Improvements for Seurat

Yun Yan

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1 Introduction

As an extension for `seurat` package, `honfleuR` has the following chases:

- Workspace before I submit pull request, and hopefully it will be merged into `seurat` package.
- Incorporate new algorithms to expand its capability of learning data.
- Design and enrich functions and parameters to make `seurat` compatible with more research of interests.
- Improve running speed while following `seurat` syntax and reproducing results.

2 Summary of changes

Category	Seurat	honfleuR	What's New
Localization	addImputedScore	fill_imputed_expr	interface for additional imputation strategies
Localization	fit.gene.k	fit_gene_k	10X faster; strict control of biological meanings
Localization	initial.mapping	initial_mapping	1X faster
Localization	refined.mapping	refined_mapping	17X faster
Clustering	jackStraw	jackStraw2	debug

`eval_seurat` function evaluates Seurat performances on landmark genes and draws ROC curves, reproducing Fig3-G&H.

3 Data imputation - `fill_imputed_expr`

Impute expression of each landmak gene (response) based on other genes with variable expressions (predictors).

`honfleuR` has three imputation strategies:

1. Lasso. Reproduce the results of `seurat`.
2. PLSR. Account for potential linear dependencies among predictors.
3. Tilling lasso. Learn the data structure and perform imputation.

Step	Lasso	PLSR	Tilling Lasso
1	Focus on specific landmark gene G.	Focus on specific landmark gene G.	Focus on specific landmark gene G.

Step	Lasso	PLSR	Tilling Lasso
2	Given the matrix with cells ID on rows and genes on columns, train **linear regression** model with **lasso** regularization.	Given the matrix with cells ID on rows and genes on columns, train a **PLSR** (Partial Least Squares Regression) model.	Given the data matrix with cells ID on rows and genes on columns, **shuffle rows randomly**.
3			Set first 20% samples as "unseen" data.
4			Use the rest 80% samples as training dataset to train a lasso model.
5			Apply the model on samples selected on Step 2, impute landmark gene expression in these cells.
6			Set second 20% samples as "unseen" data. Repeat Step 4-5 until the expression of landmark gene is imputed among all cells.
7	Apply lasso model on same matrix to impute expression of gene *G*.	Apply PLSR model on same matrix to impute expression of gene *G*.	Repeat Step 2-6 for 10 times. The average values are the imputed expressions for gene *G* among all cells.
8	Iterate Step 1-7 for other landmark genes.	Iterate Step 1-7 for other landmark genes.	Iterate Step 1-7 for other landmark genes.

3.1 Use Lasso

Run the following codes to test whether `honfleuR` reproduces results.

```
##-- Load data generated from seurat's Tutorial-2.
load('data/output_part2.Robj')
genes.sig <- pca.sig.genes(zf,pcs.use = c(1,2,3), pval.cut = 1e-2, use.full = TRUE)
insitu.genes <- colnames(zf@insitu.matrix)
lasso.genes.use <- unique(c(genes.sig, zf@var.genes))
```

```
zf@imputed <- data.frame()
zf0 <- addImputedScore(zf, genes.use = lasso.genes.use, genes.fit = insitu.genes,
                       do.print = FALSE, s.use = 40, gram = FALSE)
zf@imputed <- data.frame()
zf1 <- fill_imputed_expr(zf, genes.use = lasso.genes.use, genes.fit = insitu.genes,
                         scheme = "lasso",
                         do.print = F, s.use = 40, gram = F)
all.equal(zf0@imputed, zf1@imputed)
```

The result is (expected to be) `TRUE`.

3.2 Use PLSR

```r
zf <- fill_imputed_expr(zf, genes.use = lasso.genes.use, genes.fit = insitu.genes,
                        scheme = "plsr")
```

3.3 Use Tilling-lasso

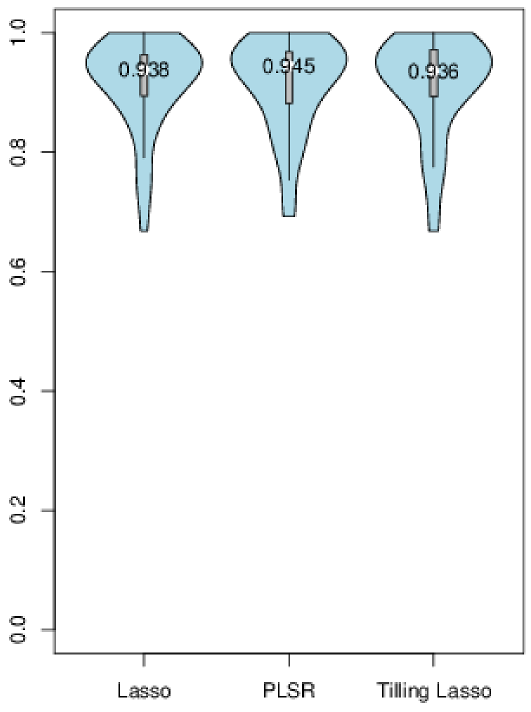
```r
zf1 <- fill_imputed_expr(zf, genes.use = lasso.genes.use, genes.fit = insitu.genes,
                         scheme = "tlasso",
                         do.print = F, s.use = 40, gram = F)
```

3.4 Quick summary

1. `honfleuR` expands `seurat` capability in addition to linear regression.
2. The interface of `honfleuR` is unified, i.e. setting parameter`scheme = c('lasso', 'plsr', 'tlasso')`.
3. `honfleuR` follows the `seurat` syntax and does not alter `seurat`'s codes frame.

3.5 ROC analysis for imputation scheems

As the way of performing ROC analysis as in Figure 3H of manuscript, the following AUC results are given by using `honfleuR`'s `eval_seurat` function.



4 Estimate bimodal distributiion of landmark gene - `fit_gene_k`

First, `honfleuR` **fixes a bug** related with biology. There is a biological issue that original `fit.gene.k` omits. There are following two scenarios:

```
fit.gene.k(zf, "SOX3", do.k = 3)
```

`do.k = 3` means it is assumed that the in situ pattern of gene G has 3 expression levels: low, med, high. In tutorial it was default as 2.

```
fit.gene.k(zf, "SOX3", start.pct=mean(zf@insitu.matrix[,"SOX3"]))
```

`start.pct` sets the initial percentage of cells in "on" state therefore the dataset is expected to be binary if `start.pct` is in action.

The above two calls are appropriate. However, current implementation of `seurat` allows the following extreme case taking place legally:

```
fit.gene.k(zf,"SOX3", do.k = 5, start.pct=mean(zf@insitu.matrix[,"SOX3"]))
```

It is conflict that five (any number greater than 2) different expression levels and "on/off" presumption coexists. Therefore `honfleuR` comes up with a patch, see `fit_gene_k`.

Furthermore, `fit_gene_k` is **10X** faster than `fit.gene.k`. Run the following codes to see the efficiency boost (on my laptop decrease from 24s down to 2s).

```
load('data/output_part2.Robj')
insitu.genes <- colnames(zf@insitu.matrix)
system.time(
  for (g in rev(insitu.genes)) {
    zf0 <- fit.gene.k(zf, g, do.k = 2, start.pct=mean(zf@insitu.matrix[, g]),
                      num.iter = 1, do.plot=FALSE)
  })
load('data/output_part2.Robj')
system.time(
  for (g in rev(insitu.genes)) {
    zf1 <- fit_gene_k(zf, g, do.k = 2, start.pct=mean(zf@insitu.matrix[, g]),
                      num.iter = 1, do.plot=FALSE)
  })
all.equal(zf0@mix.probs, zf1@mix.probs)
```

The result is `TRUE` meaning that `honfleuR` builds the model more efficiently without losing correctness. See results here estimated by using `microbenchmark` package.

5 Cells mapping - `initial_mapping` and `refined_mapping`

`initial_mapping` and `refined_mapping` exactly follows twins functions `initial.mapping` and `refined.mapping` respectively.

`initial_mapping` is **1X** faster (11s down to 5s), and `refined_mapping` is **17X** times faster (98s down to 5s). See boosting results here estimated by using `microbenchmark` package.