

# Merging Mixture Components for Cell Population Identification in Flow Cytometry Data

## The flowMerge package

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# 1 Licensing

Under the Artistic License, you are free to use and redistribute this software.

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# 2 Overview

We apply a cluster merging approach to perform automated gating of cell populations in flow cytometry data. The max BIC model fitting criterion for mixture models generally overestimates the number of cell populations (ie clusters) in flow cytometry data because the number of mixture components required to accurately model a distribution is usually greater than the number of distinct cell populations. Model fitting criteria based on the entropy, such as the ICL, provide better estimates of the number of clusters but tend to provide a poor fit to the underlying distribution. We combine these two approaches by merging mixture components from the max BIC fit based on an entropy criterion. This approach allows multiple mixture components to represent the same cell sub-population. Merged clusters are mixtures themselves and are summarized by a weighted combination of their component model parameters. The result is a mixture model that retains the good model fitting properties of the max BIC solution but the number of components more closely reflects the true number of distinct cell sub-populations.

# 3 Installation

`flowMerge` requires several packages to be pre-installed for full functionality. Specifically, `flowClust`, `flowCore`, `flowViz` and `snow` (for parallel computations) should be installed and functional.

# 4 Example: Cluster merging applied to the Rituximab data set

## 4.1 The core function

To demonstrate the functionality we use a flow cytometry data set from a drug-screening project to identify agents that would enhance the anti-lymphoma activity of Rituximab, a therapeutic monoclonal antibody. The data set is an object of class `flowFrame`; it consists of eight variables, among them only the two scattering variables (`FSC.H`, `SSC.H`) and two channels for the fluorochrome measurements (`FL1.H`, `FL3.H`) are of interest in this experiment. The `flowMerge`

package may be invoked upon the output of a call to `flowClust`, or it may be invoked on a `flowFrame` directly via the parallelized method `pFlowMerge`, which will itself call `flowClust` but throw away intermediate results. The following code will model one through 10 clusters using `flowClust` in a parallel manner using `snow`, choose the best BIC solution, merge clusters in the best BIC solution, choose the best merged solution based on the entropy criterion, then plot the results. This functionality is wrapped in the `pFlowMerge` function, with additional parallelization using `snow`. Additionally, if the `snow` cluster object passed to `pFlowMerge` is null, the non-parallel version of `flowMerge` is called.

```
> library(flowMerge)

> data(rituximab)
> summary(rituximab)
```

	FSC.H	SSC.H	FL1.H	FL2.H	FL3.H	FL1.A	FL1.W
Min.	59.0000	11.0000	0.0000	0.0000	1.000	0.0000	0.00000
1st Qu.	178.0000	130.0000	197.0000	55.0000	150.000	0.0000	0.00000
Median	249.0000	199.0000	244.0000	116.0000	203.000	0.0000	0.00000
Mean	287.0822	251.8265	349.1638	126.3994	258.345	73.4589	17.59871
3rd Qu.	331.0000	307.0000	445.0000	185.0000	315.000	8.0000	0.00000
Max.	1023.0000	1023.0000	974.0000	705.0000	1023.000	1023.0000	444.00000

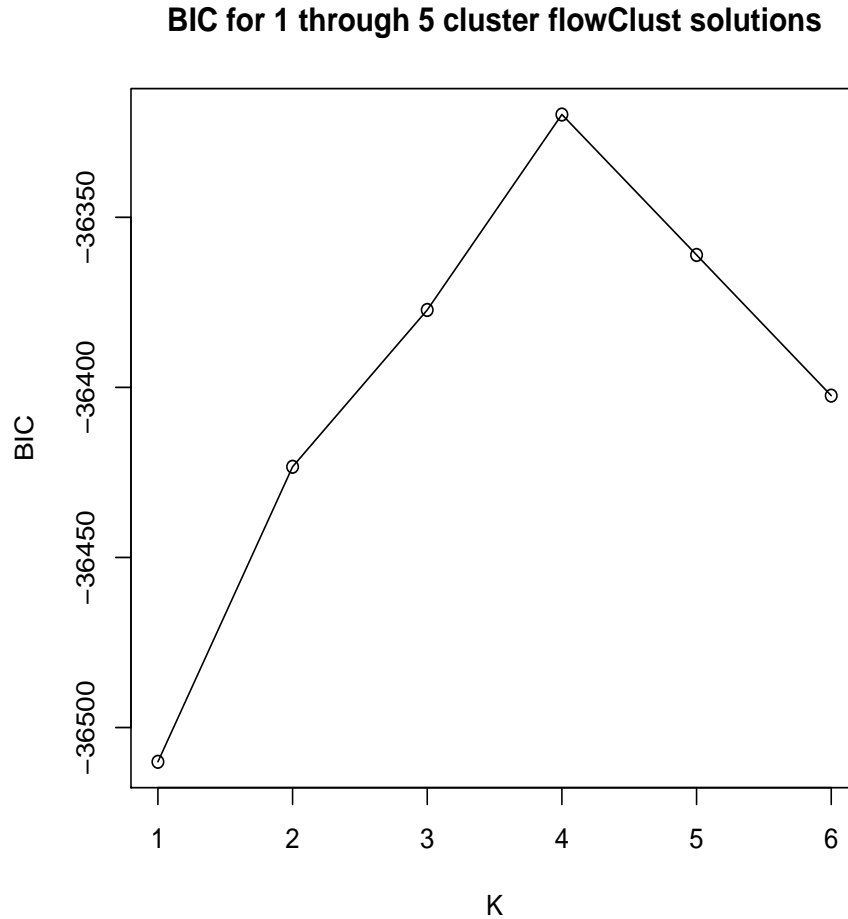
  

	Time
Min.	2.0000
1st Qu.	140.0000
Median	285.0000
Mean	294.0388
3rd Qu.	451.0000
Max.	598.0000

```
> flowClust.res <- flowClust(rituximab, varNames=c(colnames(rituximab)[1:2]), K=1:6,trans=1,
```

`flowClust.res` is a `flowClust` object containing the 5 cluster solution. We extract the BIC of each solution with the internal `flowMerge` function `BIC`:

```
> plot(flowMerge:::BIC(flowClust.res),
+ main="BIC for 1 through 5 cluster flowClust solutions",xlab="K",ylab="BIC",type="o");
> flowClust.maxBIC<-flowClust.res[[which.max(BIC(flowClust.res))]];
```



Here we have extracted the max BIC solution, found for  $K=4$ . Before we run the cluster merging algorithm on the max BIC solution, we have to create a `flowObj` object from the `flowClust` result and the `flowFrame` data. We then run the `merge` function on the `flowObj` and extract then entropy of clustering from the merged results using the `ENT` function. The list of merged cluster solutions are input to the `fitPiecewiseLinreg` function. This function fits a piecewise linear regression to the entropy vs number of clusters and locates the position of the changepoint, if appropriate. Model selection is done via the BIC criterion. The results of the fit can be optionally plotted.

```
> flowClust.flowobj<-flowObj(flowClust.maxBIC,rituximab);
> flowClust.merge<-merge(flowClust.flowobj,metric="entropy");
```

Rule of identifying outliers: 90% quantile  
 Rule of identifying outliers: 90% quantile

Rule of identifying outliers: 90% quantile

Rule of identifying outliers: 90% quantile

```
> i<-fitPiecewiseLinreg(flowClust.merge);
```

Next we extract the merged solution with number of clusters equal to the position of the changepoint found by `fitPiecewiseLinreg`. This is the optimal merged solution based on the entropy criterion. The solution can be plotted with the `plot` method.

```
> par(mfrow=c(2,2));
```

```
> flowClust.mergeopt<-flowClust.merge[[i]];
```

```
> plot(flowClust.res[[4]],data=rituximab,main="Max BIC solution");
```

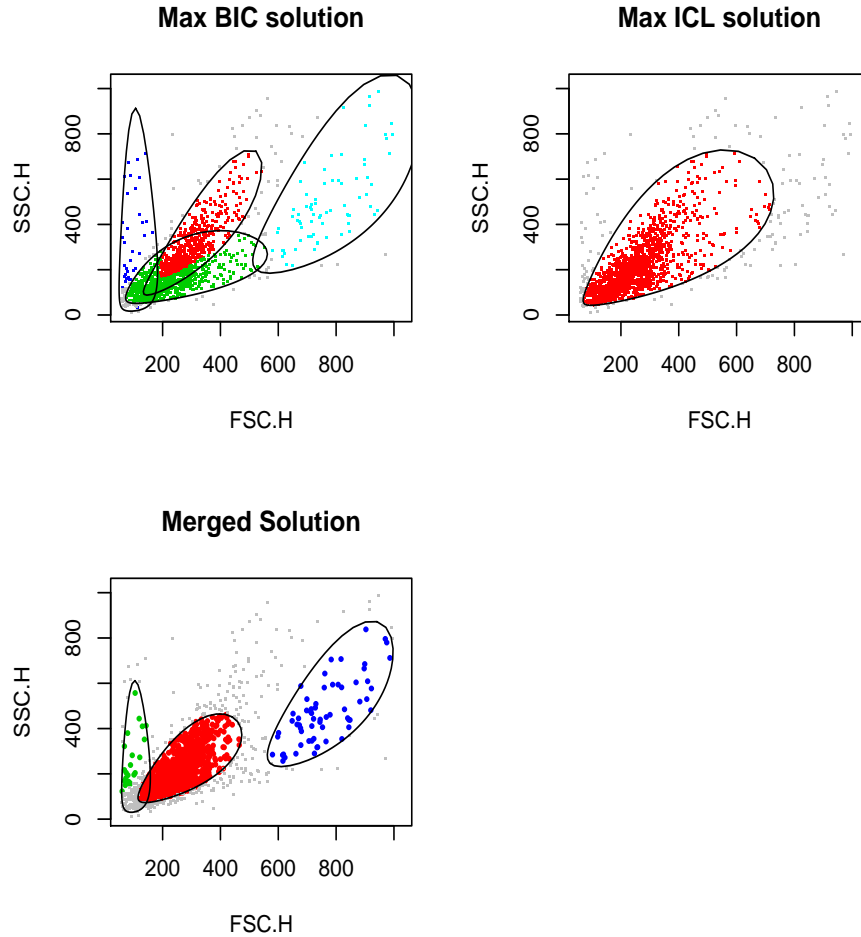
Rule of identifying outliers: 90% quantile

```
> plot(flowClust.res[[which.max(flowMerge::ICL(flowClust.res))]],data=rituximab,main="Max I
```

Rule of identifying outliers: 90% quantile

```
> plot(flowClust.mergeopt,level=0.75,pch=20,main="Merged Solution");
```

Rule of identifying outliers: 75% quantile



We see that the merged solution provides a better fit to the lymphocyte population than either the max BIC solution, or the max ICL solution. Additionally, debris and granulocytes are also clearly identified, in contrast to the ICL solution. We can extract the lymphocyte population and re-run `flowClust` and `flowMerge` on the fluorescence channels of the lymphocytes only.

```
> pop<-which(apply(apply(getEstimates(flowClust.mergeopt)$locations,2,function(x)order(x,dec
> lymphocytes<-split(flowClust.mergeopt,population=list("lymphocytes"=pop))$lymphocytes;
> lymphocytes<-lymphocytes[,c(3,5)];
> l.flowC<-flowClust(lymphocytes,varNames=c("FL1.H","FL3.H"),K=1:8,B=1000,B.init=100,tol=1e-
```

The lymphocyte population is between the debris and the granulocytes in the forward and side-scatter dimensions. We can easily select it by examining the means of the three populations. We're only interested in the fluorescence channels, so we subset those with the `[]` operator inherited from `flowClust`. Finally,

another call to `flowClust` performs a round of clustering on the lymphocyte sub-population.

```
> par(mfrow=c(2,2));  
> l.flow0<-flowObj(l.flowC[[which.max(flowMerge:::BIC(l.flowC))]],lymphocytes);  
> plot(l.flow0,main="max BIC solution",new.window=F,pch=20,level=0.9);
```

Rule of identifying outliers: 90% quantile

```
> plot(flowObj(l.flowC[[which.max(flowMerge:::ICL(l.flowC))]],lymphocytes),main="max ICL sol
```

Rule of identifying outliers: 90% quantile

```
> l.flowM<-merge(l.flow0);
```

Rule of identifying outliers: 90% quantile

Rule of identifying outliers: 90% quantile

Rule of identifying outliers: 90% quantile

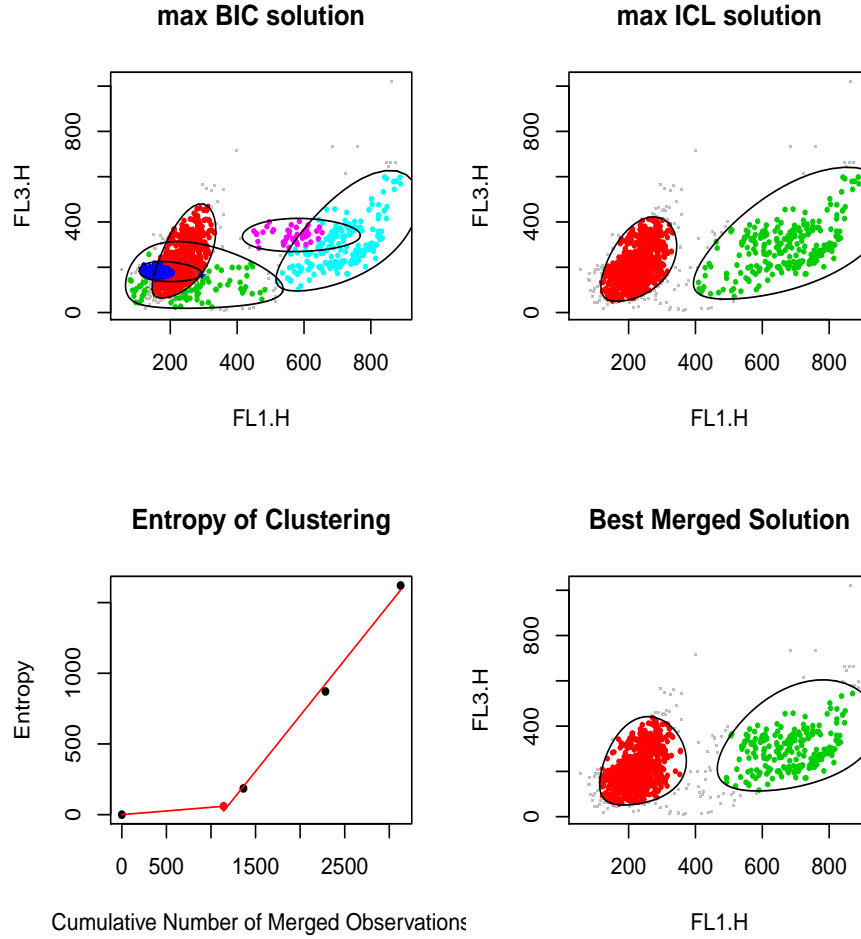
Rule of identifying outliers: 90% quantile

Rule of identifying outliers: 90% quantile

```
> i<-fitPiecewiseLinreg(l.flowM,plot=T);
```

```
> plot(l.flowM[[i]],new.window=F,main="Best Merged Solution",pch=20,level=0.9);
```

Rule of identifying outliers: 90% quantile



We complete the analysis by choosing the best fitting merged solution and comparing it to the max BIC and max ICL solutions.

## 4.2 Parallel computations with the snow package

The methodology described in the first section is wrapped in a single function call with the additional benefits of utilizing the parallel processing capabilities of the `snow` package to speed up model fitting using `flowClust`. The function `pFlowMerge` can be passed multiple `flowFrames` in the form of a `flowSet`, or a list of `flowFrames`, as well as a `snow` cluster object, and the usual set of parameters required by `flowClust` to specify the model. The call parallelizes the computation of multiple models for each `flowFrame`. For example if one wants to fit all models starting from the one cluster model through to the ten cluster model for each of ten `flowFrames`, these will be distributed amongst

the number of processors defined in the `snow` cluster object. Each processor will be assigned the calculation for a single `flowFrame` and model combination, until all processors are occupied. This speeds up computations significantly in a high-throughput analysis setting.

The resulting models are evaluated for the max BIC solutions for each `flowFrame`, the merging algorithm is run on each max BIC solution, the optimal merged solution is found as described in the example and returned to the user. The non-parallel version can be called via `pFlowMerge` with the “`cl`” argument equal to `NULL`.

### 4.3 Recent updates

The `flowMerge` algorithm has been updated to increase speed and now supports parallelization when the `foreach` package is installed. `FlowMerge` now also supports merging on the mahalanobis distance metric as well as the entropy. These can be specified to the merging algorithm via the optional `metric` argument (`metric=c("entropy","mahalanobis")`) to the `merge()` function. The default is to use “entropy”.

We have added some new features for plotting the tree of merged populations, and highlighting the nodes/populations according to marker expression. For the fluorescent markers above:

```
> require(Rgraphviz)
> f<-ptree("l.flowM",fitPiecewiseLinreg(l.flowM));
> par(mfrow=c(1,2))
> f(1);
```

A graphNEL graph with directed edges

Number of Nodes = 3

Number of Edges = 2

```
> f(2);
```

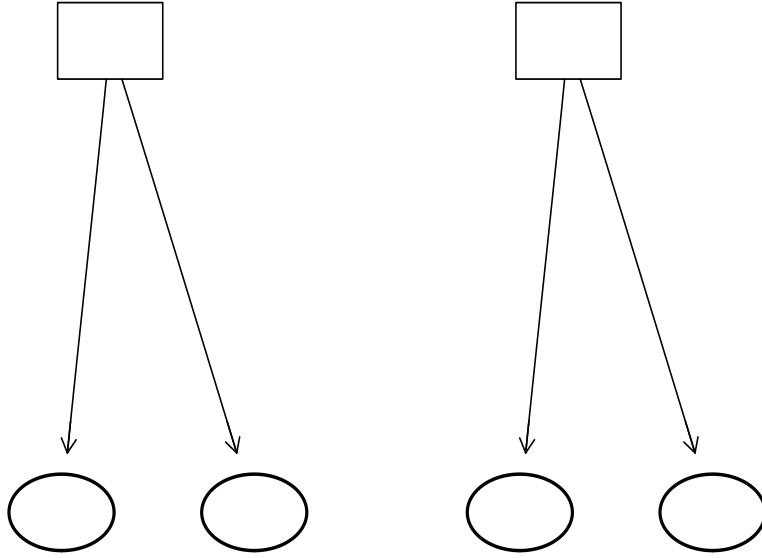
A graphNEL graph with directed edges

Number of Nodes = 3

Number of Edges = 2

Anti-BrdU FITC

7 AAD



Circular nodes with dashed borders show merged clusters. Elliptical nodes show the chosen populations in the best fitting model. Square nodes show the rest of the complete merging to a single cluster. Red indicated higher expression of the marker, and blue/green shows lower expression. Note that it is normalized between 0 and 1 on the range of the data.

#### 4.4 Upcoming improvements

FlowMerge is undergoing continuous usability improvements, specifically with respect to the parallel computation framework. Figures of merit and other statistics will be output to allow the user to monitor the success or failure of the merging algorithm as it works through a large data set. A framework for semi-supervised selection of lymphocyte populations across multiple samples is also in the works. pFlowMerge does not do any sophisticated memory management,

as such if you have a large data set, we suggest feeding it to `pFlowMerge` piece by piece, depending on the amount of RAM available to your machine / cluster.

## 4.5 Frequently Asked Questions

Q: How do I use `pFlowMerge`?

A: `pFlowMerge` is implemented to parallelize computations for `flowSets` or lists of `flowFrames`, rather than for multiple clusters in a single `flowFrame`. For example, if `data` is `flowSet` of ten `flowFrames` and `cl` is a `snow` cluster of ten nodes, then, running `pFlowMerge(cl,flowSet,varNames=c("A","B","C"),K=1:10)` will distribute the ten `flowFrames` across the ten nodes, and each node will evaluate the model for `K=1:10` clusters. If you wish to parallelize the `K=1:10` cluster computations across multiple nodes, you would need to make function call that utilizes `snow` functionality directly, such as: `clusterMap(cl,function(...)try(flowClust(...)),list(flowFrames,K=1:10))`. This will distribute the calculation of `flowFrame` for each value of `K=1` through `K=10` components across the ten nodes of `cl`. The addition of the `try()` wrapper ensures the function will return even if an individual model fails to converge for a given value of `K`. Alternately, if you wish to do the above for a `flowSet` you will need to replicate each element of the `flowSet`  $K_{max}$  times, where  $K_{max}$  is the total number of model components to evaluate per `flowFrame`. Again, an example: `clusterMap(cl,function(...)try(flowClust(...)),rep(as(flowSet,"list"),each=length(Kvector)))`. Please remember to ensure `flowClust` is loaded in each R work environment on the nodes by calling `clusterEvalQ(cl,library(flowClust))`.