

```
In [1]: # activate inline plotting
%matplotlib inline
```

```
In [1]: import sys
import scipy as SP
import pylab as PL
from matplotlib import cm
import h5py
#import scLVM
sys.path.append('../scLVM')
from scLVM import scLVM
```

Import data and filter sets of genes

```
In [2]: data = '../data/Tcell/normCountsMMus_final.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())
cellcyclegenes_filterCB600 = f['ccCBall_gene_indices'][:, :].ravel() - 1
```

```
In [3]: # filter cell cycle genes
idx_cell_cycle = SP.union1d(cellcyclegenes_filter, cellcyclegenes_filterCB600)
Ymean2 = Y.mean(0) ** 2 > 0
idx_cell_cycle_noise_filtered = SP.intersect1d(idx_cell_cycle, SP.array(Ymean2))
Ycc = Y[:, idx_cell_cycle_noise_filtered]
```

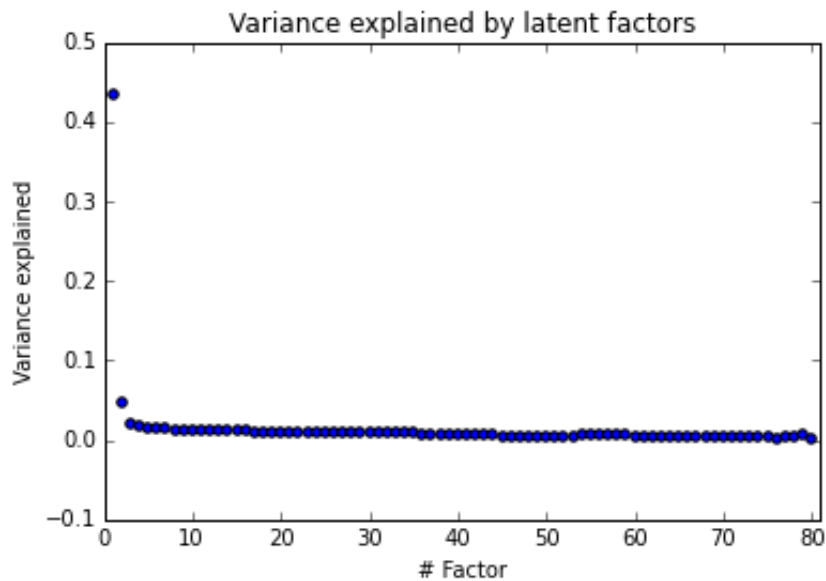
Fit GPLVM to the data

```
In [27]: 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)
X_ARD, Kcc_ARD, varGPLVM_ARD = sclvm.fitGPLVM(idx=idx_cell_cycle_noise_filtered)
```

```
In [28]: #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')
```

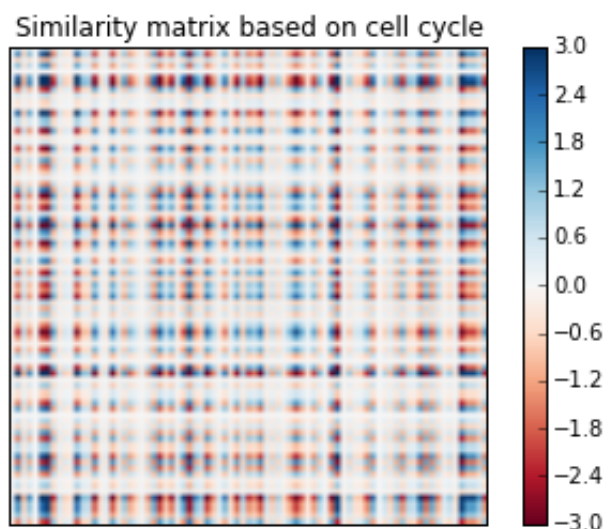
Out[28]: <matplotlib.text.Text at 0x10fbd3850>



```
In [29]: #Fit rank 1 covariance matrix (large gap between first and second latent factors)
X,Kcc,varGPLVM = sclvm.fitGPLVM(idx=idx_cell_cycle_noise_filtered,k=1,only_eigen=1)
```

```
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)
PL.colorbar()
plt.set_xticks([])
plt.set_yticks([])
```

Out[30]: []



Variance Decomposition and Corrections

```
In [25]: # considers only heterogeneous genes
Ihet = genes_het_bool==1
Y      = Y[:,Ihet]
tech_noise = tech_noise[Ihet]
geneID = geneID[Ihet]
```

```
In [8]: i0 = 0      # gene from which the analysis starts
        i1 = 50     # gene at which the analysis ends

        # define sclvm
sclvm = scLVM(Y,geneID=geneID,tech_noise=tech_noise)

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

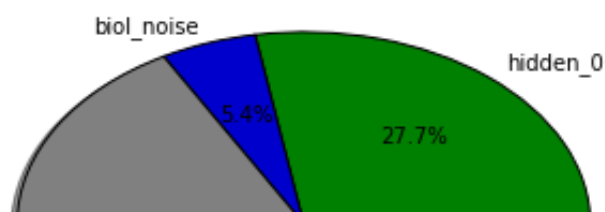
```
In [9]: normalize=True      # variance components are normalizaed to sum up to one

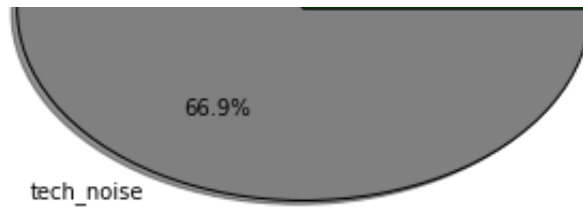
        # get variance components
var, var_info = sclvm.getVarianceComponents(normalize=normalize)
var_filtered = var[var_info['conv']] # filter out genes for which vd has no variance

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

```
In [10]: #normalize variance component and average across genes
var_mean = var_filtered.mean(0)
colors = ['Green','MediumBlue','Gray']
PL.pie(var_mean,labels=var_info['col_header'],autopct='%1.1f%%',colors=
        shadow=True, startangle=0)
```

```
Out[10]: ([<matplotlib.patches.Wedge at 0x111c2a950>,
            <matplotlib.patches.Wedge at 0x111c38cd0>,
            <matplotlib.patches.Wedge at 0x111c45fd0>],
          [<matplotlib.text.Text at 0x111c38390>,
            <matplotlib.text.Text at 0x111c456d0>,
            <matplotlib.text.Text at 0x111c529d0>],
          [<matplotlib.text.Text at 0x111c38890>,
            <matplotlib.text.Text at 0x111c45b90>,
            <matplotlib.text.Text at 0x111c52e90>])
```





Fitting Linear Mixed Model for correlations

```
In [11]: 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=True)
         # fit lmm with correction
         pv1,beta1,info1 = sclvm.fitLMM(K=Kcc,i0=i0,i1=i1,verbose=True)

         .. fitting gene 0
         .. fitting gene 1
         .. fitting gene 2
         .. fitting gene 3
         .. fitting gene 4
         .. fitting gene 5
         .. fitting gene 6
         .. fitting gene 7
         .. fitting gene 8
         .. fitting gene 9
         .. fitting gene 0
         .. fitting gene 1
         .. fitting gene 2
         .. fitting gene 3
         .. fitting gene 4
         .. fitting gene 5
         .. fitting gene 6
         .. fitting gene 7
         .. fitting gene 8
         .. fitting gene 9
```

```
In [28]: PL.subplot(2,2,1)
         PL.title('Without Correction')
         PL.imshow(beta0[:,i0:i1],cmap=cm.RdBu,vmin=-0.6,vmax=+1)
         PL.colorbar()
         plt.set_xticks([])
         plt.set_yticks([])
         PL.subplot(2,2,2)
         PL.title('With Correction')
         PL.imshow(beta1[:,i0:i1],cmap=cm.RdBu,vmin=-0.6,vmax=+1)
         PL.colorbar()
         plt.set_xticks([])
         plt.set_yticks([])
```

Out[28]: 0

