tcell_demo

October 16, 2014

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
from IPython.display import Latex
```

```
Populating the interactive namespace from numpy and matplotlib
```

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:

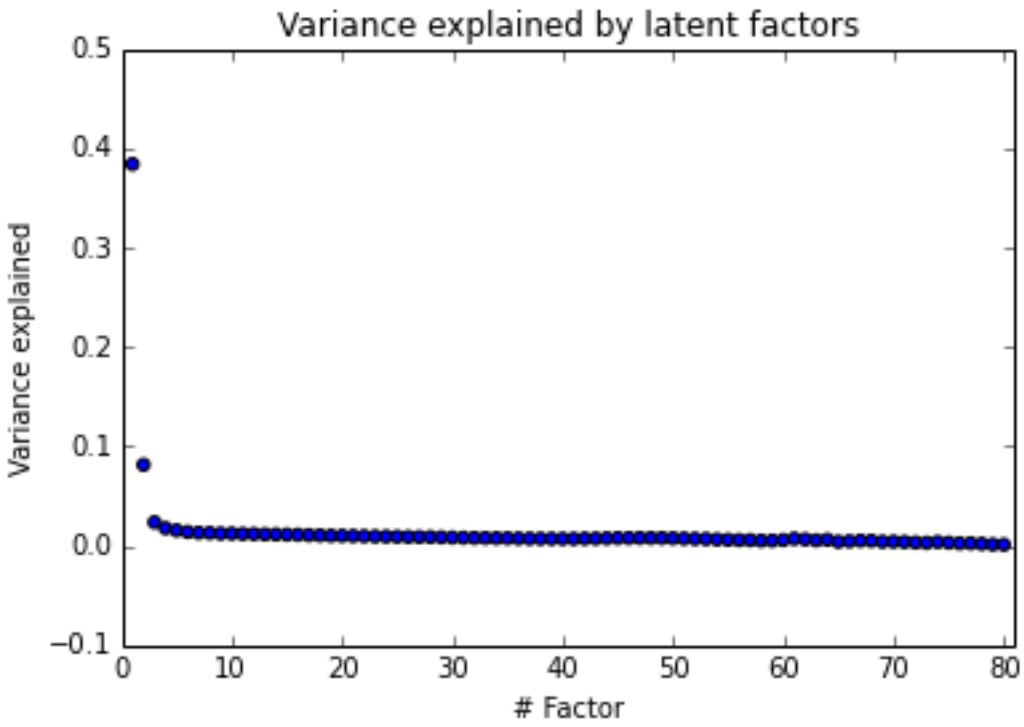
```
In [18]: # filter cell cycle genes
         idx_cell_cycle = SP.union1d(cellcyclegenes_filter,cellcyclegenes_filterCB)
         # determine non-zero counts
         idx_nonzero = SP.nonzero((Y.mean(0)**2)>0)[0]
         idx_cell_cycle_noise_filtered = SP.intersect1d(idx_cell_cycle,idx_nonzero)
         # 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 [21]: 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
```

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

```
In [49]: #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[49]: <matplotlib.text.Text at 0x113540a50>
```

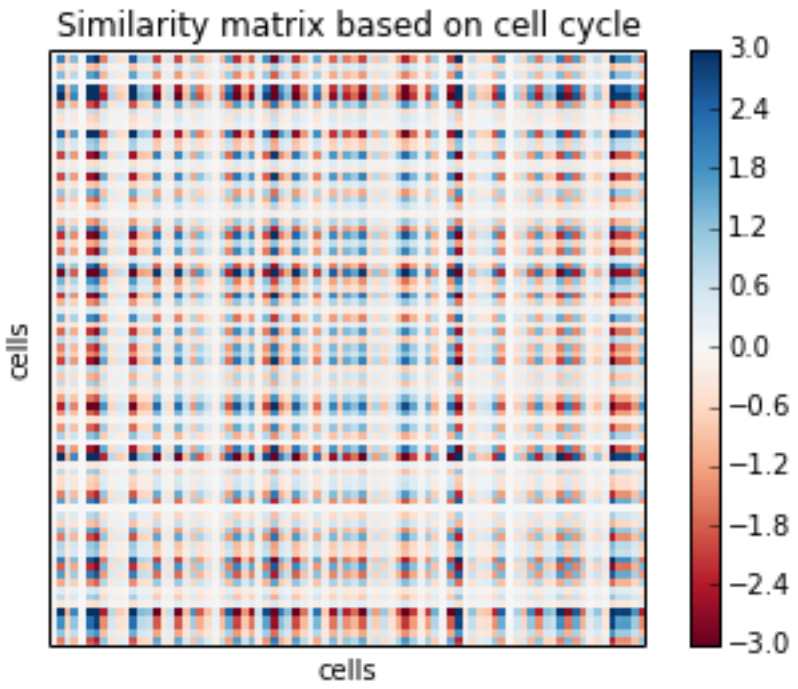


In this example (and generally when considering cell cycle as the confounding factor), there is a large gap in the proportion of explained variance between the first and the second factor. This suggests, that a single latent factor underlies the variation captured by the cellcycle genes. Consequently,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

```
In [24]: #Fit model with a single factor (rank 1 covariance matrix)
         X,Kcc,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 [30]: #Plot inferred similarity matrix
         plt = PL.subplot(1,1,1)
         PL.title('Similarity matrix based on cell cycle')
         PL.imshow(Kcc,cmap=cm.RdBu,vmin=-3,vmax=+3,interpolation='None')
         PL.colorbar()
         plt.set_xticks([])
         plt.set_yticks([])
         PL.xlabel('cells')
         PL.ylabel('cells')

Out[30]: <matplotlib.text.Text at 0x11570b4d0>
```



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 [31]: # 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.

```
In [32]: #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)
```

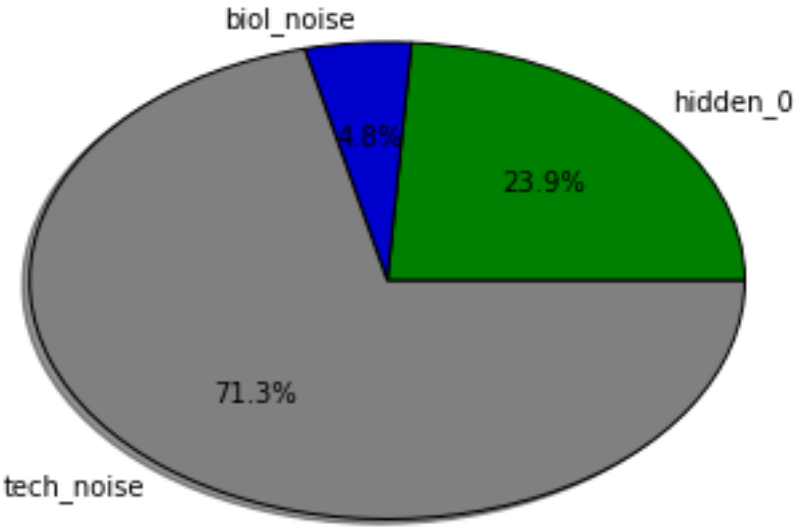
Once the contribution of cell cycle to the observed variance is estimated, cell-cycled corrected gene expression levels can be obtained. The variance component estimates calculatd by scLVM are normalised such that they sum uo to 1. There may be a small number of genes where the maximum likelihood fit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

```
In [33]: 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
```

```
Out[33]: (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 [36]: #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)
```



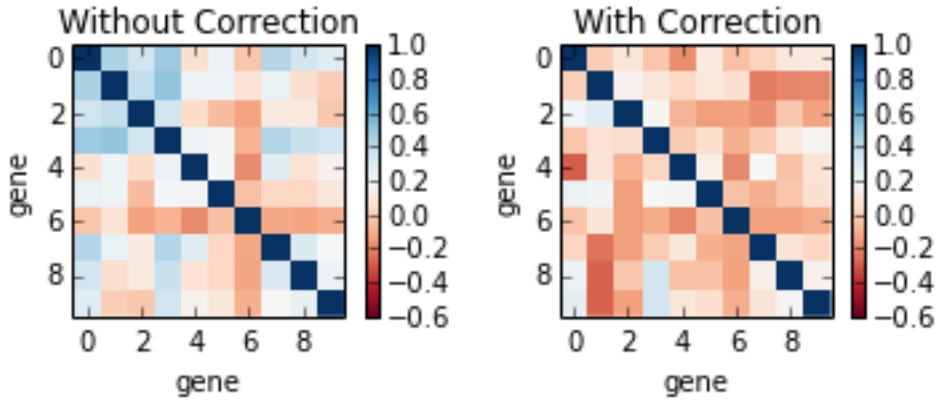
1.3 Gene correlation analysis

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 PCA model, therbey visualizing hidden substructures between cells.

```
In [51]: import GPy
```

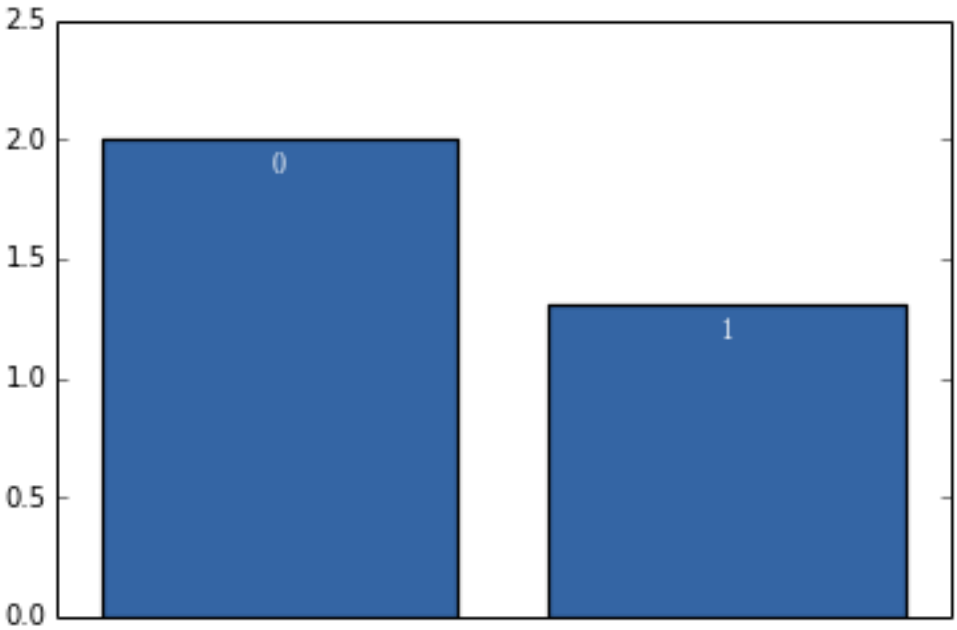
In [45]:
```
# Model optimization
Ystd = Ycorr-Ycorr.mean(0)
Ystd/=Ystd.std(0)
input_dim = 2 # How many latent dimensions to use
kern = GPy.kern.rbf(input_dim,ARD=True) # ARD kernel
m = GPy.models.BayesianGPLVM(Ystd, input_dim=input_dim, kernel=kern, num_inducing=40)
m.optimize('scg', messages=1, max_iters=2000)
```

```
Warning: adding jitter of 1.0000000000e-03
 I      F              Scale          |g|
0003   1.216795e+05   2.000000e+00   2.481229e+07
0011   1.154778e+05   5.120000e+02   1.775456e+06
0019   1.149963e+05   2.000000e+00   5.965779e+04
0024   1.149829e+05   6.250000e-02   6.449038e+03
0067   1.144602e+05   2.980232e-08   5.225704e+03
0099   1.143645e+05   2.741651e-01   5.960441e+02
0276   1.141038e+05   1.322471e-03   7.664661e+03
0445   1.140142e+05   2.093781e-06   9.669417e+01
0912   1.138157e+05   1.000000e-15   9.929305e+00
1057   1.138147e+05   1.000000e-15   8.562801e-02
1147   1.138147e+05   1.000000e-15   8.022690e-03
1156   1.138147e+05   1.000000e-15   6.995103e-03
converged - relative reduction in objective

/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/core/transf
  return np.where(x>lim_val, x, np.log(1. + np.exp(x)))
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  X = X / self.lengthscale
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._K_dist2 = -2.*tdot(X) + (Xsquare[:, None] + Xsquare[None, :])
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._psi2_Zdist_sq = np.square(self._psi2_Zdist / self.lengthscale) # M,M,Q
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._psi2_Zdist_sq = np.square(self._psi2_Zdist / self.lengthscale) # M,M,Q
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._psi1_denom = S[:, None, :] / self.lengthscale2 + 1.
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._psi1_dist_sq = np.square(self._psi1_dist) / self.lengthscale2 / self._psi1_denom
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/kern/parts/
  self._psi2_denom = 2.*S[:, None, None, :] / self.lengthscale2 + 1. # N,M,M,Q
/opt/local/Library/Frameworks/Python.framework/Versions/2.7/lib/python2.7/site-packages/GPy/util/linalg
  if np.any(diagA <= 0.):
```

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

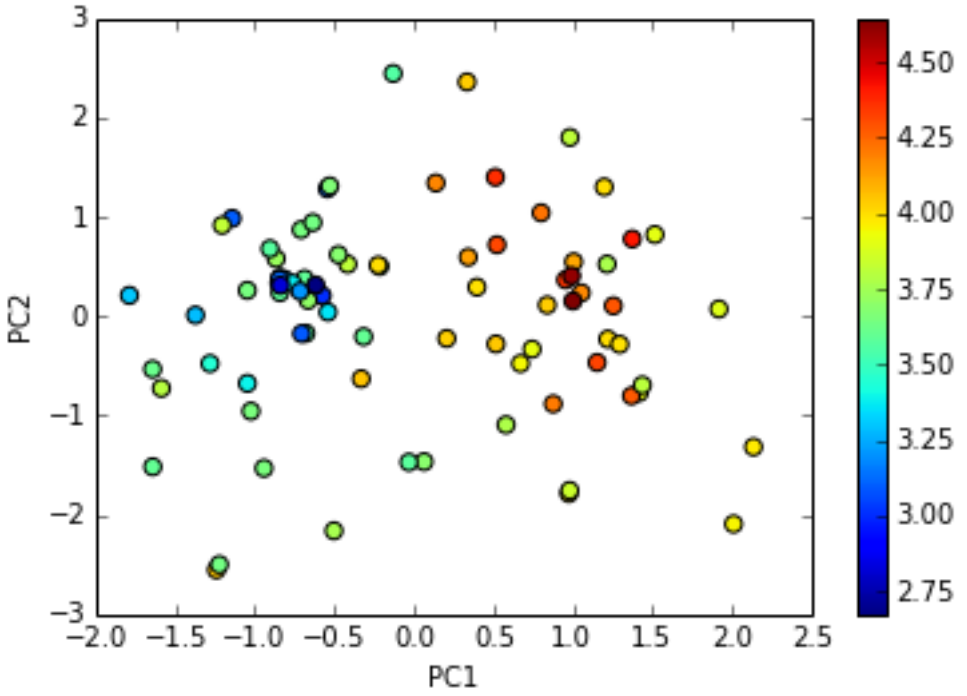
In [52]:
```
m.kern.plot_ARD()
```

Out[52]:
```
<matplotlib.axes.AxesSubplot at 0x11351a210>
```



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

Out[53]: <matplotlib.colorbar.Colorbar instance at 0x11370d050>
```


PC2
PC1
3
2
1
0
−1
−2
−3
−2.0
−1.5
−1.0
−0.5
0.0
0.5
1.0
1.5
2.0
2.5
4.50
4.25
4.00
3.75
3.50
3.25
3.00
2.75

In []: