In [1]:
```
# Many fantastic pieces of free and open-source software can be used a
s key components to enable single cell analysis
# using python notebook. This scripts showed how to import scanpy resu
lt into single cell explorer. The analytic code parts
# are modified from scanpy tutorial "Clustering 3K PBMCs", the re-impl
emntation of Seurat’s (Satija et al., 2015)
# guided clustering tutorial. We gratefully acknowledge all authors fo
r Suerat and Scanpy and their contribution.
# we use 10K healthy donor's PBMCs data obtained from 10x Genomics

# uncomment the following codes if you need to download the data

#!mkdir data
#!wget http://cf.10xgenomics.com/samples/cell-exp/3.0.0/pbmc_10k_v3/pb
mc_10k_v3_filtered_feature_bc_matrix.tar.gz -O data/pbmc_10k_v3_filter
ed_feature_bc_matrix.tar.gz
#!cd data; tar -xzf pbmc_10k_v3_filtered_feature_bc_matrix.tar.gz
```

In [2]:
```
import scpipeline
# other libs
import os, sys, csv,json,datetime,time,math,scipy.stats,collections,re
;
from sklearn import preprocessing;
import numpy as np;
import pandas as pd;
import os.path;
import scanpy;
import scanpy.api as sc
sc.settings.set_figure_params(dpi=80)
```

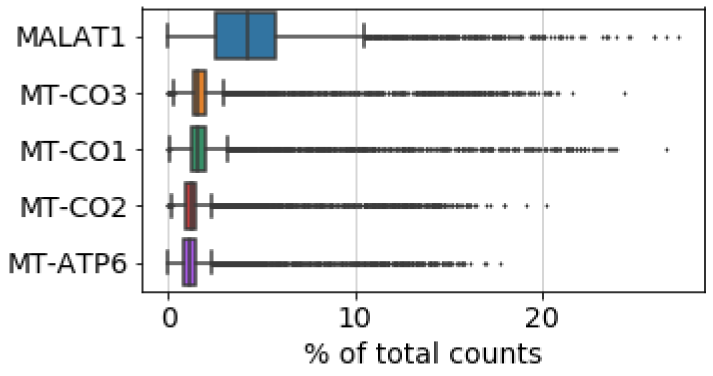
```
/home/ubuntu/.local/lib/python3.6/site-packages/numba/errors.py:105: U
serWarning: Insufficiently recent colorama version found. Numba requir
es colorama >= 0.3.9
  warnings.warn(msg)
```

In [3]:
```
###
p =  scpipeline.ProcessPipline();
dataPath='./data/filtered_feature_bc_matrix/';  # the directory with t
he `.mtx` file
p.readData(dataPath)                            # read 10X '.mtx'data,
compute mitochondra fraction, and create p.data
p.data
```

```
--> This might be very slow. Consider passing `cache=True`, which enab
les much faster reading from a cache file.
filtered out 10502 genes that are detected in less than 1 cells
```

Out[3]:
```
AnnData object with n_obs × n_vars = 11769 × 23036
    obs: 'n_genes', 'percent_mito', 'n_counts'
    var: 'gene_ids', 'n_cells'
```

In [4]:
```
### p.data is the data object for use
sc.pl.highest_expr_genes(p.data, n_top=5)
```



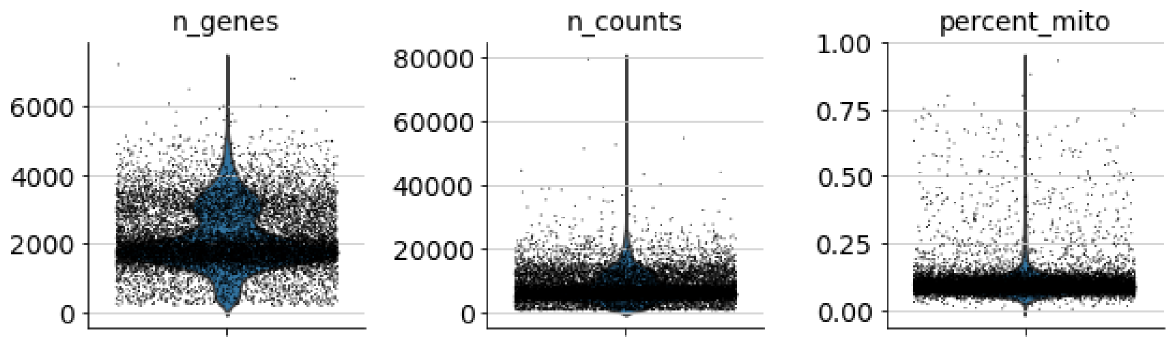
In [5]:
```
# QC function
# def QC(self,max_n_genes="" ,min_n_genes="",min_n_cells="",max_percen
t_mito="")
# scanpy tutorial QC(self,max_n_genes=2500 ,min_n_genes=200,min_n_cell
s=3,max_percent_mito=0.05)

p.QC(min_n_genes=200,min_n_cells=3)
```

```
filter cells
filtered out 232 cells that have less than 200 genes expressed
filter genes
filtered out 2684 genes that are detected in less than 3 counts
```

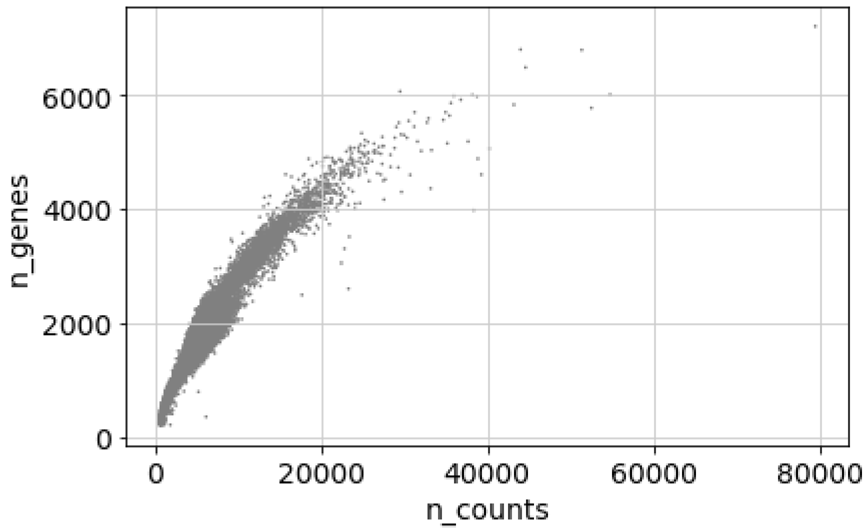
In [6]:
```
p.data
```

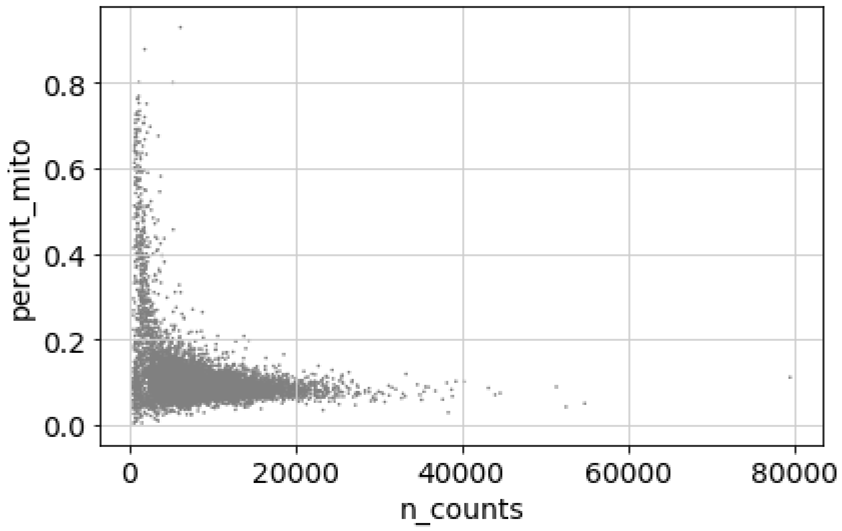
Out[6]:
```
AnnData object with n_obs × n_vars = 11537 × 20352
    obs: 'n_genes', 'percent_mito', 'n_counts'
    var: 'gene_ids', 'n_cells', 'n_counts'
```

In [7]:
```
# plot percentage of mitochondria
sc.pl.violin(p.data, ['n_genes', 'n_counts', 'percent_mito'],
             jitter=0.4, multi_panel=True)
```



In [8]:
```
sc.pl.scatter(p.data, x='n_counts', y='n_genes')
sc.pl.scatter(p.data, x='n_counts', y='percent_mito')
```





In [9]:
```
p.QC(max_n_genes=5000, max_percent_mito=0.12)

"""
# for those who are more famaliar with scanpy:
p.data = p.data[p.data.obs['n_genes'] < 5000, :]
p.data = p.data[p.data.obs['percent_mito'] < 0.12, :]
"""
```

```
filter n_genes < 5000
filter percent_mito < 0.12
```

Out[9]:
```
"\n# for those who are more famaliar with scanpy: \np.data = p.data[p.
data.obs['n_genes'] < 5000, :]\np.data = p.data[p.data.obs['percent_mi
to'] < 0.12, :]\n"
```

In [10]:
```
# QC in scanpy will remove cell barcodes. However, for database loadin
g, adata should keep same number of barcodes as original one.
# We copy data from p.data to adata, which will be used for loading to
database

adata = p.data.copy()
```

In [11]:
```
# normalization (library-size correct) the data matrix to 10,000 reads
per cell
sc.pp.normalize_per_cell(adata, counts_per_cell_after=1e4)
```

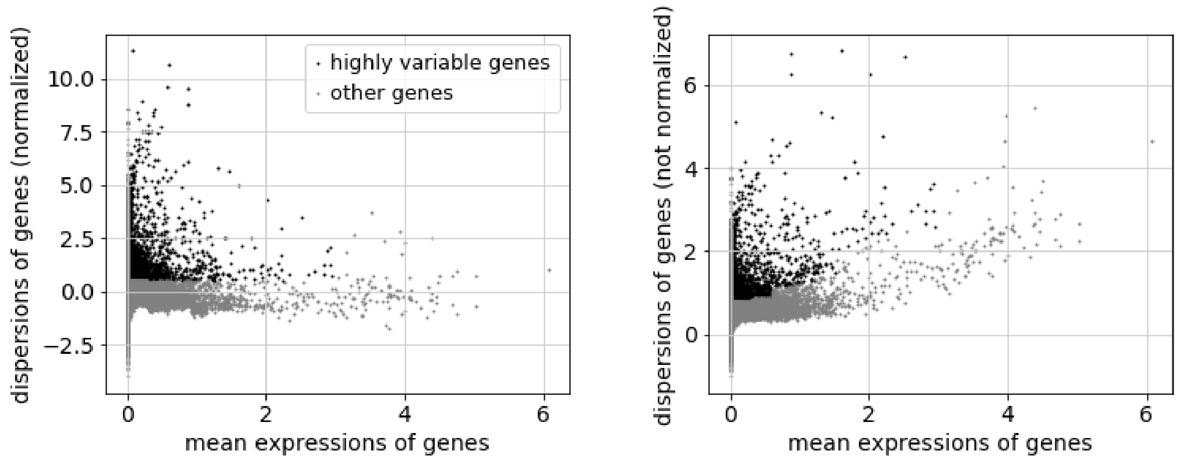
In [12]:
```
#Logarithmize the data
sc.pp.log1p(adata)
```

In [13]:
```
adata.raw = adata
```

In [14]:
```
# highly variable genes
sc.pp.highly_variable_genes(adata, min_mean=0.0125, max_mean=3, min_di
sp=0.5)
```

```
--> added
    'highly_variable', boolean vector (adata.var)
    'means', float vector (adata.var)
    'dispersions', float vector (adata.var)
    'dispersions_norm', float vector (adata.var)
```

In [15]:
```
sc.pl.highly_variable_genes(adata)
```

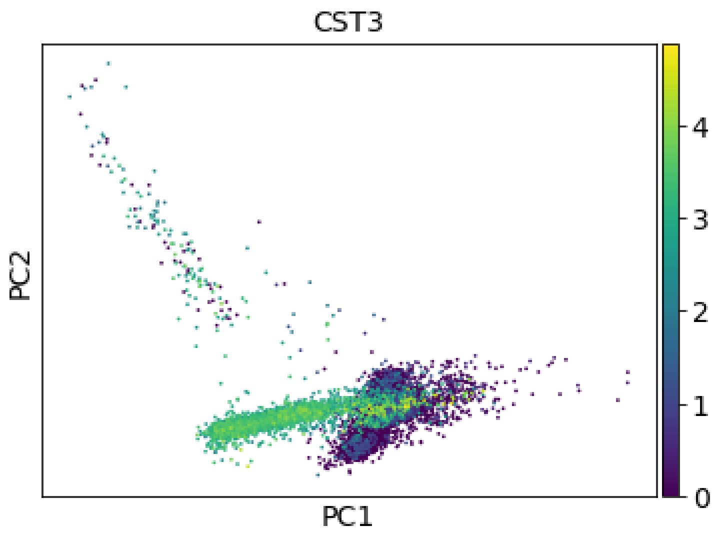


In [16]:
```
adata = adata[:, adata.var['highly_variable']]
# regress out effects of total counts per cell and the percentage of m
itochondrial genes.
sc.pp.regress_out(adata, ['n_counts', 'percent_mito'])
# Scale each gene to unit variance. Clip values exceeding standard dev
iation 10.
sc.pp.scale(adata, max_value=10)
```

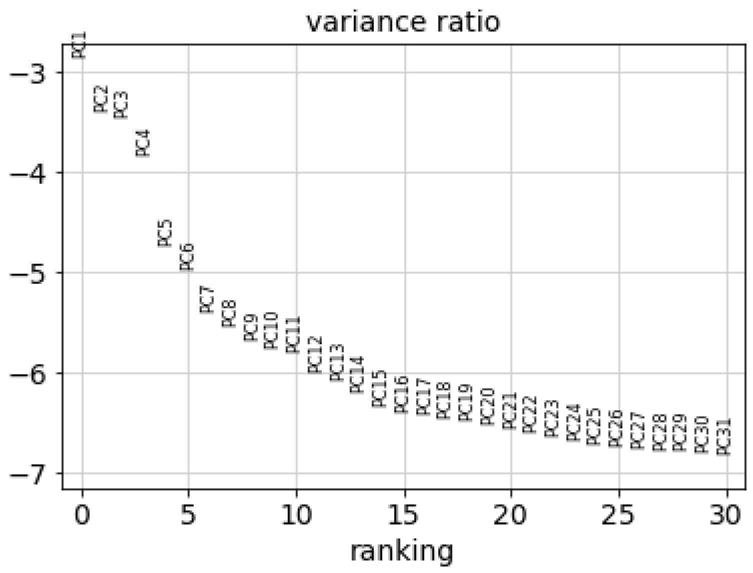
```
regressing out ['n_counts', 'percent_mito']
    sparse input is densified and may lead to high memory use
    finished (0:00:19.69)
```

In [17]:
```
# Dimention reduction: PCA as a first step
sc.tl.pca(adata, svd_solver='arpack')
sc.pl.pca(adata, color='CST3')
```

```
computing PCA on highly variable genes
```



In [18]:
```
sc.pl.pca_variance_ratio(adata, log=True)
```



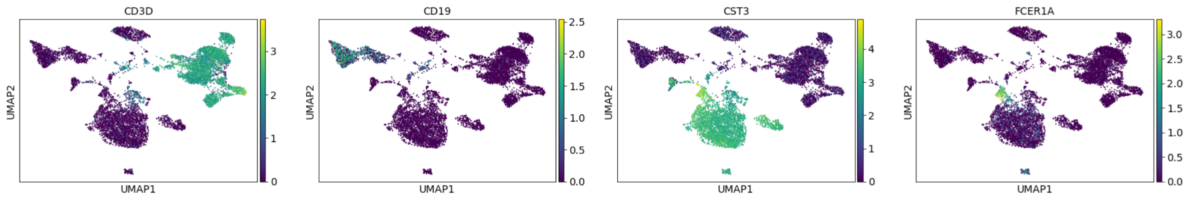
```
In [19]: sc.pp.neighbors(adata, n_neighbors=10, n_pcs=40)
```

```
computing neighbors
    using 'X_pca' with n_pcs = 40
    finished (0:00:07.82) --> added to `.uns['neighbors']`
    'distances', distances for each pair of neighbors
    'connectivities', weighted adjacency matrix
```

```
In [20]: sc.tl.umap(adata)
```

```
computing UMAP
    finished (0:00:24.77) --> added
    'X_umap', UMAP coordinates (adata.obsm)
```

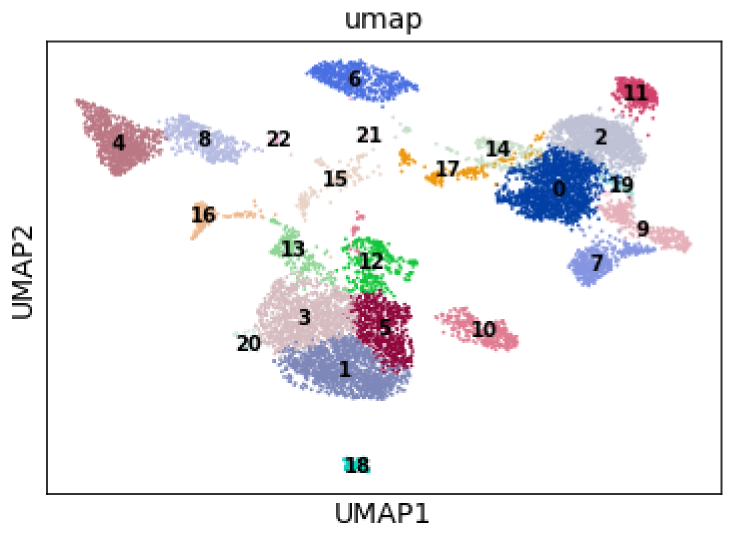
```
In [21]: #sc.pl.umap(adata, color=['CD3D', 'NKG7', 'PPBP','IRF7','CD79A','CD1
4','FCGR3A','CLEC9A',"MS4A1", "GNLY", 'FCER1A',"CD8A"])
sc.pl.umap(adata, color=['CD3D','CD19', 'CST3', 'FCER1A'])
```



```
In [22]: sc.tl.leiden(adata)
```

```
running Leiden clustering
    finished (0:00:01.47) --> found 23 clusters and added
    'leiden', the cluster labels (adata.obs, categorical)
```

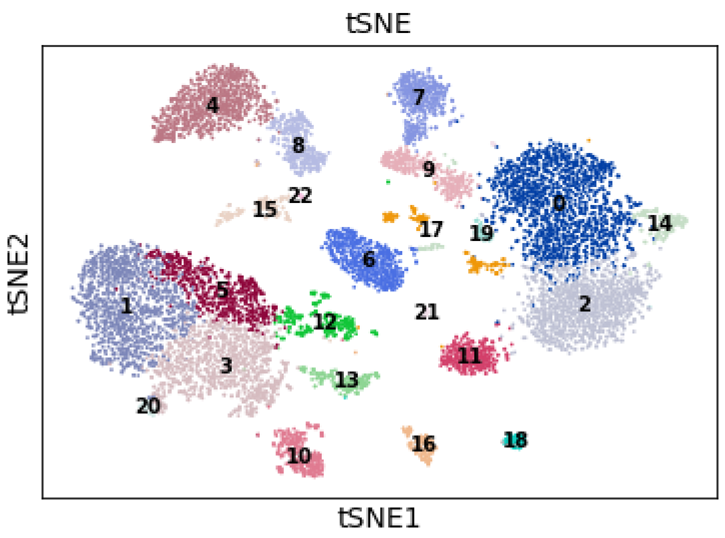
```
In [23]: sc.pl.umap(adata, color=['leiden'],legend_loc='on data',title= "umap")
```



```
In [24]: ## optional marker gene identification
# sc.tl.rank_genes_groups(adata, 'leiden', method='t-test')
# sc.pl.rank_genes_groups(adata, n_genes=25, sharey=False)
```

In [25]:
```
### t-SNE has better seperation among cell clusters, easy for single c
ell explorer users to lasso select cell clusters
sc.tl.tsne(adata,n_pcs=40)
sc.pl.tsne(adata, color='leiden', legend_loc='on data', title='tSNE')
```

```
computing tSNE
    using 'X_pca' with n_pcs = 40
WARNING: Consider installing the package MulticoreTSNE (https://githu
b.com/DmitryUlyanov/Multicore-TSNE). Even for n_jobs=1 this speeds up
the computation considerably and might yield better converged results.
    using sklearn.manifold.TSNE with a fix by D. DeTomaso
    finished (0:01:37.93) --> added
    'X_tsne', tSNE coordinates (adata.obsm)
```



In [26]:
```
## data loading into single cell explorer database
p.insertToDB(dbname= 'scDB',dbport= 27017,dbhost='localhost',
                   adata=adata,mapType="umap",    # umap, tsne
                   mapName='pmbc10k_health_umap', # this is the title
 of the map, you can label details info
                   study="Demo",
                   subjectid="",
                   disease="Healthy",
                   source="10XGenomic",
                   sample="Blood",
                   comment="",
                   author="demo"
              );
```

```
start insert to db
success
mapid: 5ccfb3c066adac0c7e7924c9
```

In [27]:
```
## data loading into single cell explorer database
p.insertToDB(dbname= 'scDB',dbport= 27017,dbhost='localhost',
                   adata=adata,mapType="tsne",
                   mapName='pmbc10k_health_tSNE', # this is the title
 of the map, you can label details info
                   study="Demo",
                   subjectid="",
                   disease="Healthy",
                   source="10XGenomic",
                   sample="Blood",
                   comment="",
                   author="demo"
              );
```

```
start insert to db
success
mapid: 5ccfb94266adac0c7e7924e3
```

In [ ]:
```

```