you can convert the image to the L*a*b* color space.

```
cform = makecform('srgb2lab');
lab_he = applycform(he,cform);
```

Step 3: Classifying the Colors in the a*b* Space Using K-Means Clustering

Clustering is a way to separate groups of objects. K-means clustering treats each object as having a location in space. It finds partitions such that objects within each cluster are as close to each other as possible, and as far from objects in other clusters as possible. K-means clustering requires that you specify the number of clusters to be partitioned and a distance metric to quantify how close two objects are to each other.

Since the color information exists in the a*b* space, your objects are pixels with a* and b* values. Use the `kmeans` function in Statistics and Machine Learning Toolbox to cluster the objects into three clusters using the Euclidean distance metric.

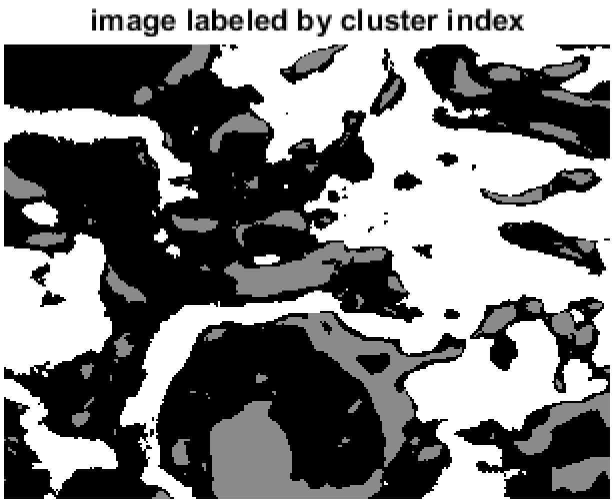
```
ab = double(lab_he(:,:,2:3));
nrows = size(ab,1);
ncols = size(ab,2);
ab = reshape(ab,nrows*ncols,2);

nColors = 3;
% repeat the clustering 3 times to avoid local minima
[cluster_idx, cluster_center] = kmeans(ab,nColors,'distance',...
                                       'sqEuclidean','Replicates',3);
```

Step 4: Labeling Every Pixel in the Image Using the Results from `kmeans`

For every object in your input, `kmeans` returns an index corresponding to a cluster. The `cluster_center` output from `kmeans` will be used later in the example. You can label every pixel in the image with its `cluster_index`.

```
pixel _ labels = reshape(cluster _ idx,nrows,ncols);
imshow(pixel _ labels,[]), title('image labeled by cluster index');
```



Step 5: Creating Images That Segment the H&E Image by Color

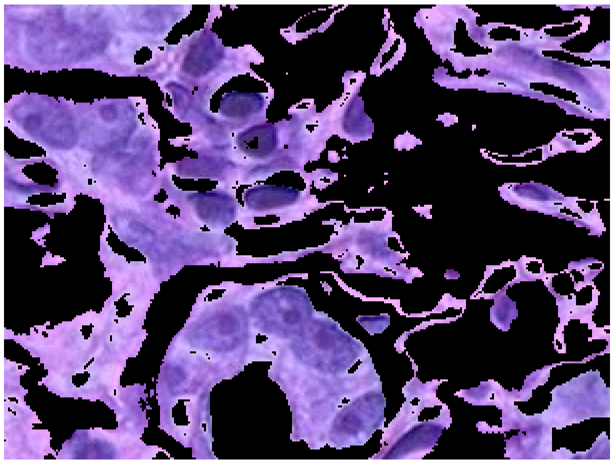
Using `pixel _ labels`, you can separate objects in `hestain.png` by color, which will result in three images.

```
segmented _ images = cell(1,3);
rgb _ label = repmat(pixel _ labels,[1 1 3]);

for k = 1:nColors
     color = he;
     color(rgb _ label ~= k) = 0;
     segmented _ images{k} = color;
end
```

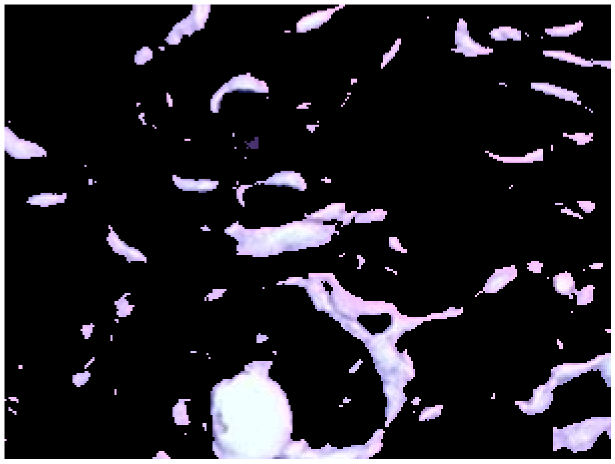
```
imshow(segmented_images{1}), title('objects in cluster 1');
```

objects in cluster 1

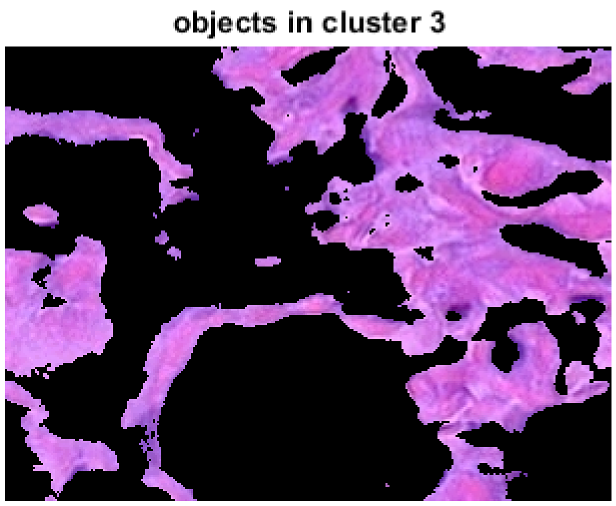


```
imshow(segmented_images{2}), title('objects in cluster 2');
```

objects in cluster 2



```
imshow(segmented_images{3}), title('objects in cluster 3');
```



Step 6: Segmenting the Nuclei into a Separate Image

Notice that there are dark and light blue objects in one of the clusters. You can separate dark blue from light blue using the L* layer in the L*a*b* color space. The cell nuclei are dark blue.

Recall that the L* layer contains the brightness values of each color. First, find the cluster that contains the blue objects. You can extract the brightness values of the pixels in this cluster and threshold them with a global threshold using `imbinarize`.

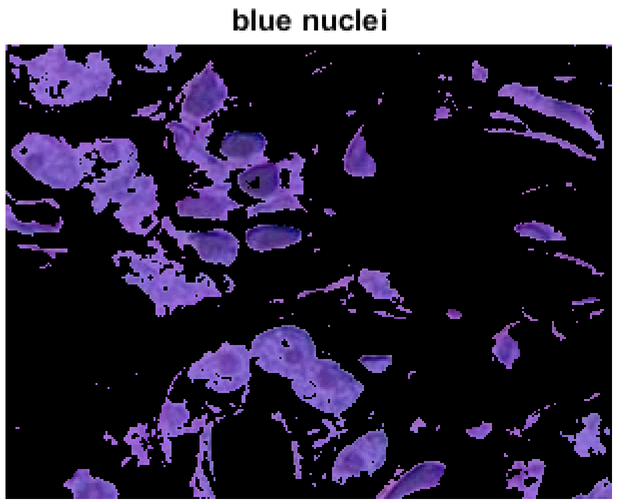
You must programmatically determine the index of the cluster containing the blue objects because `kmeans` will not return the same `cluster_idx` value every time. You can do this using the `cluster_center` value, which contains the mean a* and b* value for each cluster. The blue cluster has the smallest `cluster_center` value (determined experimentally).

```
mean_cluster_value = mean(cluster_center,2);
[tmp, idx] = sort(mean_cluster_value);
blue_cluster_num = idx(1);

L = lab_he(:,:,1);
blue_idx = find(pixel_labels == blue_cluster_num);
L_blue = L(blue_idx);
is_light_blue = imbinarize(L_blue);
```

You can use the mask `is_light_blue` to label which pixels belong to the blue nuclei, then display the blue nuclei in a separate image.

```
nuclei_labels = repmat(uint8(0),[nrows ncols]);
nuclei_labels(blue_idx(is_light_blue==false)) = 1;
nuclei_labels = repmat(nuclei_labels,[1 1 3]);
blue_nuclei = he;
blue_nuclei(nuclei_labels ~= 1) = 0;
imshow(blue_nuclei), title('blue nuclei');
```



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- *clusterData* (download)
- *SegmentTool: An Interactive GUI for Segmenting Images* (download)
- *Segment Images Interactively and Generate MATLAB Code* (download)
- *Image Processing Toolbox* (product trial)