TUM Data Innovation Lab with cellasys

How to Handle Data from Living Cells

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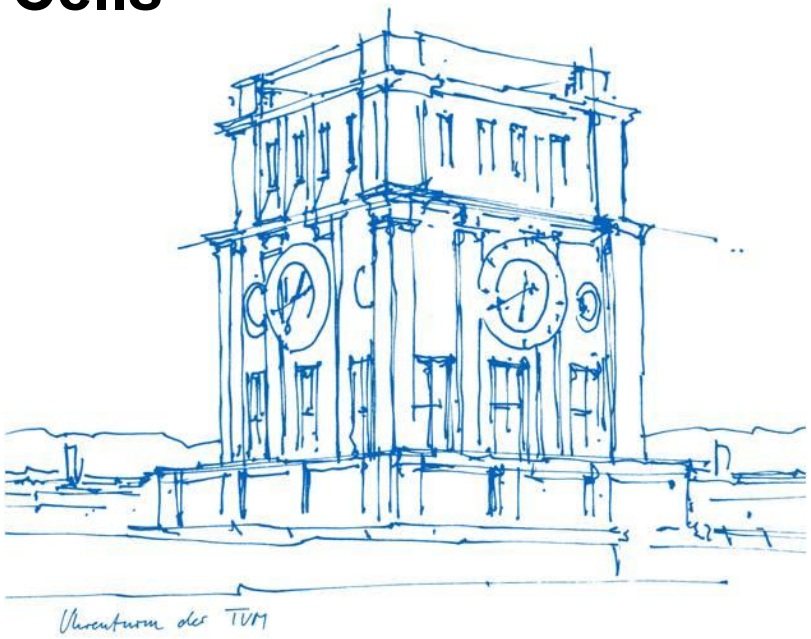
Munich, August 06, 2019



Agenda

1	Introduction
2	Data Collection & Pre-Processing
3	Data Analysis & Results
4	Summary & Conclusion

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1	**Introduction**
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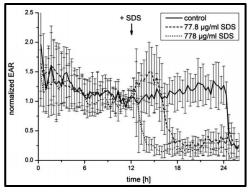
cellasys

Analysis of Living Cells

Microphysiometry

- Novel methodology of **analyzing living cells**
- Interface of **electronic engineering and life sciences** (biology, chemistry)
- Electrochemical and optochemical sensor technology to **record cell metabolism**



- Use of algorithms and models to **draw conclusions** from this raw data:
  → prediction models for toxicological effects
  → development of new drugs

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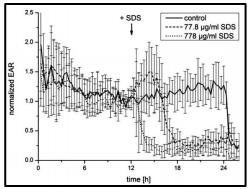

- Use of algorithms and models to **draw conclusions** from this raw data:
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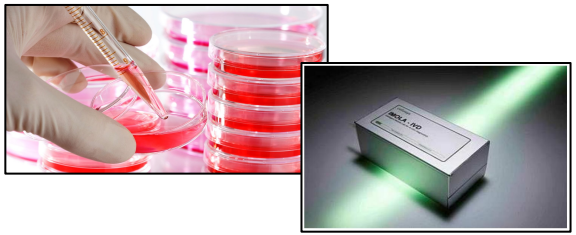
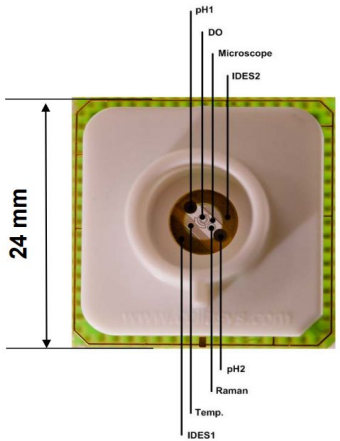
cellasys

- Founded in 2007 as TUM spin-off
- Specialized in providing systems and solutions for microphysiometry



cellasys' Solution for the Analysis of Living Cells

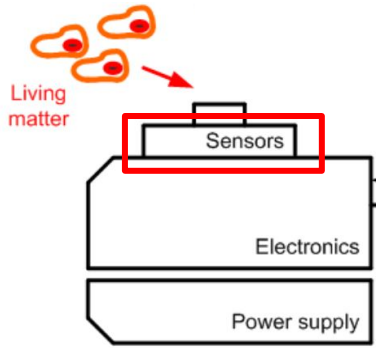
Bio-Chip



- Sensors for pH, oxygen, temperature & impedance

IMOLA-IVD*

**Intelligent Mobile Lab for in-vitro diagnostic (IMOLA-IVD)*



- Boxed in-vitro cell chip test system
- Contains the Bio-Chip

Incubator

- Assures homogeneous temperature distribution
- Contains 6 IMOLA-IVDs

Experiments Follow the ChemDef Protocol

Phase Nr.	Hours	Reference Group		Test Group
		Negative and positive control with cell culture	Blank without cell culture	4 replicates with cell culture
1	0 - 6 h	optimal medium (DMEM* + 10% FBS**)	optimal medium	optimal medium
2	6 - 12 h			test medium
3	12 - 16 h			optimal medium
4	16 - 20 h			test medium
5	20 - 24 h	toxic medium (0.2% SDS***)	toxic medium	toxic medium

** DMEM: Dulbecco's Modified Eagle Medium, **FBS: Fetal Bovine Serum, ***SDS: Sodium Dodecyl Sulfate*

Project Goals

Optimize existing approaches of preparation & analysis

Develop methods to **reduce the noise** in the data

Develop methods to **assess the validity** of the data

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Dataset Description

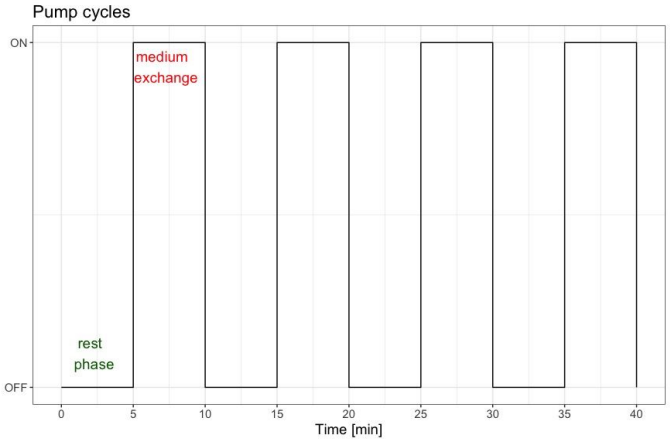
Data from **two 24h experiments**:

- Each experiment uses **6 IMOLAs**
- Data comes as .exp (text format) file
- Contains measurement recordings, air bubble detections and current configuration information
- Information about **valid and invalid IMOLAs provided** (see table)

Exp.	Valid	Invalid
1	IMOLA 1, 2, 3, 4	IMOLA 5, 6
2	IMOLA 2, 3, 5	IMOLA 1, 4, 6

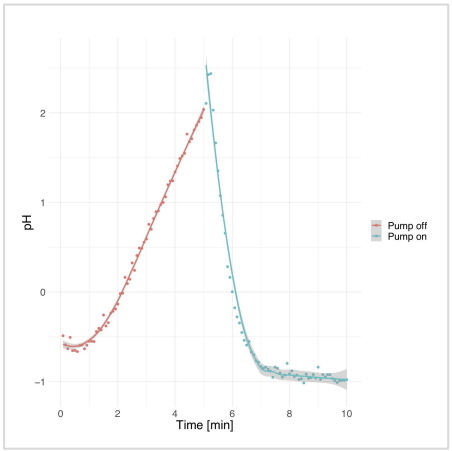
Pump Intervals

- **Fluidic system provides fresh cell culture medium** to cells on chips
- **Pump cycle:** pump switches between OFF (rest phase) and ON (medium exchange)
- 1 interval = 5 min rest + 5 min pump



Pumping Interval

- **Rest phase:** cells start to metabolize (pH increases)
- **Pump phase:** re-calibration of pH



→ pH change (slope) during rest phase as indirect measure for the **extracellular acidification rate (EAR)**

Speed-Up of the Existing Data Preparation Script “Bubble”

Steps of data preparation:

- Reads the .exp input file
- Performs formatting & restructuring steps, joins the different data
- Outputs one table per IMOLA

Implemented the routine again from scratch:

- Exploitation of R functionalities (lapply)
- Faster reading (readr)
- Parallelization of independent operations (parLapply, foreach)
- Profiling the code (profvis)

Script	Runtime
Bubble (old)	1,800 s (30 min)
Bubble (new)	180 s (3 min)
Bubble_Cluster	60 s (1 min)
Bubble_Online	6 s

10x times faster with 2 cores
30x times faster with 6 cores

Normalizing the Data for Validation

Problem:

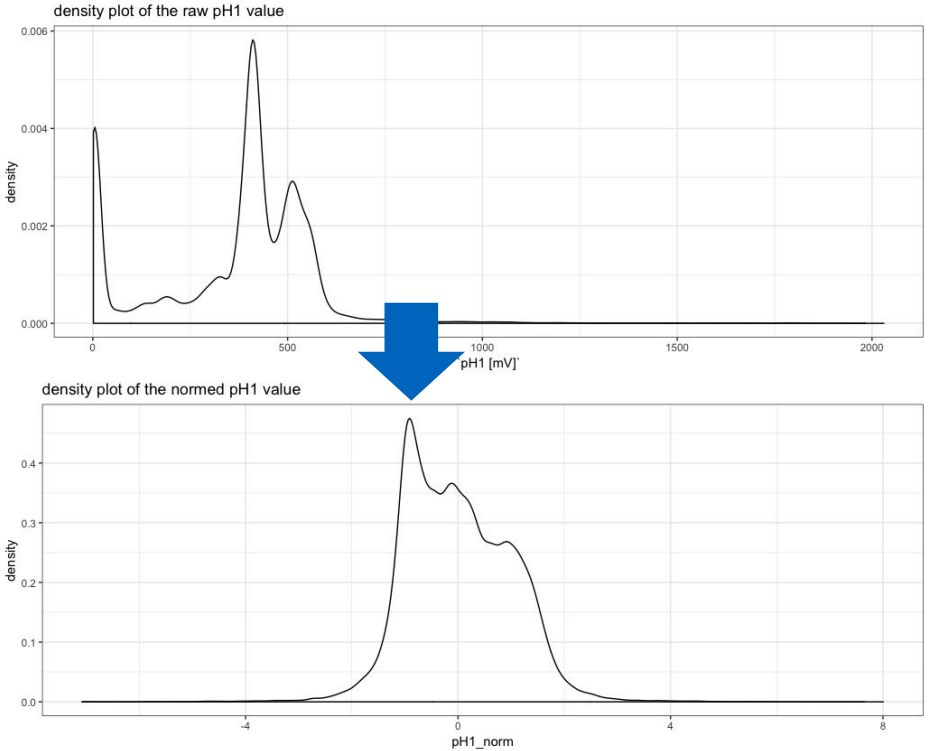
- Sensor are not calibrated and reveal drifting behavior → data not suitable for comparison

Solution:

- Normalize the data X of each interval to

$$\frac{X - \mu}{\sigma}$$

where
μ = mean of one interval
σ = standard deviation of one interval



Check for Dummy Chips and Open Circuits

- Used to **test the system's electronic components**
- Original cell culture and sensors are **replaced** by a Dummy Bio-Chip or **removed completely** (Open Circuit)
- For both cases **expected ranges** for the sensor readings are known

→ **Check** whether an IMOLA is Open Circuit, contains a Dummy Bio-Chip or real cell culture

Sensor	Dummy Bio-Chip Range	Open Circuit Range
pH	300 mV +/- 30 mV	0mV +/- 30 mV
Temperature	1500 mV +/- 150 mV	0 mV +/- 150 mV
O_2	1850 mV +/- 185 mV	2075 +/- 185 mV
Impedance_real	130 Ω +/- 13 Ω	0 Ω +/- 5 Ω
Impedance_imag.	-35 Ω +/- 5 Ω	0 Ω +/- 5 Ω

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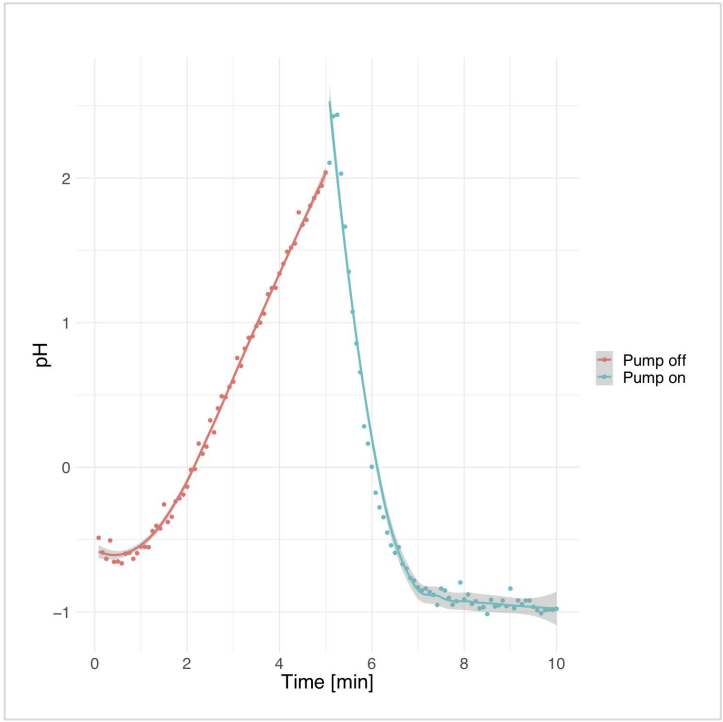
Criterion Based Validation

Why validation?

- Only curves of pH values that follow a valid curve pattern depict normal cellular metabolism
- Interpretations about cellular activity can be made only from valid pH curves

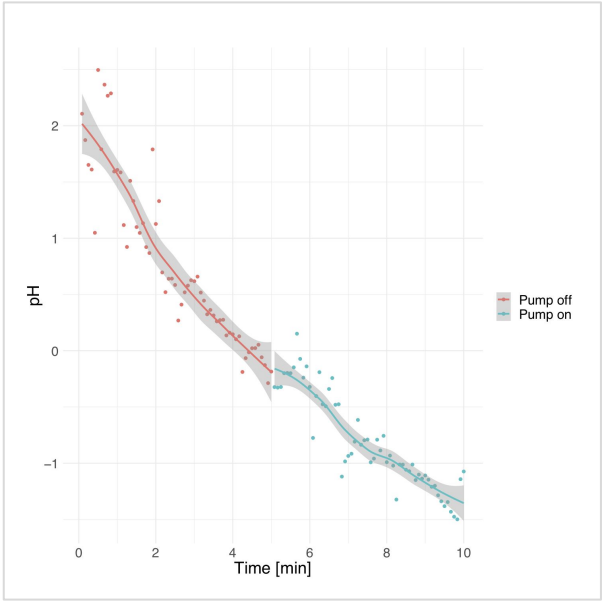
What is a good / valid pH curve?

- Curves which resemble a shark fin structure as shown in figure
- pump-off phase (rest): increasing pH value
- pump-on phase: first decreasing and then constant pH value

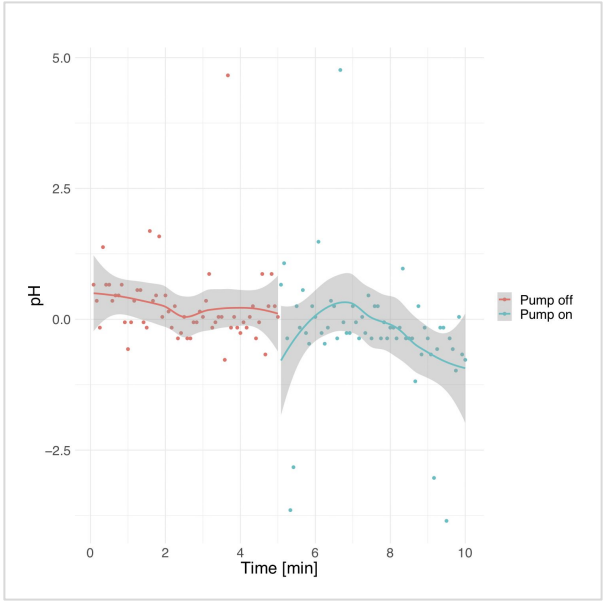


Criterion Based Validation: Invalid Intervals

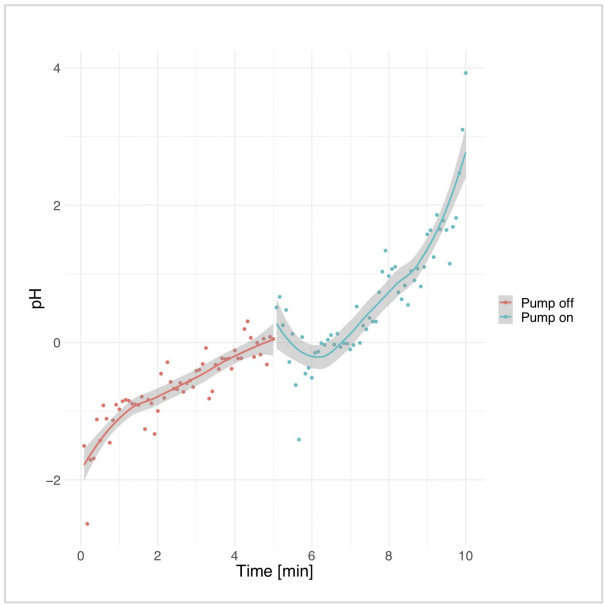
Negative pH slope in pump-off phase



High loss (MSE) in pump-on phase



Mean pH of pump-on phase higher than corresponding 75% quantile of pump-off phase



Input Data and Expected Results

What data do we have ?

- Data from two 24h experiments: Experiment_1 & Experiment_2
- Each experiment has 6 IMOLAs

What are the expected results?

Experiment	Valid	Invalid
1	IMOLA 1 IMOLA 2 IMOLA 3 IMOLA 4	IMOLA 5 IMOLA 6
2	IMOLA 2 IMOLA 3 IMOLA 5	IMOLA 1 IMOLA 4 IMOLA 6

Results of the Criterion Based Validation

Experiment_1

IMOLA	High MSE	Negative Rest	Mean Pump	Valid IMOLA [%]
1	4	8	6	88.811189
2	3	16	34	74.825175
3	5	3	10	90.909091
4	3	4	29	76.923077
5	16	87	64	4.195804
6	47	72	32	18.881119

Experiment_2

IMOLA	High MSE	Negative Rest	Mean Pump	Valid IMOLA [%]
1	22	39	28	44.755245
2	2	2	4	95.804196
3	1	4	4	94.405594
4	58	94	2	24.475524
5	2	2	4	95.804196
6	8	120	4	3.496503

If a 50% benchmark is set for number of valid intervals for an IMOLA to be valid:

- IMOLA 1,2,3, and 4 are valid from Experiment_1
- IMOLA 2,3 and 5 are valid from Experiment_2

Results from criterion based validation matches expected results!

Expected Results		
Exp	**Valid**	**Invalid**
1	IMOLA 1,2,3,4	IMOLA 5,6
2	IMOLA 2,3,5	IMOLA 1,4,6

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Validation Using Clustering of Functional Data

What is 'Functional Data Analysis (FDA)'?

- 'FDA' deals with the analysis of curves or functions
- Curves are estimated from data as being linear combinations of basis functions (with the assumption that they are intrinsically smooth)

What is 'Clustering'?

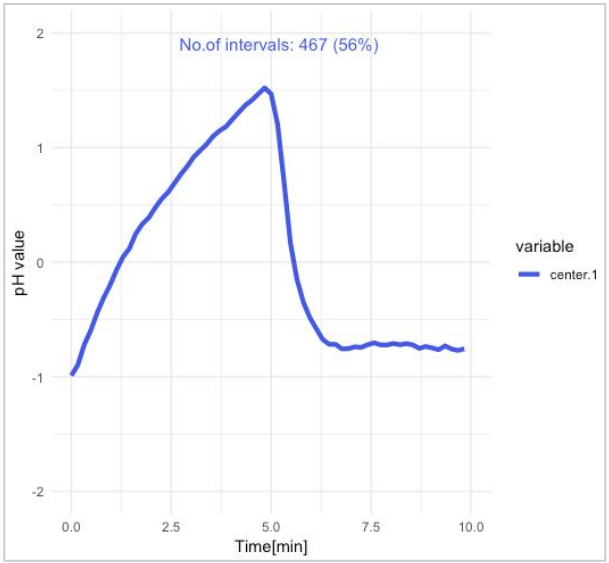
- 'Clustering' is a technique that groups together a set of data objects, such that the objects within the same cluster are more similar (w.r.t. a distance metric) than to those objects in other clusters

How did we use 'FDA' and 'Clustering'?

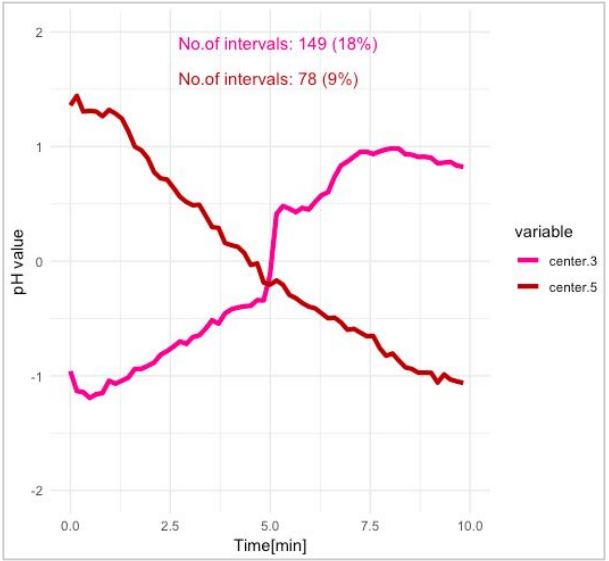
- We aim to do validation, i.e classifying intervals as being (in)valid based on the shape of the pH curves
- We estimate the functional data (curves) using splines which are polynomial curves
- We use a clustering algorithm to find similar functional data (similar curves)
- We validate the intervals using the cluster patterns observed

Observed Cluster Patterns

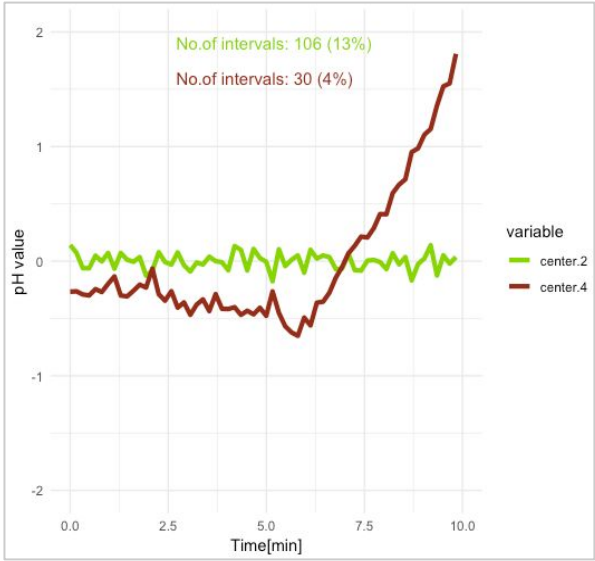
Valid pH curve



Invalid pH curves (increasing & decreasing pH)



Invalid pH curves (constant pH & pH pump off < pH pump on)



Results for Experiment_1

IMOLA	1	2	3	4	5	Valid IMOLA [%]
1	99	3	5	1	7	86.086957
2	107	1	29	5	1	74.825175
3	129	2	7	3	2	90.209790
4	111	0	29	2	1	77.622378
5	5	27	58	7	46	3.496503
6	16	73	21	12	21	11.188811

- Intervals belonging to cluster 1 are considered valid
- If a 50% benchmark is set for number of valid intervals for an IMOLA to be valid:

  → IMOLA 1, 2, 3 and 4 are valid

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Results from validation based on clustering matches the expected results!

Expected Results		
Exp	**Valid**	**Invalid**
1	IMOLA 1,2,3,4	IMOLA 5,6

FDA: Decision Making for New Data

What do we have?

- Pre-defined cluster patterns identified from data of Experiment_1 that represents commonly observed pH curve patterns

What do we want?

- For new data from other experiments, we need to validate the intervals

What do we do?

- Estimate the curves from the new data
- For each curve, find the closest pre-defined cluster and assign it to that cluster
- Classify the curve as being valid/invalid according to the cluster it is assigned to

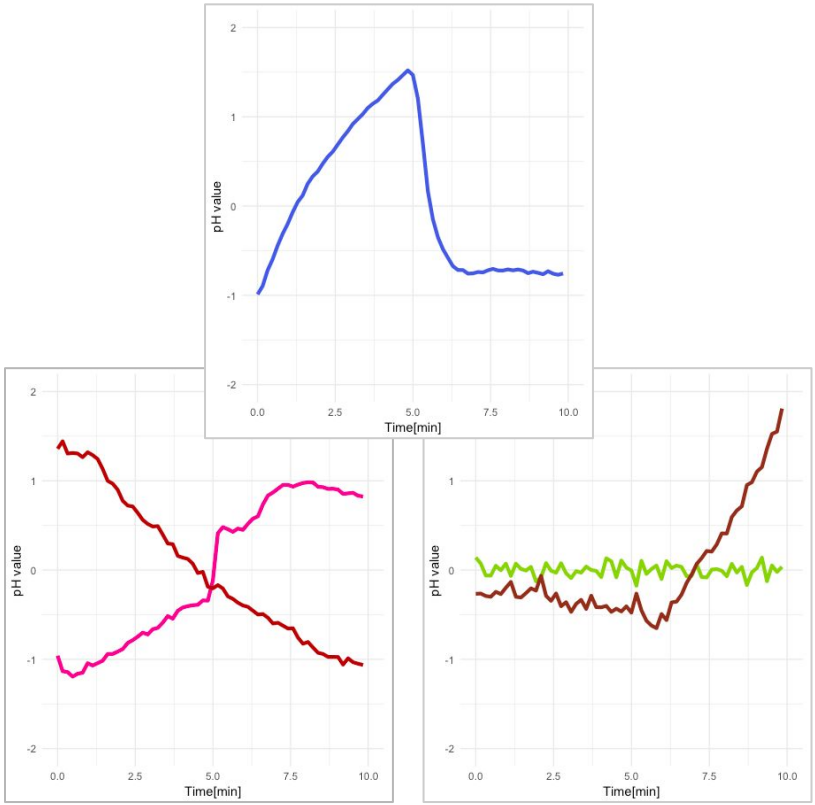

Figure: Pre-defined Cluster Patterns

Results for Experiment_2

IMOLA	1	2	3	4	5	Valid IMOLA [%]
1	42	31	20	7	37	30.656934
2	132	3	5	2	1	92.307692
3	123	14	1	1	4	86.013986
4	11	124	0	5	3	7.692308
5	134	2	3	1	3	93.706294
6	0	15	3	4	109	0.000000

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  → IMOLA 2,3 and 5 are valid

Results for Experiment_2

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Results from validation based on clustering matches the expected results!

Expected Results		
Exp	**Valid**	**Invalid**
2	**IMOLA 2,3,5**	**IMOLA 1,4,6**

Comparison of Both Validation Techniques

Experiment_2

Expected Results		
Exp	Valid	Invalid
2	IMOLA 2,3,5	IMOLA 1,4,6

Criterion Based Validation			
IMOLA	Invalid	Valid	Valid IMOLA [%]
1	75	62	45 %
2	6	137	96 %
3	8	135	94 %
4	108	35	24 %
5	6	137	96 %
6	126	5	3%

Validation Based on FDA			
IMOLA	Invalid	Valid	Valid IMOLA [%]
1	95	42	31 %
2	11	132	92 %
3	20	123	86 %
4	132	11	8 %
5	9	134	94 %
6	131	0	0 %

Both validation techniques show promising results!

Agenda

Fourier Transformation

Idea of Fourier Transformation:

- Convert a signal x from its original domain (in our case: time in sec to a frequency domain and vice versa

$$X_k = \sum_{t=0}^{N-1} x_t \exp\left(-i \frac{2\pi}{N} tk \right)$$

- Amplitude: Modulus(X_k)
  Phase: Argument(X_k)

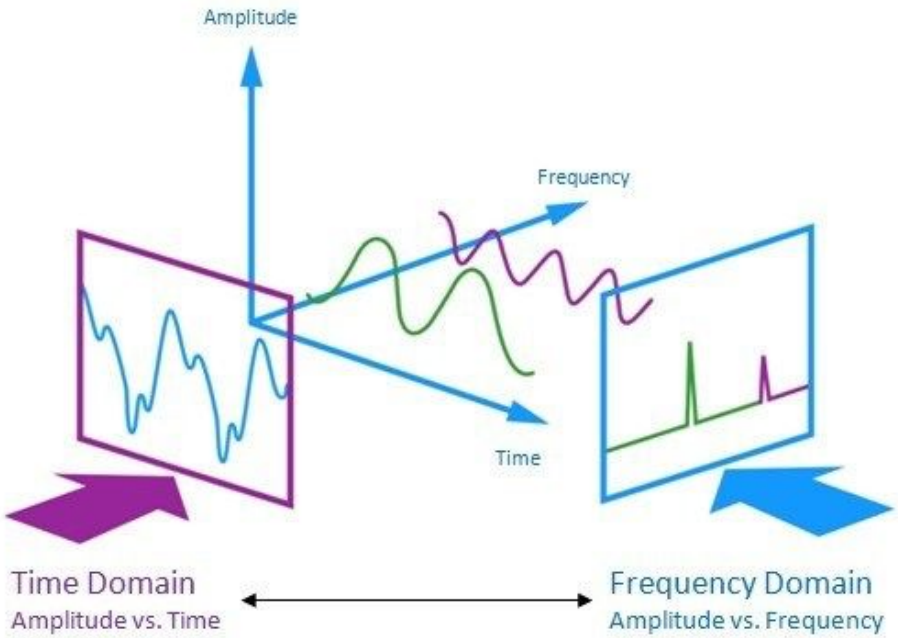
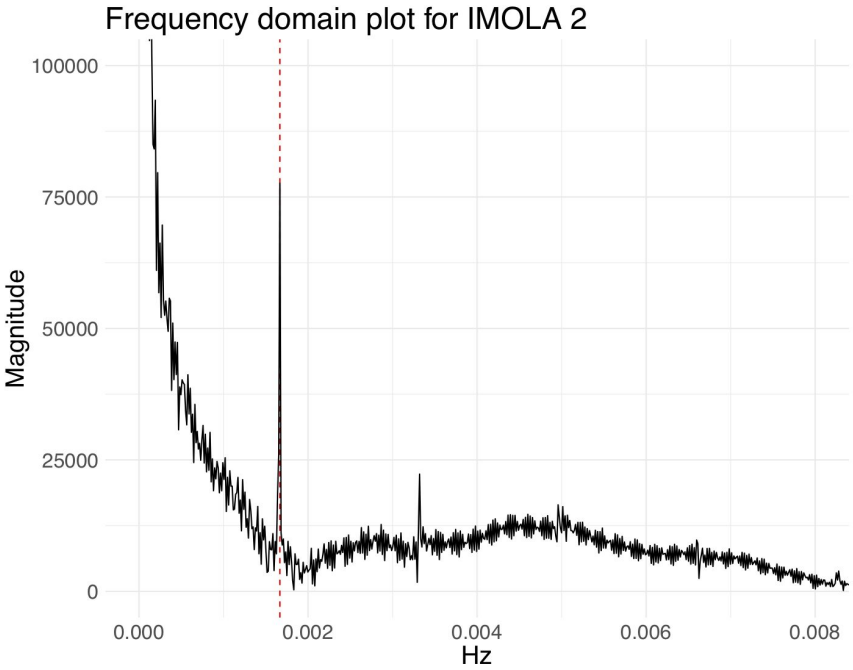


Figure: Idea of Fourier Transformation (Source)

Fourier Transformation



- Plot the **frequency domain** of a valid IMOLA to see if there is a periodic behaviour in the data
- A peak is observed at 0.00167 Hz (corresponds to **fluid cycle frequency** of 1/600s)
- All important microphysiometric information must be **stored in the frequency domain** of the fluid cycle frequency

Filter around the pump cycle frequency to eliminate the noise

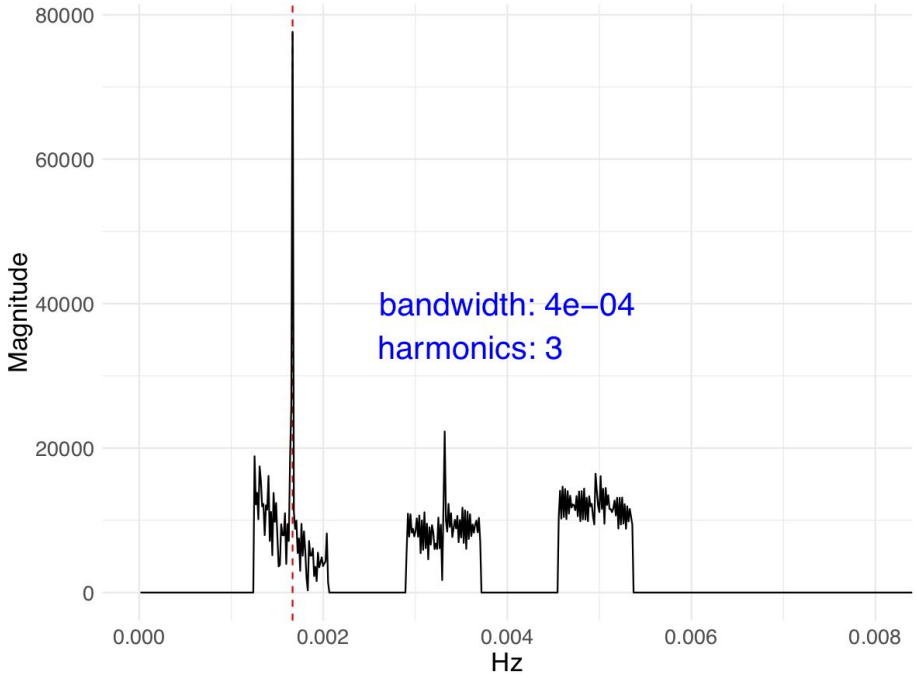
Fourier Filtering

Idea of Fourier Filtering:

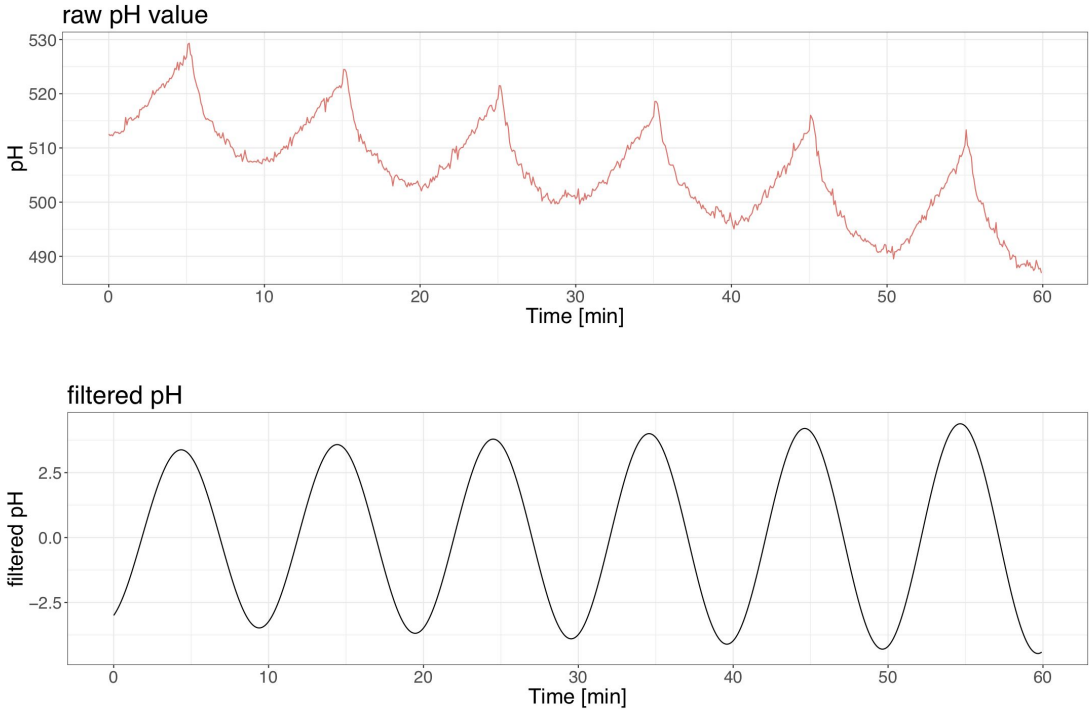
- Set the unwanted frequencies to zero
- Apply the inverse Fourier transformation to the filtered values:

$$x_t = \frac{1}{N} \sum_{k=0}^{N-1} F(X_k) \exp\left(i \frac{2\pi}{N} tk\right)$$

  where F is a filter that filters only the required frequencies
- Calculate the real part of x_t to get the filtered pH values



Fourier Filtering: Example



Evaluation of the Filters

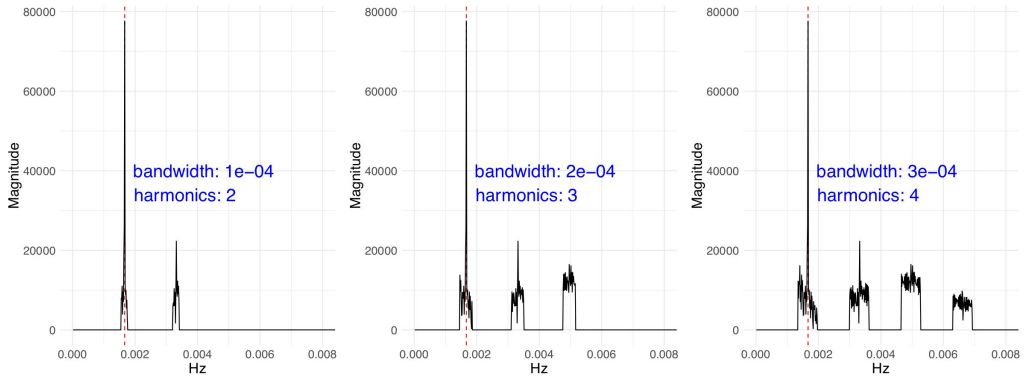
Idea of evaluation:

- Calculate the slope for each interval **before** filtering and **after** filtering
- **Normalize** the slopes to get comparable values
- Calculate the **difference** between the two slopes to see how much the filter affects the slope

Best case: slope doesn't change
(no frequency corresponding to the cell activity was filtered out)

Varying the bandwidth and number of harmonics:

- Vary the number of harmonics from 1 to 10 and the bandwidth from 0.01 mHz to 0.83 mHz
  → 420 combinations



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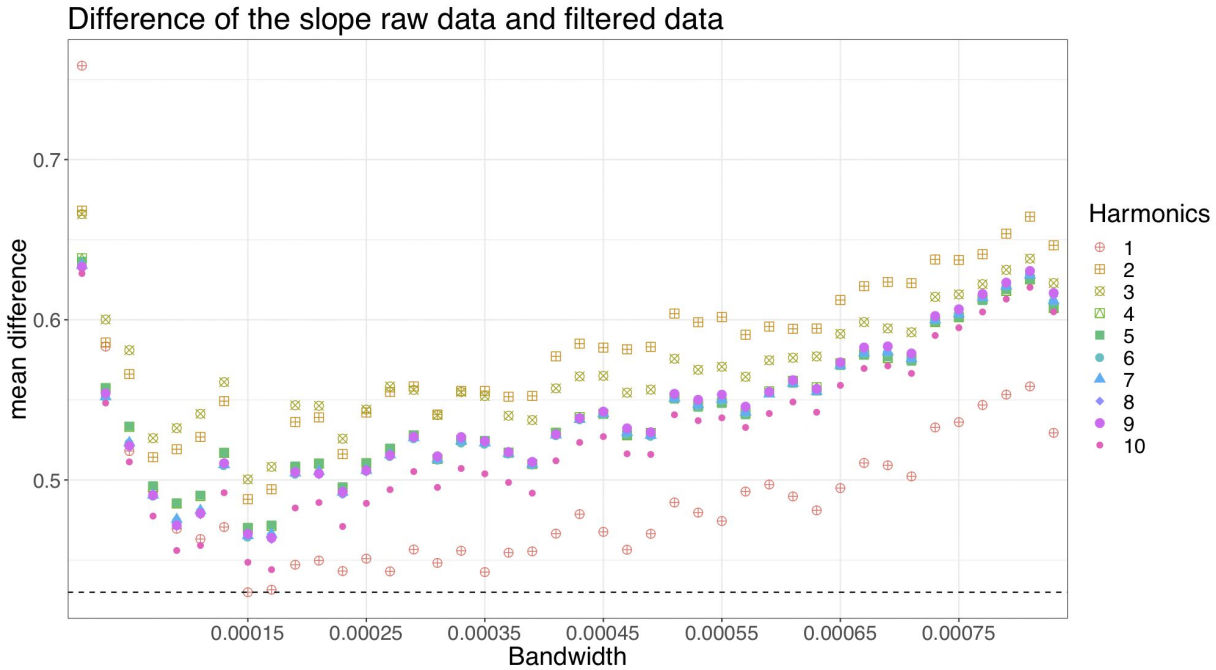

We can conclude that the best results are obtained when using:

- **Harmonics = 1** and
- **Bandwidth = 0.15 mHz**

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Apart from the pH data, we also analysed the following data:

- **Air bubble detections**
  → Either too many or too few detections

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- **Impedance data**
  → Recordings of impedance sensors did not fully match the expected behavior

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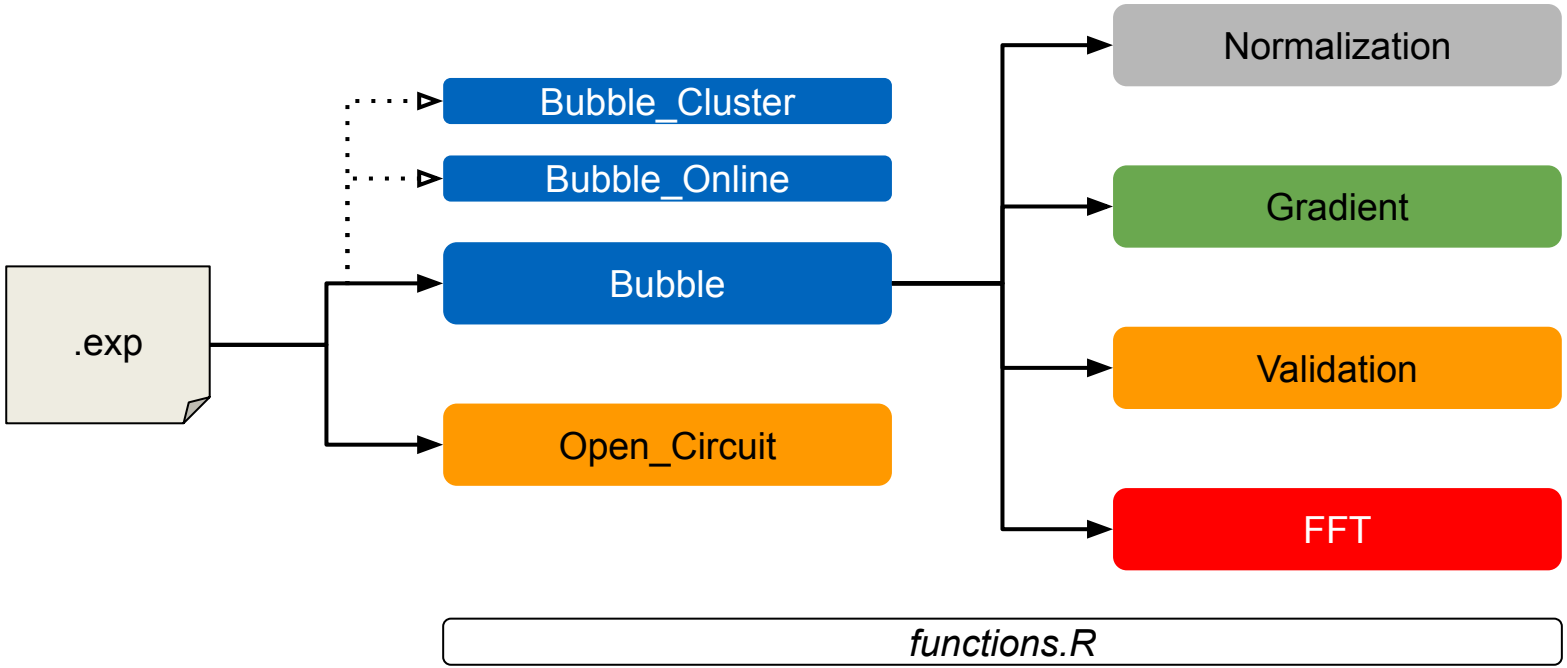
- **Air bubble detections**
  → Either too many or too few detections

- **Sensor ranges**
  → No clear indication on impact on validity

- **Impedance data**
  → Recordings of impedance sensors did not fully match the expected behavior

- **Temperature data**
  → Temperature data did not reveal further indication on cell activity

Summary of the Scripts' Workflow



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- Develop methods to **assess the validity** of the data

Integrate those methods into cellasys' software environment DALiA

cellasys

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Optimize existing approaches of preparation & analysis

- Parallelization accelerated data preparation by factor 10
- Provided cluster version to speed-up by factor 30
- Developed tools to assess chip type (Open Circuit, Dummy)

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- Fast Fourier Transformation and Filtering
- Reduced unwanted noise while keeping signal from cells

Develop methods to **assess the validity** of the data
- Criterion and FDA-Based Validation
- Results match expectation extremely well

Integrate those methods into cellasys' software environment DALiA

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- Parallelization accelerated data preparation by factor 10
- Provided cluster version to speed-up by factor 30
- Developed tools to assess chip type (Open Circuit, Dummy)

Develop methods to **reduce the noise** in the data
- Fast Fourier Transformation and Filtering
- Reduced unwanted noise while keeping signal from cells

Develop methods to **assess the validity** of the data
- Criterion and FDA-Based Validation
- Results match expectation extremely well

Integrate those methods into cellasys' software environment DALiA

- Ensured that all scripts can be run from RStudio and DALiA Analytics

TUM Data Innovation Lab with cellasys

How to Handle Data from Living Cells

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