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Fraunhofer IZI

Fraunhofer-Institut für Zelltherapie und Immunologie IZI

LIFE-KOOP 2024: Microclots as Predictive Parameters for Vascular and Neurodegenerative Changes of the Brain in Aging (SMWK TG70)

Quantification of microclots in cryo-preserved human serum via Deformability Cytometry (DC)

Dr. Jessica S. Freitag and Dr. David M Smith
Fraunhofer Institut für Zelltherapie und Immunologie (IZI), Leipzig
Integrative Biomarkers and Nanotools Group

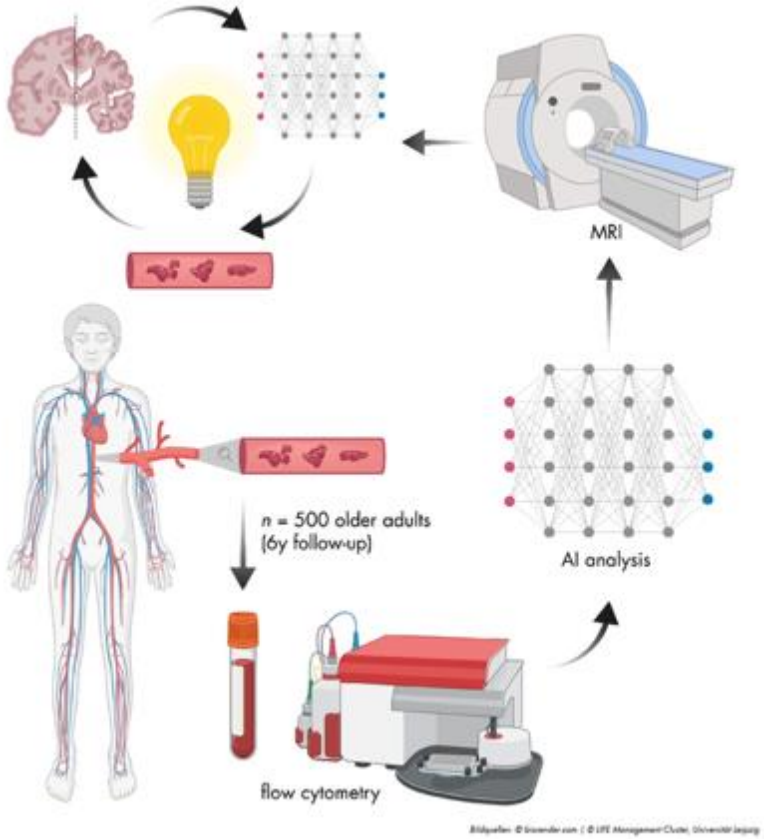
Dr. Ronny Baber
Life Management Cluster / LMB

Dr. Veronica Witte
MPI für Kognitions- und Neurowissenschaften Leipzig

Aims

Gesamtprojekt

- Establishment connection between microclots in blood, cognitive parameters and brain structure/function
- Samples obtained in LIFE between 2011-2020, longitudinal (~6 years between samples/surveys)
- Quantify microclots via cytometry (DC), compare to MRI scans, cognitive surveys
- Hypothesis: microclots are potential biomarkers associated with ageing processes in brain
  - ➢ Causative through mediation of vascular structure, leading to degenerative changes in brain



IZI Teilprojekt

- Establishment of a measurement protocol for the detection of microclots in human blood samples using deformability cytometry (DC)
- Development and validation of an appropriate gating strategy based on 48 donor samples (24 samples from the LIFE Long COVID cohort and 24 samples from the LIFE Baseline cohort) within the framework of a feasibility study
- Measurement and analysis of approximately 1,100 serum samples (550 donors)

Microclots

Potential players in long-Covid & chronic brain degeneration

- Microclots are insoluble, small blood clots overloaded with inflammatory molecules
- Evidence of increased presence in capillaries during COVID-19 infections and long-Covid (ME/CFS)
  - formation have also been observed in (cardio)vascular diseases and Type 2 diabetes
  - may also play a crucial role in dementia diseases, e.g., in vascular, amyloid, and tau pathologies
- Non-acute, but disrupt local supply of oxygen (localized hypoxia) and nutrients in tissues
- Triggers neurological-psychiatric symptoms such as muscle pain, fatigue, or brain fog


RESEARCH ARTICLE | AUGUST 20 2021

SARS-CoV-2 spike protein S1 induces fibrin(ogen) resistant to fibrinolysis: implications for microclot formation in COVID-19

In Collection Coronavirus

Lize M. Grobbelaar; Chantelle Venter; Mare Vlok; Malebogo Ngoepe ; Gert Jacobus Laubscher; Petrus Johannes Lourens; Janami Steenkamp; Douglas B. Kell ; Etheresia Pretorius

Check for updates

+ Author and Article Information

Deformability Cytometry at Fraunhofer IZI

Introduction

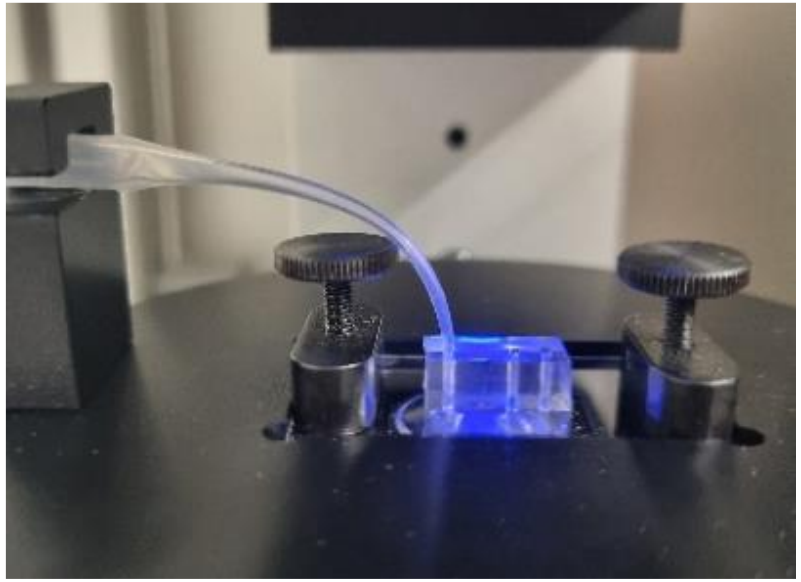
Deformability Cytometry (DC)

Imaging cytometry and hydrodynamic mechanics

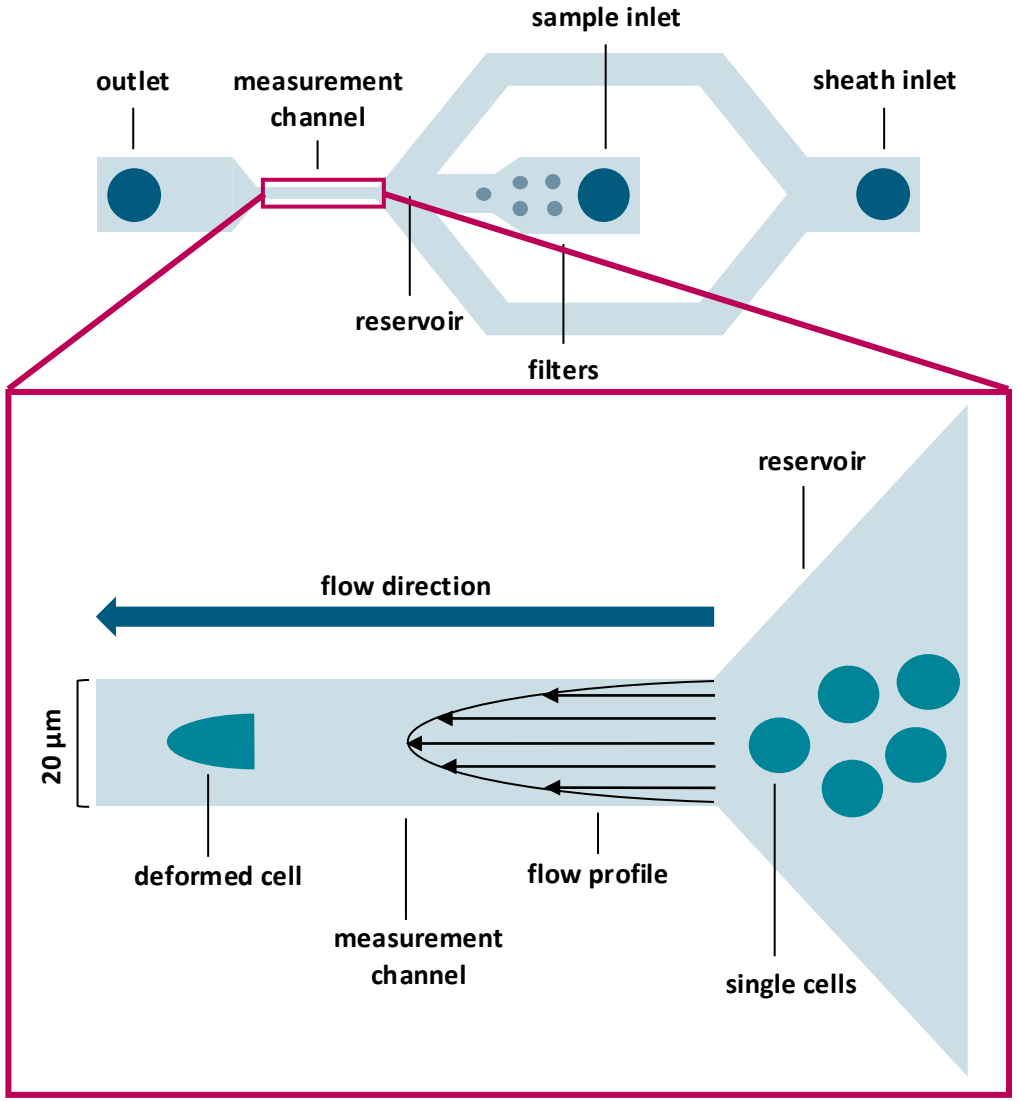
- Cellular processes (activation, transduction,…) regulate morphomechanical and biomechanical properties of cells
- **Physical phenotyping**: characterization of single living cells by their physical and morphological features such as **size, shape and stiffness**
  - Technique for doing this is **deformability cytometry (DC)**
- **Label-free** method based purely on **brightfield images of cells**
- Thousands of single cells flow quickly through a narrow microfluidic channel, where the cells deform due to the forces applied; an **image of each cell** is taken and analyzed (classical heat maps)
- Various substrates: whole blood, cell cultures, dissociated tissue, mixtures of cells, plasma, serum, …

Deformability Cytometry (DC)

Naiad 1.0 Deformability Cytometer

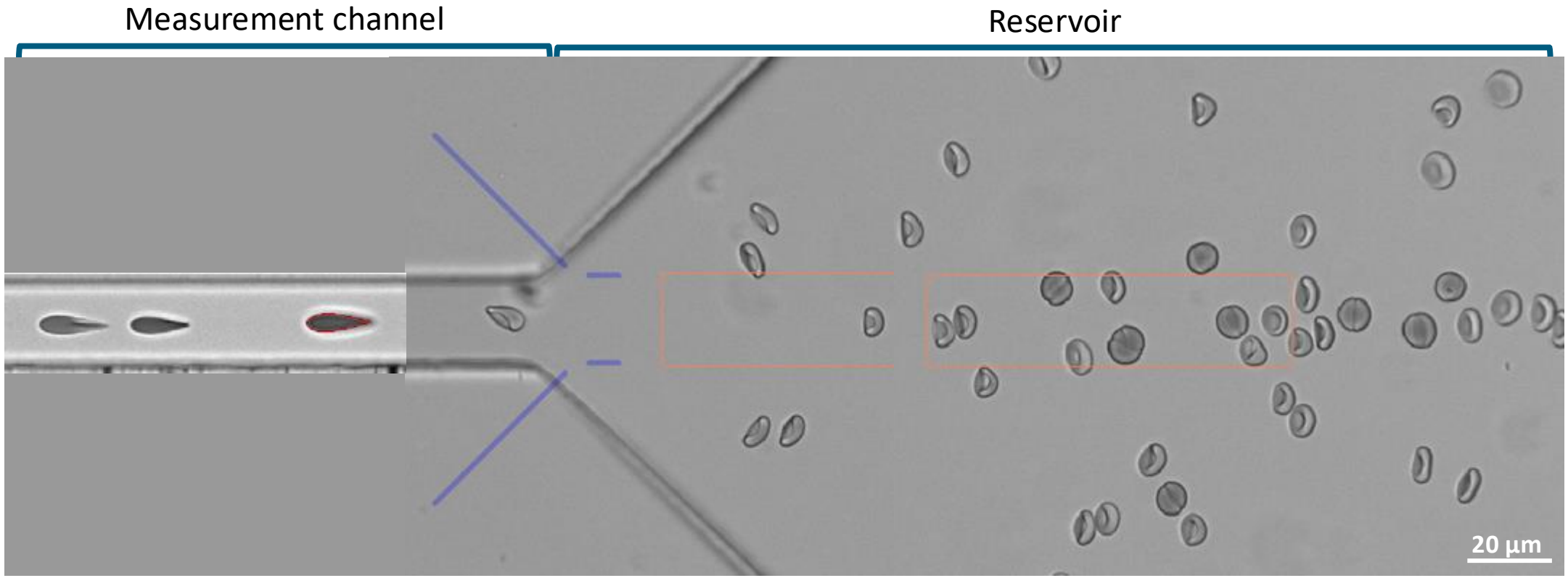


- Leipzig: imaging cytometry (high-throughput)



Deformability Cytometry (DC) Analysis

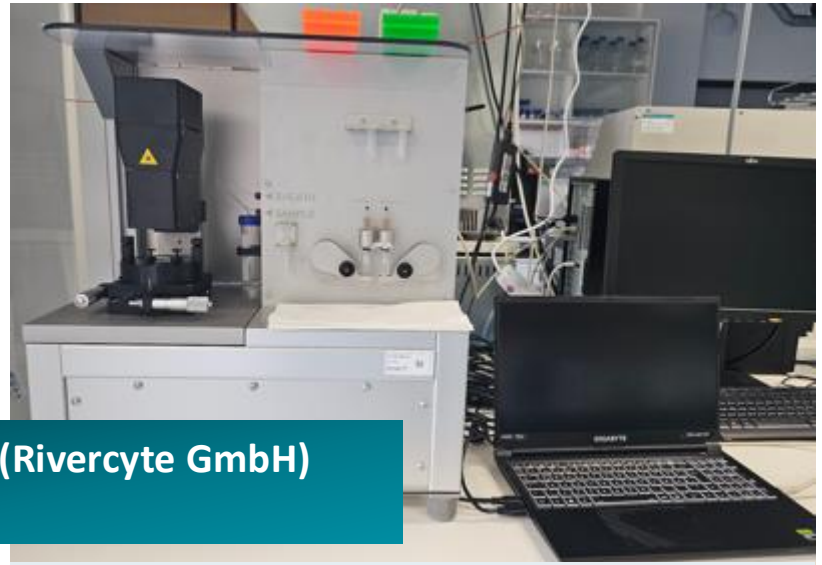
Measurement Chip



Schematic representation of the measurement process performed with the Naiad1.0 deformation cytometer using fresh whole blood . Cell deformation takes place within the measurement channel.

Deformability Cytometry (DC)

Two devices in AG Integrative Biomarkers and Nanotools



Naiad1.0 (Rivercyte GmbH) Leipzig

Deformability Cytometry (DC)
- „blind“ image recording (3400 images/sec)
- segmentation (20-50Gb per dataset)
- no fluorescence, no temperature control
- designed for clinical use (>20/day) (high-troughput)

AcCellerator (Zellmechanik Dresden GmbH) Erfurt

Real-Time Deformability Cytometry (RT-DC)
- real-time analysis/segmentation
- Raw data: 500Mb-2Gb
- 3-color fluorescence (Ex: 488/561/641; Em:525/591/700)
- temperature control
- research device (5-10 samples/day)

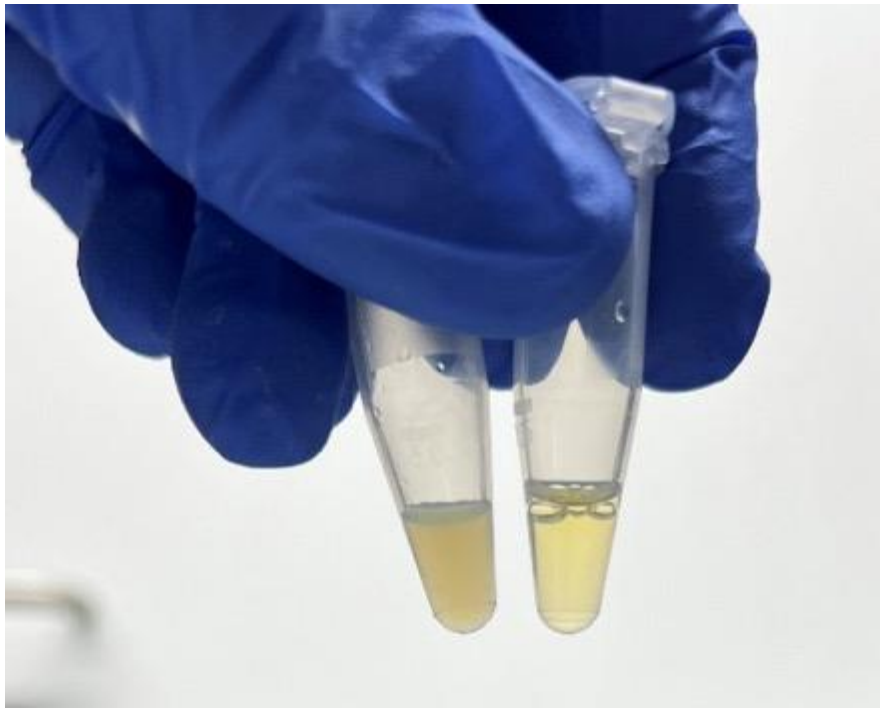
Deformability Cytometry at Fraunhofer IZI

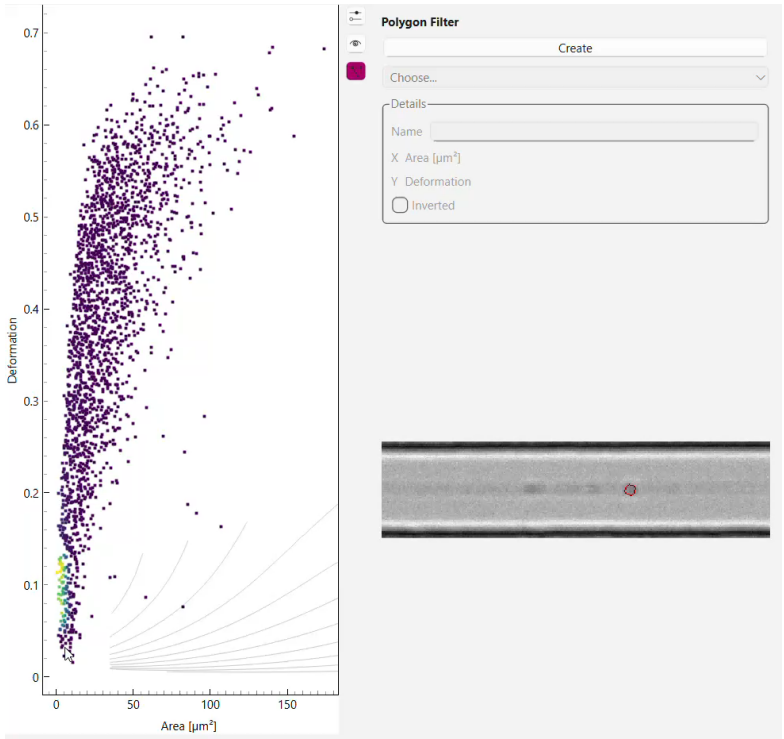
Microclot detection

Preliminary experiments

Microclot measurements: „Mettwurst“ samples

- Measurement and analysis of three serum samples

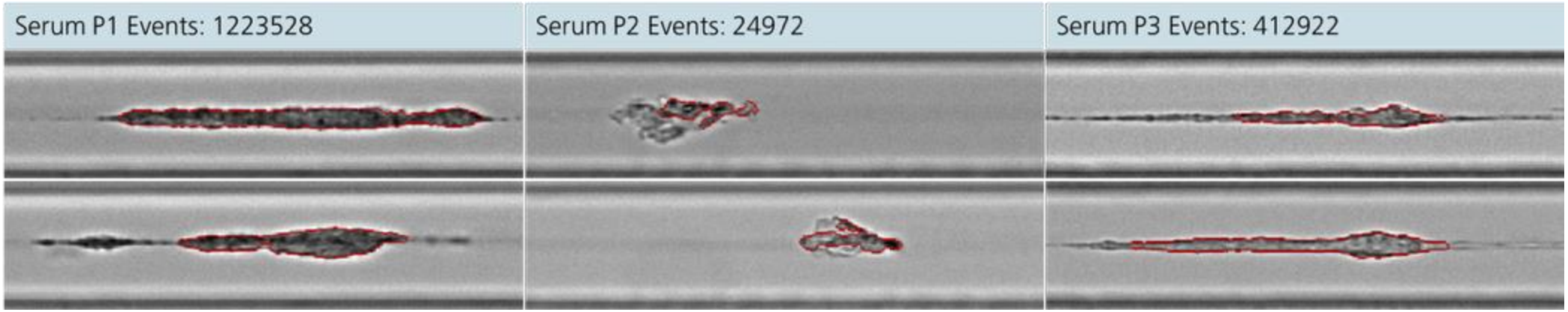




Preliminary experiments

Microclot measurements: „Mettwurst“ samples

- Measurement and analysis of three serum samples

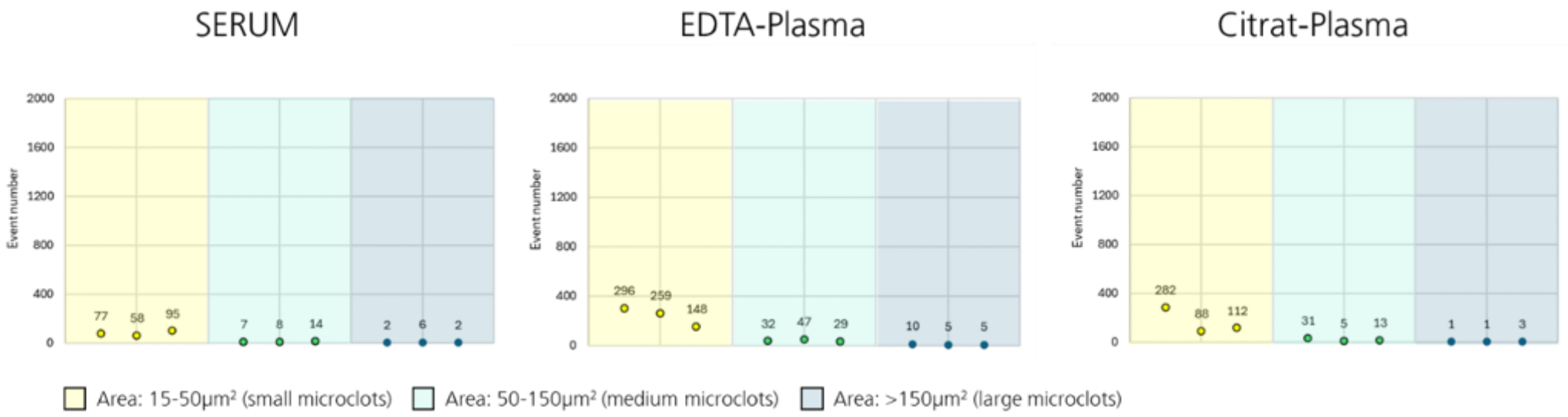


→Lipemic samples will be excluded from analysis in future measurements

Preliminary experiments

Microclot measurements: Serum vs. EDTA-Plasma vs. Citrat-Plasma

- Measurement and analysis of three substrates (Serum/EDTA-Plasma/Citrat-Plasma) of 5 donors



→ Higher serum availability in the LMB: Future DC measurements will exclusively use serum

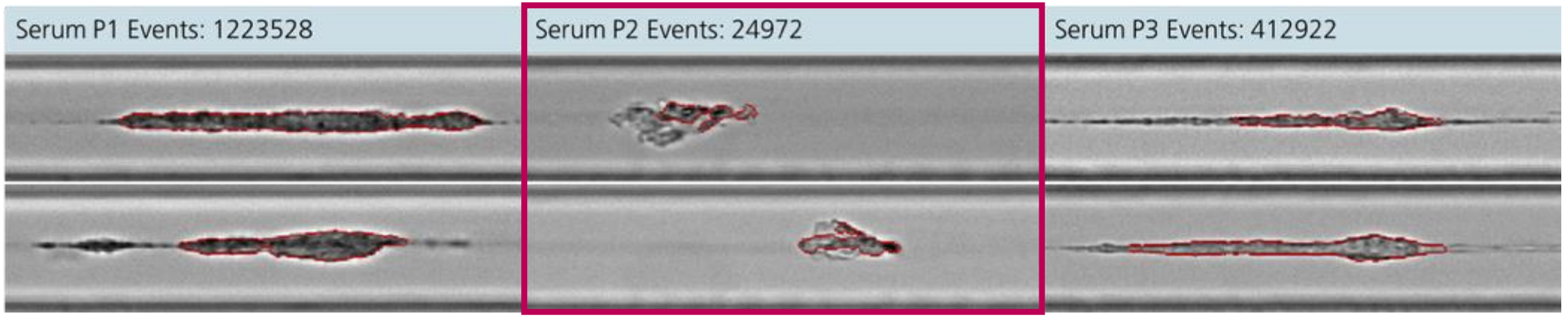
Deformability Cytometry at Fraunhofer IZI

Feasibility study (LC cohort)

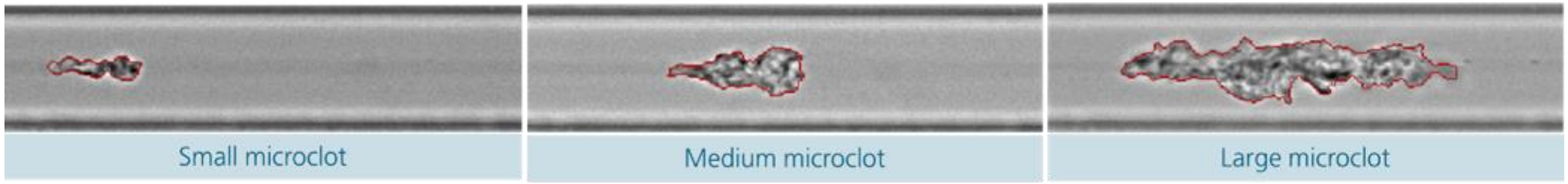
Feasibility study

Threshold-based segmentation vs. AI-based segmentation

- Threshold-based segmentation



- AI-based segmentation



Feasibility study

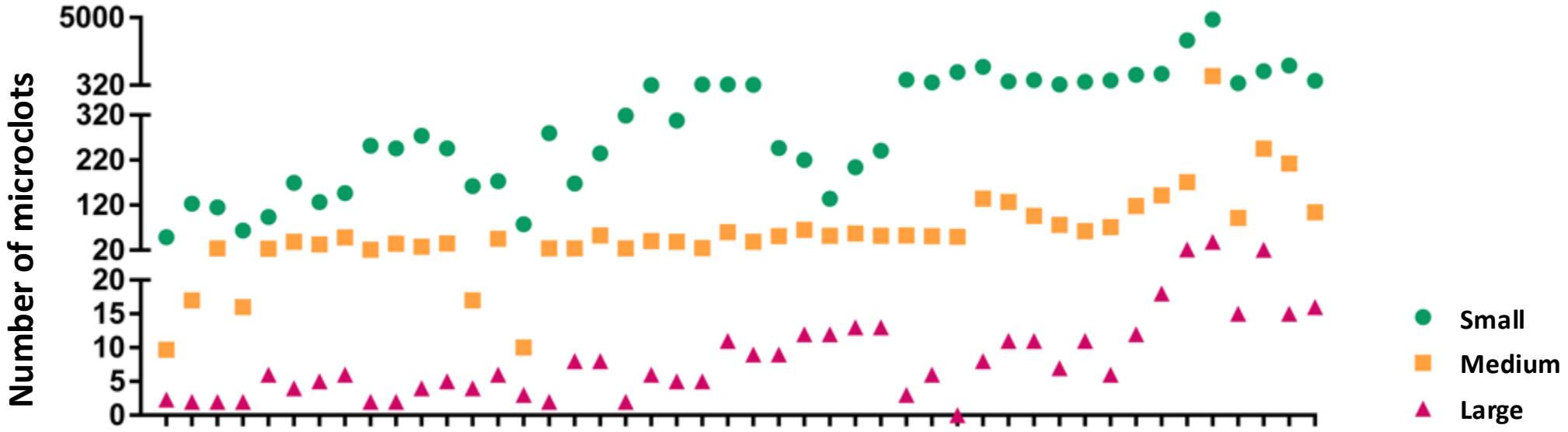
Gating strategy

- Applied box filters for the analysis of microclots

Gating Parameters	Properties
Area [µm^2] of raw contour	Size, internal structure, focus
Texture inverse difference moment (avg)	
Texture variance (ptp)	
Polygon Filter	
Position lateral in channel [µm]	Position within channel
Deformation	Differentiation between cells,debris and microclots
Porosity (convex to measured area ratio)	

Feasibility study

Analysis of 48 samples of Long-COVID cohort



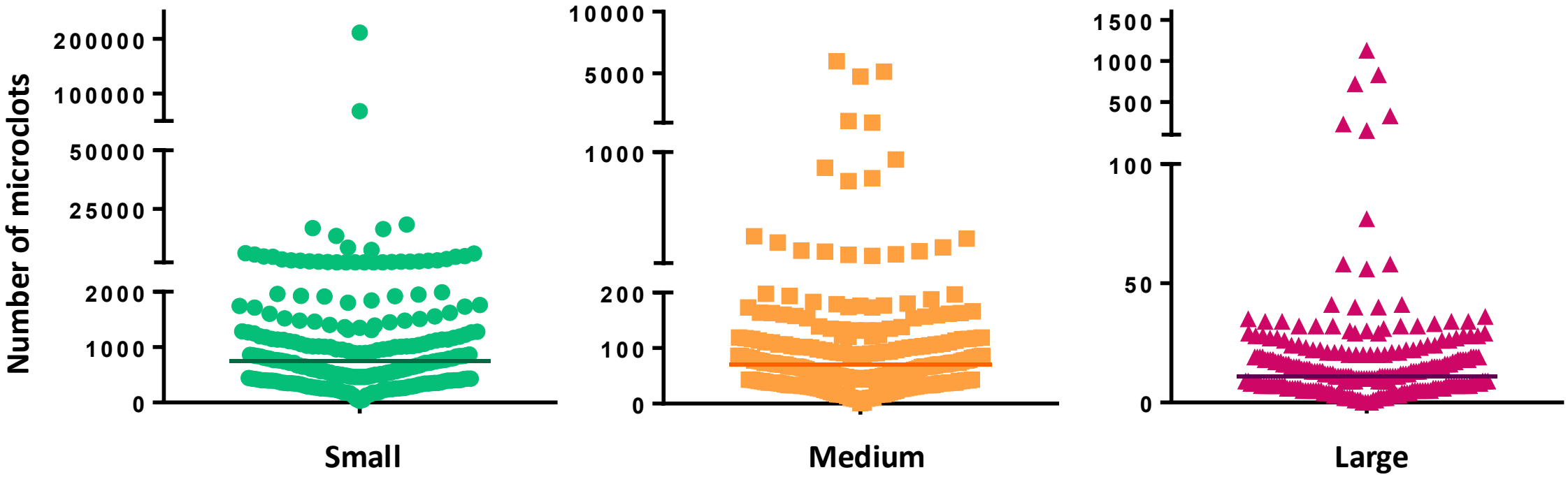
Deformability Cytometry at Fraunhofer IZI

Life-KOOP cohort

Life-KOOP measurements

256 samples

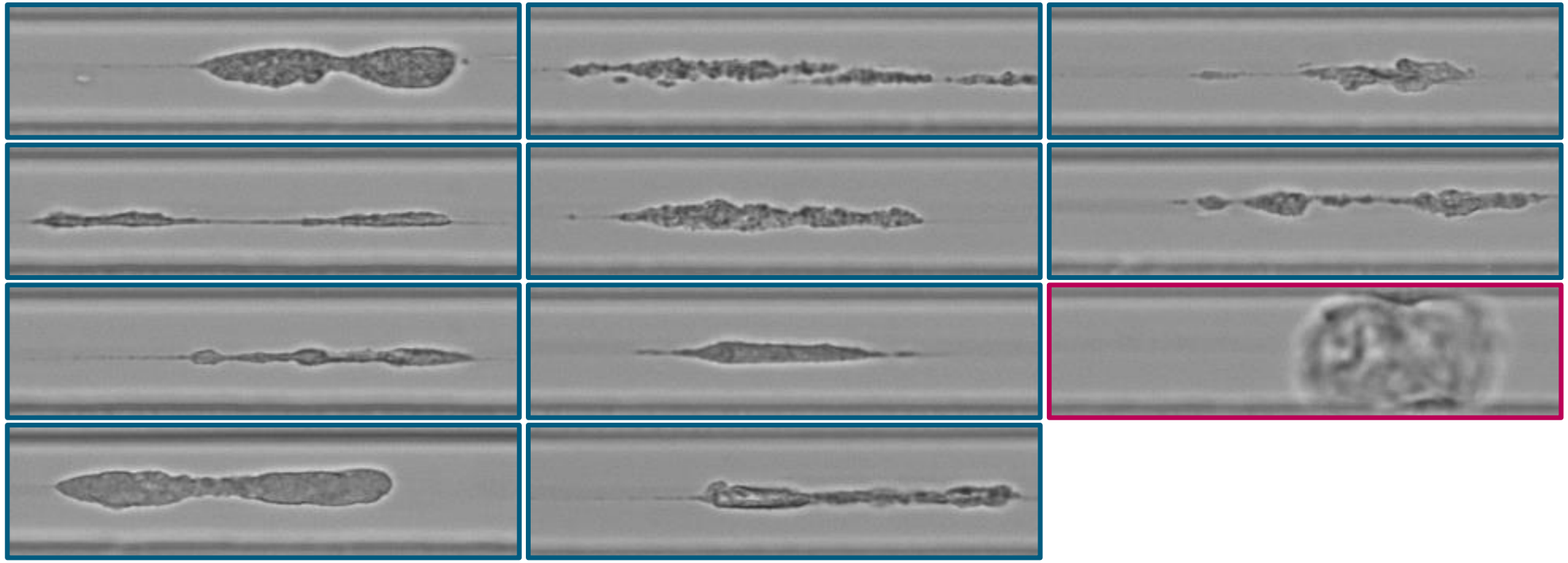
- Samples were analyzed using the previously determined gating matrix



Life-KOOP measurements

256 samples: 11 outlayers

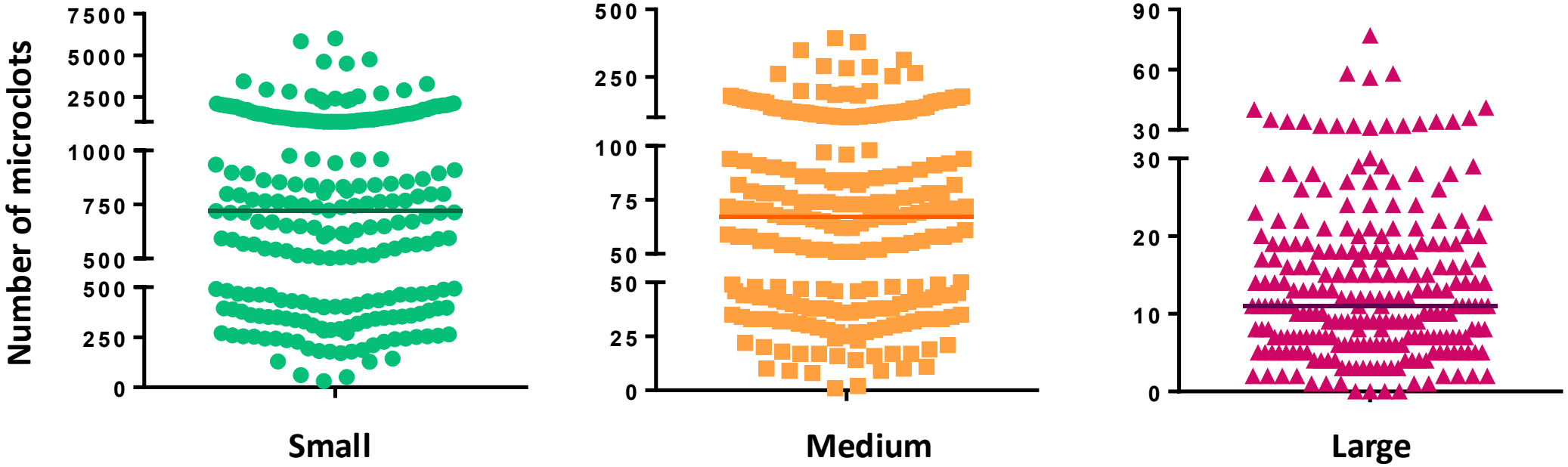
- 11 outlayers were identified and excluded from analysis
  →10 fatty samples, 1 channel clogging (both scenarios give a large amount of (large) microclots)



Life-KOOP measurements

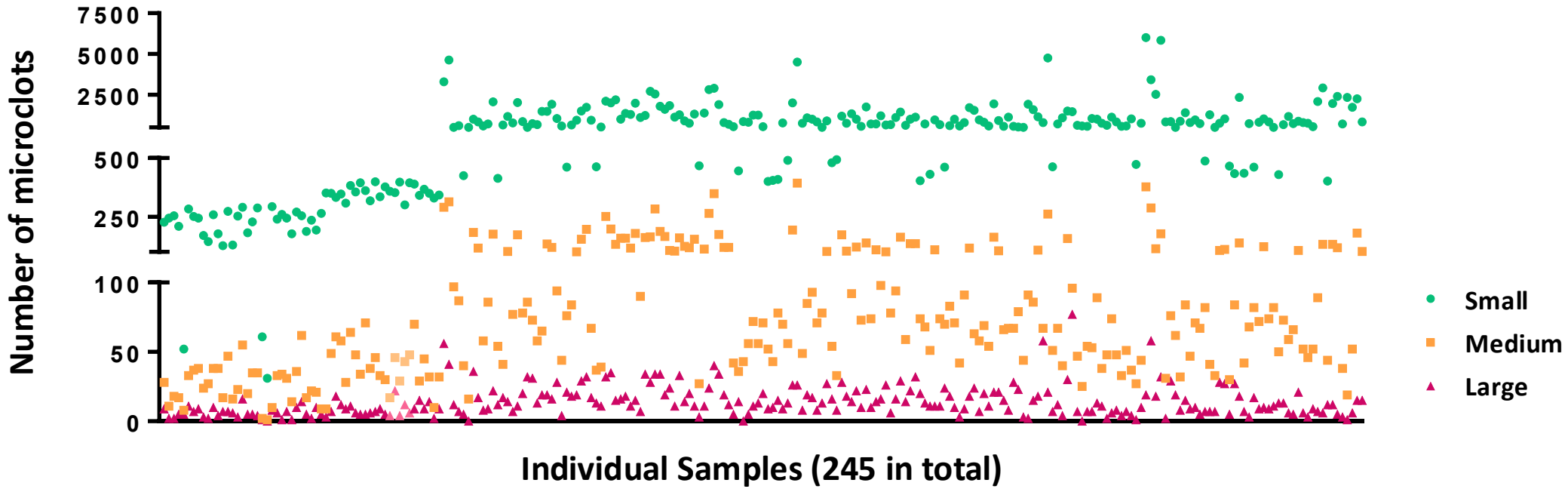
245 Samples (outlayers excluded)

- 11 outlayers were identified and excluded from analysis („Mettwurst-samples“)



Life-KOOP measurements

256 Samples: 11 outlayers

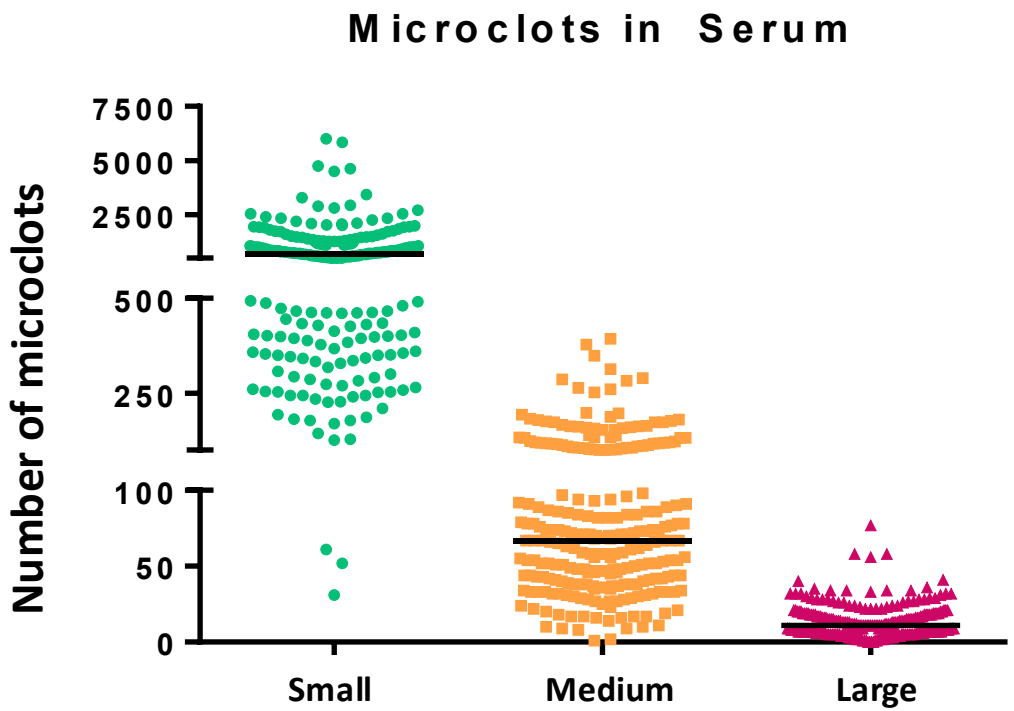


- A large number of small microclots often correlates with a large number of medium and large microclots (and vice versa)

Life-KOOP measurements

Outlook

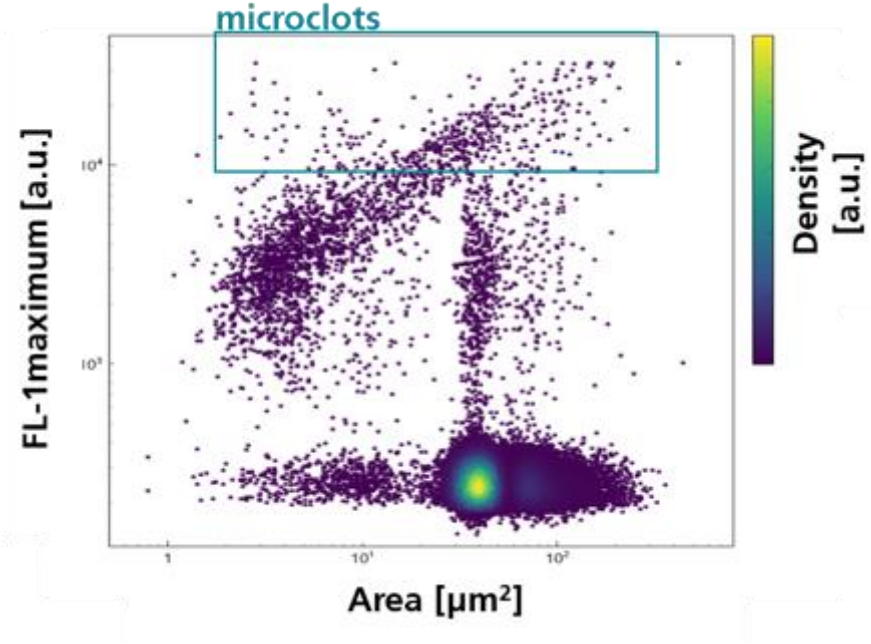
- Combine results from MPI, Fraunhofer IZI and LMB
- „unblind“ samples to correlate possible diseases

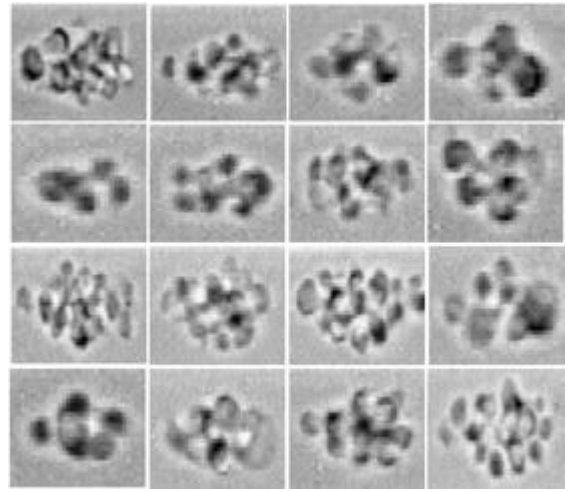


Outlook

AI-based analysis

- Antibody-based detection of microclots using RT-DC of whole blood (FITC Anti-CD61)
- Gating of microclots
- Training of a neuroal network (*AIDeveloper*)
- Aim: Implementation of an *AI*-based data evaluation





Microclots at 40x magnification using RT-DC

Kontakt

Dr. David M. Smith
AG Integrative Biomarkers and Nanotools
Tel. +49 341 35536-9311
david.smith@izi.fraunhofer.de

Fraunhofer-Institut für Zelltherapie und Immunologie IZI
Perlickstraße 1
04103 Leipzig
www.izi.fraunhofer.de

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LIFE KOOP Team

Ronny Baber (LIFE/LMB)

Matthias Nüchter (LIFE/LMB)

Anja Sorge (LIFE/LMB)

Matthias Reusche (LIFE/LMB)

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