

A close-up photograph of a laboratory workspace. In the foreground, several clear plastic microcentrifuge tubes are visible, some containing a pink liquid. A black pipette tip is positioned near the tubes. The background shows more laboratory equipment and containers, slightly out of focus.

Additional value of cell differentiation in the course of DHI testing

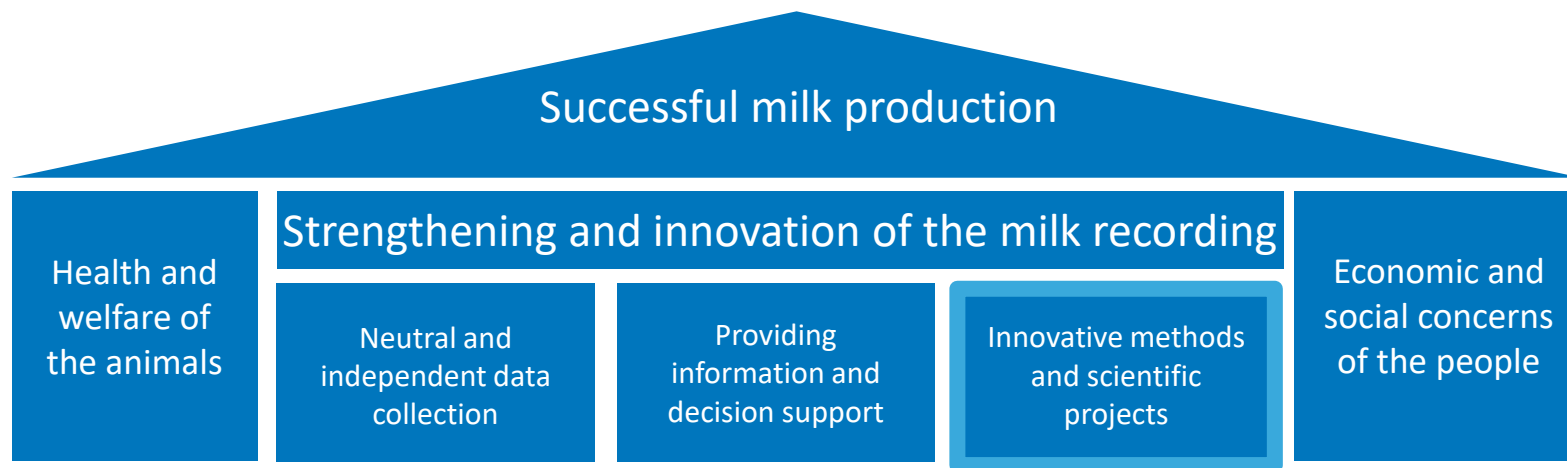
F. Onken¹, E. Gaß¹, A. Bartel², C. Baumgartner³, F. Querengässer², M. Doherr²

¹ German Association for Performance and Quality Testing

² Institute for Veterinary Epidemiology and Biostatistics, Free University Berlin

³ Bavarian Association for raw milk testing

- German Association for Performance and Quality Testing
- Group of milk recording organizations, associations for raw milk testing and the national calculation center vit



Objective of project:

Integration of cell differentiation information into the DHI testing

- New parameter CDI (cell differentiation index)
- Prognosis of udder health
- Udder health report



Innovation

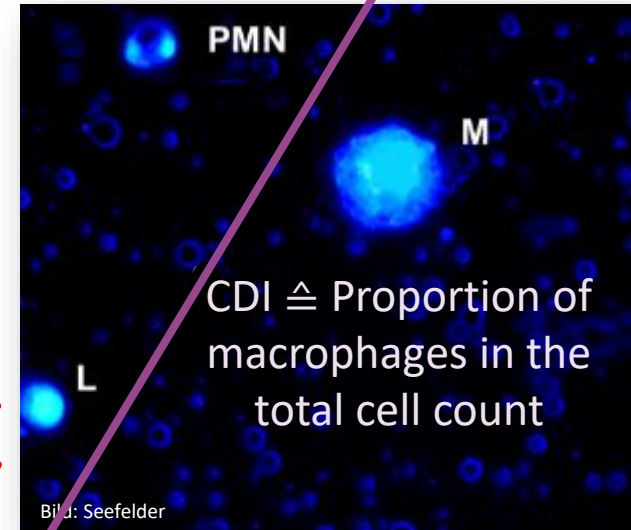


Development



Added value

- Somatic cells in the milk
 - Polymorphonuclear neutrophils (PMN)
 - Macrophages (M)
 - Lymphocytes (L)
- Healthy udder → Total cell count ↓
Macrophages ↑
- Inflammation → Total cell count, PMN ↑
Macrophages ↓



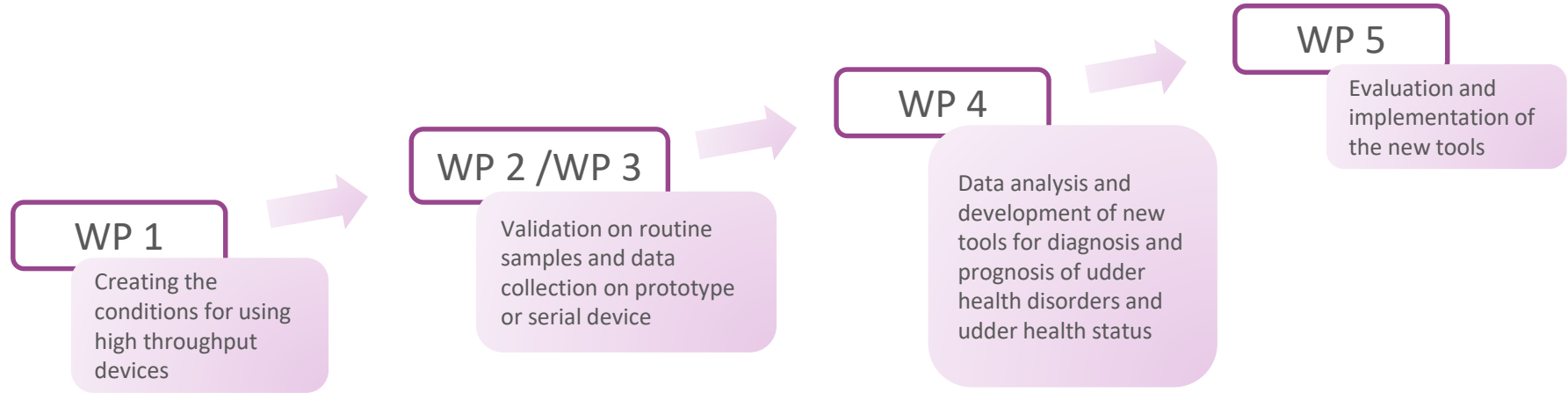
So far

- Microscopic or flow cytometric implementation
 - Small scale
 - Research on physiological processes
 - Quarter milk samples
-
- Project *milch* **Q** *plus* 2012 – 2016 → Initial work on small-scale cell differentiation to examine the possibilities under the prevailing conditions (e.g. conservation)
-
- For routine use in the context of milk recording, a high throughput method is required

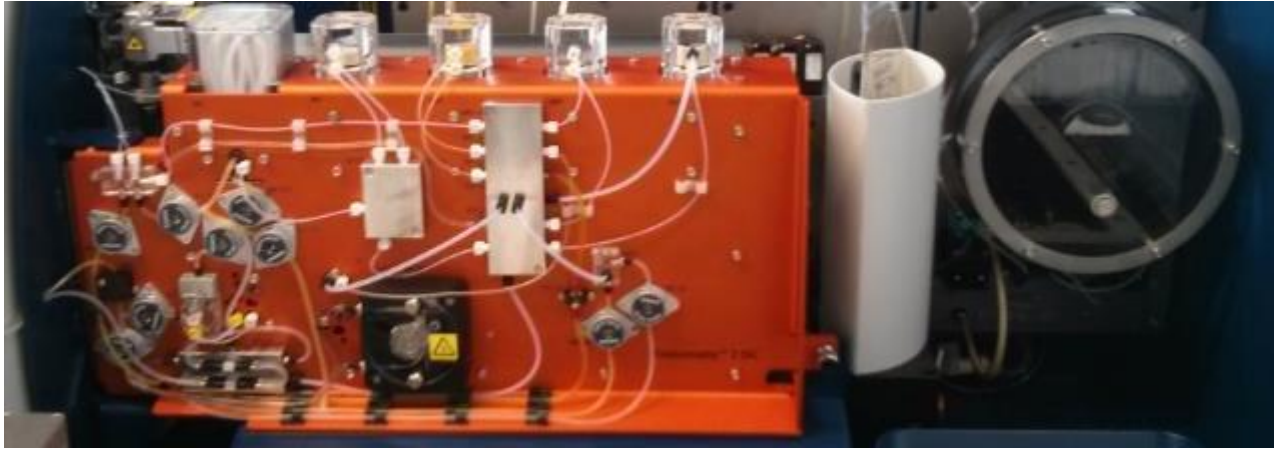


Work packages

- Kickoff ZellDiX in April 2016



- First prototype for cell differentiation at mpr in January 2017
- First serial device for cell differentiation at mpr in November 2017
- Five series units in total at the mpr since January 2018
- Further installations in Germany



- Bavaria: Routine DHI samples
- Berlin-Brandenburg: Routine DHI and quarter milk samples
- North Rhine-Westphalia: Routine DHI samples
- Weekly samples on a smaller scale (from other projects within the DLQ)

→ Data analysis by FU Berlin

Bavaria, as of Jan. 2019

	Number
CDI measurements	9.260.894
Farms	17.259
Farm size	Median: 58 Tiere
Cows overall	743.315
Simmental	571.578
Brown Swiss	87.841
Holsteins bw	62.675
Holsteins red	16.953
Other	4.268

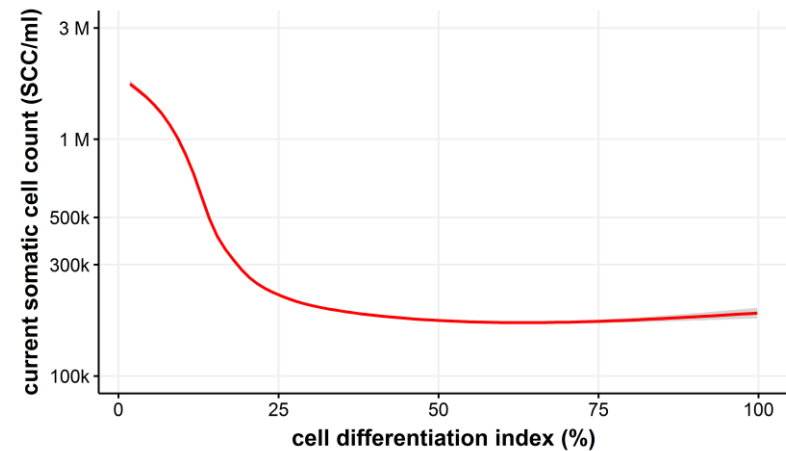
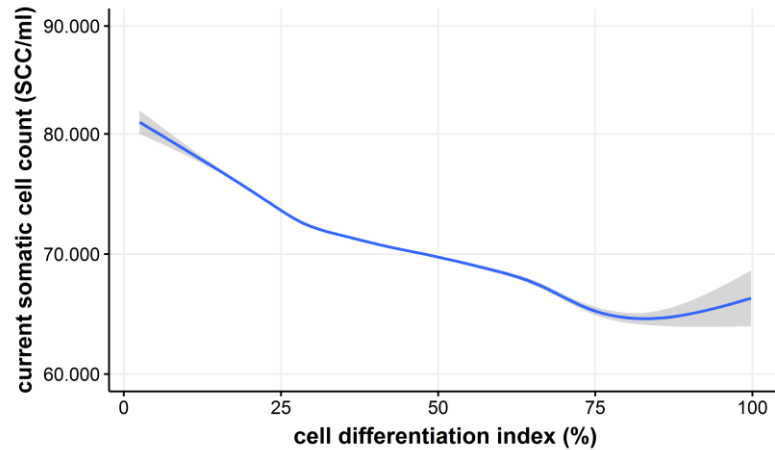
North Rhine-Westphalia, as of Jan. 2019

	Number
CDI measurements	979.303
Farms	1.901
Farm size	Median: 114 Tiere
Cows overall	180.259
Simmental	8.228
Brown Swiss	956
Holstein bw	134.971
Holstein red	25.616
Other	10.488

- How do CDI and somatic cell count correlate?
- Can the future trajectory of the cell count be estimated?
- For which animals are prognoses interesting?
 - < 100.000 SCC/ml \rightarrow Stable udder health?
 - > 100.000 SCC/ml \rightarrow Sustained SCC increase?



Data analysis

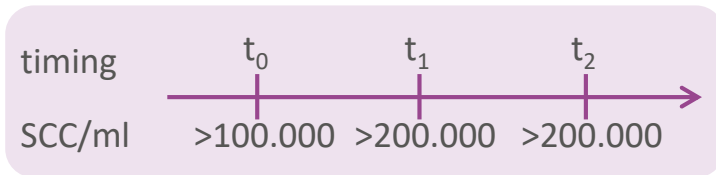
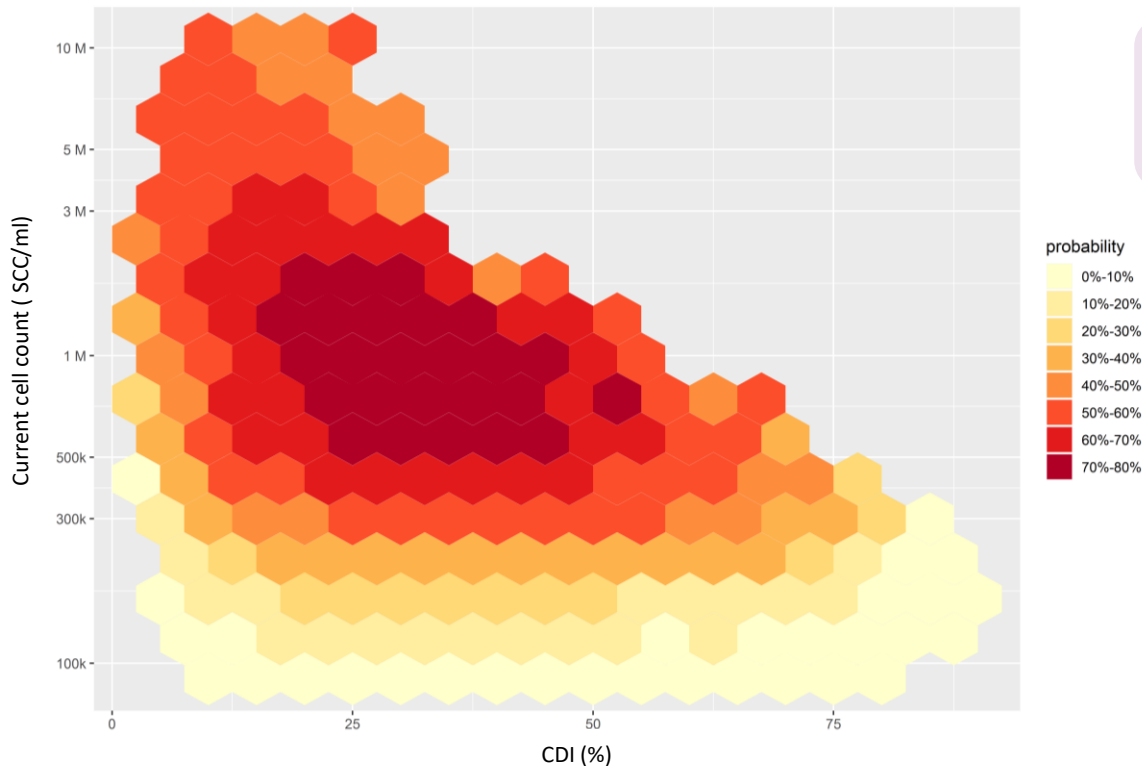


→ The higher the cell count, the lower the CDI

• Healthy udder	→	Total cell amount ↓ Macrophages ↑
• Inflammation	→	Total cell amount, PMN ↑ Macrophages ↓

Prognosis

Presentation by heat map: probability of a chronic event



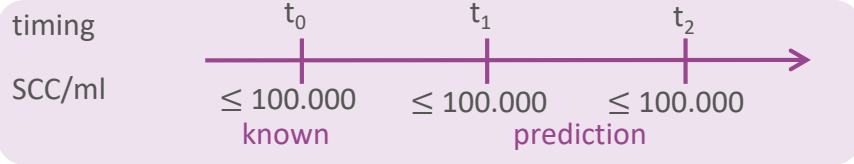
- Different probabilities depending on the CDI
- The range 600,000 - 3 million is particularly interesting

Interim conclusion

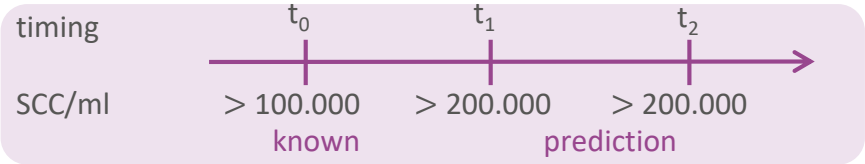
- The complex interaction of the CDI with the cell count must be taken into account
 - In models for persistent cell count elevations, the CDI can provide new information about udder health
 - The interpretation of the CDI as a pure numerical value is not possible
- The information needs to be contextualized for the user (models)

- „Stable model“
 - Initial situation at time t_0 : < 100.000 SCC/ml
 - Probability of cell counts < 100.000 SCC/ml over the next two months
 - Variables in the model: interaction between cell count and CDI, milk yield and DIM interaction, age, f/p ratio, lactose, percentage of udder healthy cows, and rate of new infections
- „Chronic model“
 - Initial situation at time t_0 : > 100.000 SCC/ml
 - Probability of increased cell count in the next two months (> 200 ; 400 ; 700.000 SCC/ml)
 - Same variables (see above), different weighting

„Stable model“



„Chronic model“

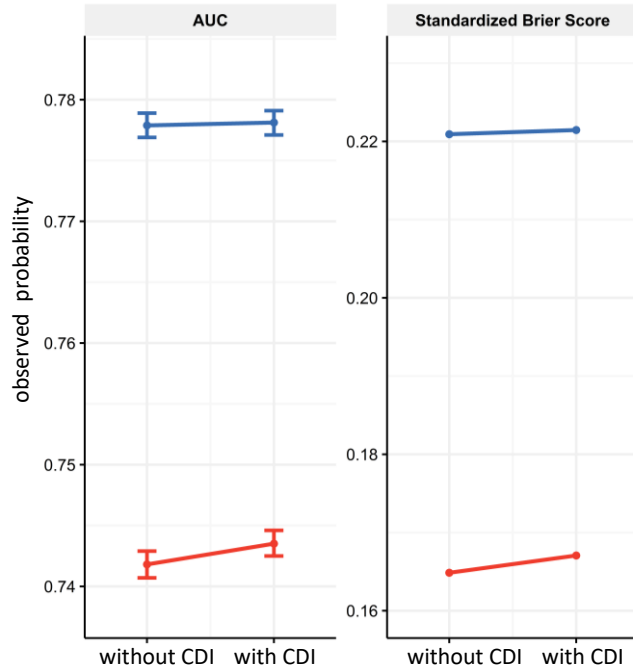
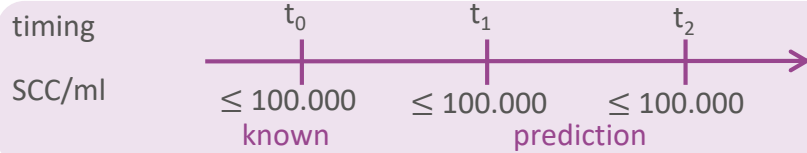


Added value of the CDI in the model?

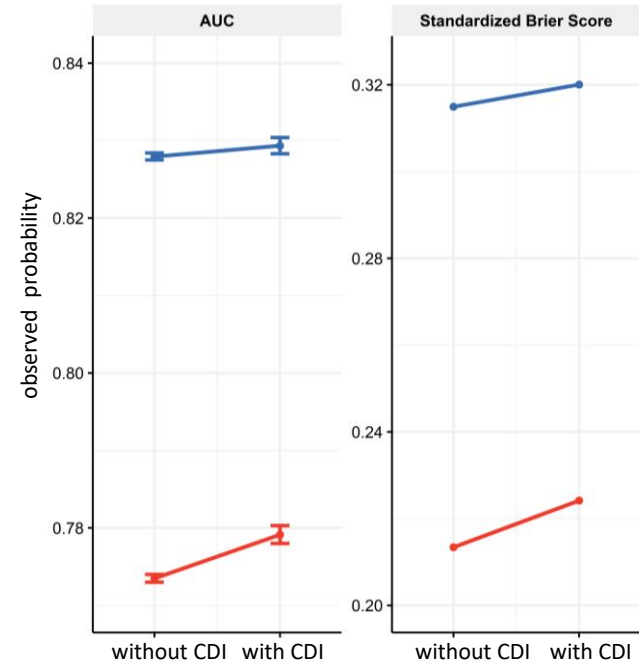
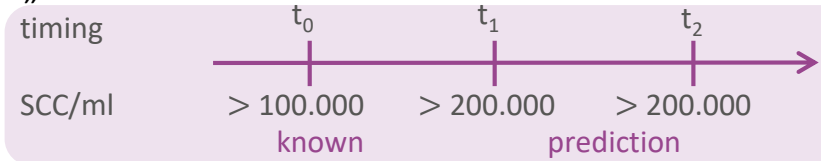
- Comparison of the model quality (AUC and Standardized Bier Score)
- Simple model: total cell count **with/without** CDI
- Extended model: total cell count, additional milk control parameters (e.g., milk yield, DIM, age, etc.) **with/without** CDI

Models

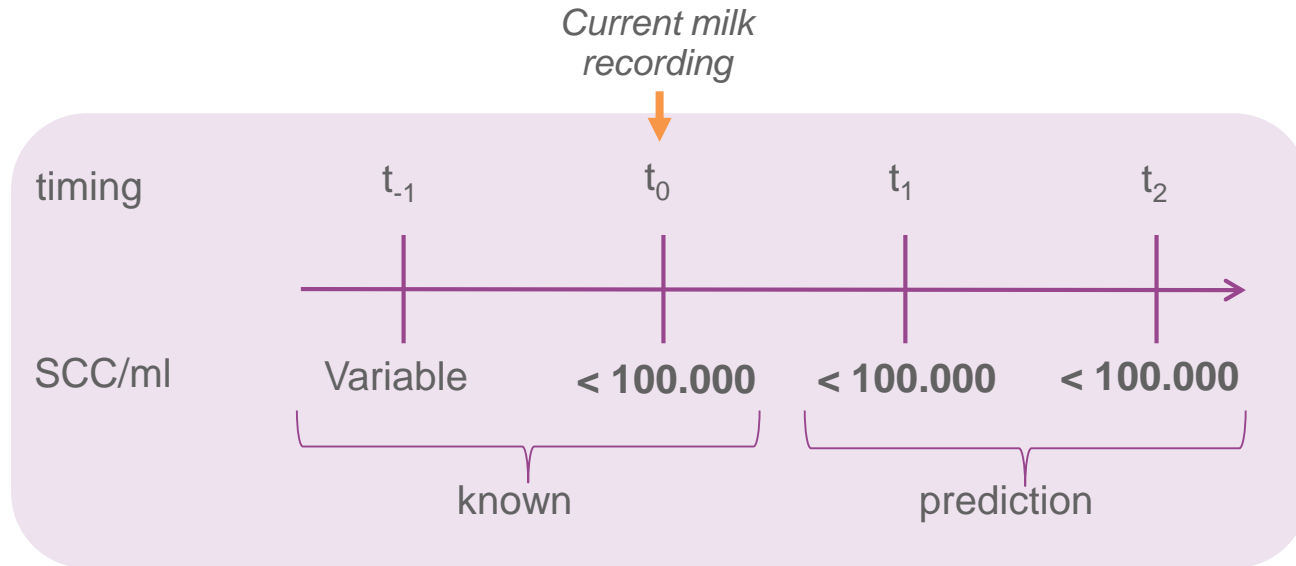
„Stable model“



„Chronic model“



„Stable model“

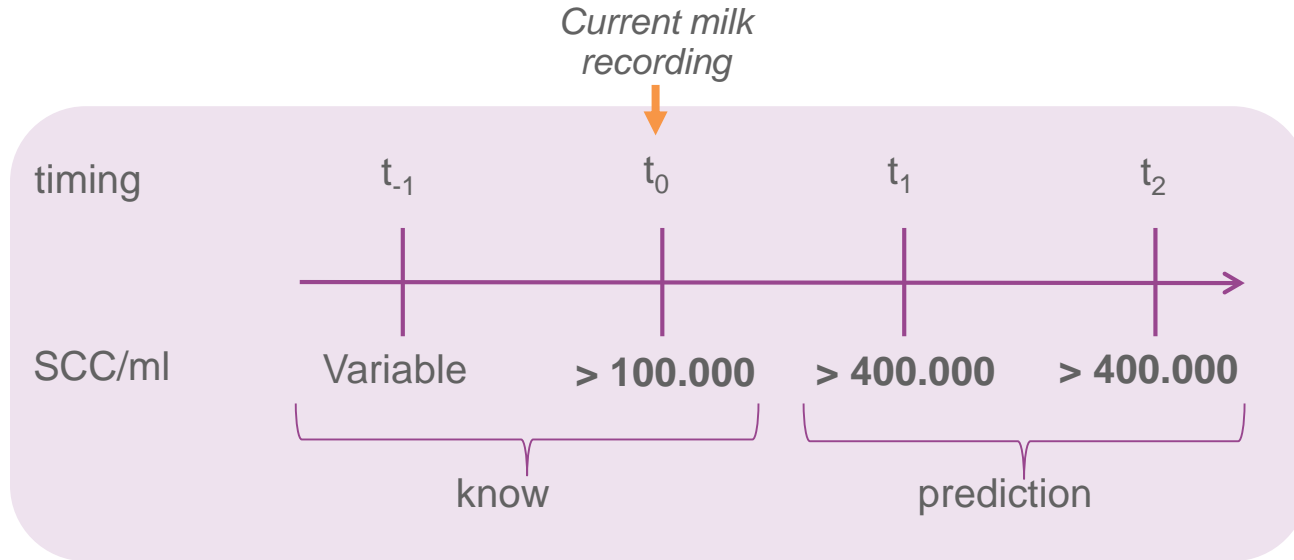


- Individual prediction of whether the cell count will remain below 100,000 SCC/ml in the next two milk recordings
- To be interpreted as resistance to infections
- No pathogen contact = no infection
- It is highly probable that the cow should recover quickly and heal completely after pathogen contact

Examples „stable model“

Lactation number	DIM	Milk yield	last month's SCC	current SCC	stable prediction	Observed SCC next month	Observed SCC second next month
3	154	33,3	9.000	10.000	90,2%	31.000	18.000
1	184	26,6	300.000	18.000	85,4%	22.000	78.000
Short-term elevations							
2	25	42,1	205.000	18.000	87,8%	583.000	43.000
1	186	24,0	111.000	21.000	80,5%	1.597.000	32.000
Sustained elevations							
1	113	19,0	88.000	100.000	39,2%	128.000	446.000
6	73	32,6	225.000	60.000	23,1%	4.997.000	1.034.000

„Chronic model“



- Prediction of whether the cell count will remain above 400,000 SCC/ml in the next two milk recordings
- Support for intervention decision
 - Animals with low risk for sustained SCC don't need intervention
 - Medium risk animals profit from antibiotic treatment (treatment)
 - High risk animals relapse after antibiotic treatment (culling)

Examples „chronic model“

Lactation number	DIM	Milk yield	last month's SCC	current SCC	chronic prediction	Observed SCC next month	Observed SCC second next month
6	88	46,0	3.607.000	2.926.000	74.2%	524.000	4.559.000
6	105	40,8	928.000	612.000	63,5%	577.000	877.000
Low risk							
4	190	44,0	138.000	2.749.000	23,6%	1.483.000	131.000
2	186	27,2	110.000	2.204.000	11,0%	153.000	113.000
2	175	20,4	1.665.000	214.000	8,4%	1.454.000	97.000

- Started May 2018
- Ended Jan 2019
- 6 farms, in total approx. 2,500 animals
- Sampling up to 6 months
- Monthly measurement of DHI samples on FM 7 DC → SCC, CDI
- In parallel: quarter milk samples as reference base → SCC, CDI, bacteriological status (= intramammary infection, IMI)
- Additional information, e.g. diagnoses, treatments

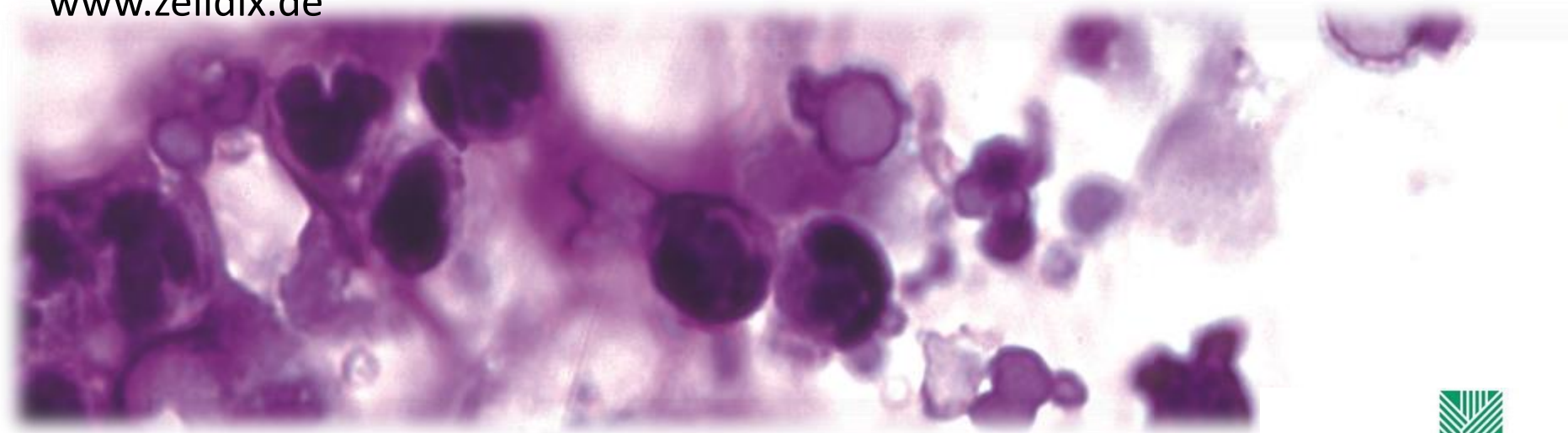
- Cooperation with pilot farms
- Evaluation of the field test
- Elaboration of further information / benefits of the CDI
- International cooperation



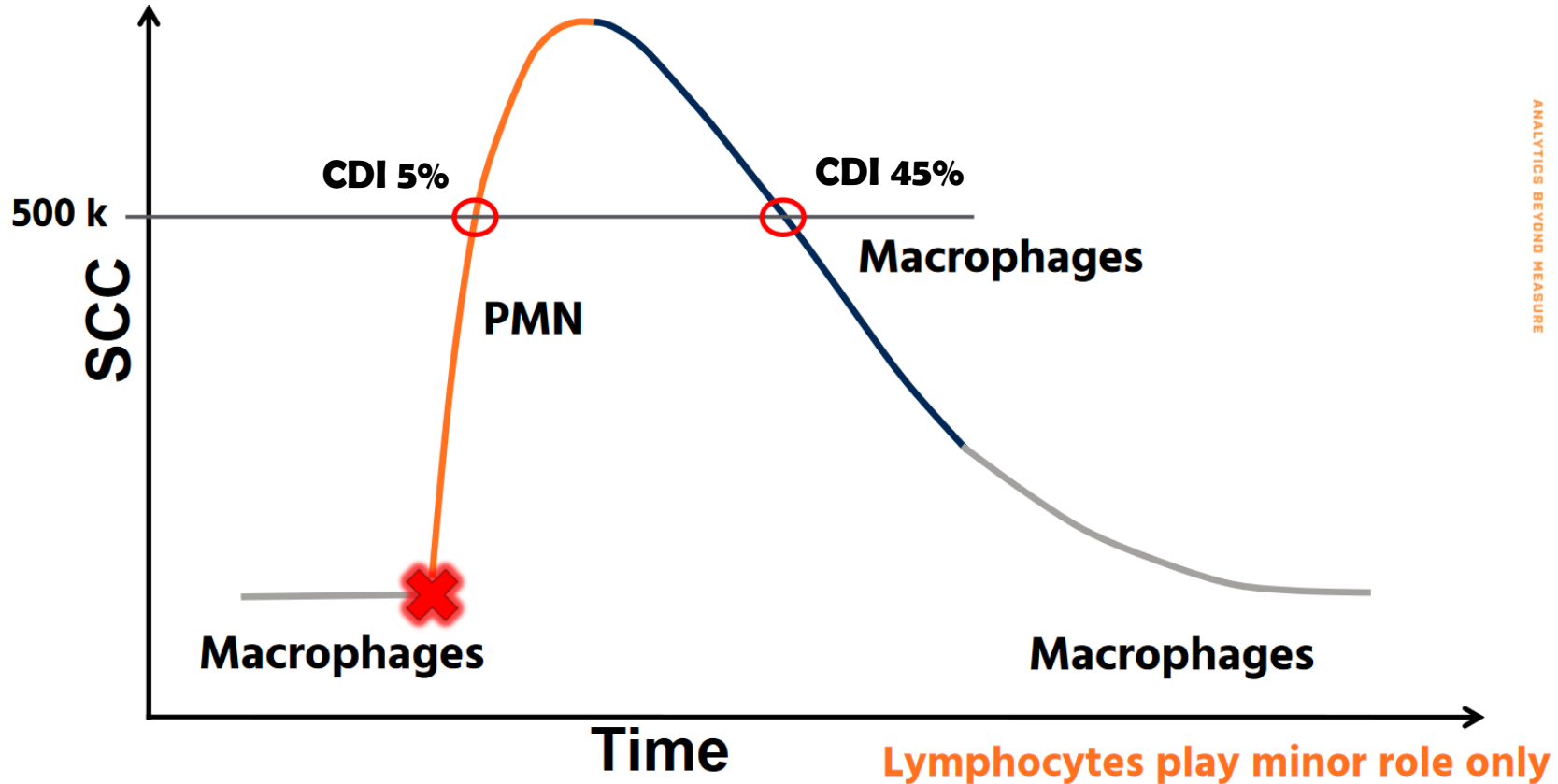
- For the first time, high-throughput devices for measuring cell differentiation were put in operation
- Incomparably large data pool including CDI from the routine
- The pure numerical value of the CDI can not be interpreted
- CDI has added value, but currently this is minimal for large models
- So far establishment of two models for the prediction of the risk at individual animal and farm level

Many thanks for your attention!

www.zelldix.de

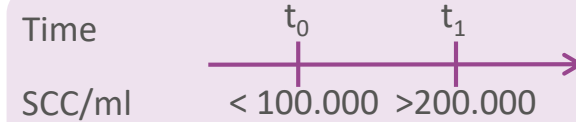
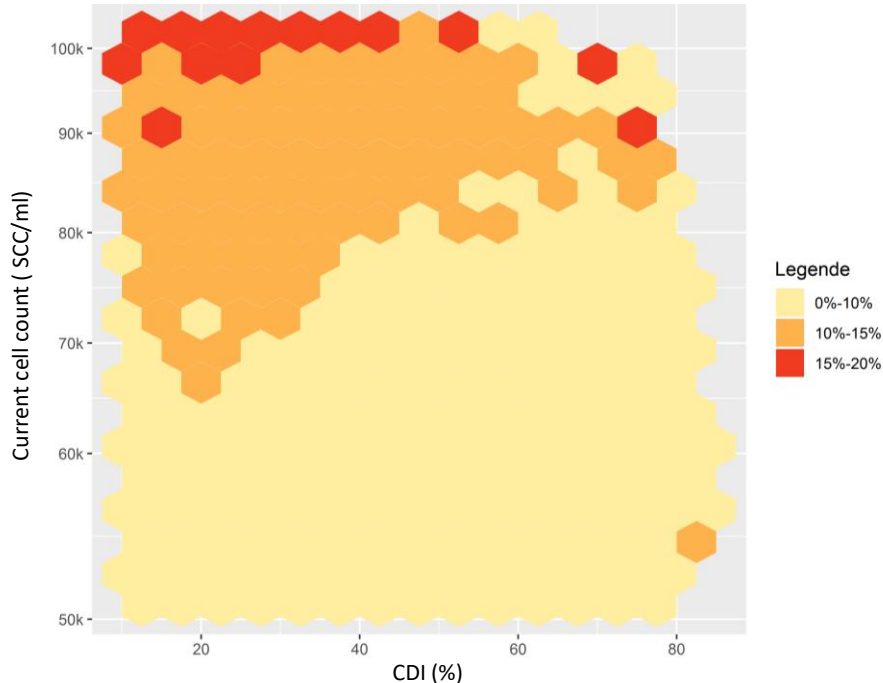


Cell differentiation



Prognosis

Presentation by heat map: probability for new infection



- Low frequency of this situation
- Current cell count already has high significance
- Tendency: Low CDI increases susceptibility to cell count elevation