Cluster Analysis

This lab will demonstrate how to perform the following in Python:

- Hierarchical clustering
- K-means clustering
- Internal validation methods
  - Elbow plots
  - Silhouette analysis
- External validation method: Adjusted Rand Index

You will need:

- Python
- Anaconda
  - numpy
  - pandas
  - matplotlib
  - scipy
  - sklearn
  - csv
  - mpl_toolkits

Hierarchical clustering of cancer gene expression data

NCI-60 RNAseq dataset filname: 'nci_var_filtered.txt'
This file is normalized RNA abundance data (TPM). It has been filtered to keep only the genes with variability below a certain threshold. You can open it in your desired spreadsheet program to see what it looks like.

Read about the NCI-60:
https://dtp.cancer.gov/discovery_development/nci-60/

Let's cluster the data to see how similar these cancers are on the basis of gene expression. Is their similarity related to tissue of origin?

1. Load the data. I like using pandas for this.

```
import pandas as pd
datafile = 'nci_var_filtered.txt'
df = pd.read_csv(datafile, sep='\t')
```

If doing these steps interactively (in a Python Console), you can check out what the dataframe (df) looks like by entering `df`

Get a list of the columns in the df with:

```
list(df.columns.values)
```

Find the size of the df with:

```
df.shape
```

How many genes and cell lines does the NCI-60 data have?

2. Process the data for clustering. Change the index from the numerical index (default when you load a pandas df) to the first column (gene names).

```
df = df.set_index('gene')
```

Get a list of all the cell lines.

```
cells = list(df.columns.values)
```

3. Create a function that contains everything needed for performing hierarchical clustering. I'm calling mine dendrogrammer. Once it is written, call the function like so:

```
dendrogrammer(df, cells)
```

But before we can call it, we must create the function somewhere (in a new .py file, or at the top of a .py script):

```
# display dendrogram
# give it the labels for the data you want as leaves

def dendrogrammer(df, leaf_labels):

      # all the things that dendrogrammer should do will go in here
```

4. More rearranging of the data. Dendrogrammer will take a pandas df as input. But we don't need all that. Let's import numpy to help with this processing. We'll go ahead and import scipy for clustering and matplotlib for visualizing the results.

At the top of the file where you have def dendrogrammer, add these things:

```
import numpy as np
from scipy.cluster.hierarchy import dendrogram, linkage
from matplotlib import pyplot as plt

      #get just the numerical data from the dataframe in a numpy array
      D = df.values
```

Plus, scipy's clustering algorithm clusters the rows, not the columns. If we want to cluster the cell lines, we'll need to transpose the data.

```
      # Check to see if we need to transpose D
      # Length of leaf labels should be same as the number of rows in D
      if len(leaf_labels) != len(D):
            D = np.transpose(D)
```

5. Perform hierarchical clustering. You can specify different linkage methods and distance metrics.

```
      Z = linkage(D, method='ward', metric='euclidean')
```

Linkage methods could be 'single', 'average', 'complete', 'median', 'centroid', 'weighted', or 'ward'

There are many possible distance metrics (e.g., 'cityblockk', 'yule', 'hamming', 'dice', 'kulsinski', 'correlation', 'jaccard', and many more), or you can create your own. See the scipy documentation for **pdist** for more info.

6. Plot the dendrogram.

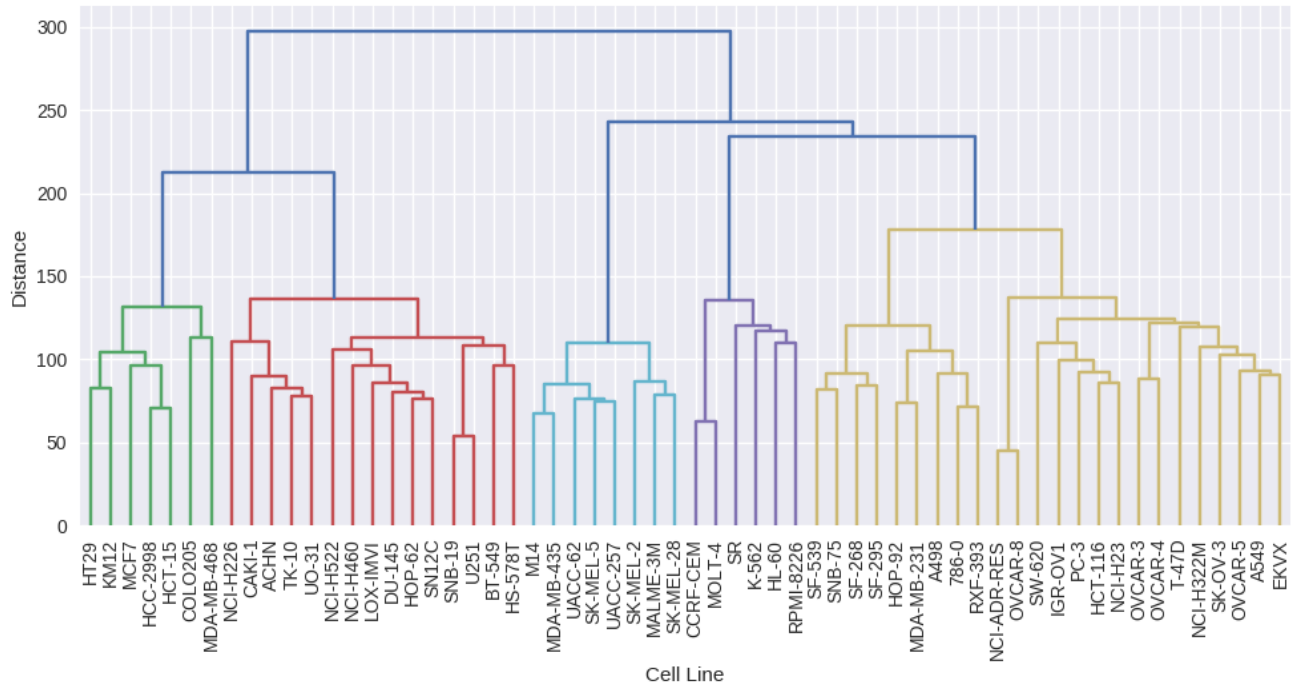
```
      # plot dendrogram
```

```
plt.figure(figsize=(10, 6))
ax = plt.subplot()
plt.subplots_adjust(left=0.07, bottom=0.3, right=0.98, top=0.95,
wspace=0, hspace=0)
plt.xlabel('Cell Line')
plt.ylabel('Distance')

dendrogram(Z, leaf_rotation=90., leaf_font_size=10.,
labels=leaf_labels)

plt.savefig('dendrogram_nci60.png')
```

Mine looks like this:



What does this tell us? Who knows!?

We can get a rough idea of which cell lines have similar global gene expression profiles. For instance, we see many of the ovarian cancer cell lines in the yellow cluster (OVCAR-3, OVCAR4, OVCAR8, SKO-OV-3) and some melanomas in the blue cluster (SK-MEL-28, MALME3M, SK-MEL-2, M14, MDA-MD-435).

If we knew something about the mutational background, we could start looking for other rational explanations for the clusters. e.g., do any of the clusters share a driving mutation in RAS? BRAF? EGFR?

Go ahead and adjust the **linkage method** and/or **distance metric** to see how the dendrogram changes.

K-means clustering of NCI-60 cancer gene expression data

1. Create a function that performs Principle Component Aanalysis. We will do this just because we want to visualize the data. This is a dataset with >9000 dimensions. We'll use PCA to project into three dimensions.

```
from sklearn.decomposition import PCA

# Perform PCA on the data, for dimensionality reduction
def PCAer(df):
    D = df.values
    D = np.transpose(D)
    pca = PCA()
    pca.fit(D)
    projected = pca.fit_transform(D)
    return projected
```

2. Create a function for K-means analysis. I'll call mine kmeanser. We'll call kmeanser like so:

```
[proj, labels, centroids] = kmeanser(df,k)
```

And kmeanswer should contain:

```
from sklearn.cluster import KMeans

# k-means clustering
# user supplies k
def kmeanser(df,k):

      # we'll perform a PCA just so we can plot the clustering results
      Dpc = PCAer(df)

      # Now kmeans
      kmeans = KMeans(n_clusters=k) # initialize
      kmeans = kmeans.fit(Dpc) # compute K-means clustering
      labels = kmeans.predict(Dpc) # get cluster labels for data points
      C = kmeans.cluster_centers_ # get cluster centers
      out = [Dpc, labels, C]
      return out
```

3. Call the K-means function and cluster the NCI-60 data into six groups.

Call the function a few times, and write all the results to a file. How do the cluster assignments change from run to run? How do they compare to the groups from the hierarchical clustering? How do they change if we don't run a PCA?

```
import csv

# Export list of cluster labels
matrix = zip(cells, labels1, labels2, labels_nopca)
with open('kmeans_clusters.txt', 'wb') as f:
    writer = csv.writer(f, delimiter='\t')
    writer.writerows(matrix)
```

4. Plot the data

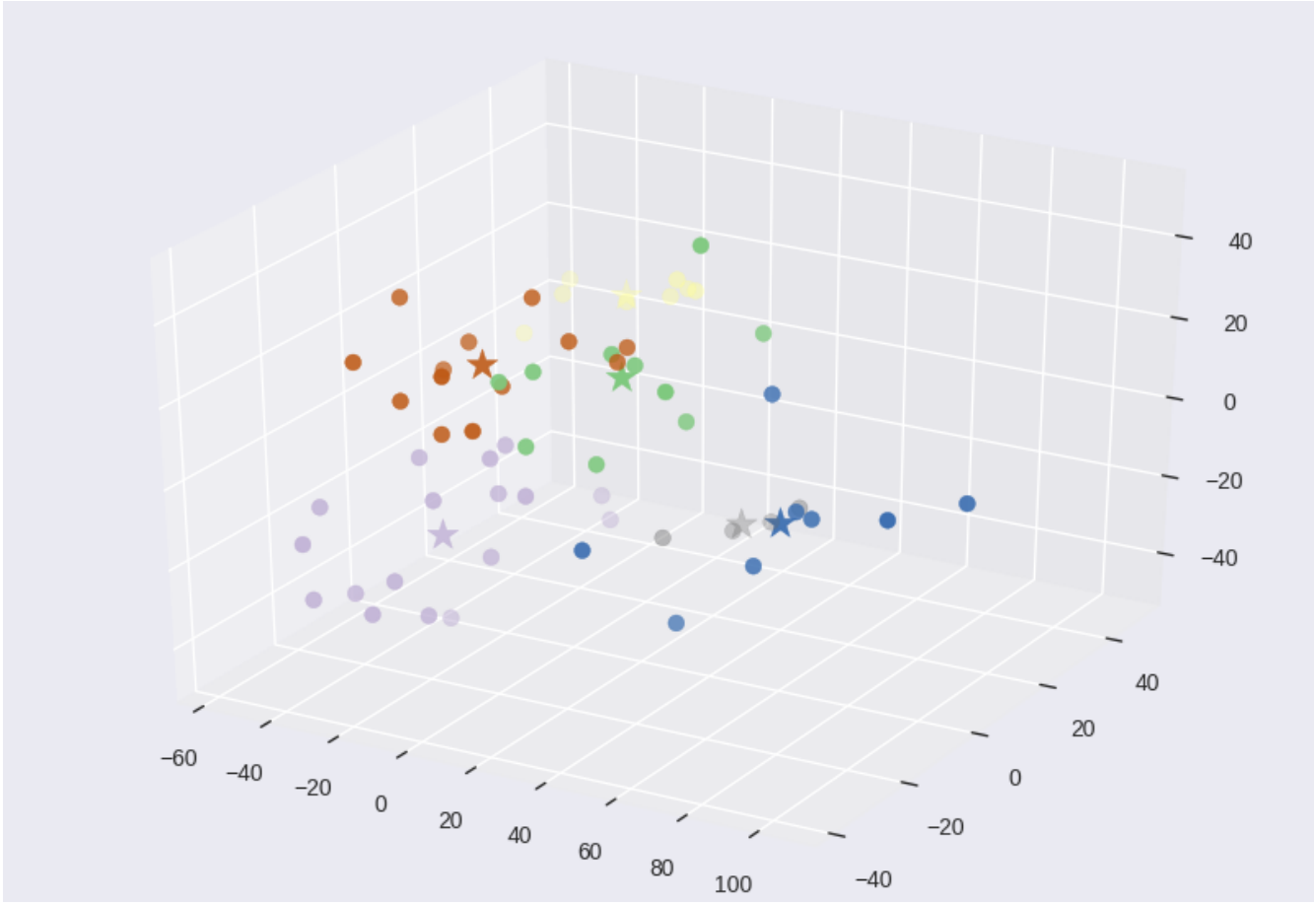
```
from mpl_toolkits.mplot3d import Axes3D
```

```
# plot the clusters
fig1 = plt.figure()
ax1 = fig1.add_subplot(111)
ax1 = Axes3D(fig1)

# plot the projected data with assigned clusters
ax1.scatter(proj[:, 0], proj[:, 1], proj[:, 2], c=labels, s=50, cmap='Accent')

# plot the centroids
ax1.scatter(centroids[:,0], centroids[:,1], centroids[:,2], c=range(k), s=200,
marker='*', cmap='Accent')
fig1.show()
```

Show or save the figure: `fig1.savefig('kmeans_nci60.png')`



K-means clustering (k=6) on the NCI-60 RNAseq data. Stars mark the cluster centroids. Axes are the first three principle components.

Try changing the number of clusters.

Cluster evaluation

How many clusters should we have?
Does cluster assignment match tissue of origin?

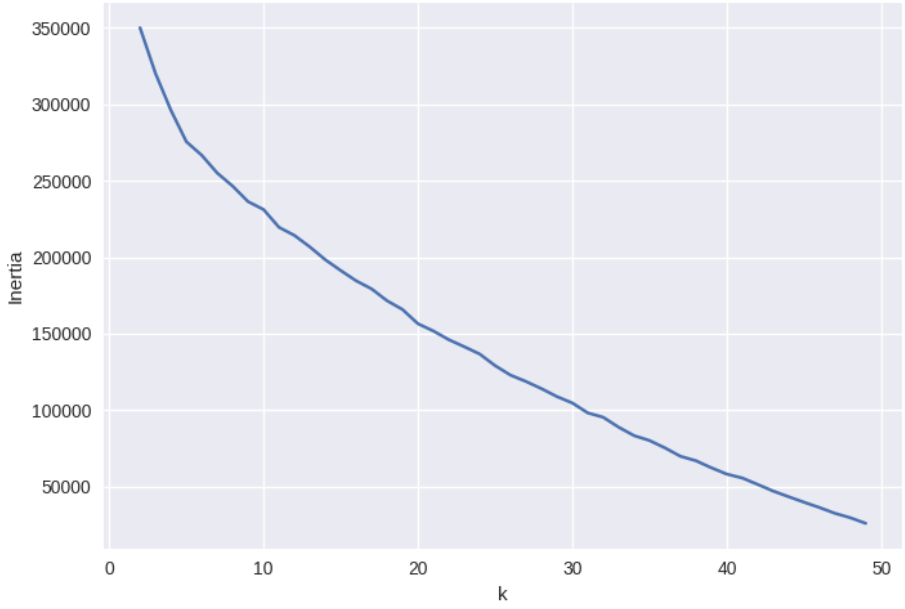
1. Elbow plot. One common way to gauge the number of clusters (*k*) is with an elblow plot, which shows how compact the clusters are for different *k* values. This assumes that we want clusters to be as compact as possible.

Write a function that runs a K-means analysis for a range of *k* values and generates an Elbow plot.

This function should take the `df` as input
You should generate a vector of *k* values and a measure of the cluster compactness.

Hint: The within-cluster sum-of-squares is a good metric for how "internally coherent" the clusters are. This measure can be obtained, after running a K-means clustering as shown previously, with:

```
kmeans.inertia_
```



Elbow plot for k = 2 to 50 clusters of the NCI-60 RNAseq data, clustering by cell line

This dataset generates a very smooth curve for the elbow plot, without a clear elbow. This indicates that the clusters aren't very compact for any *k*. We can try other methods of evaluating the optimal *k*.

2. Average Silhouette score. The average Sihouette score measure cluster compactness and cluster separation.

For each data point:

- **a**: the mean distance between the data point and all other data points in the same cluster
- **b**: the mean distance between a data point and all other points in the next nearest cluster

The Sihouette score for a single data point is then:

$$s = \frac{b - a}{max(a, b)}$$

The average Silhouette score for a dataset is the mean of the scores for all data points. The score can range between -1 (for incorrect clustering) and +1 (for highly dense clustering). Scores close to 0 indicate overlapping clusters. The score is higher as clusters are dense and well separated.

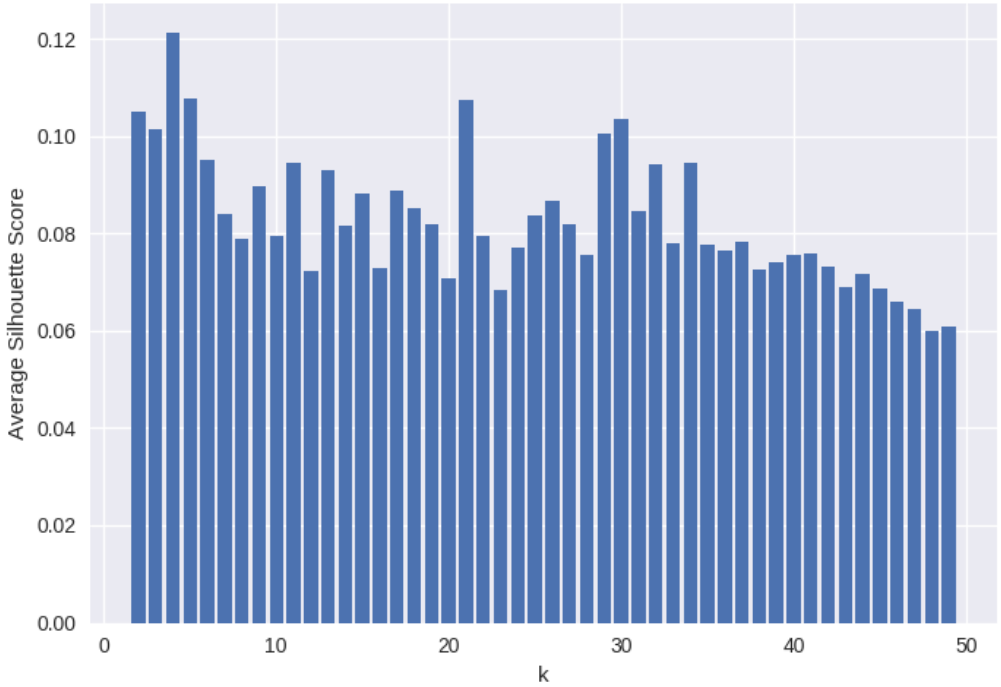
Write a function that calculates the average Silhouette score for a range of values of k.

Hint:

```
from sklearn.metrics import silhouette_score

# average silhouette score across all points in dataset Dpc (PCA transformed
data), with labels from K-means clustering:

sil = silhouette_score(Dpc, labels)
```



Average Silhouette Score for NCI-60 RNAseq data, clustering by cell line

The highest Silhouette score is with k=4 clusters. But a score around ~ 0.12 indicates that clusters aren't very well defined or well separated. So we can make the conclusion that all of these human cancer cell lines have similar expression across this set of ~9,000 genes. It's not surprising, really. None of these cells were being treated with drug or environmental stress when their RNA was extracted. They were all just happily growing in a plastic dish.

3. Adjusted Rand Index (ARI). The ARI is a measure of external validation. It will allow us to compare a set of known labels to the labels assigned with K-means clustering. In this case, we will use the tissue of origin as the known label.

Tissue of origin is in the file: "nci_var_filtered_type.csv"

The ARI ranges between -1 and 1. A value of ARI=1 indicates that the predicted labels (from clustering) perfectly match the known labels. The ARI will be close to 0 for random labeling independent of the number of clusters. The ARI

The ARI is a version of the Rand Index (RI) that has been adjusted for chance.

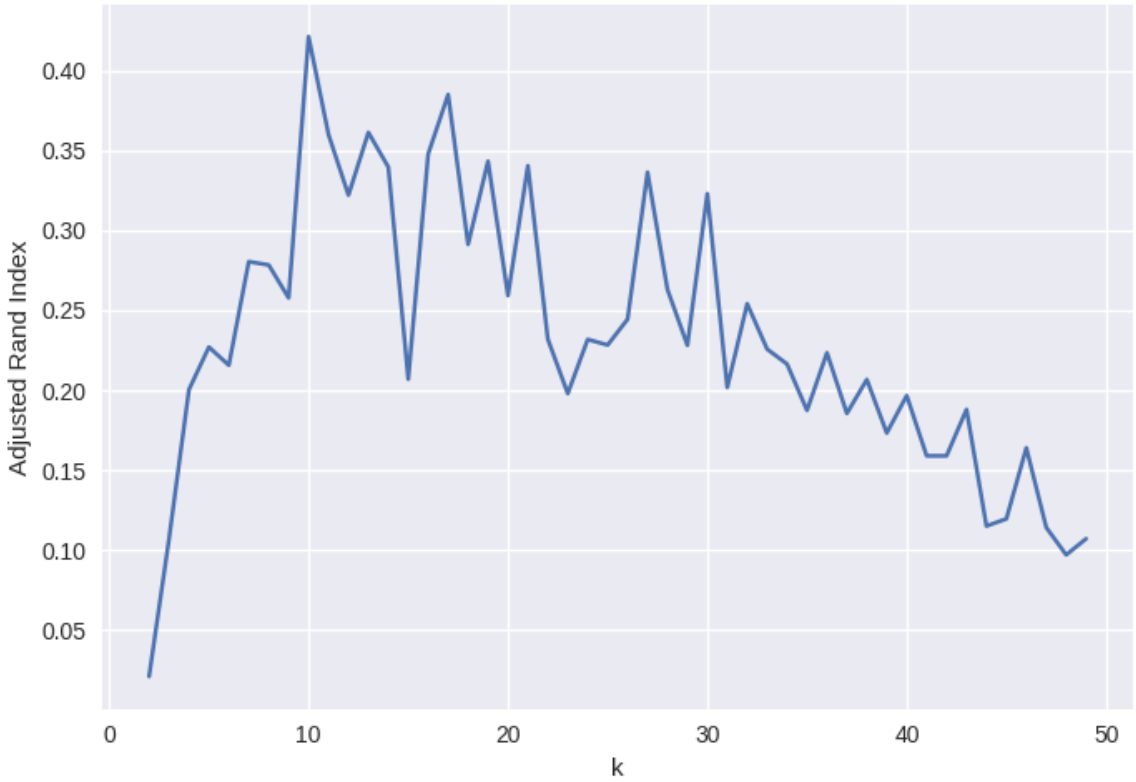
```
ARI = (RI - Expected_RI) / (max(RI) - Expected_RI)
```

Write a function that computed the ARI for a range of k values.

Hint:

```
from sklearn.metrics.cluster import adjusted_rand_score

rand = adjusted_rand_score(predicted_labels, known_labels)
```



Adjusted Rand Index (ARI) for a range of k, for the NCI-60 RNAseq data, clustering by cell line

The plot above shows that when the number of clusters k = 10, the known tissue types are best sorted into clusters.

Additional exercises

Perform the above clustering and validation for the Cancer Cell Line Encyclopedia (CCLE) proteomics dataset.

- Clustering by cell line
- Clustering by protein
