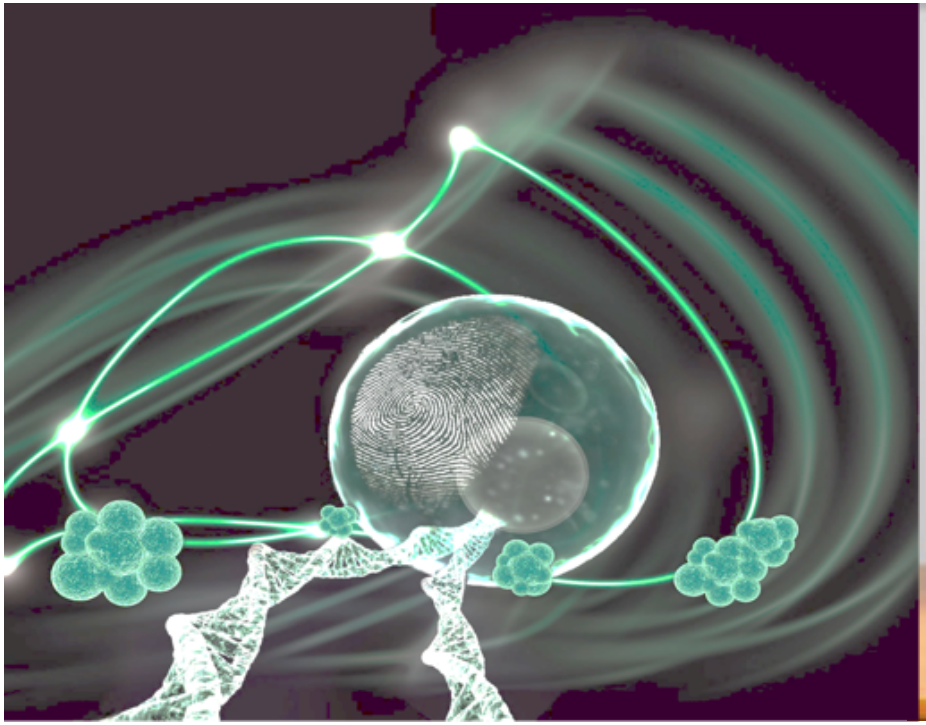


Label-free single cell analysis to monitor cell development and surveil quality of ATMP products

Dr. Karin Schütze

June 2020

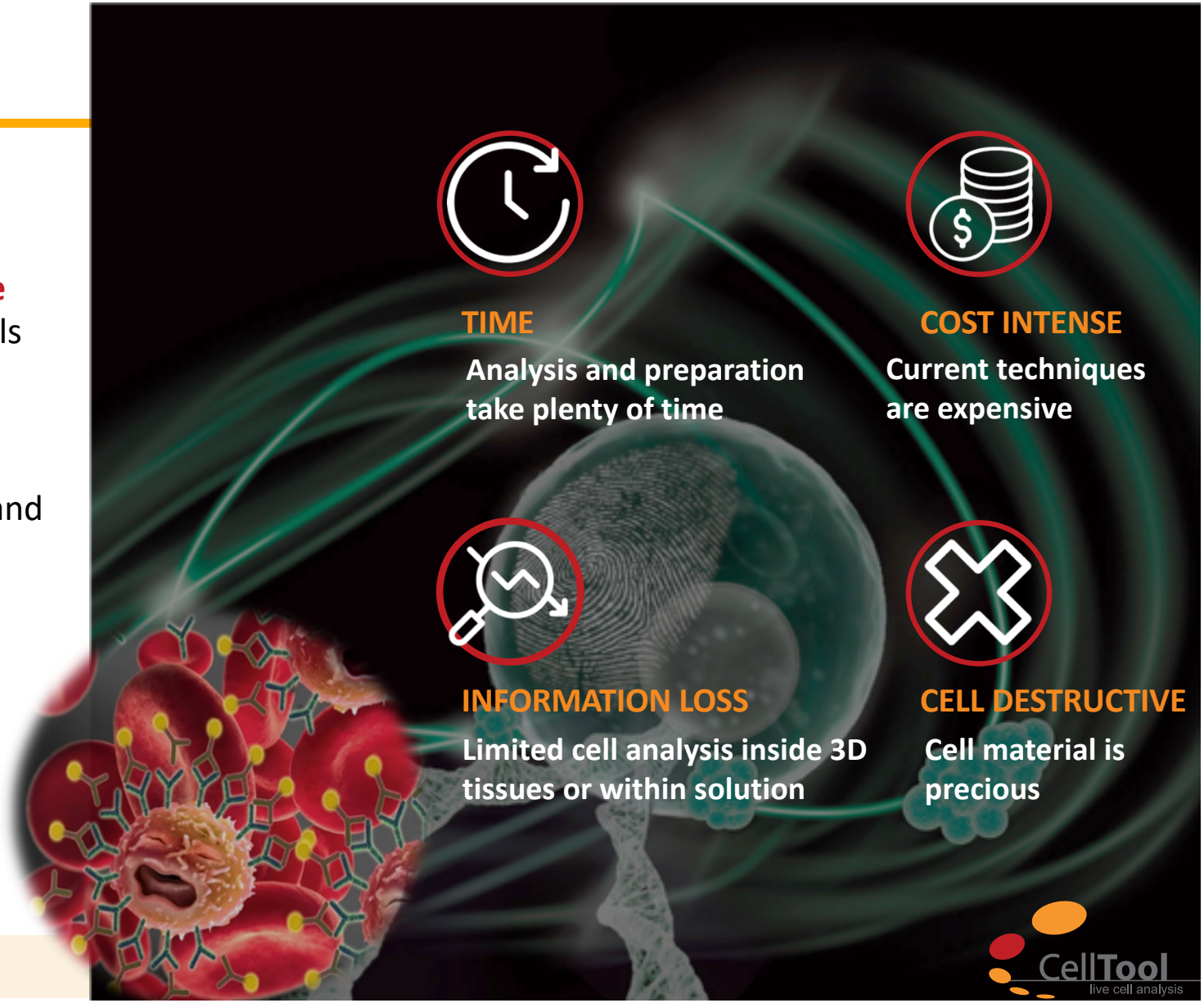
CellTool
single cell analysis

Unmet Need

In biomedical sciences and therapies there is an increasing demand for **label-free**, **real time** and **non-invasive analysis** of cells and tissue.

Raman technology can provide necessary information in a fast and reliable way, to **meet the requirements** of biotech and pharmaceutical market.

Current Cell Analysis Challenge (FACS, MACS)



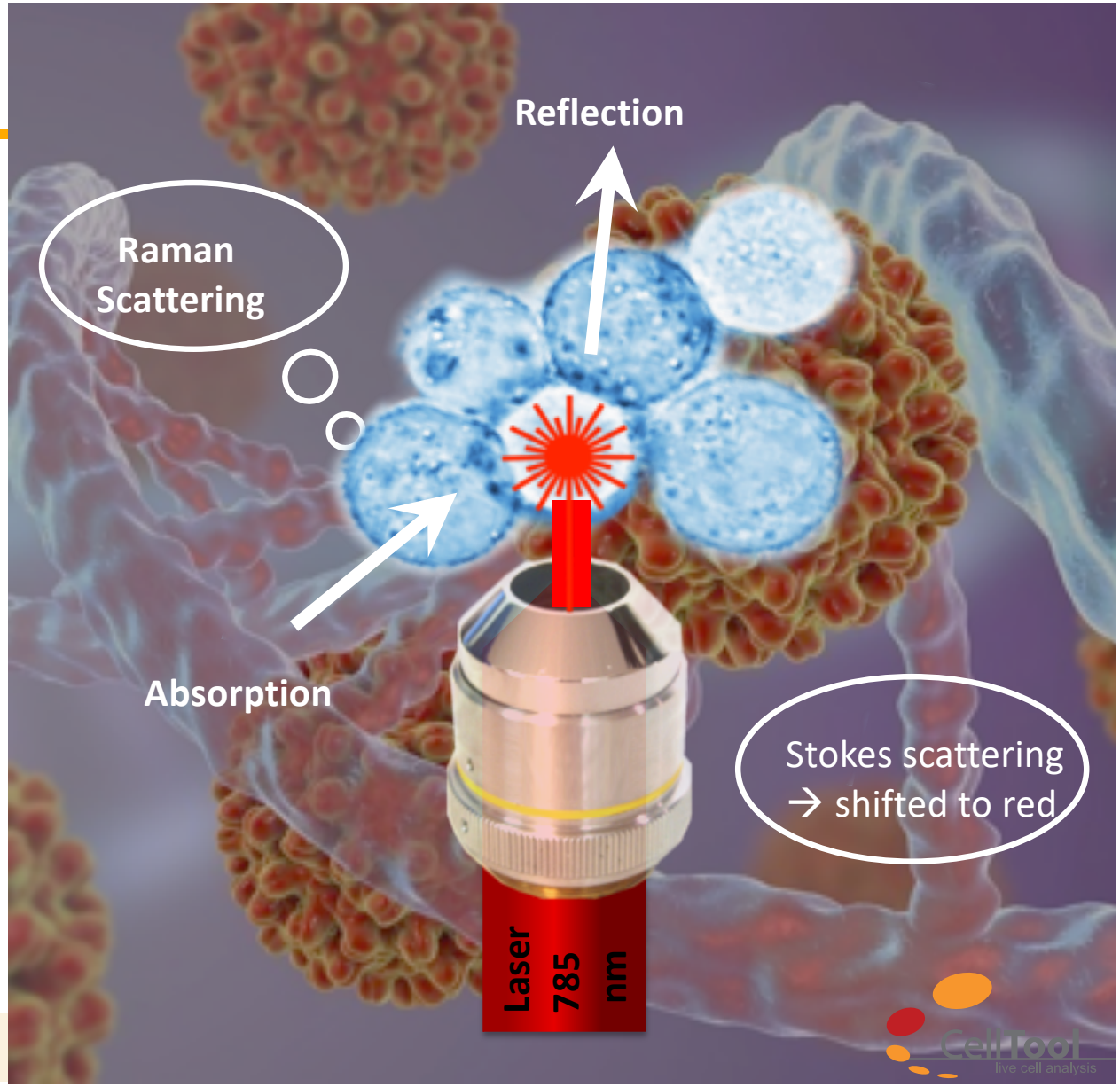
RAMAN TECHNOLOGY

Raman spectroscopy characterizes cells **solely** by their interaction with focused laser light.

Raman spectra are associated with biochemical features

They are as characteristic as a “fingerprint”

- ✓ focus on single cell analytics -> wide range of applications
- ✓ quality control in cell-based therapeutics
- ✓ Diagnosis of cancer and disease



Two Nobel prize awarded physical technologies inside

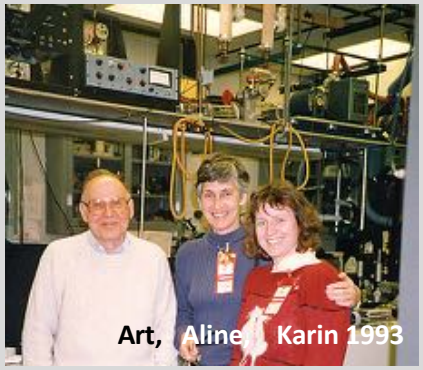
2 Nobel Prize technologies inside

Optical trapping

Special laser coupling and focusing create electro-magnetic forces that enable to catch& move and arrest single cells within the laser focus.

Simultaneous:
as soon as the Raman excitation laser is switched on trapping features arise

Unique:
- analyze cells, motile bacteria, and even exosomes or virus in solution (i.e. liquid biopsies: blood, urine, sputum) - in a highly automated manner



Dr. Art Ashkin
Nobel Prize 2018

Simultaneous Optical Trapping

RamACS:

Raman activated Cell Sorting

Ashkin A, Schütze K, et al.: Force generation of organelle transport measured in vivo by an infrared laser trap. ature, 1990, 348(6299): 346-348.

Cutting Edge Technology for Next Generation Cell Analysis

Tool-of-choice:

combining **Raman** spectroscopy, **Optical Tweezers** with **Fluorescence** microscopy

valuable:

- if labels (antibodies) are **unspecific** or not present
- when only small sample amounts available (i.e.ATMPs)

-> cells are not affected - remain undisturbed for further use

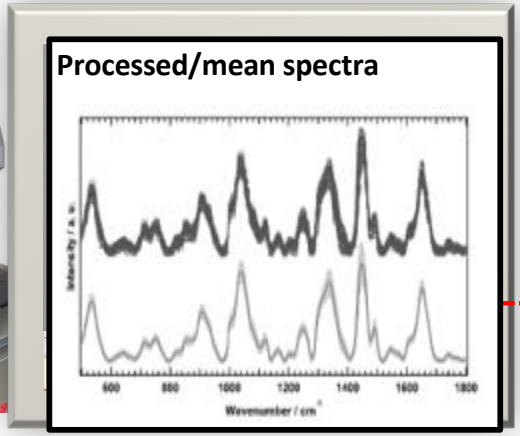
saving:

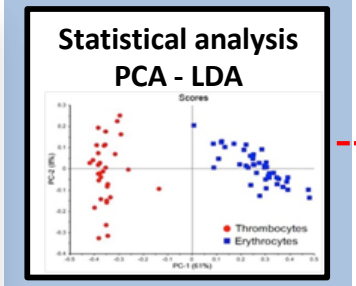
RTM saves **time** (no expansion <100 cells) and **costs** (no labeling)

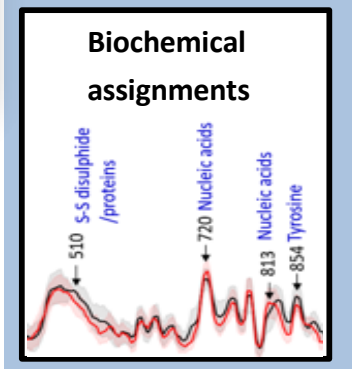
BioRam®: Raman-Trapping-Microscopy

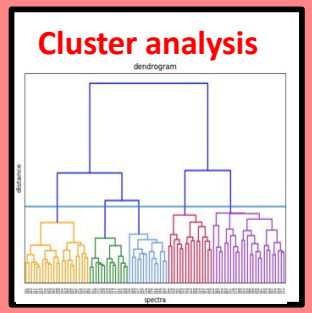
CellTool Demo Video: http://celltool.de/en/news

BioRam® workflow









QC and Homogeneity of cell products

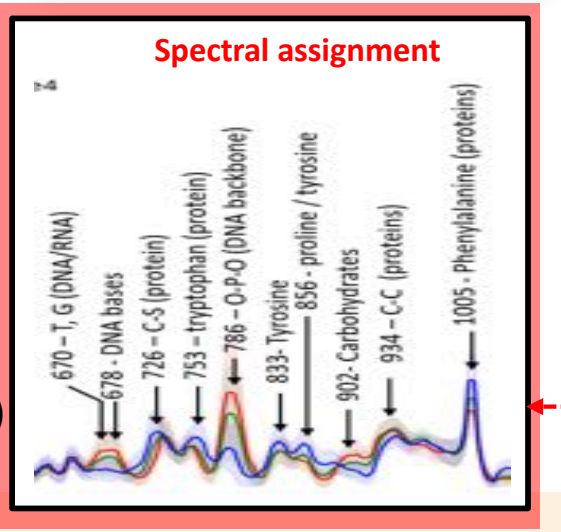
Identify subpopulations

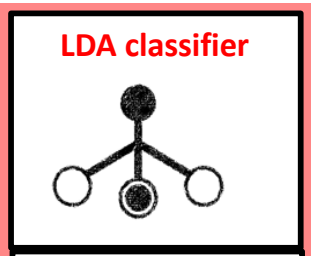
Automatic classification and quantification

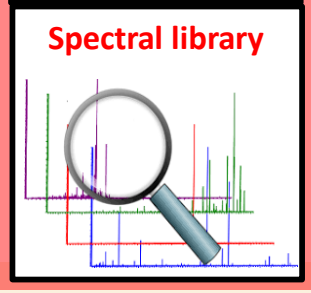
Molecular profiling of cells

Quantitative and semi quantitative analysis.

Biochemical changes (Band ratios & thresholds)







Spectral library inputs and matching search engine

Automatic identifications of bacteria and infections

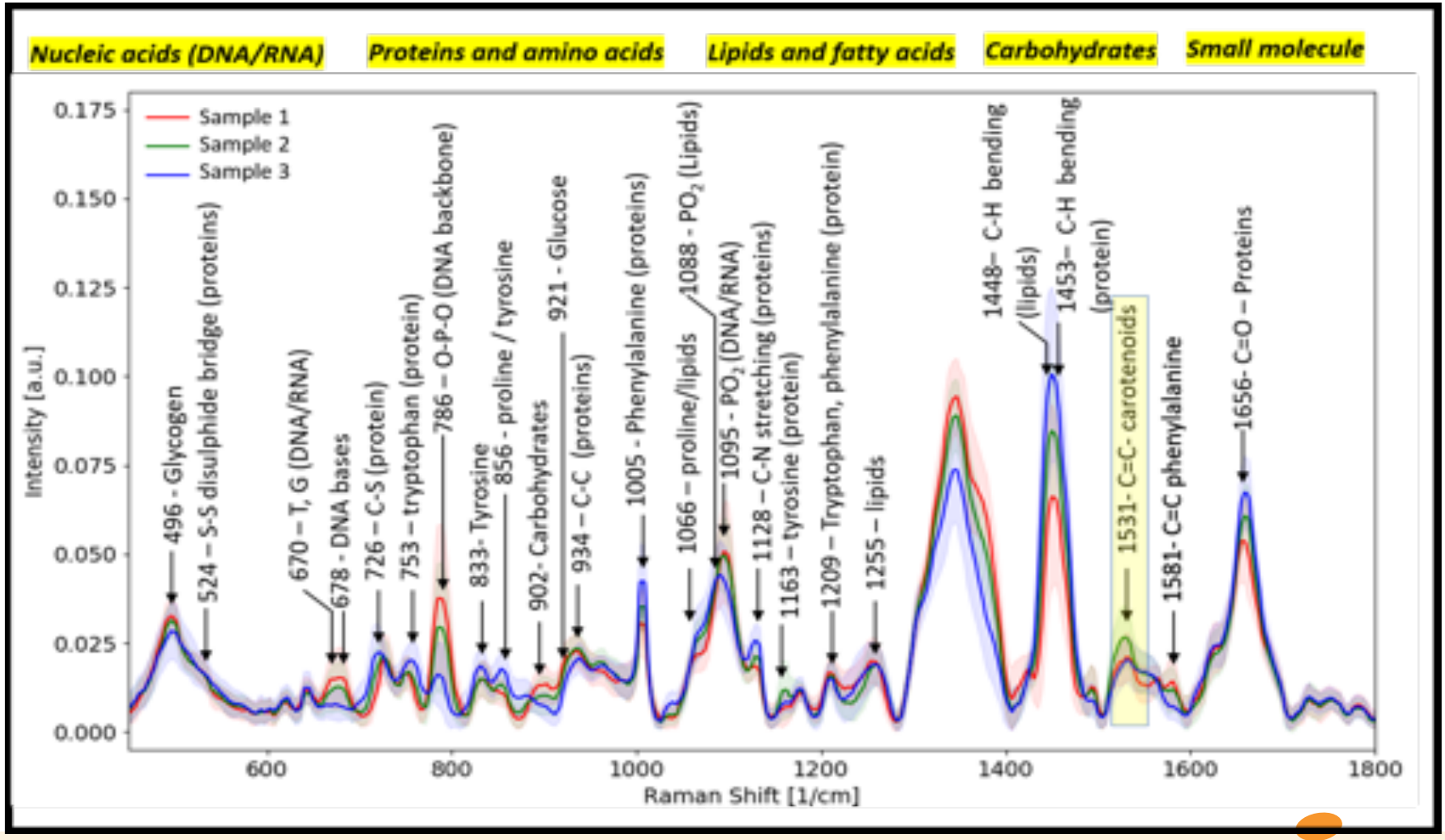
Food safety

GMP of cell-based products

Raman-Multi-omics profile of cells

Functional genomics

Multi-omics information of the cell



BioRam®: Cutting-edge technology

See the whole spectrum of cell development

Cell biology, cell culture, stem cell research

Safe and sound with BioRam®

Quality control of cell based therapeutics

Successful therapy - just a laser beam away

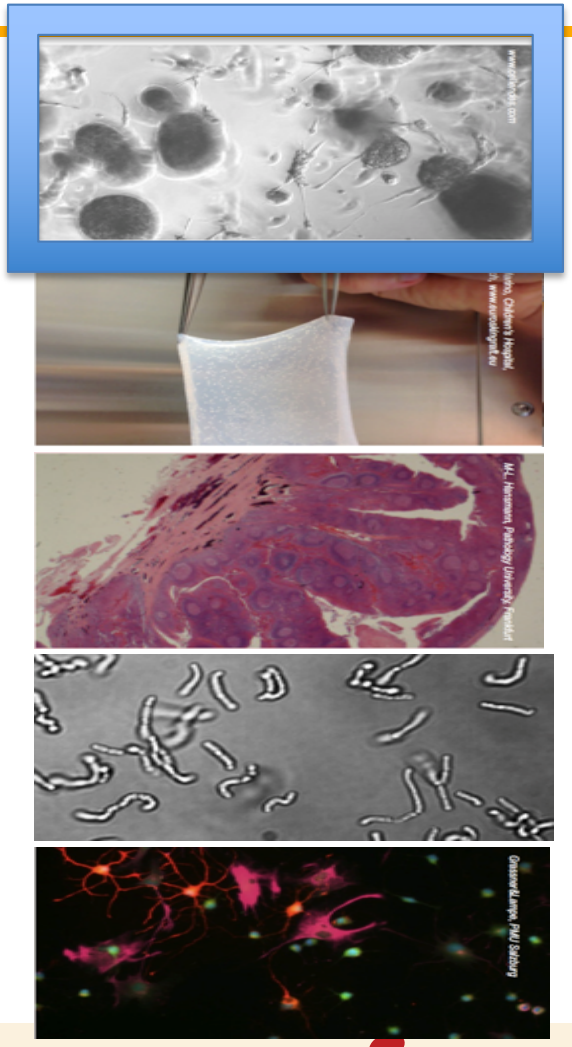
Cancer research, pathogens, exosomes, tumor therapy

Surf and trap across liquids

Microbiology, food contamination, pollution

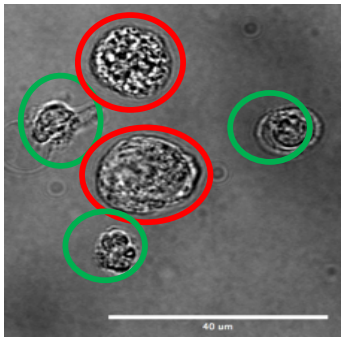
Shed light into cell behavior

Drug & tox screening, environmental impact



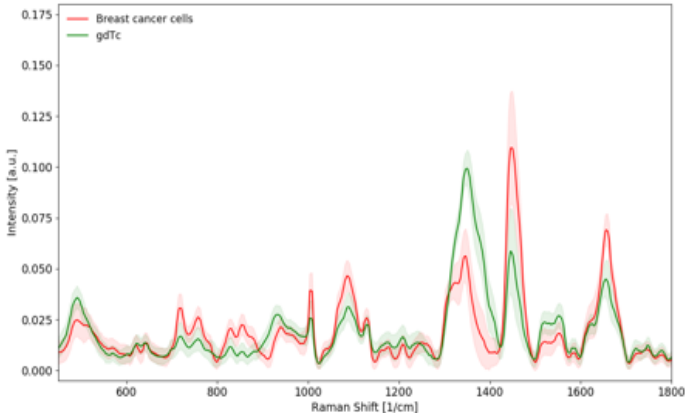
Discriminate cell types

Breast cancer cells differ from T cells

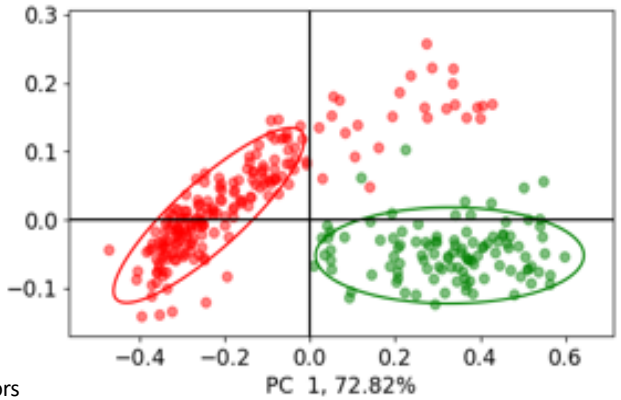


Breast cancer cells have significant different Raman pattern as compared to T-cells **(gdTc)**

eurostars™



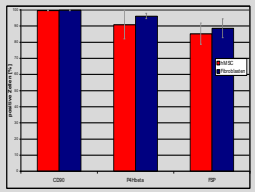
Cell Type discrimination

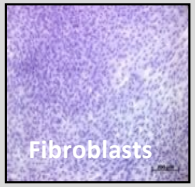


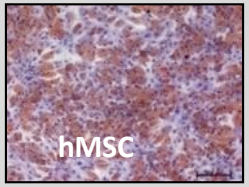
Eurostars E11497 – TEST: Developing Tools to assess Efficacy and Safety of the promising TEG treatment for solid tumors

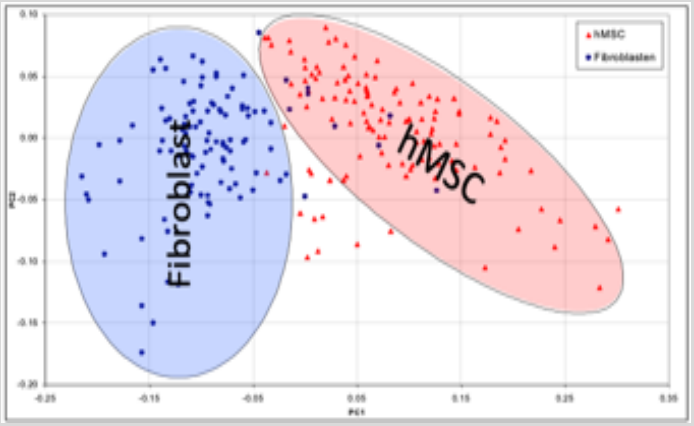
Identify fibroblasts within mixed culture: in Flow cytometry discrimination using CD90, FSP and PH4beta is not significant

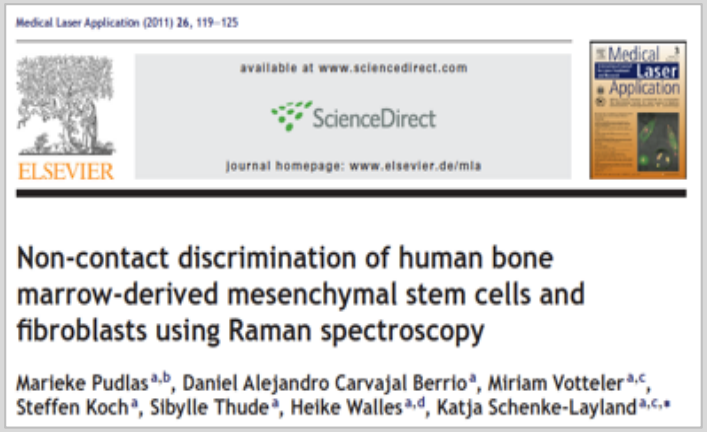
Final discrimination requires long-term cultivation










Medical Laser Application (2011) 26, 119–125

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.de/mla

ELSEVIER

Non-contact discrimination of human bone marrow-derived mesenchymal stem cells and fibroblasts using Raman spectroscopy

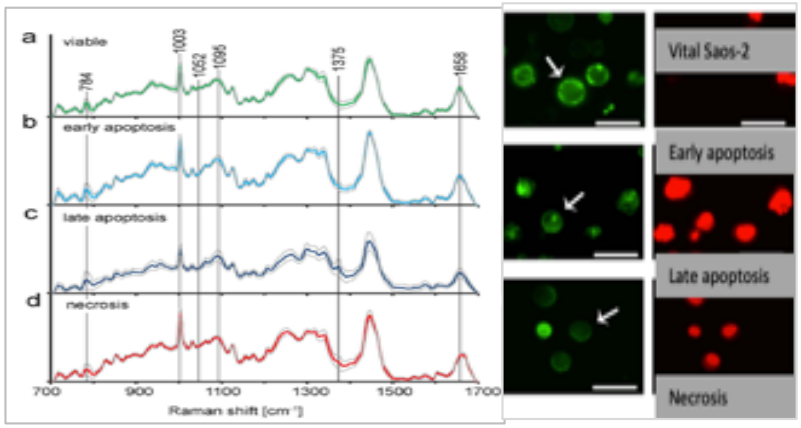
Marieke Pudlas[a,b], Daniel Alejandro Carvajal Berrio[a], Miriam Votteler[a,c], Steffen Koch[a], Sibylle Thude[a], Heike Walles[a,d], Katja Schenke-Layland[a,c,*]

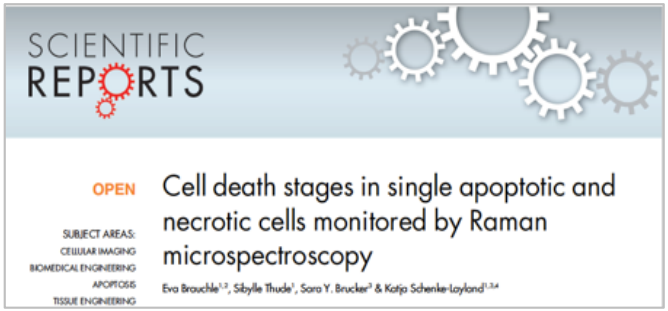
CellTool single cell analysis

Cell growth - differentiation - decay

Identification and discrimination of apoptotic and necrotic cell death in vitro:
fast - continuous monitoring

highly reliably: viable vs dead cells: 99.7%; cell states: 92,3%



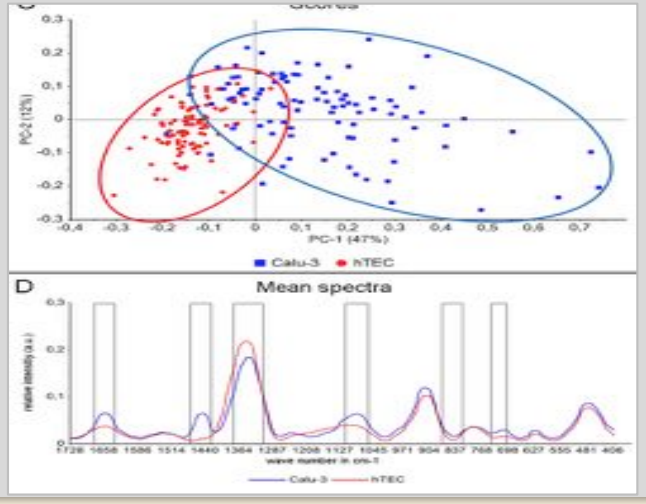


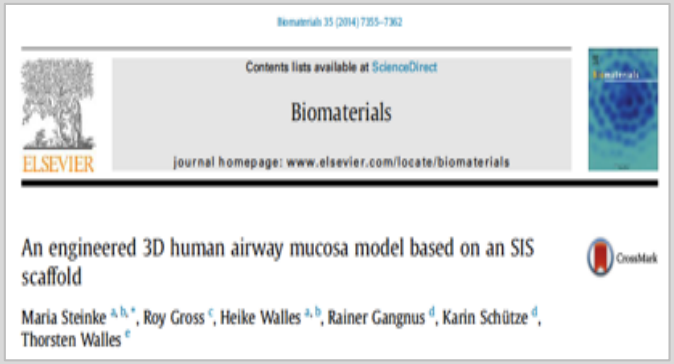
Apoptose studies: S. Koch et al, (2013) Proc. SPIE 8798; Brauchle et al. (2014) Nature Scientific Reports 4 : 4698

Check for cancer cell contamination in engineered tissue:

3D test system for infection studies with human airway pathogens

-> Adenocarcinoma cells vs tracheobronchial epithelial cells.





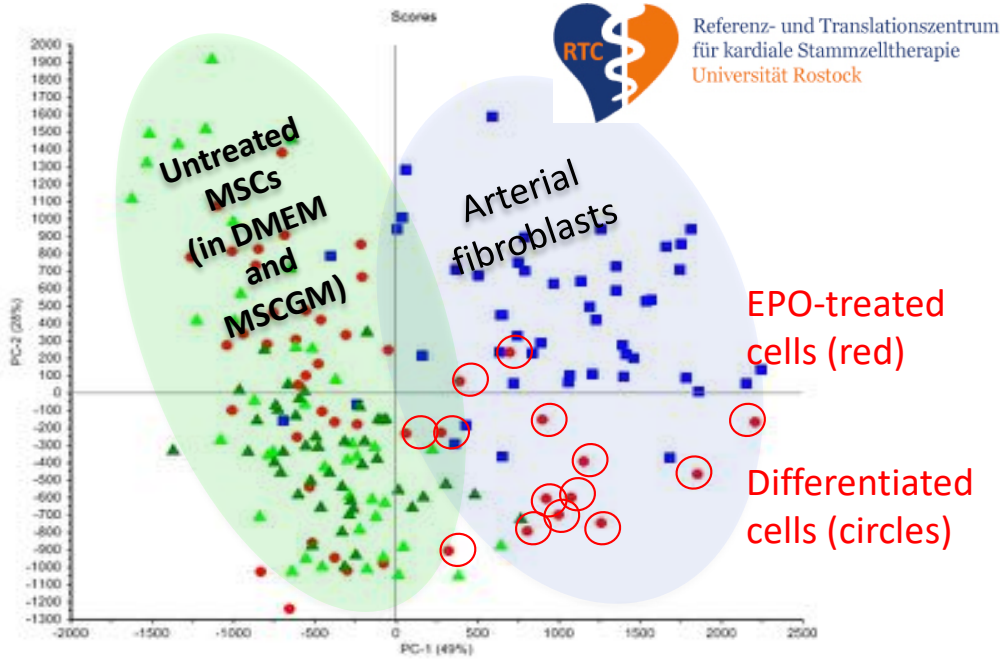
Discriminate cells – quantify differentiation

Improve treatment of ischemic heart failure

Boosting intracardiac regenerative key mechanisms could improve myocardial infarction healing.

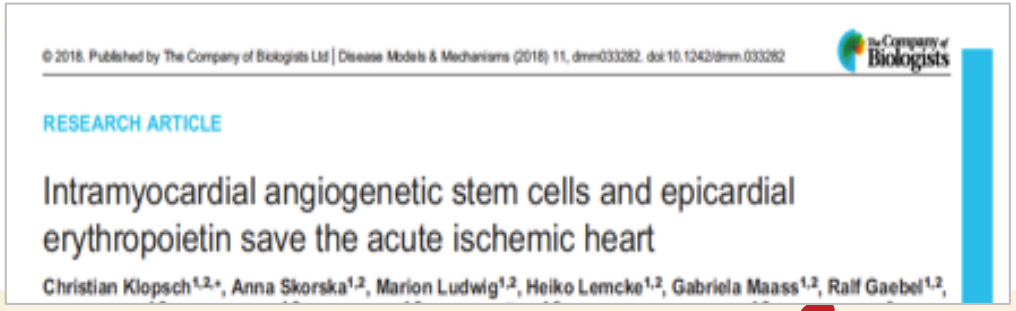
Mesenchymal stem cells treated with Epicardial erythropoietin (EPO) differentiate towards arterial fibroblasts.

Up to now **no discriminating antibody** available!



Referenz- und Translationszentrum für kardiale Stammzelltherapie
Universität Rostock

- 35% of EPO treated cells are differentiated
- results are confirmed **with gene profiling**
- **fast, efficient and reliable quality control of successful differentiation**


© 2018. Published by The Company of Biologists Ltd | Disease Models & Mechanisms (2018) 11, dmm033282. doi:10.1242/dmm.033282

RESEARCH ARTICLE

Intramyocardial angiogenetic stem cells and epicardial erythropoietin save the acute ischemic heart

Christian Klopsch[1,2,*], Anna Skorska[1,2], Marion Ludwig[1,2], Heiko Lemcke[1,2], Gabriela Maass[1,2], Ralf Gaebel[1,2],

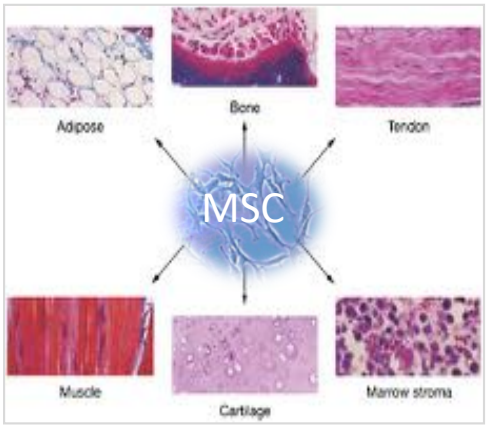
Klopsch et al. Disease Models & Mechanisms (2018) 11

CellTool single cell analysis

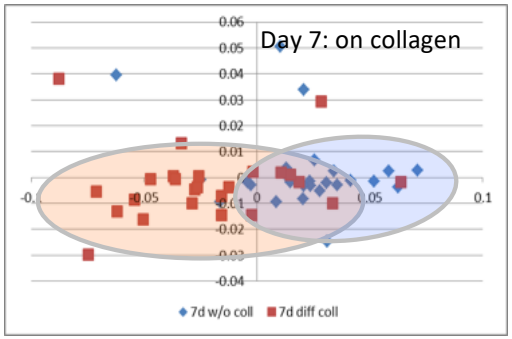
Osteogenic and chondrogenic differentiation

Osteogenic differentiation

How early can Raman detect differentiation?



BulletKit™

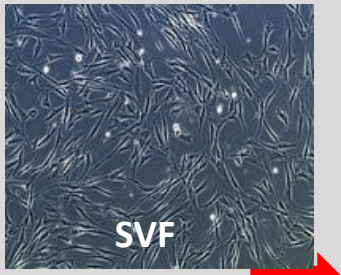


Common: 3 weeks of cultivation (expensive media)

Raman clearly identifies **osteogenic** differentiation on day 7 in cells plated on collagen.

Subcutaneous human adipose tissue:
an abundant source of mesenchymal stromal/stem cells (MSC) with potency to develop to adipocytic, osteoblastic and chondrocytic lineages.

LIPOASPIRATE

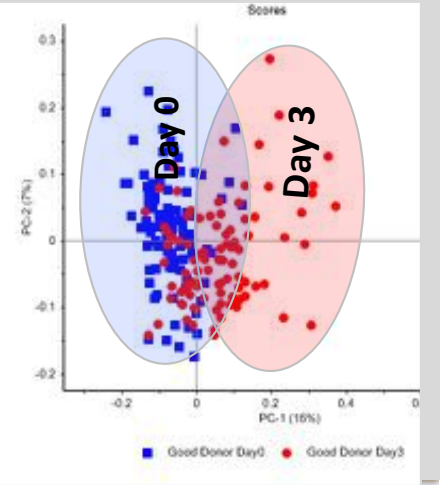


COLLAGENASE

ASC

Common: 3 weeks required to see differentiation.

Raman depicts differentiation at day three of cultivation with media
(70 % of the cells differ from day 0)



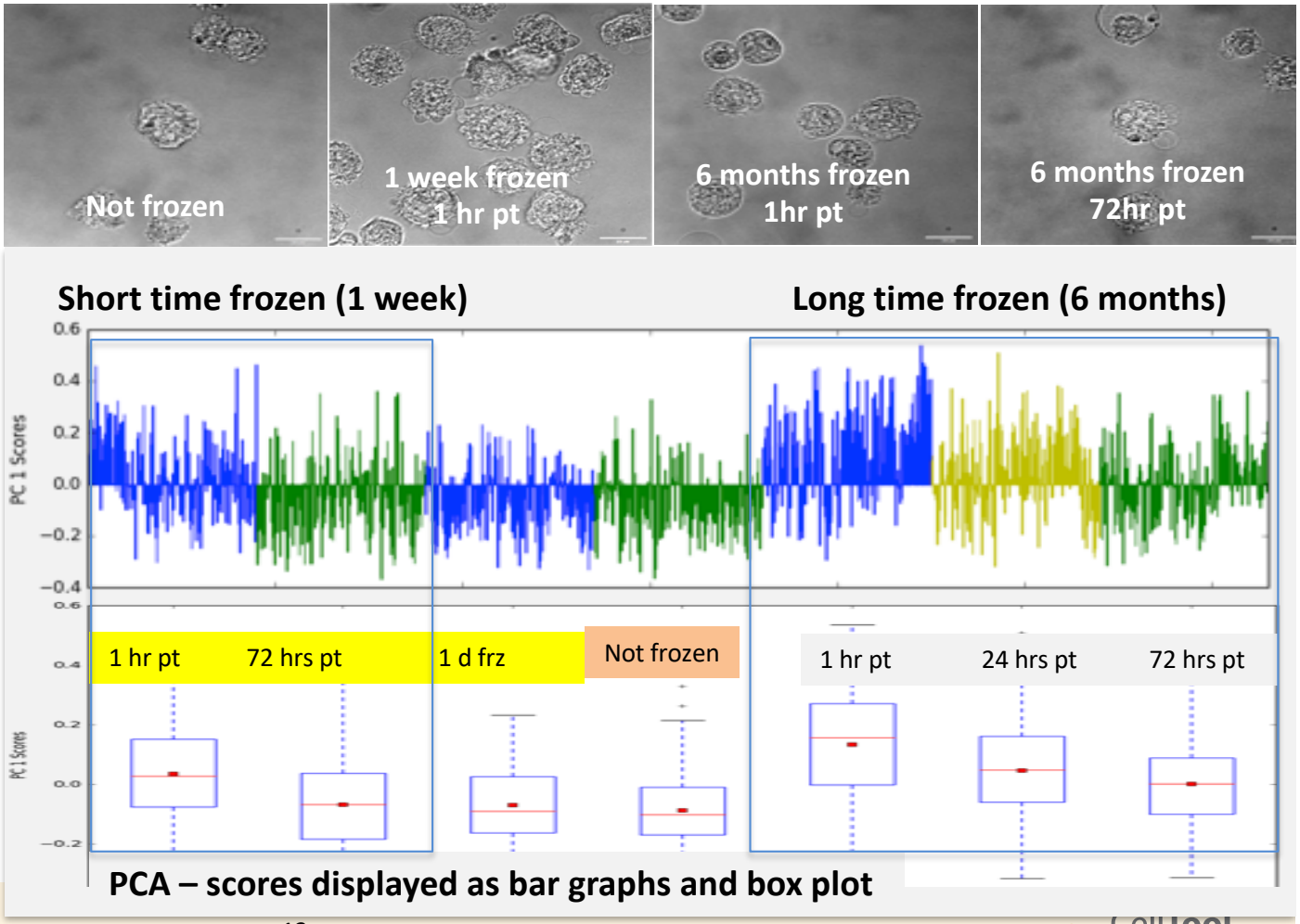
Freezing-Thawing Effects

Mesenchymal Stem Cells

Long term frozen: (6 months):
It takes 72 hrs to get comparative spectra as unfrozen samples.

No differences between samples frozen only for 1 day

-> time of freezing might require elongated time of regeneration in culture



PCA – scores displayed as bar graphs and box plot

Guido Moll AG Geißler

BioRam®: Cutting-edge technology

See the whole spectrum of cell development

Cell biology, cell culture, stem cell research

Safe and sound with BioRam®

Quality control of cell based therapeutics

Successful therapy - just a laser beam away

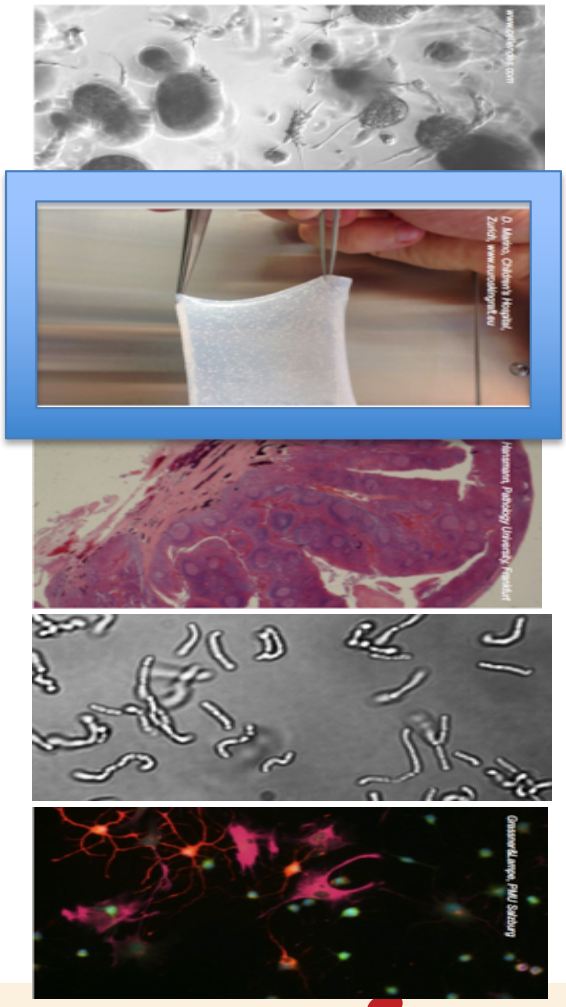
Cancer research, pathogens, exosomes, tumor therapy

Surf and trap across liquids

Microbiology, food contamination, pollution

Shed light into cell behavior

Drug & tox screening, environmental impact



ATMP – THE FUTURE IN MEDICINE

Advanced Therapy Medicinal Products (ATMP)

a new category of medicines with a wide therapeutic potential for treating different types of diseases such as cancer, neuro-degenerative and cardiovascular diseases including

- Tissue Engineered Products (TEP)

  - skin, bone, cartilage grafts

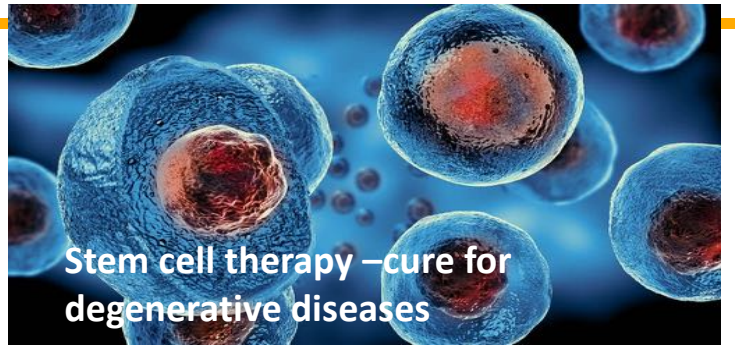
- Cell Therapy Medicinal Products (CTMP)

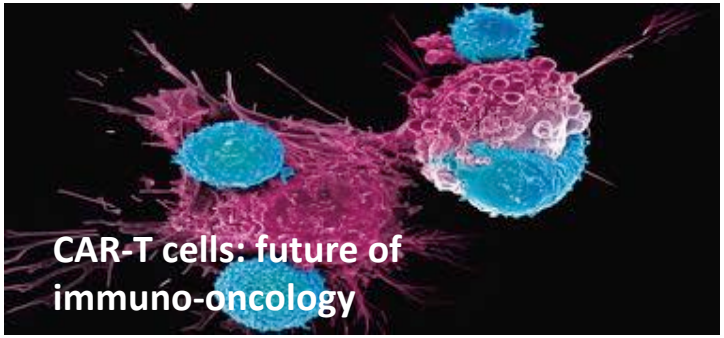
  - blood products, TiLs, immuno-therapeutics

- Gene Therapy Medicinal Products (GTMP)

  - iPS ,CAR-T cells

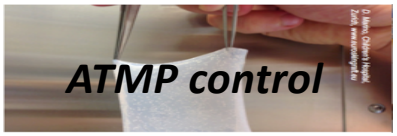
Quality control plays a pivot role to warrant safety of cell-based products -> huge potential for BioRam®


Stem cell therapy –cure for degenerative diseases


CAR-T cells: future of immuno-oncology


iPS cells - induced pluripotent stem cell lines derived from human somatic

Functional and quality control of blood products

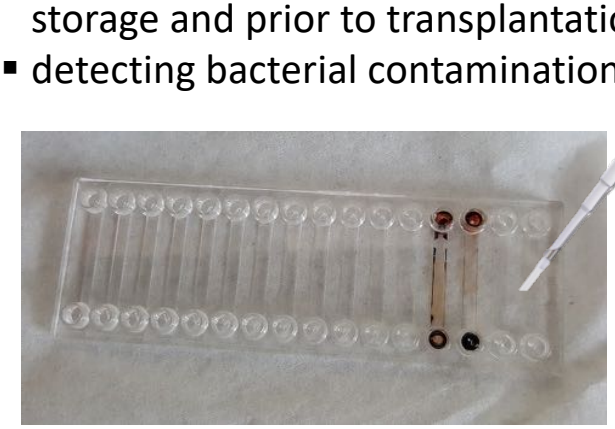


Increase of patient safety – erythrocyte product

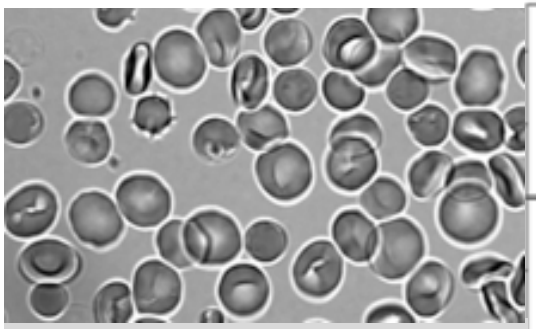
Today: only **spot test - 1%** during production
(12 different routine tests performed)

Need: increased patient safety

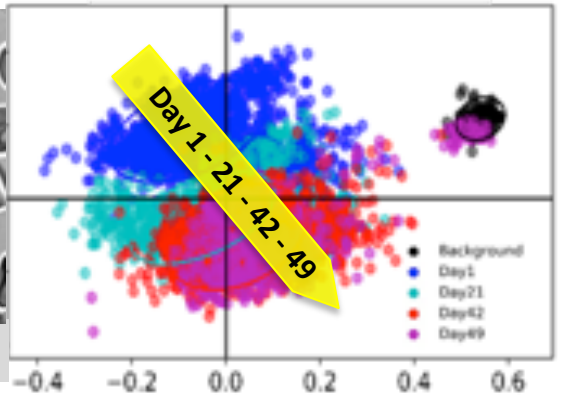
- monitoring stability and functionality duri storage and prior to transplantation
- detecting bacterial contamination



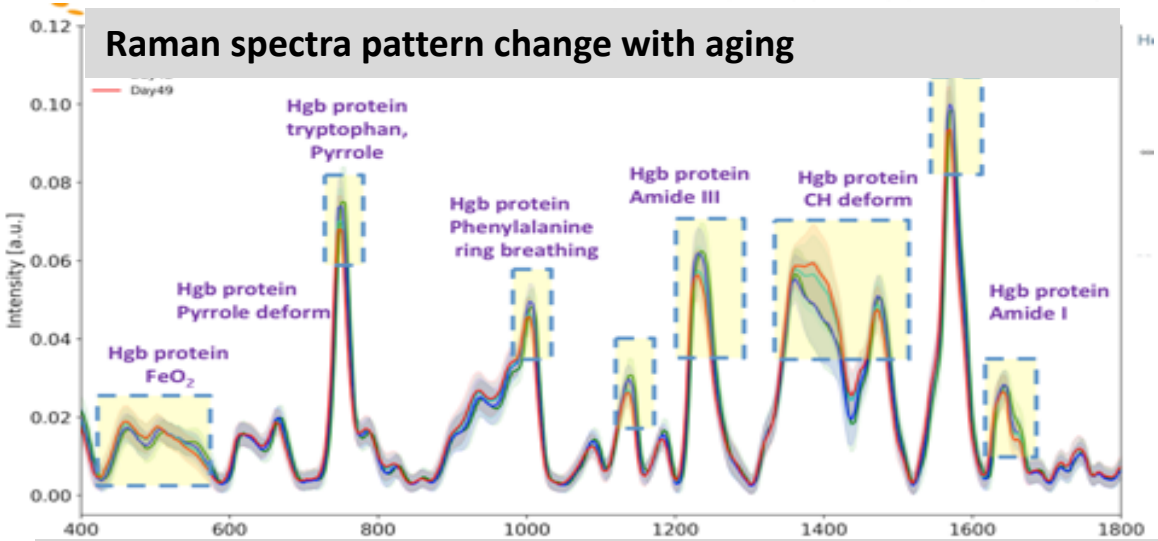
Bundesministerium für Bildung und Forschung



Erythrocytes: shelf live 48 days



Raman spectra pattern change with aging



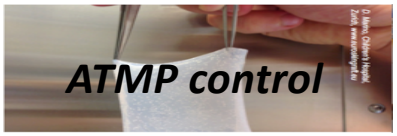
Spectral change corresponds to structural changes of hemoglobin

BioRam® could check functionality of blood products immediately prior transfusion

BMBF-"KMU-innovative: Medizintechnik: HämatoRam" 13GW0112A

CellTool single cell analysis

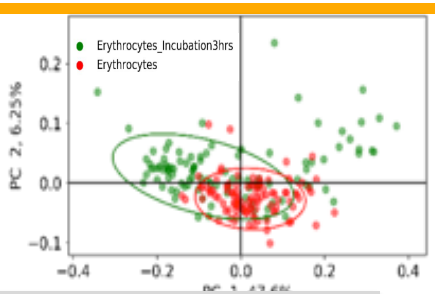
Identify bacterial contamination in minutes

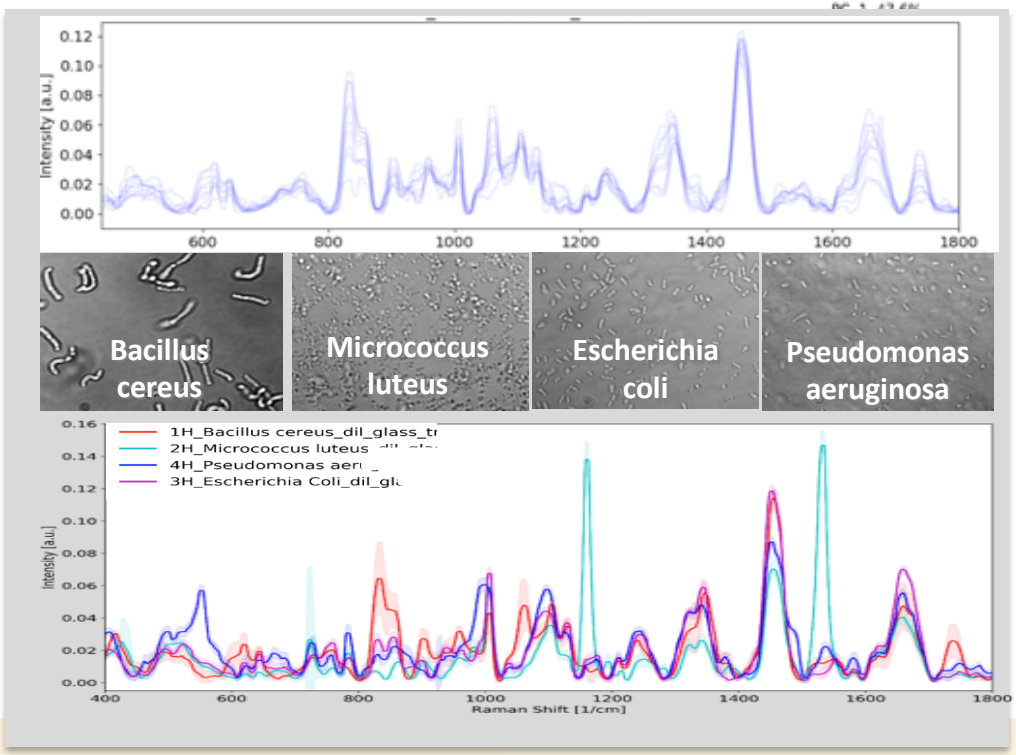


Sterility testing

Blood product spiked with bacteria:

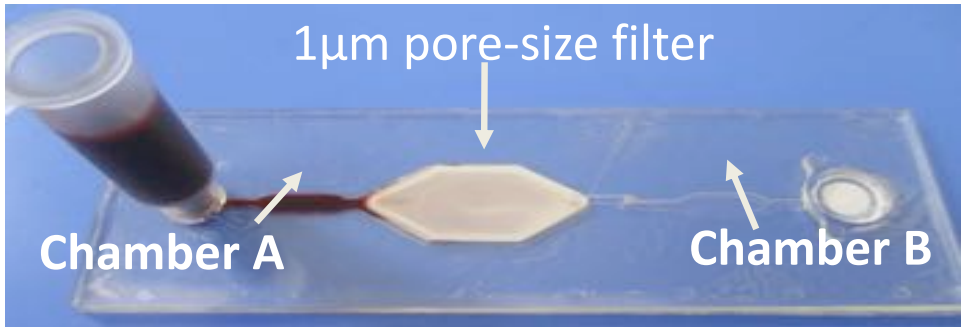
Raman pattern of erthrocytes change immediately (< than 3 hrs).

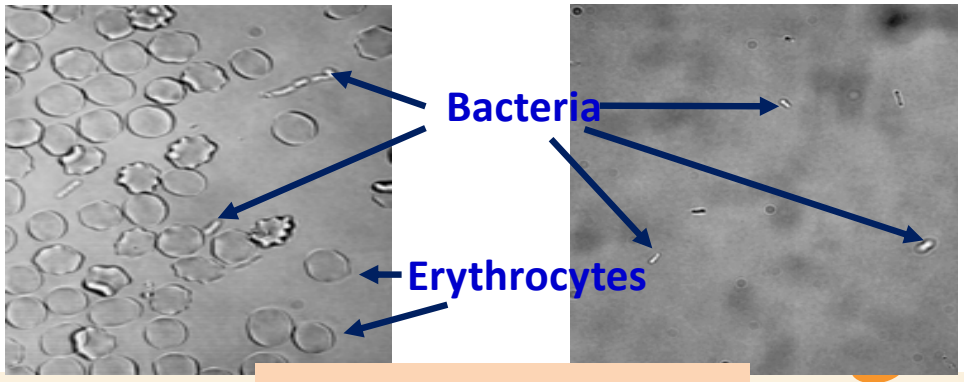




Fast contamination test:

A droplet of whole blood mixed with bacteria is pipetted into a special membrane chip (ChipShop, Jena). Blood cells are retained within the meshwork and only bacteria are found in chamber B.





> Preparation time 5 min !!

“KMU-innovative: Medizintechnik: HämatoRam” 13GW0112A

CellTool single cell analysis

Skin graft – increase patient safety & reduce costs

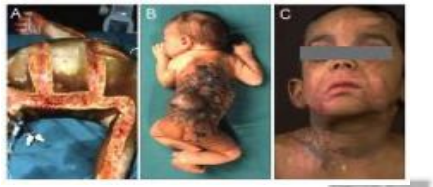
Quality control during production

Standard: FACS analysis to exclude cross-contamination of expanded cells (<5%)
-> vast amount of cells required
-> specific antibody is not available

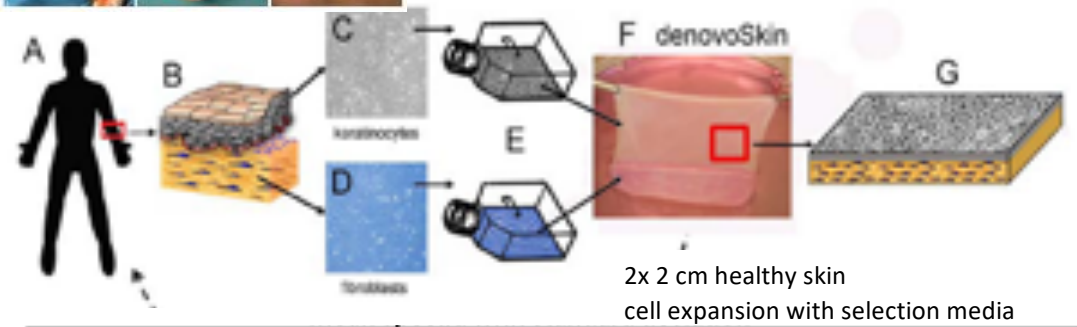
(*expansion and staining requires > 48 hrs;*
cell culture material & antibodies are expensive)

Needs:
- reduction of costs necessary
- immediate results desirable

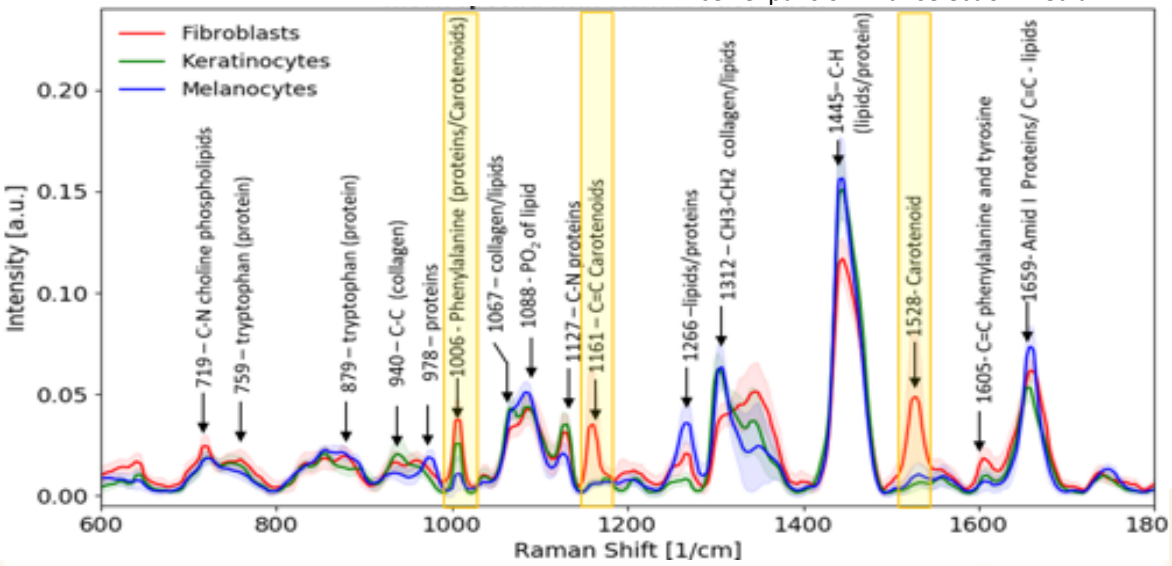
CUTISS personalized skin



tissue biology research unit
Kinderspital Zürich · Chirurgische Klinik



2x 2 cm healthy skin
cell expansion with selection media



BioRam® checks for purity and functionality of expanded skin cells in less than 2 hrs !!

Photonic marker: carotenoid peaks for fibroblasts!

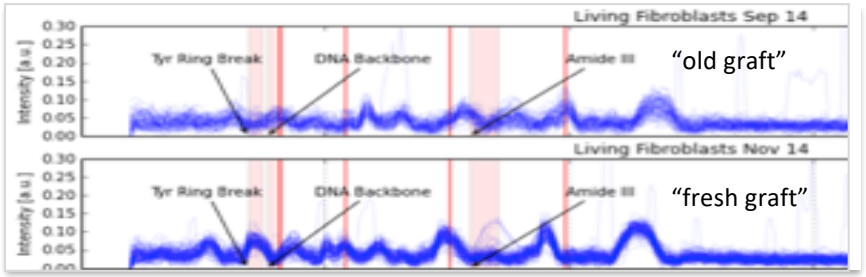
Autologous skin graft – increase patient safety

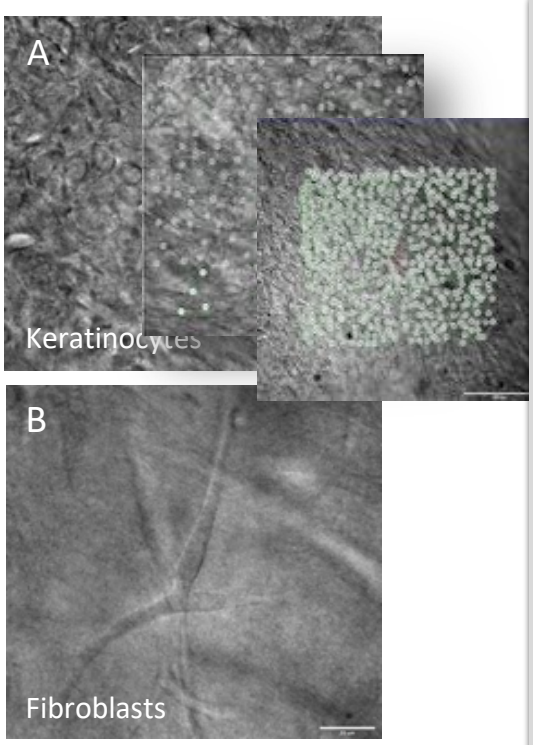
Quality control of the final graft

Standard: DNA count – 24 hrs
Provides total number of cells

BioRam®: discriminate fibroblasts and keratinocytes within 3D graft
Check for **purity** and **functionality**
Cell counting via DAPI fluorescence (4-6 hrs)

Cost and time saving, increased safety
Including reduced time at intense care
BioRam® could save up to €3.000 per graft !





tissue biology research unit
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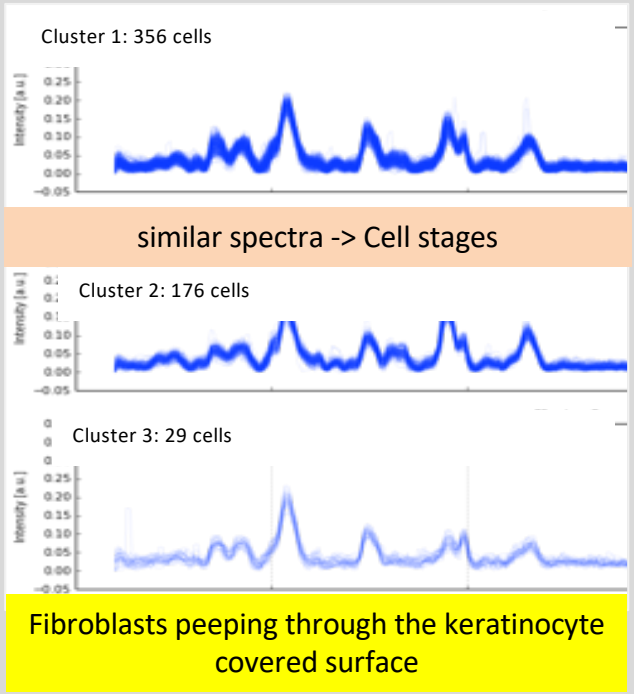
Cluster analysis: Keratinocytes - 561 cells

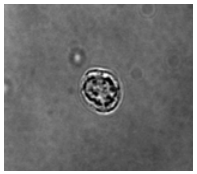


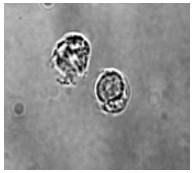
Fibroblasts peeping through the keratinocyte covered surface

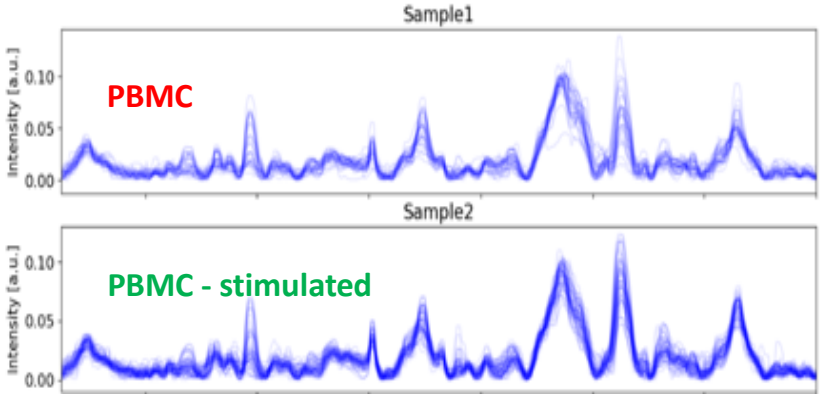
Raman microscopy identifies activated T-Cells

PBMC differ from stimulated PBMCs

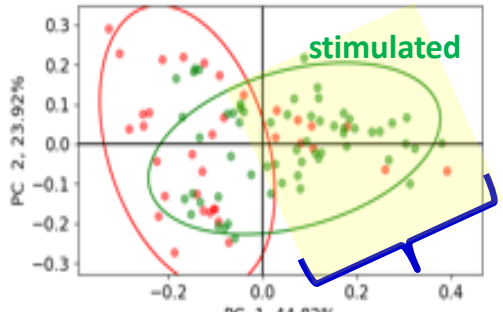
PCA loadings indicate a decrease in DNA intensity and an increase of proteins upon stimulation of PBMCs.





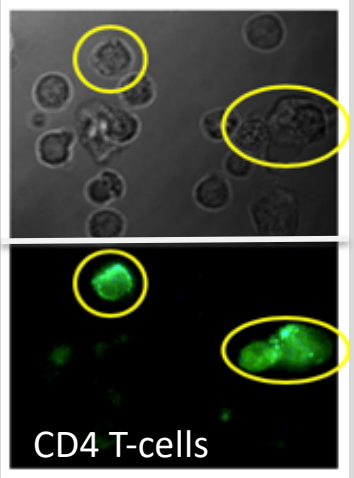


PCA-scores plot



58% of the cells are stimulated

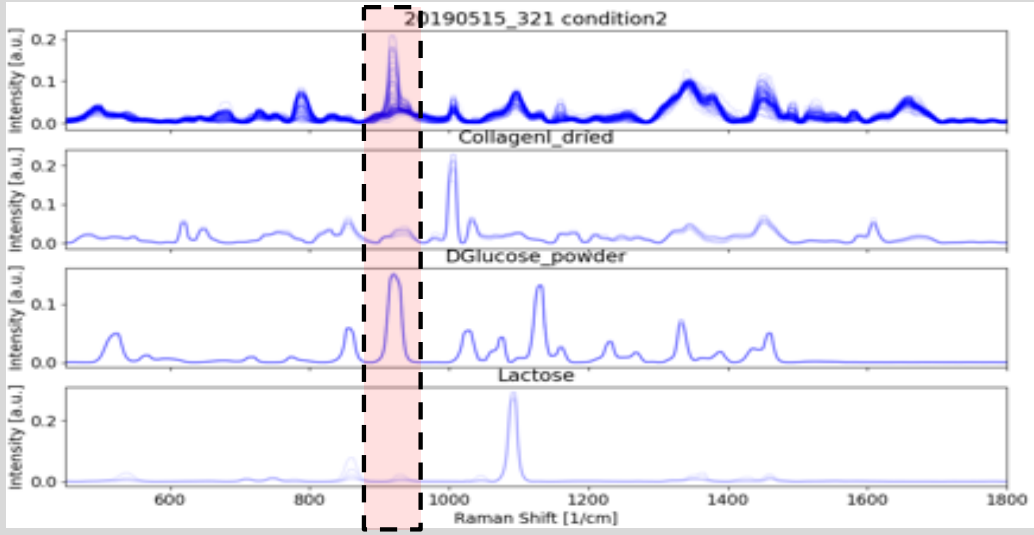
Photonic marker for activated T-cells



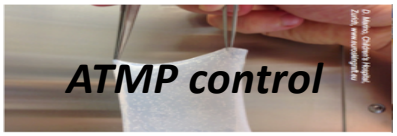
A spectral marker for T-cell activation??
Activated T-cells show a distinct peak at 920 cm^{-1} (C-C stretching vibration).

Spectra were compared with collagen type I, D-glucose, and lactose.

D-glucose has the band at 920 cm^{-1} similar to the T cells samples.



Raman-Trapping-Microscopy in Immunotherapy



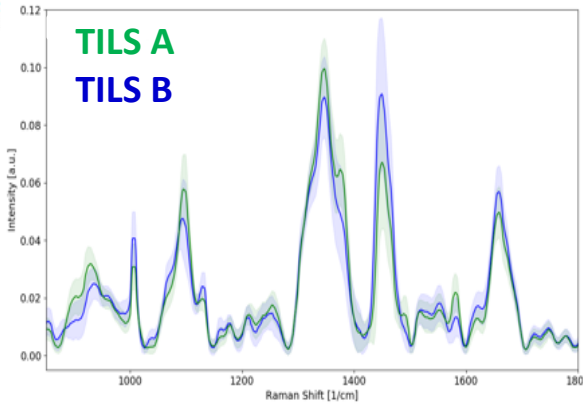
Tumor infiltrating lymphocytes (TiLs)

Zellwerk GmbH·HiPer-Gruppe

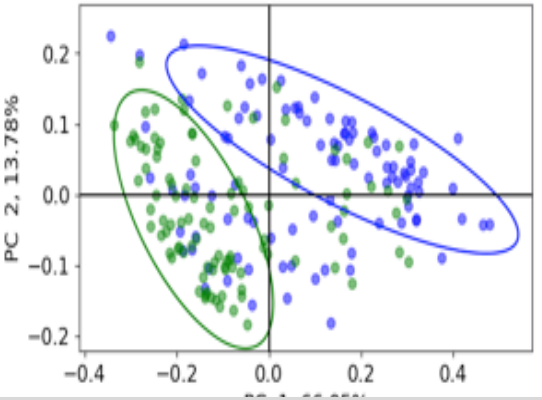
Effective cancer immunotherapy depend on the presence of large numbers of anti-tumor lymphocytes. Those are isolated from tumor biopsies, identified and grown ex vivo.

Raman discriminates various TiLs and might be used to identify most efficiently therapeutic TiLs.

Raman mean spectra



PCA-score plot

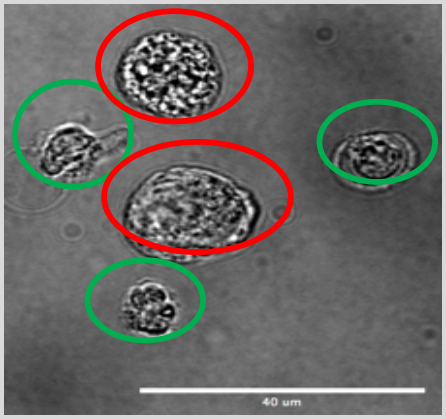


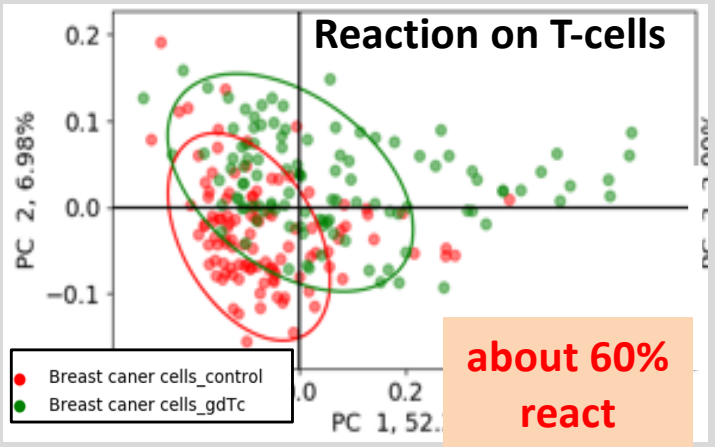
T-cells induce apoptosis in breast cancer cells

Breast cancer cells react on gδ-T cells.

- decrease in intensity of proteins, increase in nucleic acids after 24 hrs of gδ-T cell incubation.

Change in Raman spectra correlate with typical signs of **early and late apoptosis**





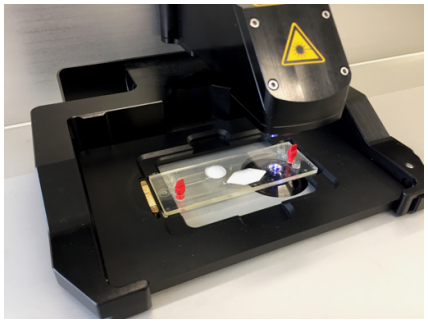
S. Koch et al, (2013) Proc. SPIE 8798;
Brauchle et al. (2014) Nature Scientific Reports 4 : 4698
Steinke, M., *et al*, (2018) *Angew. Chem. Int. Ed. 57, 4946 –4950*

Fast examination of exosomes and pathogens

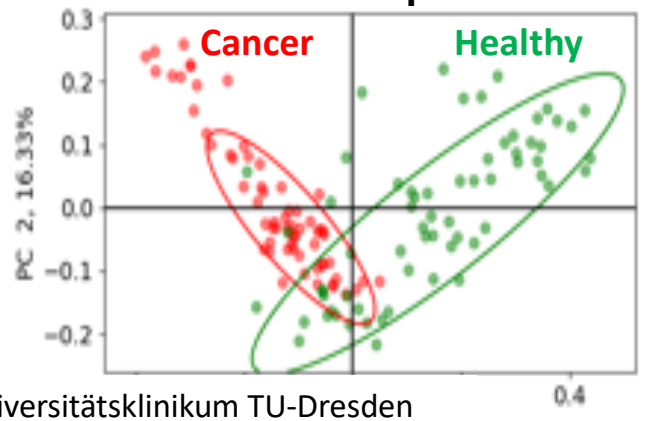
Comparison exosomes from tumor patients & from patients with vascular disease

Laser trapping was applied to collect and enrich exosomes within the laser focus.

30 Raman spectra were collected from each sample revealing clear differences between both cases.



PCA-score plot

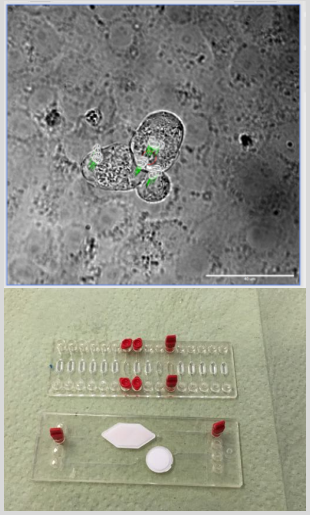


Promising marker for disease detection and therapy follow-up

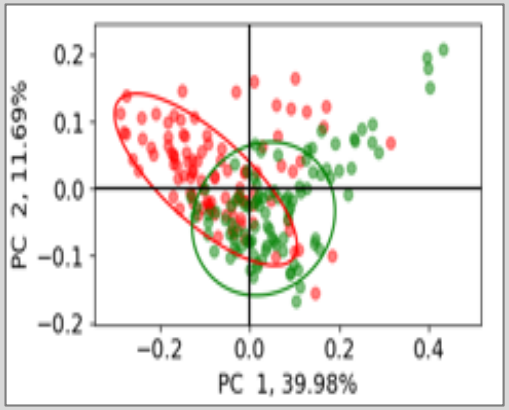
C.Kahlert Universitätsklinikum TU-Dresden

Covid and Influenca virus infected cells *(Preliminary findings):*

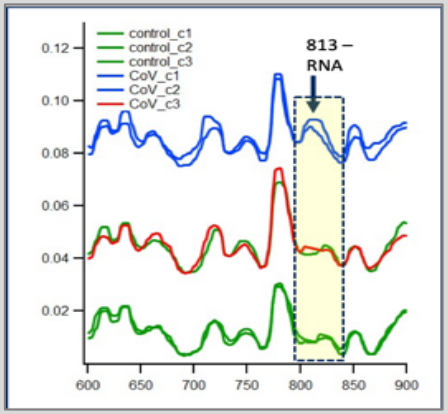
- influencaA (nucleus): clear changes in **IVA infected A549 lung** cancer cells after 24h incubation.
- impact of **SARS-CoV2 virus** on the nucleus (increase of RNA content)
- Measuring virus directly within solution is possible due to trapping



A549 cells incubated 24 hrs with IVA



Custer analysis of Vero cells incubated 24 hrs with Covid



Raman Trapping of bacteria and erythrocytes

Raman-Trapping Microscopy for deeper product insight

- purity
- potency
- functionality

during production and of the final product & monitor therapeutic success

Support healthiness – save life

FAST, GENTLE, EFFICIENT

specific information about the overall chemical composition of cells and fluidics

precise results – in 2D and 3D cell culture, and tissues

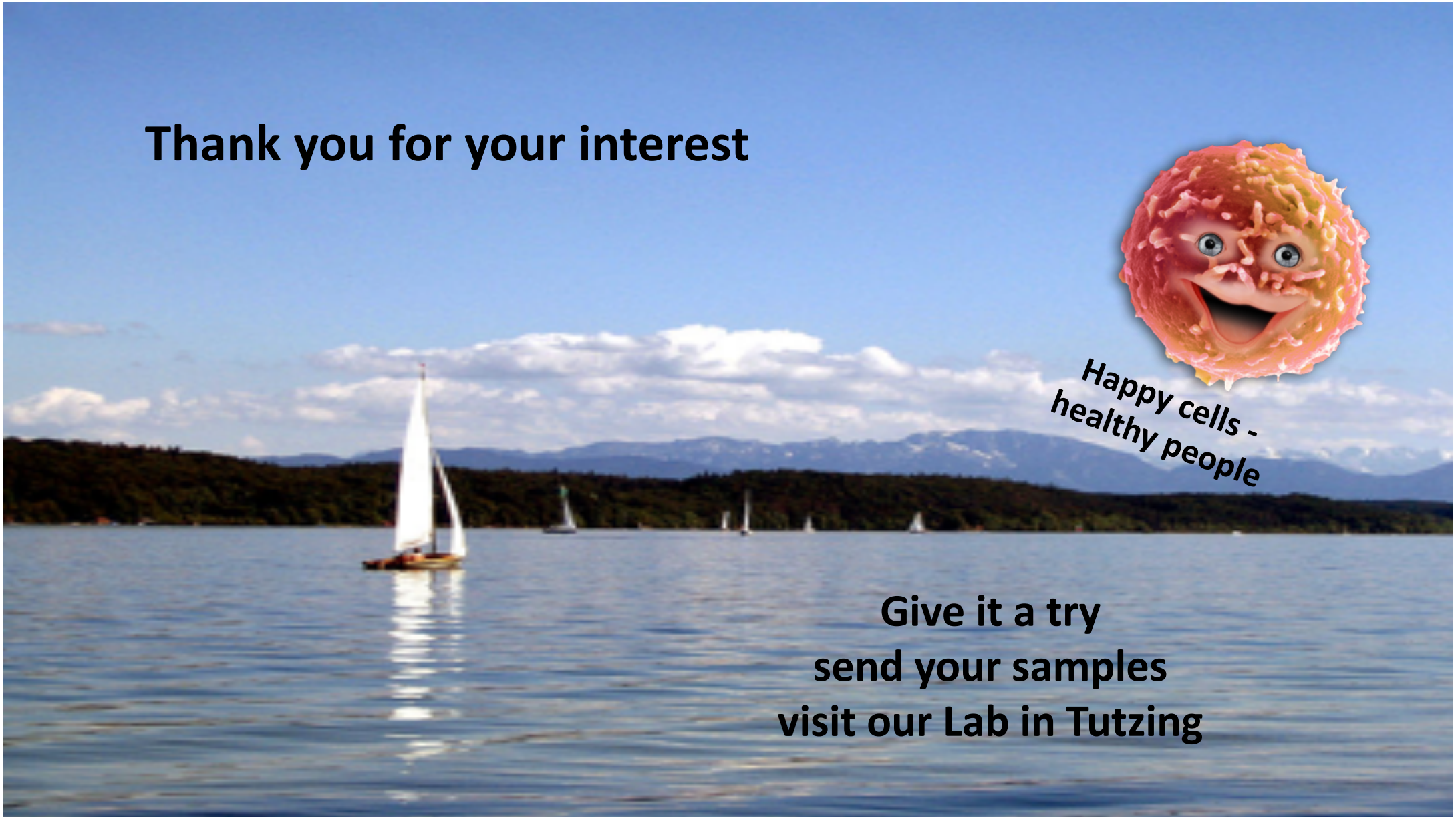
work with **small sample** amounts (100 cells)

investigate eukaryotic, prokaryotic, plant cells …

Unique **trapping features** to analyze cells bacteria, exosomes, or virus in solution

Live cell sorting of Raman identified cells

CellTool single cell analysis


Thank you for your interest
Happy cells -
healthy people
Give it a try
send your samples
visit our Lab in Tutzing