Constructing Simulation Data with Dependence Structure for Unreliable Single-Cell RNA-sequencing Data using Copulas

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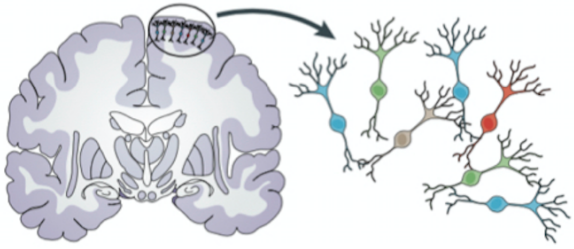
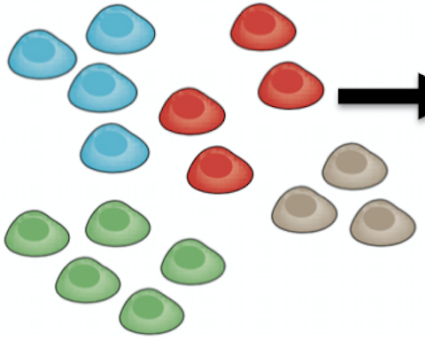
Working group

Biological application

Tissue (e.g. tumor)



Isolate and sequence individual cells

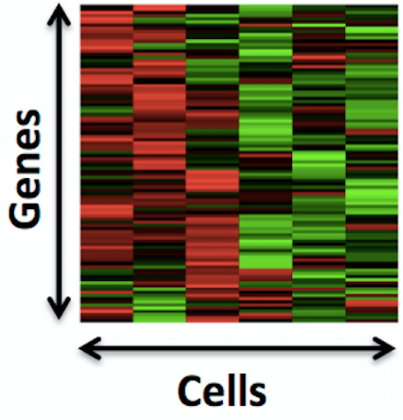


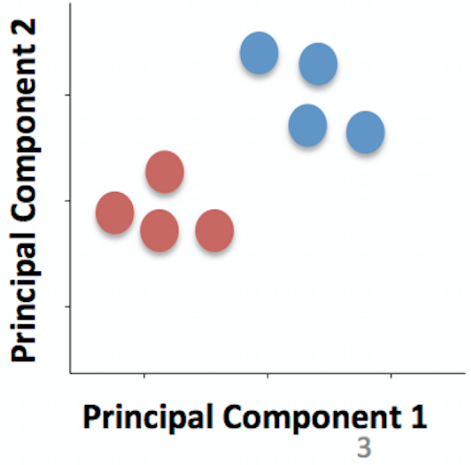
Gene 1
Cell 1

Read Counts

	Cell 1	Cell 2	…
Gene 1	18	0	
Gene 2	1010	506	
Gene 3	0	49	
Gene 4	22	0	
…			

Compare gene expression profiles of single cells





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1. Construction of Simulation Data
2. Incorporation of Dependence Structure
3. Consequences with regard to Application

Outline

Distribution Approximation of the Distribution of Read Counts

Best distribution approximation of read counts:
Zero Inflated Negative Binomial (ZINB) Zeileis et al. (2008), Wagner et al. (2013) and Kleiber and Zeileis (2016):

Zero Inflated Negative Binomial (ZINB):

$$f_{ZINB}(X_j = x) = \begin{cases} \pi_j + (1-\pi_j) f_{NB}(0) & \text{if } x = 0 \\ (1-\pi_j) f_{NB}(x) & \text{if } x \in \mathbb{N} \end{cases}$$

Generalisation of the negative binomial distribution:
Mixture of Poisson distributions with a gamma distributed poisson rate

$$f_{NB}(X_j = x) = \frac{\Gamma(x+\phi)}{\Gamma(\phi) \cdot x!} \cdot \frac{\mu^x \cdot \phi^\phi}{(\mu+\phi)^{x+\phi}} \cdot \mathbf{I}_{\mathbb{N}}(x)$$

Different Degrees of Heterogeneity

Basis of the Simulation Design:
Quantiles of the estimated parameters
Based on the 7225 genes of the real data set Kolodziejczyk et al. (2015)

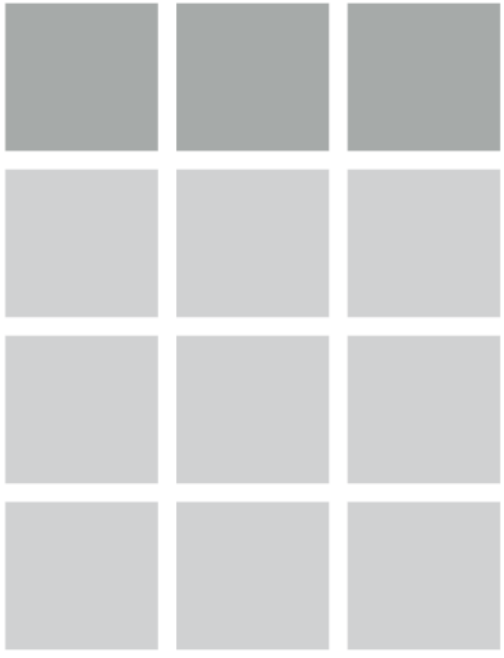
Scenario 1 Most homogeneous scenario
$\Rightarrow$ Narrowest parameter interval
Scenario 3 Most heterogeneous scenario
$\Rightarrow$ Broadest parameter interval

	μ		ϕ	π
Sc.	Group 1	Group 2	Group 1, Group 2	Group 1, Group 2
1	[35%-80%]	[15%-60%]	[45%-55%]	[45%-55%]
2	[25%-85%]	[10%-70%]	[40%-60%]	[40%-60%]
3	[20%-90%]	[5%-75%]	[35%-65%]	[35%-65%]

Table: Quantiles of the estimated ZINB parameters of the reference data that are used for the construction for each scenario of target group 1 and target group 2.

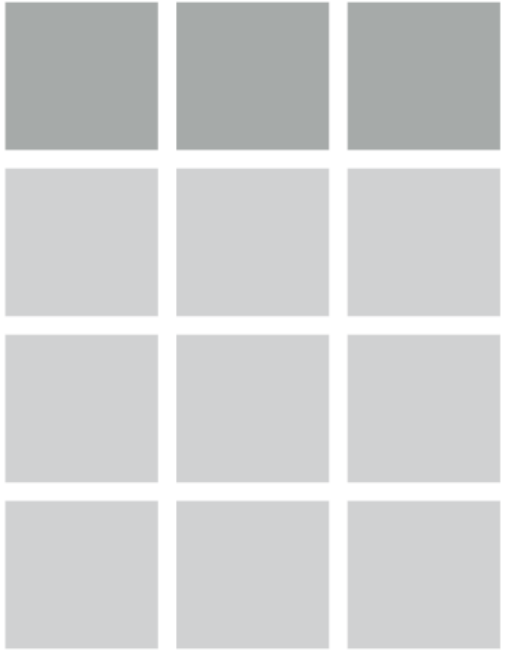
Undistorted Simulation Data - No dependence structure

Scenario 1:
Homogenous



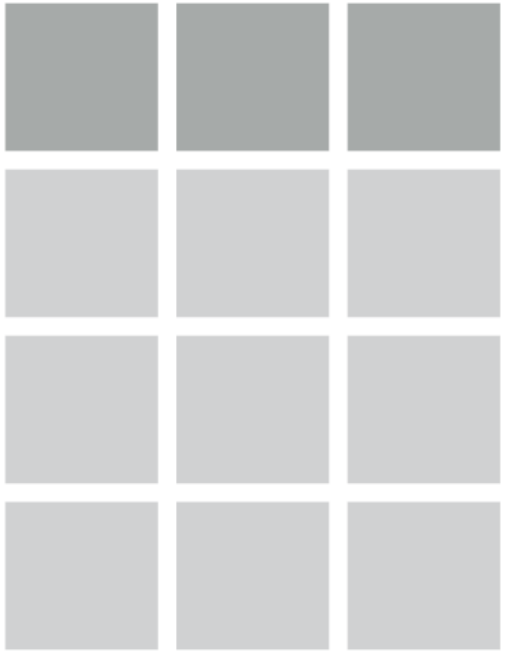
$(n^{(1)} + n^{(2)}) \times m$

Scenario 2:
Transition



$(n^{(1)} + n^{(2)}) \times m$

Scenario 3:
Heterogeneous



$(n^{(1)} + n^{(2)}) \times m$

Constructing Distorted Data via Lower and Upper Distribution Functions

Upper distribution function: Measuring tendencially decreased read counts
Lower distribution function: Measuring tendencially increased read counts

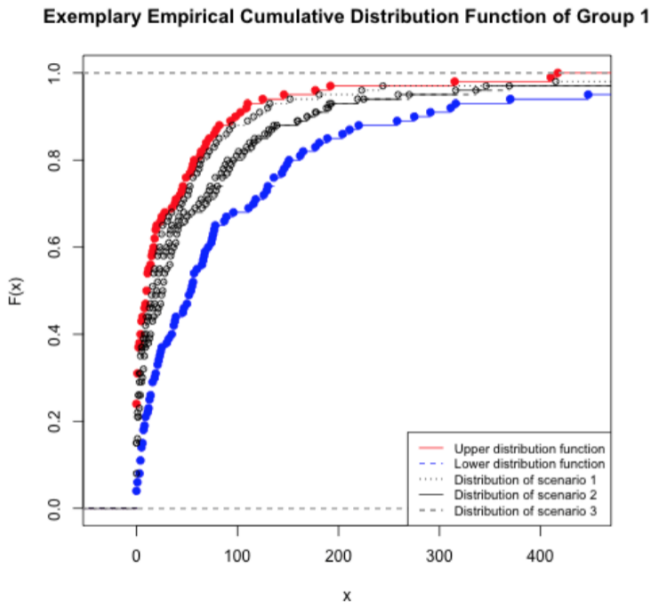


Figure: Lower and upper cumulative distribution function of simulated gene 3 for group 1 using the statistical software R of the R Core Team (2014).

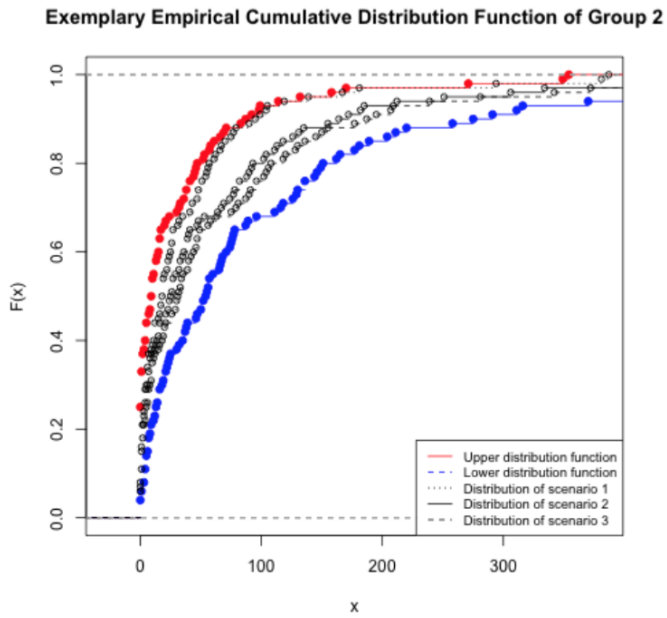
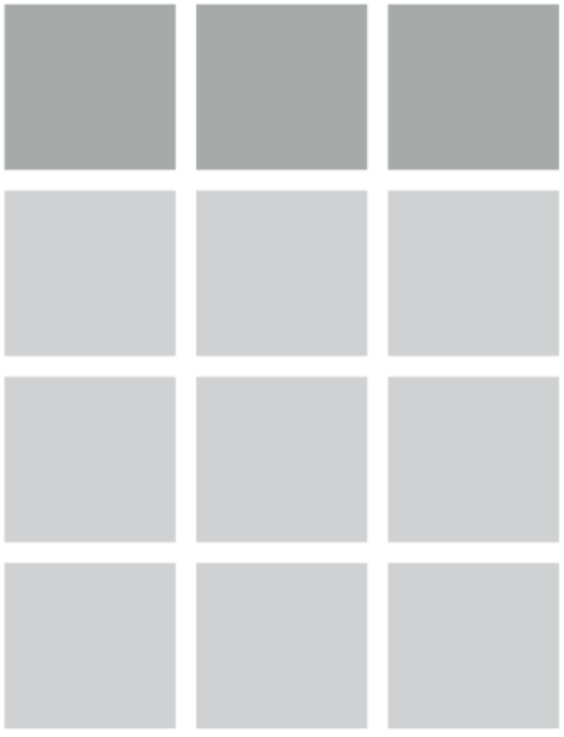


Figure: Lower and upper cumulative distribution function of simulated gene 3 for group 2 using the statistical software R of the R Core Team (2014).

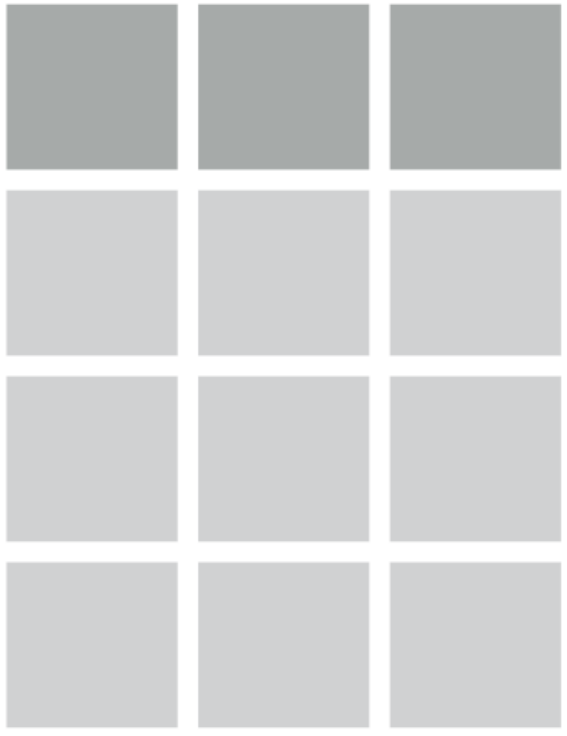
Distorted Simulation Data - No dependence structure

Upper Distribution:



$(n^{(1)} + n^{(2)})$ x m

Lower Distribution:



$(n^{(1)} + n^{(2)})$ x m

Outline

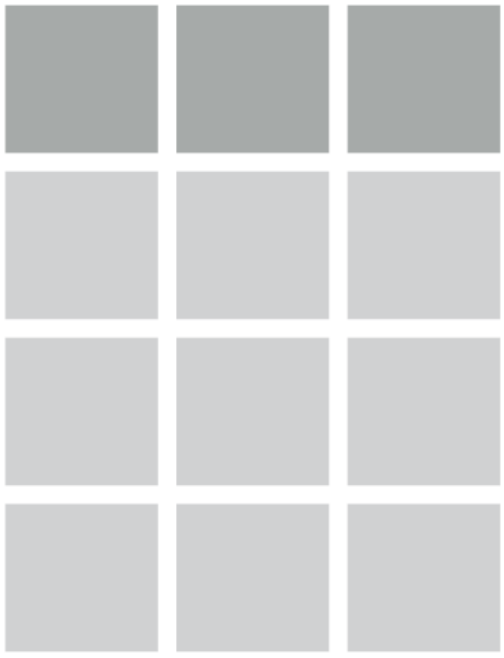
Dependence Structure using Copulas

Sklar (1959) states that one can find a copula function of family v over all marginal distributions, which leads to the joint distribution function that keeps the univariate marginal distributions:

$$F_{\mathbf{X}}^{(g)}(x_1, ..., x_m) = C_v(F_1^{(g)}(x_1), F_2^{(g)}(x_2), ..., F_m^{(g)}(x_m))$$

Undistorted Simulation Data - With dependence structure

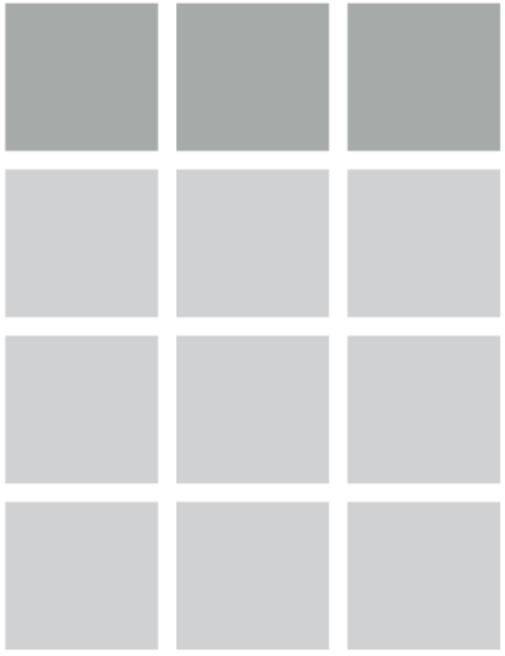
Scenario 1:
Homogenous



$(n^{(1)} + n^{(2)}) \times m$

Gaussian Copula
Clayton Copula
Frank Copula

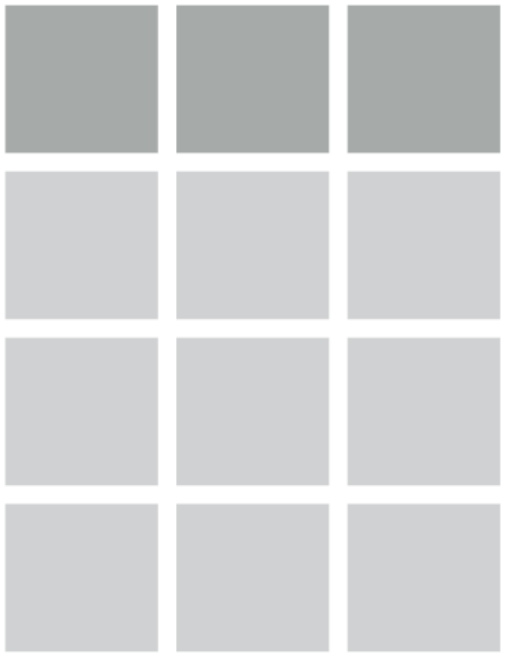
Scenario 2:
Transition



$(n^{(1)} + n^{(2)}) \times m$

Gaussian Copula
Clayton Copula
Frank Copula

Scenario 3:
Heterogeneous



$(n^{(1)} + n^{(2)}) \times m$

Gaussian Copula
Clayton Copula
Frank Copula

Distorted Data with Dependence Structure

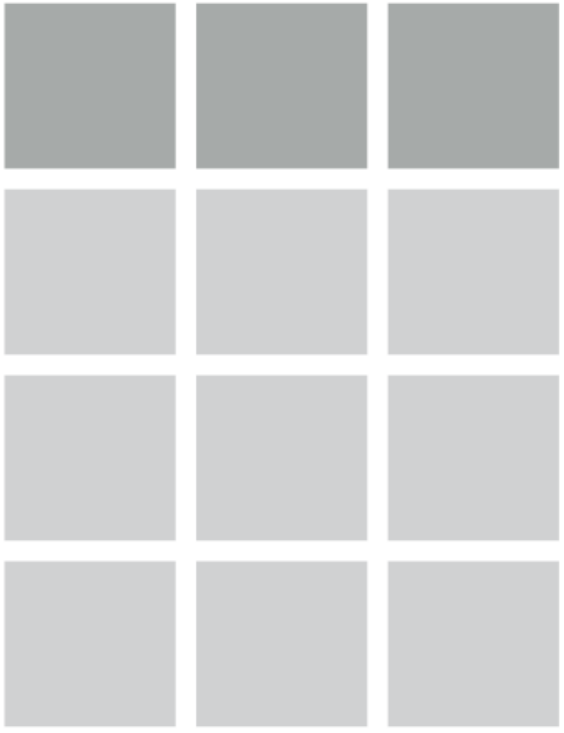
Distorted data are no longer ZINB distributed:

$\Rightarrow$ No parametric marginals anymore
$\Rightarrow$ Computation of upper and lower cumulative distribution function in order to sample from the joint distribution, keeping the same marginals:

$$\underline{\hat{F}}^{(g)}_{\mathbf{X}}(x_1, ..., x_m) = C_v(\underline{\hat{F}_1}^{(g)}(x_1), \underline{\hat{F}_2}^{(g)}(x_2), ..., \underline{\hat{F}_m}^{(g)}(x_m))$$
$$\overline{\hat{F}}^{(g)}_{\mathbf{X}}(x_1, ..., x_m) = C_v(\overline{\hat{F}_1}^{(g)}(x_1), \overline{\hat{F}_2}^{(g)}(x_2), ..., \overline{\hat{F}_m}^{(g)}(x_m))$$

Distorted Simulation Data - With dependence structure

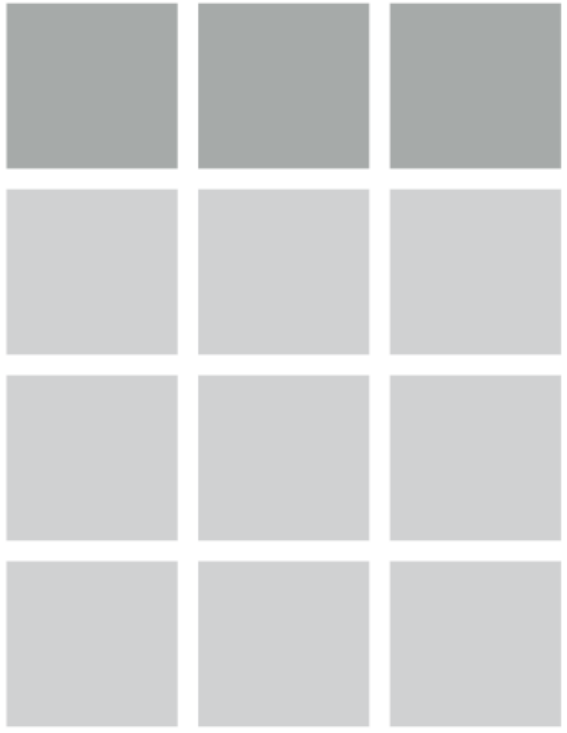
Upper Distribution:



$(n^{(1)} + n^{(2)})$ x m

Gaussian Copula
Clayton Copula
Frank Copula

Lower Distribution:



$(n^{(1)} + n^{(2)})$ x m

Gaussian Copula
Clayton Copula
Frank Copula

Outline

Results of the application

Undistorted data:

- Classification improvement with a higher number of genes

Distorted data:

- Upwards distorted (Lower Distribution):
  A lot of variation possible due to ($\mathbb{W} \in [0, \infty)$)
  $\Rightarrow$ Easier distinctions of the target groups
- Downwards distorted (Upper Distribution):
  Less variation possible due to $\mathbb{W} \in [0, \infty)$
  $\Rightarrow$ Difficult distinctions of the target groups
- Upwards distortion results in better accuracy than downwards distortion

Discussion

Intention of simulation data:

- Reflection of measurement error of an instrument
- Allowance for calibration of measuring instruments in the appropriate direction (Current state-of-the-art: tends to miss low read counts)

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