tcell_demo

1 scLVM - Accounting for cell-to-cell heterogeneity in single-cell RNA-Seq data

scLVM requires preprocessed and normalize signle-cell RNA-Seq data as input. This example assumes that the data have already been processed appropriately. For an example of how the input file for this notebook can be generated form raw counts, see R/transform_counts_Tcells.R

1.1 Stage 1: Fitting process

scLVM use the Gaussian Process Latent variable model to fit a cell-cell covariance matrix, which is induced by a specified number of hidden factor (typically low rank). This approach resembles a Principal Component Analysis on genes annotated to a hidden factor (such as cell cycle). However, instead of explicitly reconstructing PCA loadings and scores, the GPLVM approach fits a low-rank cell-to-cell covariance to the empirical covariance matrix of these genes. Moreover, scLVM accounts for the technical noise estiamtes during the fitting.

```
In [1]: # activiate inline plotting
        %pylab inline
        # load modules
        import sys
        import scipy as SP
        import pylab as PL
        from matplotlib import cm
        import h5py
        #adjust path
        sys.path.append('./..')
        from scLVM import scLVM
        sys.path.append('../include')
        sys.path.append('../CFG')
        from misc import *
        from barplot import *
        from default import *

        from IPython.display import Latex
```

```
Welcome to pylab, a matplotlib-based Python environment [backend: module://IPython.zmq.pylab.backend_inl
For more information, type 'help(pylab)'.
```

First, the required data have to be loaded. These includes: * Normalised gene expression data: LogNcountsMmus * Technical noise (in log space): LogVar_techMmus * Gene symbols: gene_names * Heterogeneous genes (boolean vector): genes_geterogen * Cell cycle genes (vector of indices): cellcyclegenes_filter

```
In [2]: data = './../data/Tcell/data_Tcells_normCounts.h5f'
        f = h5py.File(data,'r')
        Y = f['LogNcountsMmus'][:]                # gene expression matrix
```

```
tech_noise = f['LogVar_techMmus'][:]       # technical noise
genes_het_bool=f['genes_heterogen'][:]     # index of heterogeneous genes
geneID = f['gene_names'][:]             # gene names
cellcyclegenes_filter = SP.unique(f['cellcyclegenes_filter'][:].ravel() -1) # idx of cell cycle
cellcyclegenes_filterCB = f['ccCBall_gene_indices'][:].ravel() -1        # idx of cell cycle ge
```

First, for the fitting process, we need the gene matrix of cell cycle genes:

In [3]:
```
# filter cell cycle genes
idx_cell_cycle = SP.union1d(cellcyclegenes_filter,cellcyclegenes_filterCB)
# determine non-zero counts
idx_nonzero = SP.nonzero((Y.mean(0)**2)>0)[0]
idx_cell_cycle_noise_filtered = SP.intersect1d(idx_cell_cycle,idx_nonzero)
# subset gene expression matrix
Ycc = Y[:,idx_cell_cycle_noise_filtered]
```

scLVM can now be fit using the cell cycle expression matrix. The user needs to define the number of latent factors to be fitted. Initially, we fit a model assuming a large numbers of factos:

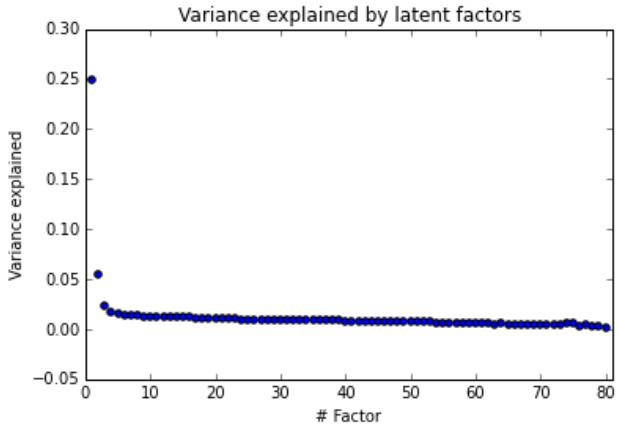
In [4]:
```
k = 80                    # number of latent factors
out_dir = './cache'       # folder where results are cached
file_name = 'Kcc.hdf5'    # name of the cache file
recalc = True             # recalculate X and Kconf
use_ard = True            # use automatic relevance detection
sclvm = scLVM(Y)
#Fit model with 80 factors
X_ARD,Kcc_ARD,varGPLVM_ARD = sclvm.fitGPLVM(idx=idx_cell_cycle_noise_filtered,k=k,out_dir='./ca
```

```
./../scLVM/core.py:84: FutureWarning: comparison to `None` will result in an elementwise object compari
  assert idx!=None, 'scLVM:: specify idx'
```

In order to determine an appropriate number of hidden factors, it is instructive to visualize the variance contributions of the individual latent factors.

In [5]:
```
#Plot variance contributions from ARD
plt = PL.subplot(1,1,1)
PL.title('Variance explained by latent factors')
PL.scatter(SP.arange(k)+1,varGPLVM_ARD['X_ARD'])
PL.xlim([0,k+1])
PL.xlabel('# Factor')
PL.ylabel('Variance explained')
```

Out[5]:
```
<matplotlib.text.Text at 0x1102ee9d0>
```



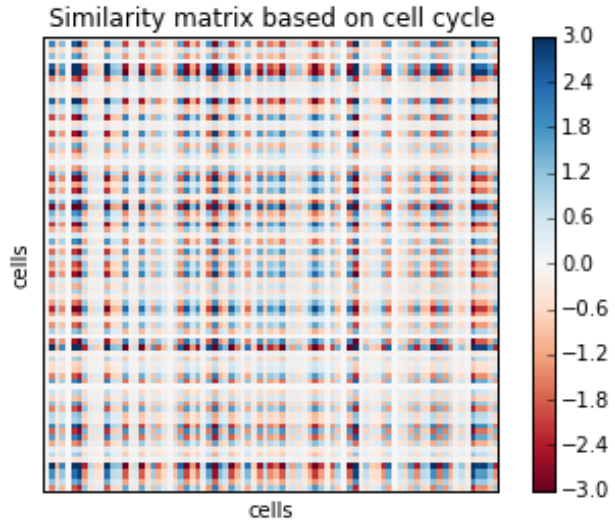
In this example (and generally when considering cell cycle as the confounding factor), there is a large gap in the proportion of explained variance between the first and the second factor. This suggests, that a single latent factor underlies the variation captured by the cellcycle genes. Consequently, we choose to re-fit the scLVM mdoel with one latent factor only.

```
In [6]: #Fit model with a single factor (rank 1 covariance matrix)
        X,Kc,varGPLVM = sclvm.fitGPLVM(idx=idx_cell_cycle_noise_filtered,k=1,out_dir='./cache',file_na
```

The inferred cell to cell covaraince matrix can be visualized:

```
In [7]: #Plot inferred similarity matrix
        plt = PL.subplot(1,1,1)
        PL.title('Similarity matrix based on cell cycle')
        PL.imshow(Kc,cmap=cm.RdBu,vmin=-3,vmax=+3,interpolation='None')
        PL.colorbar()
        plt.set_xticks([])
        plt.set_yticks([])
        PL.xlabel('cells')
        PL.ylabel('cells')
```

```
Out[7]: <matplotlib.text.Text at 0x10d7502d0>
```



1.2 Stage 2: Variance decomposition and cell cycle correction

First, we use the fitted scLVM model to decompose the source of variance for each gene.

```
In [8]: # considers only heterogeneous genes
        Ihet = genes_het_bool==1
        Y    = Y[:,Ihet]
        tech_noise = tech_noise[Ihet]
        geneID = geneID[Ihet]
```

The computatioan time for the next step can be substantial. If large datasets are considerd, it may be advisable to distribute these calculations on a high performance compute cluster. In this case i0 and i1 determine the range of genes for wich this anlaysis is performed. Here, we fit the model on 1,000 genes only in order to limit computation times.

In [9]:
```
#optionally: restrict range for the analysis
i0 = 0    # gene from which the analysis starts
i1 = 1000 # gene at which the analysis ends

# construct sclvm object
sclvm = scLVM(Y,geneID=geneID,tech_noise=tech_noise)

# fit the model from i0 to i1
sclvm.varianceDecomposition(K=Kcc,i0=i0,i1=i1)
```

```
./../scLVM/core.py:62: FutureWarning: comparison to `None` will result in an elementwise object comparis
  if tech_noise!=None:
./../scLVM/core.py:146: FutureWarning: comparison to `None` will result in an elementwise object compari
  assert self.tech_noise!=None, 'scLVM:: specify technical noise'
./../scLVM/core.py:147: FutureWarning: comparison to `None` will result in an elementwise object compari
  assert K!=None, 'scLVM:: specify K'
/Users/florian/Code/python_code/limix-0.6.4/build/release.darwin/interfaces/python/limix/modules/varianc
  assert K!=None or is_noise, 'VarianceDecomposition:: Specify covariance structure'
/Users/florian/Code/python_code/limix-0.6.4/build/release.darwin/interfaces/python/limix/modules/varianc
  if scales0!=None and ~perturb:         init_method = 'manual'
/Users/florian/Code/python_code/limix-0.6.4/build/release.darwin/interfaces/python/limix/modules/varianc
  if scales0!=None:
/Users/florian/Code/python_code/limix-0.6.4/build/release.darwin/interfaces/python/limix/modules/varianc
  if scales==None:
/Users/florian/Code/python_code/limix-0.6.4/build/release.darwin/interfaces/python/limix/modules/varianc
  if fixed0!=None:
./../scLVM/core.py:200: FutureWarning: comparison to `None` will result in an elementwise object compari
  if self.geneID!=None:        geneID[count] = self.geneID[ids]
./../scLVM/core.py:211: FutureWarning: comparison to `None` will result in an elementwise object compari
  if geneID!=None:        var_info['geneID'] = SP.array(geneID)
./../scLVM/core.py:213: FutureWarning: comparison to `None` will result in an elementwise object compari
  if geneID!=None:        Ystar_info['geneID'] = SP.array(geneID)
```

Once the contribution of cell cycle to the observed variance is estimated, cell-cycled corrected gene expression levels can be obtained. The variance component estimates calculatd by scLVM are normalised such that they sum uo to 1. There may be a small number of genes where the maximum likelihood fit does not converge propperly. We suggest to remove these in downstream analyses.

In [10]:
```
normalize=True    # variance components are normalizaed to sum up to one

# get variance components
var, var_info = sclvm.getVarianceComponents(normalize=normalize)
var_filtered = var[var_info['conv']] # filter out genes for which vd has not converged

# get corrected expression levels
Ycorr = sclvm.getCorrectedExpression()
Ycorr.shape
```

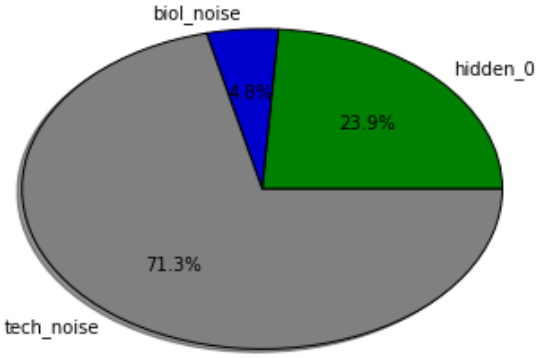
```
./../scLVM/core.py:234: FutureWarning: comparison to `None` will result in an elementwise object compari
  assert self.var!=None, 'scLVM:: use varianceDecomposition method before'
```

```
./../scLVM/core.py:262: FutureWarning: comparison to `None` will result in an elementwise object compari
  assert self.var!=None, 'scLVM:: use varianceDecomposition method before'
```

Out[10]: (81, 1000)

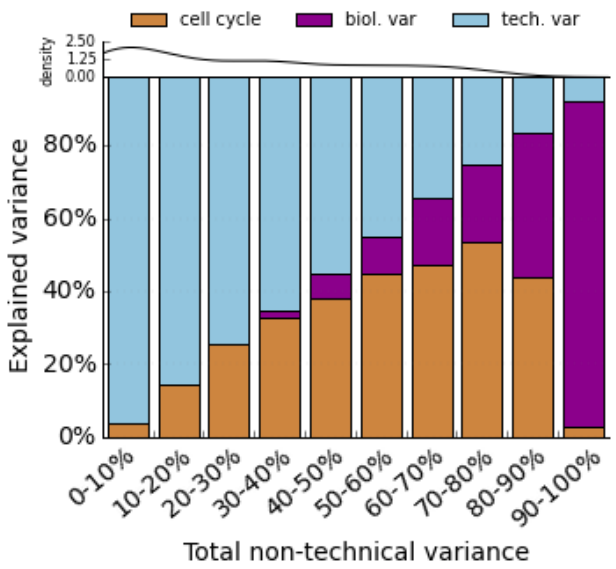
Here, we visualize the resulting variance component using a pie chart. Shown are the average contributions of varaince (across genes) for different categories: * Hidden_0: the first hidden factor, here the the cell cycle * bio_noise: the residual biological variation * techh_noise: the technical noise level

In [11]:
```
#calculate average variance components across all genes and visualize
var_mean = var_filtered.mean(0)
colors = ['Green','MediumBlue','Gray']
pp=PL.pie(var_mean,labels=var_info['col_header'],autopct='%1.1f%%',colors=colors,
       shadow=True, startangle=0)
```



We can also visualize this stratifying for different levels of technical noise.

In [20]:
```
H2=1-var_filtered[:,2]
var_plot(var_filtered,H2,CFG['var_comp_fields'][SP.array([0,1,2])],filename='./cache/var_plot.
```



1.3 Gene correlation analysis

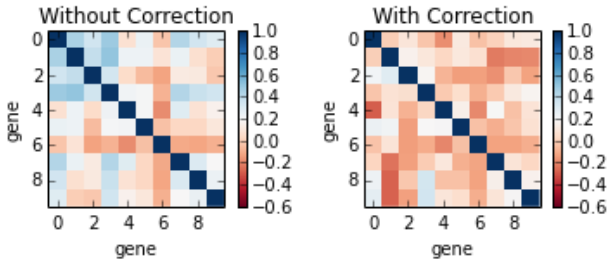
The fitted cell cyle covariance matrix can also be used in a range of other analyses. Here, we illustrate it's use to improve the estimation of pairwise correlation coefficients between genes, while accounting for the cell cycle. For each gene i, we fit a linear mixed model with a fixed effect representing the contribution of a second gene j and random effect representing the contribution of the cell cycle. Gene correlations can then be determined by testing the significance of the fixed effect. Again, the computational complexity of this analysis can be substantial, requiring distributing these analyses on a parallel compute cluster. For illustration, we here consider the gene-gene correlation network of the first 10 genes.

```
In [37]: i0 = 0      # gene from which the analysis starts
         i1 = 10     # gene to which the analysis ends

         # fit lmm without correction
         pv0,beta0,info0 = sclvm.fitLMM(K=None,i0=i0,i1=i1,verbose=False)
         # fit lmm with correction
         pv1,beta1,info1 = sclvm.fitLMM(K=Kcc,i0=i0,i1=i1,verbose=False)
```

```
In [39]: PL.subplot(2,2,1)
         PL.title('Without Correction')
         p=PL.imshow(beta0[:,i0:i1],cmap=cm.RdBu,vmin=-0.6,vmax=+1,interpolation='None')
         PL.colorbar()
         plt.set_xticks([])
         plt.set_yticks([])
         PL.xlabel('gene'),PL.ylabel('gene')
         PL.subplot(2,2,2)
         PL.title('With Correction')
         p=PL.imshow(beta1[:,i0:i1],cmap=cm.RdBu,vmin=-0.6,vmax=+1,interpolation='None')
         PL.colorbar()
         plt.set_xticks([])
         plt.set_yticks([])
         PL.xlabel('gene'),PL.ylabel('gene')
```

```
Out[39]: (<matplotlib.text.Text at 0x1189bf790>, <matplotlib.text.Text at 0x1189a0390>)
```



1.4 Downstream analysis

The cell-cycle corrected gene expression matix can used for various kinds of downstream analysis. This includes clustering, visualisation, network analysis etc. To use the correct expression matrix in other programmes, it is straightforward to export the correct expression matrix as CSV file:

```
In [50]: SP.savetxt('Ycorr.txt',Ycorr)
```

As an example for downstream analyses using corrected exprssion levels, we here consider GPy to fit a non-linear Bayeisan PCA model, therbey visualizing hidden substructures between cells.

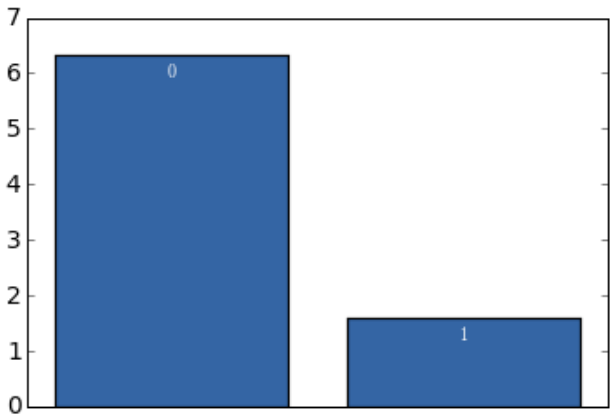
```
In [37]: import GPy
```

```
In [38]: # Model optimization
         Ystd = Ycorr-Ycorr.mean(0)
         Ystd/=Ystd.std(0)
         input_dim = 2 # How many latent dimensions to use
         kern = GPy.kern.RBF(input_dim,ARD=True) # ARD kernel
         m = GPy.models.BayesianGPLVM(Ystd, input_dim=input_dim, kernel=kern, num_inducing=40)
         m.optimize('scg', messages=0, max_iters=2000)
```

The model assumes two principle components. Here, we visualize the relative importance of the two components.

```
In [39]: m.kern.plot_ARD()
```

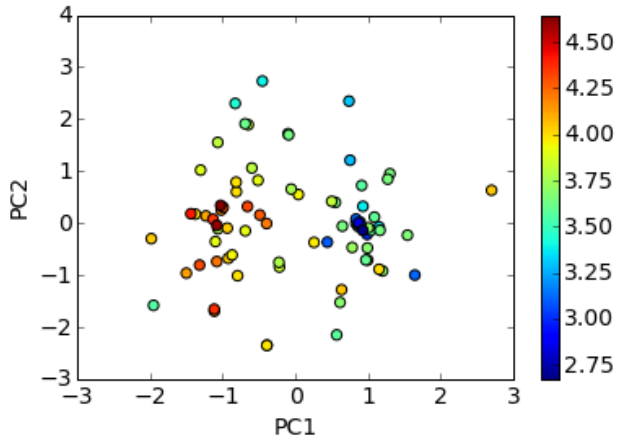
```
Out[39]: <matplotlib.axes._subplots.AxesSubplot at 0x10c2606d0>
```



Finally, the position of individual cells in the principle component space can be visualized. Cells are colour coded by GATA3 expression, a canonical T-cell differentiation marker gene.

```
In [40]: i_Gata3 = SP.where(geneID=='ENSMUSG00000015619')
         PL.scatter(m.X[:,0]['mean'], m.X[:,1]['mean'], 40, Ycorr[:,i_Gata3])
         PL.xlabel('PC1')
         PL.ylabel('PC2')
         PL.colorbar()
```

```
Out[40]: <matplotlib.colorbar.Colorbar instance at 0x10cdb1440>
```



In []: