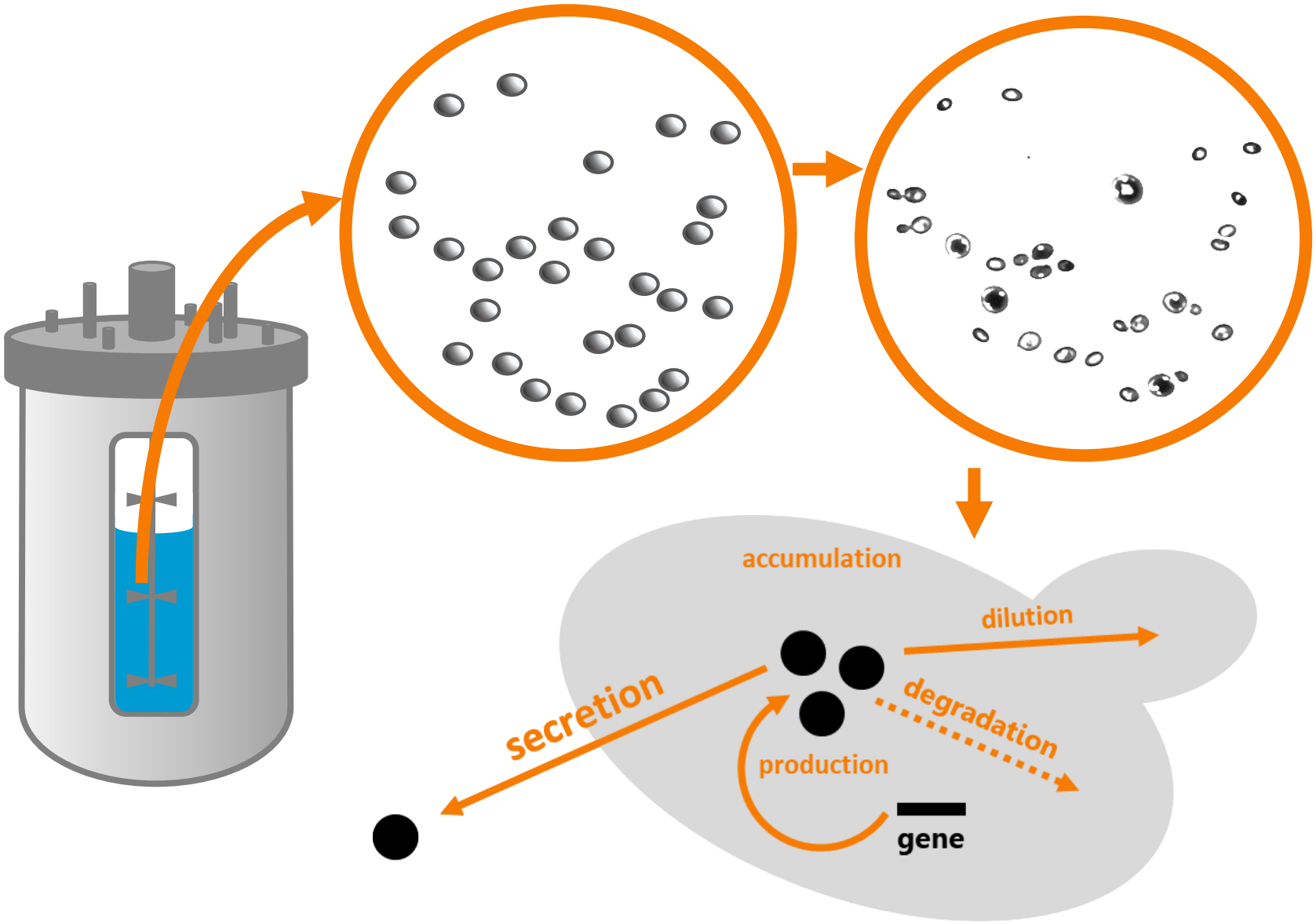
UNIVERSITY OF CHEMISTRY AND TECHNOLOGY PRAGUE

Heterogeneity of microbial populations in bioprocesses

Clusters with different behaviour patterns develop within microbial populations during bioprocesses. (How) can biotechnologists handle the „rebels“?

Hana Raschmanová
Department of Biotechnology
UCT Prague

4th PhD_Post.doc meeting
06.06.2019



Relevant information can be lost in conventional and average-based process analysis.

Outline

- Sources of heterogeneity
- Troubles linked to intra-population heterogeneity
- Techniques for detection of heterogeneity (bioreactors)
- Approaches to eliminate heterogeneity in bioprocesses

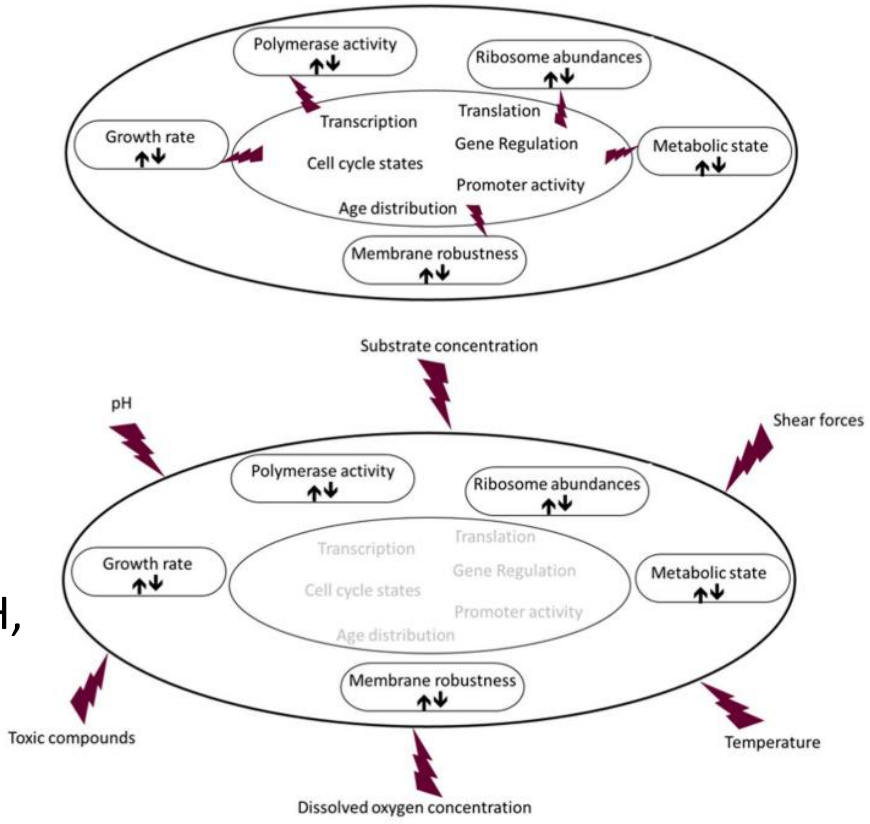
Sources of heterogeneity

Intrinsic

- Not caused by environmental conditions (also in homogenous environments)
- Differences in gene expression and metabolism (cell cycle, age)

Extrinsic

- Caused by the **fluctuation of environmental conditions (large scale!)**
- Spatial and temporal gradients (T, pH, nutrients, oxygen)
- Stochasticity of metabolic state, growth rate

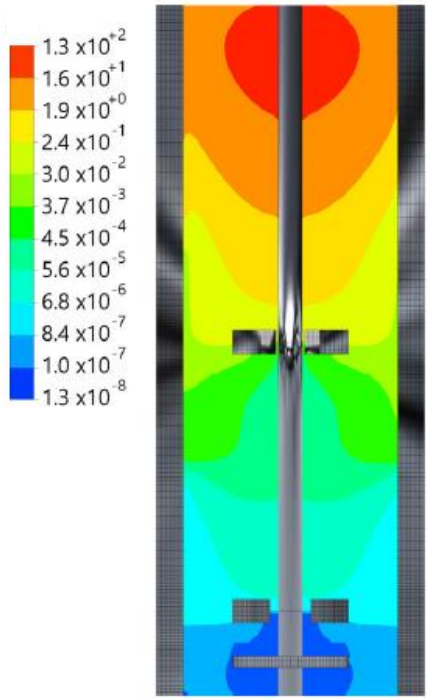


(Heins and Weuster-Botz 2018)

Sources of heterogeneity

Extrinsic

- e.g. cultivation of *Penicillium chrysogenum* in industrial-scale bioreactor (54 m^3, two Rushton turbines, n = 1.63 s^{-1}, constant feeding rate)



Substrate zone	% volume	Substrate concentration (µM)
Depletion	55-60	3.5×10^{-3}
Limitation	30-40	34.7
Excess	5-10	294

(*Haringa, Tang et al. 2016*)

Troubles linked to intra-population heterogeneity

- Different states than the desired productive phenotype
- Subpopulations – different rates of growth/ production/ secretion, non-producing/ non-secreting (up to 60%), non-viable
- Calculations and modelling for the whole population – do not actually fit the single cells → „over-fed“ and „under-fed“ cells)
- Toxic product – high-producers may die

→ **decreased productivity**

„…**quantitative single-cell analysis** promises to give insight into the cell dynamics occurring during large-scale bio-process operation and **may lead to significant process improvements**."

(Demling, Westerwalbesloh et al. 2018)

„…**enormous potential** of single cell-based control strategies **for bioprocessing**."

(Delvigne, Baert et al. 2017)

„…**population heterogeneity** and its underlying mechanism needs to be better understood before we can use it as a fitness advantage or function **to make bioprocesses** more **robust** or to totally eliminate it from bioprocesses to **avoid losses in yield**…"

(Heins and Weuster-Botz 2018)

„…**industry** needs to be able to **engineer heterogeneity** to obtain **better yields** and more **robust processes**. This requires both quantitative evaluation of the change of **individual cells** in time and of their interaction with the environment…"

(Gonzalez-Cabaleiro, Mitchell et al. 2017)

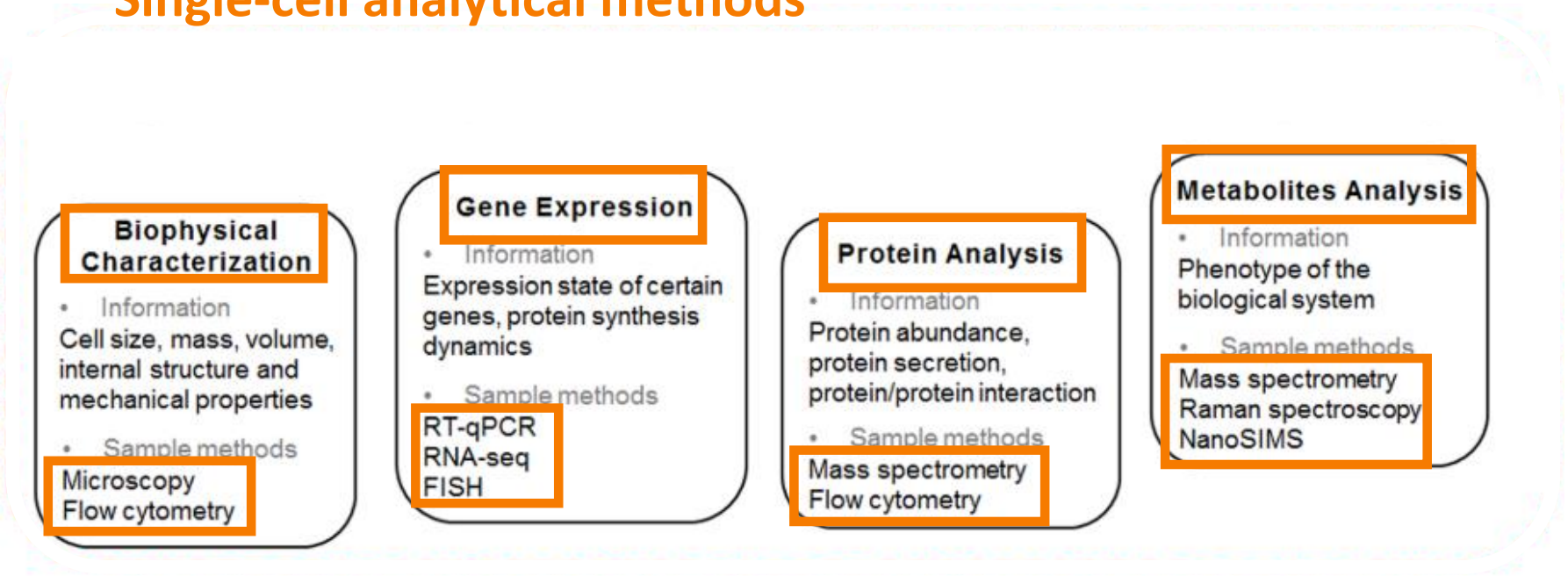
But *how* ?

Techniques for detection of heterogeneity

- Reporter strains (biosensors)/ fluorescent dyes
  - e.g. promoter-based biosensors – fluorescent tags
  - Stress, metabolic activity, viability, metabolite production, cell cycle, cell age…
- Suitable **single-cell** technique for detection (e.g. flow cytometry, microscopy)

Techniques for detection of heterogeneity

Single-cell analytical methods

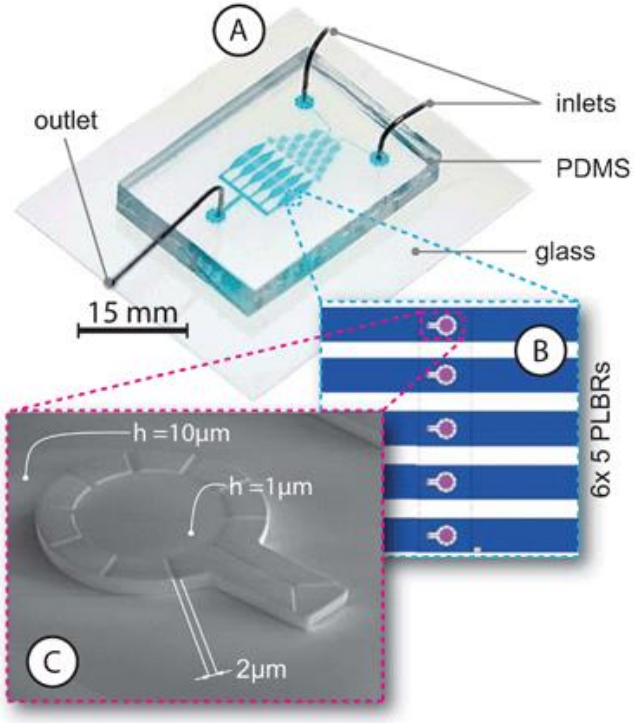


(Gonzalez-Cabaleiro, Mitchell et al. 2017)

Techniques for detection of heterogeneity

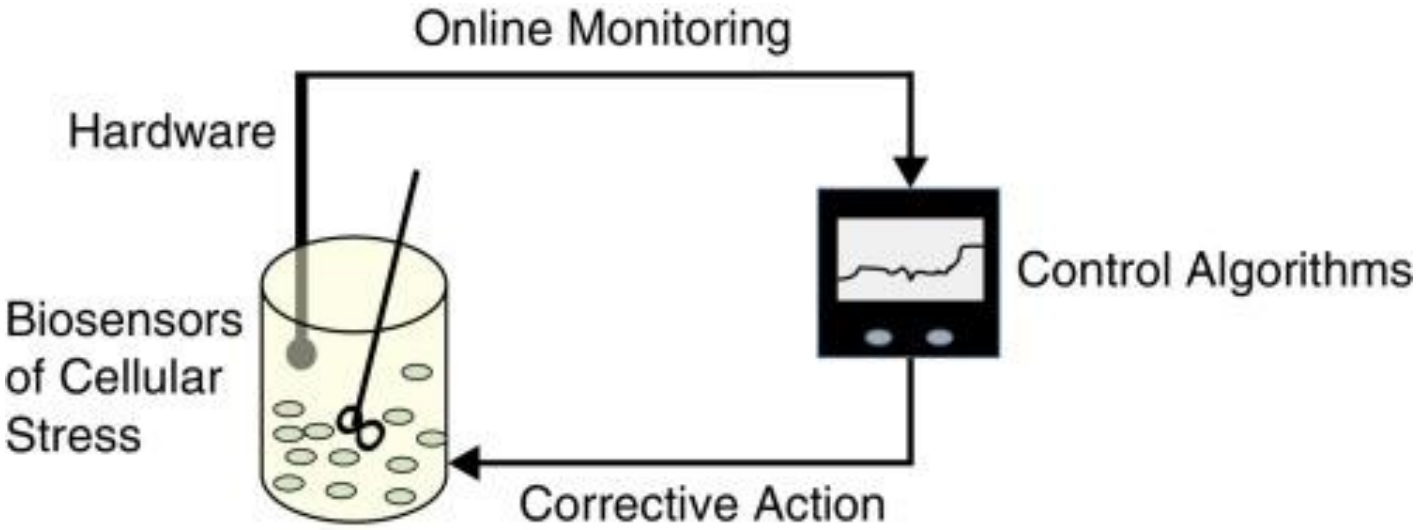
Microfluidic single-cell analysis (lab on chip devices)

- Microfluidic cultivation device (to pL) + single-cell analysis on one chip
- Precisely controlled environmental conditions
- To unravel mechanisms in population and environmental heterogeneity
- Growth and production kinetics of single cells
- Massive parallelisation, high-throughput
- Basic research, bioprocess development, strain characterization



(Grunberger, Paczia et al. 2012)

Techniques for detection of heterogeneity in bioreactors



(Polizzi and Kontoravdi 2015)

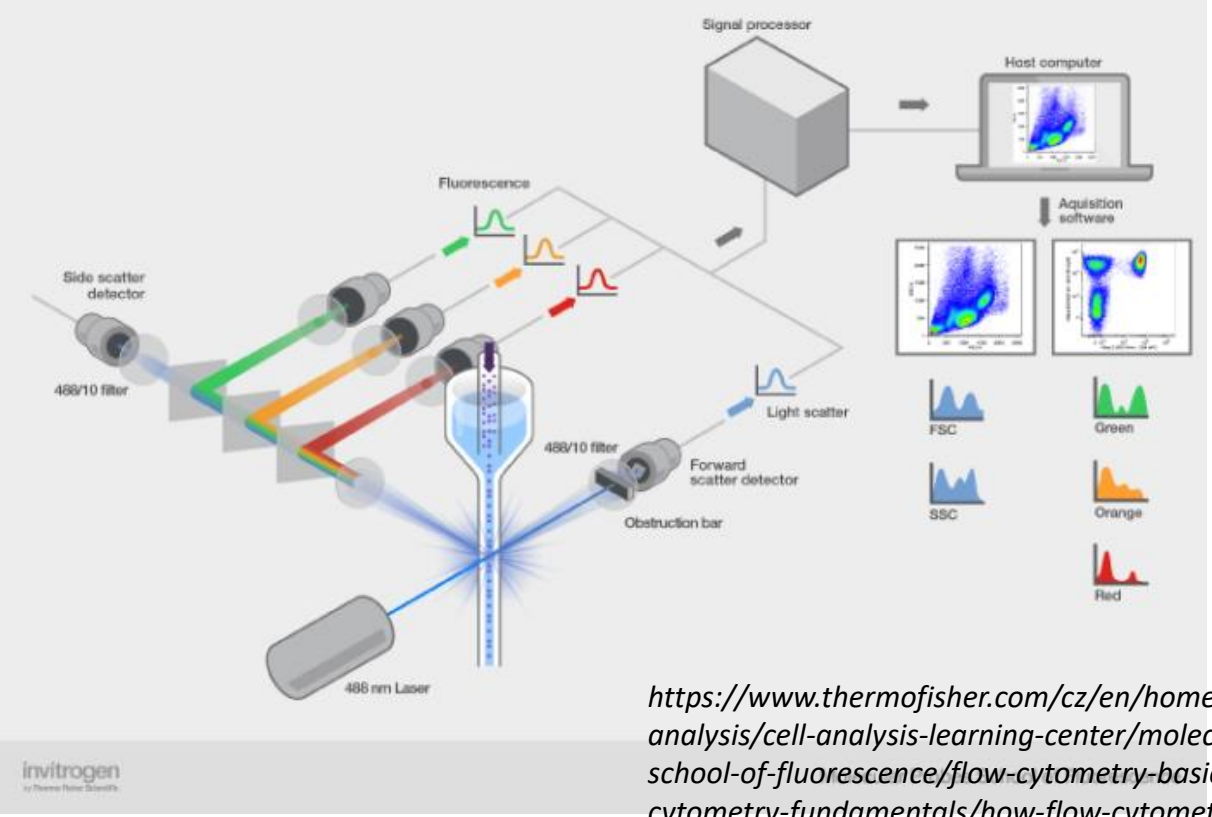
Techniques for detection of heterogeneity in bioreactors

Challenges & troubles

- **Technical adaptation** of single-cell methods for microbial bioprocesses – automation, short processing time, high-throughput
- **Software** – automatic acquisition of the analysis, data interpretation
- **Knowledge, mathematical models** – how to integrate data in control loops
- **Non-desired alterations** of industrial strains and processes – e.g. fluorescent protein markers in the strains, improvisation in process operation

Techniques for detection of heterogeneity in bioreactors

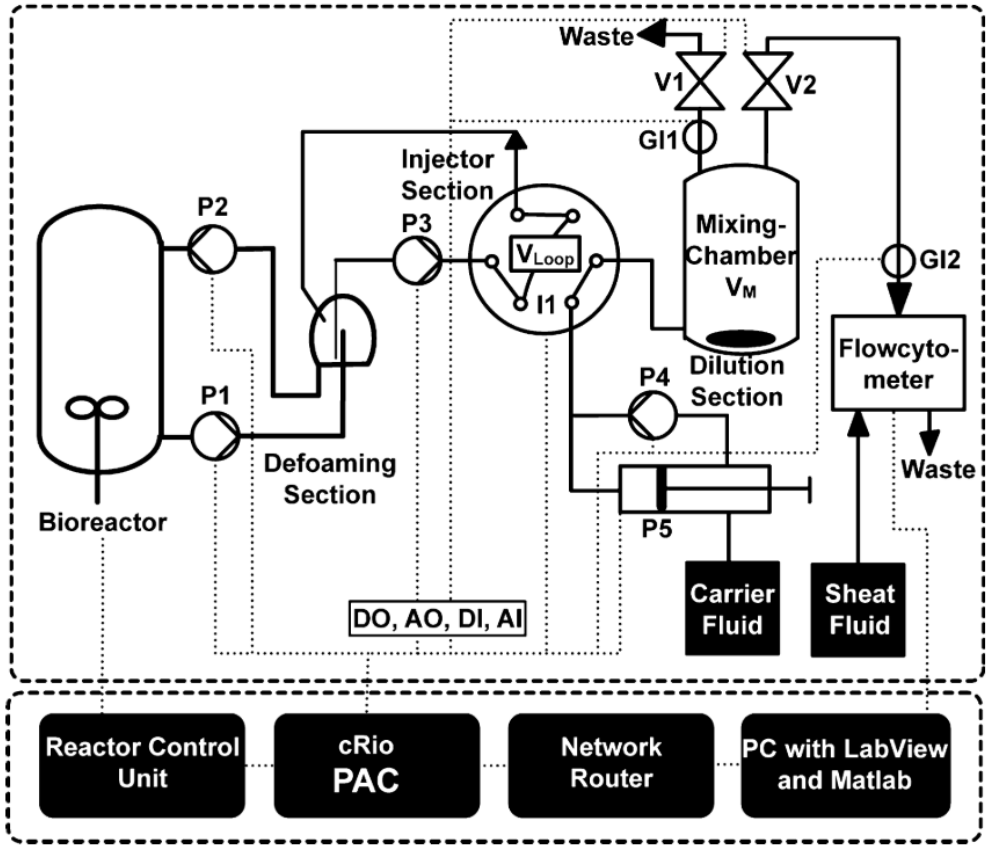
Flow cytometry



https://www.thermofisher.com/cz/en/home/life-science/cell-analysis/cell-analysis-learning-center/molecular-probes-school-of-fluorescence/flow-cytometry-basics/flow-cytometry-fundamentals/how-flow-cytometer-works.html

Techniques for detection of heterogeneity in bioreactors

Automated flow cytometry (laboratory setups)



(Broger, Odermatt et al. 2011)

Techniques for detection of heterogeneity in bioreactors

Automated flow cytometry (commercially available interface)

https://www.securecell.ch/en/process-analytics_(PAT)/Numera.html

Techniques for detection of heterogeneity in bioreactors

Automated flow cytometry (laboratory setups)

- **Real-time monitoring** of bioprocess
  - Production of GFP
  - Production of GFP-tagged proteins
  - Viability
  - Cell concentration
  - Cell size
- **Loop control**
  - Optogenetic control of GFP expression
  - Maintaining feed rate according to cell count

- **Utilization of single-cell information?**

significant process improvements

enormous potential for bioprocessing

to make bioprocesses robust

But *how* ?

to avoid losses in yield

better yields

We can analyse it.
We know about it.
So what next?

Loop control – at the whole population level
At single-cell level?
Autoregulation = mending?
Series of bioreactors?
Cell sorting?

Approaches to eliminate heterogeneity in bioprocesses

Strain development

- Appropriate combination strain-expression cassette
- Gene deletions → increased tolerance to environmental fluctuations
- Single-cell-based screening (+sorting) of stable/over-producing clones

Process development and optimization

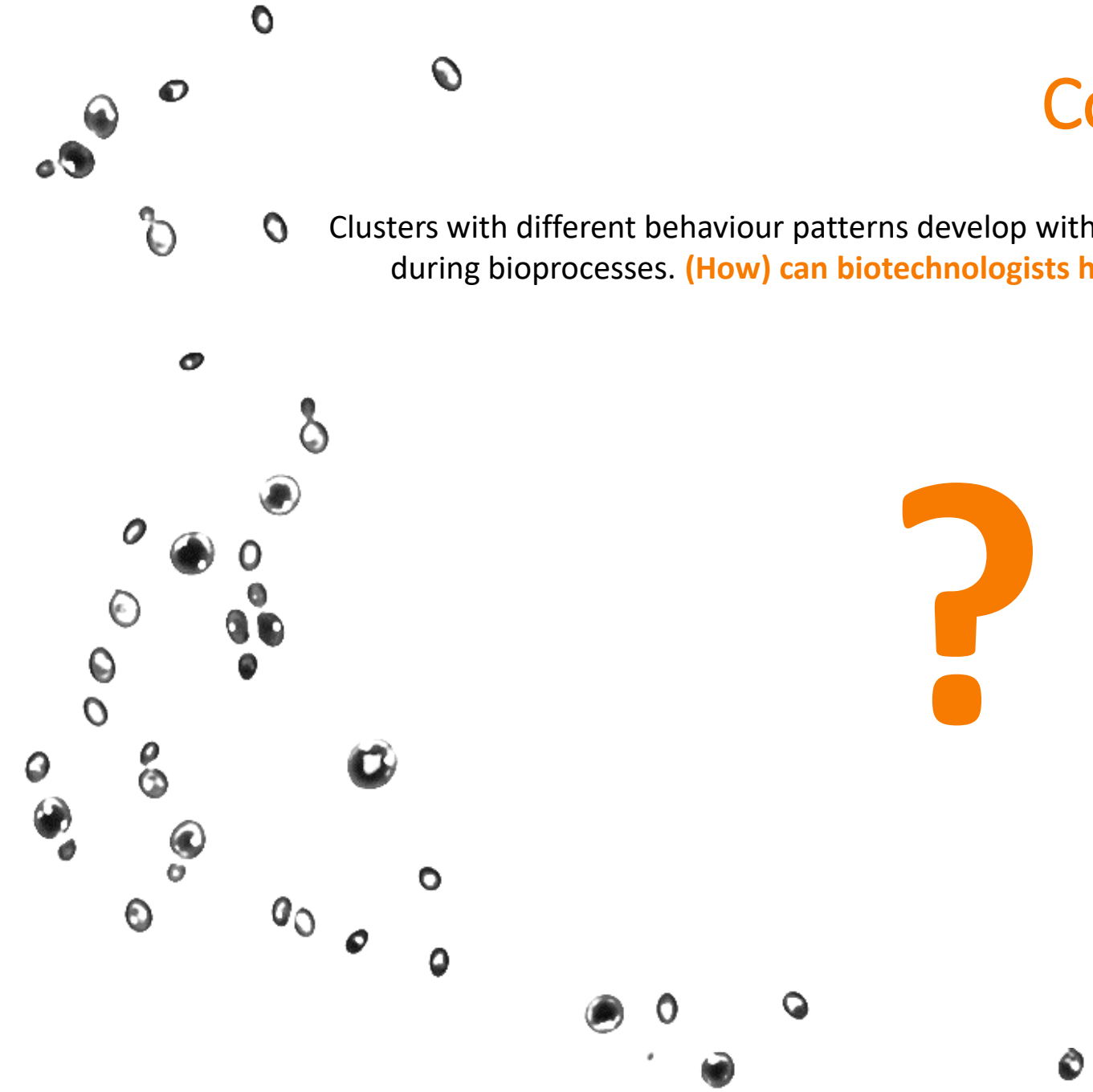
- Appropriate cultivation conditions (microfluidics)

Process monitoring & control

- Control of substrate feeding or aeration according to fluorescence signal (dye Redox Sensor Green)
- Control of product accumulation by addition of production inducer (fluorescent fusion partner?)

Conclusions

- Clusters with different behaviour patterns develop within microbial populations during bioprocesses. **(How) can biotechnologists handle the „rebels“?**



References

- Demling, P., C. Westerwalbesloh, S. Noack, W. Wiechert and D. Kohlheyer (2018). "Quantitative measurements in single-cell analysis: towards scalability in microbial bioprocess development." Curr Opin Biotechnol **54**: 121-127.
- Delvigne, F., J. Baert, H. Sassi, P. Fickers, A. Grunberger and C. Dusny (2017). "Taking control over microbial populations: Current approaches for exploiting biological noise in bioprocesses." Biotechnology Journal **12**(7): 17.
- Gonzalez-Cabaleiro, R., A. M. Mitchell, W. Smith, A. Wipat and I. D. Ofiteru (2017). "Heterogeneity in Pure Microbial Systems: Experimental Measurements and Modeling." Frontiers in Microbiology **8**: 8.
- Heins, A. L. and D. Weuster-Botz (2018). "Population heterogeneity in microbial bioprocesses: origin, analysis, mechanisms, and future perspectives." Bioprocess and Biosystems Engineering **41**(7): 889-916.
- Polizzi, K. M. and C. Kontoravdi (2015). "Genetically-encoded biosensors for monitoring cellular stress in bioprocessing." Current Opinion in Biotechnology **31**: 50-56.
- Broger, T., R. P. Odermatt, P. Huber and B. Sonnleitner (2011). "Real-time on-line flow cytometry for bioprocess monitoring." J Biotechnol **154**.
- Haringa, C., W. J. Tang, A. T. Deshmukh, J. Y. Xia, M. Reuss, J. J. Heijnen, R. F. Mudde and H. J. Noorman (2016). "Euler-Lagrange computational fluid dynamics for (bio)reactor scale down: An analysis of organism lifelines." Engineering in Life Sciences **16**(7): 652-663.
- Grunberger, A., N. Paczia, C. Probst, G. Schendzielorz, L. Eggeling, S. Noack, W. Wiechert and D. Kohlheyer (2012). "A disposable picolitre bioreactor for cultivation and investigation of industrially relevant bacteria on the single cell level." Lab on a Chip **12**(11): 2060-2068.