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► **To cite this version:**

V Vandewalle, Camille Ternynck, Guillemette Marot. Linking different kinds of Omics data through a model-based clustering approach. IFCS 2019, Aug 2019, Thessalonique, Greece. hal-02400525

**HAL Id: hal-02400525**

**<https://hal.science/hal-02400525>**

Submitted on 9 Dec 2019

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# Linking different kinds of Omics data through a model-based clustering approach

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IFCS Meeting  
Thessaloniki  
August 28<sup>th</sup>, 2019

# Heterogeneous Omics data application

## Central dogma of molecular biology

DNA  $\rightarrow$  **RNA**  $\rightarrow$  Proteins

## Gene expression measurements

- microarray measurements: continuous variables
- RNAseq measurements: count variables

For gene  $g$

		Patient 1	Patient 2	$\dots$	Patient $n$
Gene $g$	microarray 1	$X_{g11}$	$X_{g12}$	$\dots$	$X_{g1n}$
	$\vdots$				
	microarray $n_g$	$X_{gn_g1}$	$X_{gn_g2}$	$\dots$	$X_{gn_gn}$
	RNAseq 1	$Y_{g11}$	$Y_{g12}$	$\dots$	$Y_{g1n}$
	$\vdots$				
	RNAseq $m_g$	$Y_{gm_g1}$	$X_{gm_g2}$	$\dots$	$X_{gm_gn}$

## Usual related questions

- High dimensional framework with many genes
- Low number of subjects
- Differential expression analysis: find gene with expression related to some disease
- Normalization of the data: remove some undesirable bias
- Often RNA transformations performed (log-transformation, normalized log-transformation, variance stabilizing transformation, . . . ) to treat this count variable as a continuous one

# Considered framework

## Initial questions

- Data normalisation framework: make RNAseq and microarray on the same "scale".
- How can we detect that some RNAseq and microarray measurements belong to the same genes (group of genes)?

## Clustering of genes

1. Use the genes memberships information of the measurements,
2. Not use the genes membership information (in many settings gene information not available)  $\Rightarrow$  how to cluster variables from the same gene?

## Similarity criterion

Cluster together genes which have the same distribution with respect to both microarray and RNAseq measurements.

# Considered framework

## Initial questions

- Data normalisation framework: make RNAseq and microarray on the same "scale".
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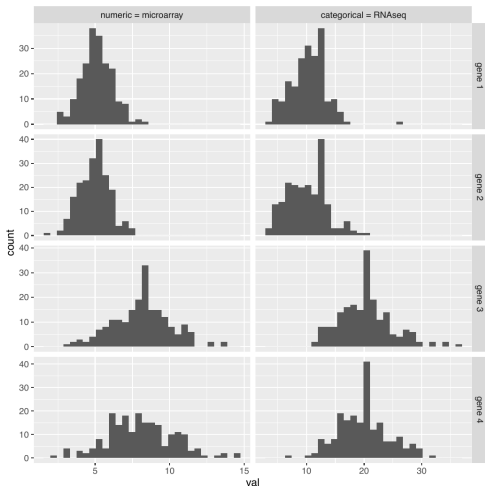
## Clustering of genes

1. **Use the genes memberships information of the measurements,**
2. Not use the genes membership information (in many settings gene information not available)  $\Rightarrow$  how to cluster variables from the same gene? **Semi-supervised setting, partial gene membership information!**

## Similarity criterion

Cluster together genes which have the same distribution with respect to both microarray and RNAseq measurements.

# Illustration



- Information on the distribution
- ⇒ forget the individual level
- ≈ symbolic data in some sense

## Data

- $n$  patients, described by  $G$  genes
- for each patient  $i$ , each gene  $g$  is described by:
  - $n_g$  continuous microarray measurements  $x_{g1i}, \dots, x_{gn_gi}$
  - $m_g$  count RNAseq measurements  $y_{g1i}, \dots, y_{gm_gi}$
- assume that the genes come from  $K$  different clusters
- $z_g \in \{0, 1\}^K$  denoting the cluster for gene  $g$  in binary coding.

## Model

- For each gene  $g$ ,  $z_g$  is a realization of the random variable  $Z_g \sim \mathcal{M}(1; \pi_1, \dots, \pi_K)$
- $Z_1, \dots, Z_G$  are assumed independent and identically distributed
- $x_{gji}$  the microarray measurement for the measure number  $j$  ( $j \in \{1, \dots, n_g\}$ ) of the patient number  $i$  for the gene  $g$  is a realization of the random variable  $X_{gji}$ .
- $y_{g\ell i}$  the RNAseq measurement for the measure number  $\ell$  ( $\ell \in \{1, \dots, m_g\}$ ) of the patient number  $i$  for the gene  $g$  is a realization of the random variable  $Y_{gji}$ .



# Detail of the model assumptions

## Parametric model

- measures on each patient are assumed independent
- given the gene cluster each measure are independent
- $X_{gji}|Z_g = k \sim \mathcal{N}(\mu_k; \sigma_k)$
- $Y_{g\ell i}|Z_g = k \sim \mathcal{P}(\lambda_k)$

## Remarks

1.  $\lambda_k$  should be an increasing function of  $\mu_k$ , but this constraint is not imposed to simplify the estimation process (no need of a link function between  $\lambda_k$  and  $\mu_k$ )
2. gene membership of each probe assumed to be known: often the case for human expression data, however not for bacterial data ...
3. model able to cope with partial gene information, however for identifiability reasons it is needed that each cluster contains at least one gene for which we have observed both RNAseq and microarray measurements.

## Parameters estimation (1/2)

Parameters estimated by maximum likelihood through the EM algorithm

### E-step

$$t_{gk}^{(r)} = P(Z_{gk} = 1 | \mathbf{x}_g, \mathbf{y}_g; \theta^{(r)})$$

with

$$P(Z_{gk} = 1 | \mathbf{x}_g, \mathbf{y}_g) \propto \pi_k \prod_i^n \left[ \left( \prod_{j=1}^{n_g} f(x_{gji}; \alpha_k) \right) \times \left( \prod_{\ell=1}^{m_g} h(y_{g\ell i}; \beta_k) \right) \right]$$

where  $f$  is the pdf for microarray data and  $h$  the pdf for RNAseq data.

### M-step

$$\pi_k^{(r+1)} = \frac{\sum_{g=1}^G t_{gk}^{(r)}}{G}$$

$$\alpha_k^{(r+1)} = \arg \max_{\alpha_k} \sum_{g=1}^G t_{gk}^{(r)} \sum_{i=1}^n \sum_{j=1}^{n_g} \ln f(x_{gji}; \alpha_k)$$

$$\beta_k^{(r+1)} = \arg \max_{\beta_k} \sum_{g=1}^G t_{gk}^{(r)} \sum_{i=1}^n \sum_{\ell=1}^{m_g} \ln h(y_{g\ell i}; \beta_k),$$

### Remarks

- the model collapses all the data of a same gene  $g$
- clustering of genes  $\simeq$  clustering of distributions

## Parameters estimation (2/2)

Computations considerably speed up by the use of sufficient statistics:

$$\mu_k^{(r+1)} = \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \frac{1}{n \times n_g} \sum_{i=1}^n \sum_{j=1}^{n_g} x_{gji} = \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \bar{x}_g$$

$$\begin{aligned} \sigma_k^{2(r+1)} &= \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \frac{1}{n \times n_g} \sum_{i=1}^n \sum_{j=1}^{n_g} (x_{gji} - \mu_k^{(r+1)})^2 \\ &= \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \sigma_{x_g}^2 + \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} (\mu_k^{(r+1)} - \bar{x}_g)^2 \end{aligned}$$

$$\lambda_k^{(r+1)} = \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \frac{1}{n \times m_g} \sum_{i=1}^n \sum_{\ell=1}^{m_g} y_{g\ell i} = \frac{1}{n_k^{(r+1)}} \sum_{g=1}^G t_{gk}^{(r)} \bar{y}_g$$

gene  $g$  can be summarized by:

- M-step:  $(\bar{x}_g, \sigma_{x_g}^2, \bar{y}_g)$ .
- E-step:  $n_g$  and  $m_g$  additionally needed.
- log-likelihood:  $\sum_{\ell=1}^{m_g} \sum_{i=1}^n \log(y_{g\ell i}!)$  additionally needed.

# Presentation of the data

## The Cancer Genome Atlas (TCGA) dataset

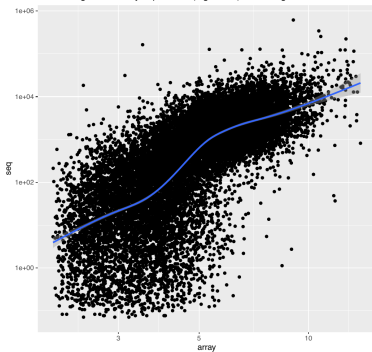
- Data about the Acute Myeloid Leukemia <sup>1</sup>
- 169 patients
- Keep genes with:
  - Only one RNAseq measure
  - Non null RNAseq values for at least 9 patients
  - At least one microArray measure
  - Keep probes related to only one gene
- At the end: 16,741 genes with an RNAseq measure and one or more microarray measures

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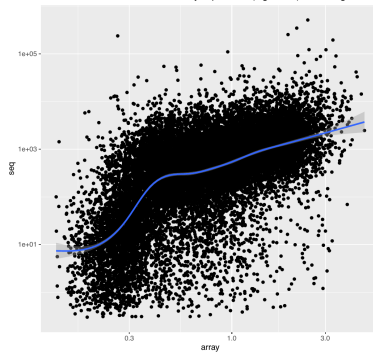
<sup>1</sup><https://portal.gdc.cancer.gov/projects/TCGA-LAML>

# Link RNAseq / microarray

Average RNAseq expression (log-scale) according to the average microarray expression (log-scale) for each gene

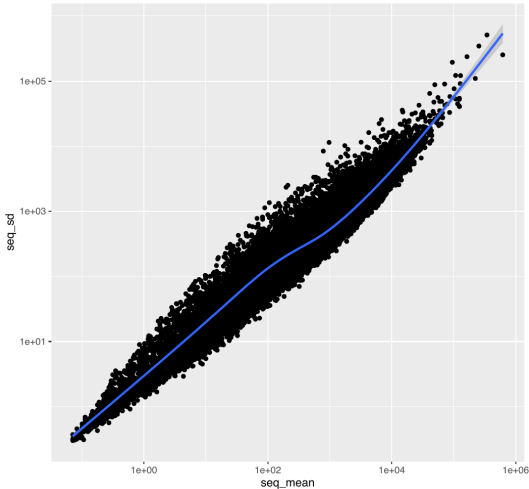


Standard deviation of RNAseq expression (log-scale) according to the standard deviation of microarray expression (log-scale) for each gene



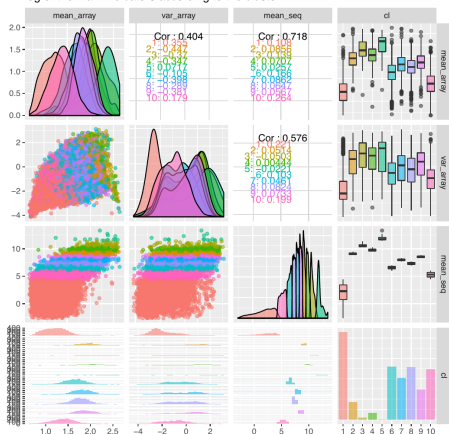
# Link between standard deviation and average for RNAseq data

Standard deviation of RNAseq expression (log-scale) according to the mean of RNAseq expression (log-scale) for each gene



# Results of a clustering in 10 clusters (1/2)

log of the main indicators according to the cluster



## Remarks

- It is essentially the RNAseq which determines the cluster membership!
- How to explain this particularly big influence?

## Results of a clustering in 10 clusters (1/2)

Model parameters:

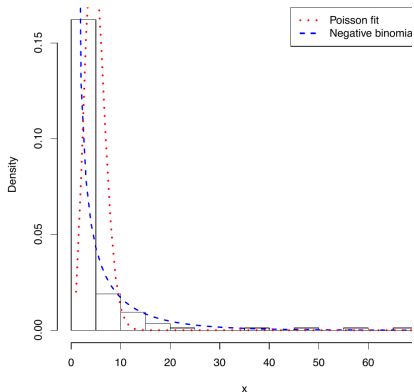
cluster	prop	mean_array	var_array	mean_seq
1	0.26	3.46	1.14	19.53
2	0.15	5.27	2.64	736.30
3	0.15	5.92	2.98	1577.26
4	0.15	4.13	1.74	220.13
5	0.12	6.32	3.57	2938.47
6	0.09	6.60	3.88	5207.63
7	0.05	7.15	4.96	9332.41
8	0.02	7.80	6.45	18148.48
9	0.01	8.79	8.98	41936.76
10	0.00	9.61	12.76	179799.30

- Small clusters have an higher mean
- The mean array value is monotetic according to the mean seq value
- Huge differences according to the cluster for mean seq value

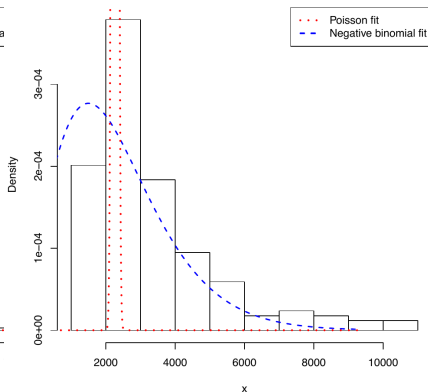


# Explanation of the influence of RNAseq

Histogram of RNAseq data for gene ABCB10



Histogram of RNAseq data for gene NA



- Count for RNAseq overdispersed
- ⇒ Poisson not accurate, the mixture model prioritizes to improve this bad fit,
- ⇒ better fit using negative binomial distribution.

## Negative binomial distribution fit

$$p(y|r, p) = \frac{\Gamma(r+y)p^r(1-p)^y}{\Gamma(r)\Gamma(y+1)}$$

$r$ : size,  $p$ : probability,  $\mu = r(1-p)/p$ : expectation

### Maximum likelihood estimation

Expression of  $p$  according to  $r$  and the data

$$p = \frac{Nr}{Nr + \sum_{i=1}^N y_i}$$

Partial derivate according to  $r$

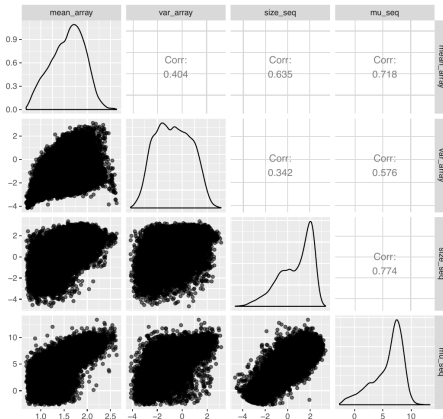
$$\frac{d\ell(r|y_1, \dots, y_N)}{dr} = N \ln \left( \frac{Nr}{Nr + \sum_{i=1}^N y_i} \right) - N\psi(r) + \sum_{i=1}^N \psi(r + y_i) = 0,$$

$\psi$ : digamma function, *i.e.* derivate of the Gamma function.

- Non-closed form, found by numerical optimisation
- Algorithm implemented in `fitdistrplus`
- No sufficient statistic available due to the digamma function

# New preliminary analysis based on a negative binomial fit

log of the main parameters according to the gene  
using negative binomial fit



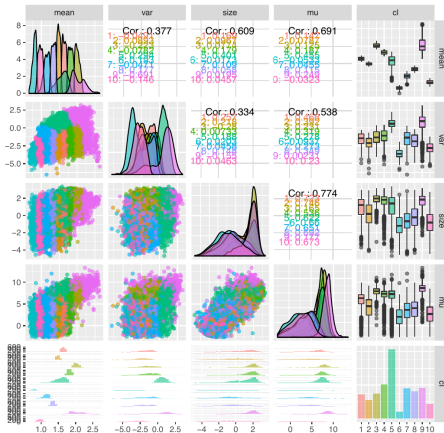
Outlier removed (log-value) :

gene	mean_array	var_array	size_seq	mu_seq
TBC1D21	1.26	-2.26	12.55	-2.24

# Results with the model based clustering approach

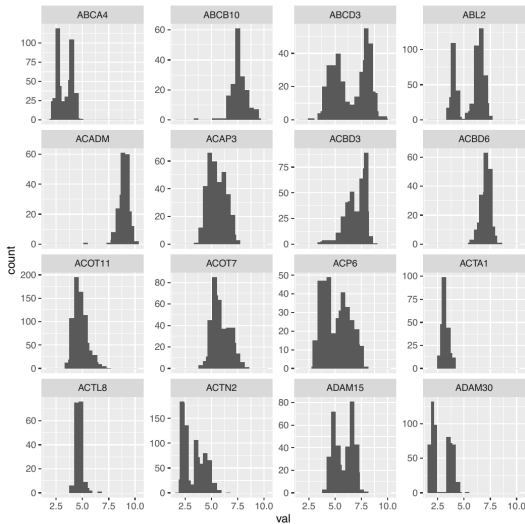
## Clustering in 10 clusters

log of the main indicators according to the cluster



- Now it seems that microarray data dominates the clustering process
- It should be mainly due to bad fit of Gaussian distribution

# Explication of the influence of microarray data

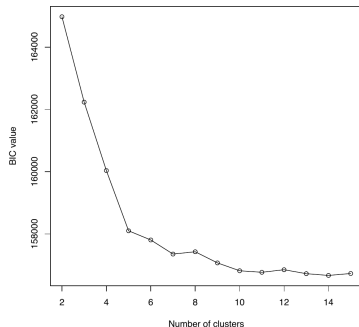


- Unimodal distribution not accurate for a lot of variables.
  - Consider mixture of Gaussian
- ⇒ Increase the cost of the algorithm, work in progress
- ...

# Clustering of genes based on summary of each gene

- **Rationale: as the number of patients  $n$  increases, the summary of each gene becomes consistent.**
- Use clustering of genes based on summaries provided by Gaussian and negative binomial fit for each gene.
- Model based clustering with mixture of gaussian of these summaries
- Easier to balance the influence of each kind of data.

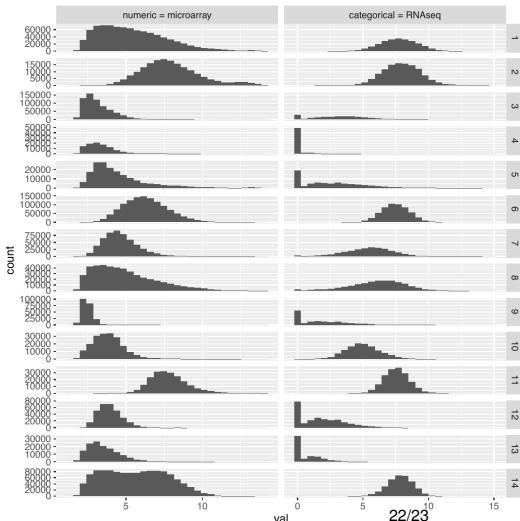
BIC value according to the number of clusters



BIC indicates approximately 14 clusters



# Resulting empirical distribution in each cluster on the initial data



- Can be used to cluster new data based only on RNAseq
- Then deduce the distribution of microarray data given the observed RNAseq data



# Conclusion and perspective

## Conclusion

- Approach very sensitive to model assumptions
- Difficulty to balance the influence of each kind of variables
- Empirical solution through clustering of summaries

## Perspectives

- Work in progress to stabilize the approach
- Take into account dependency of the measurements for a patient
- Simultaneous clustering of patients and genes