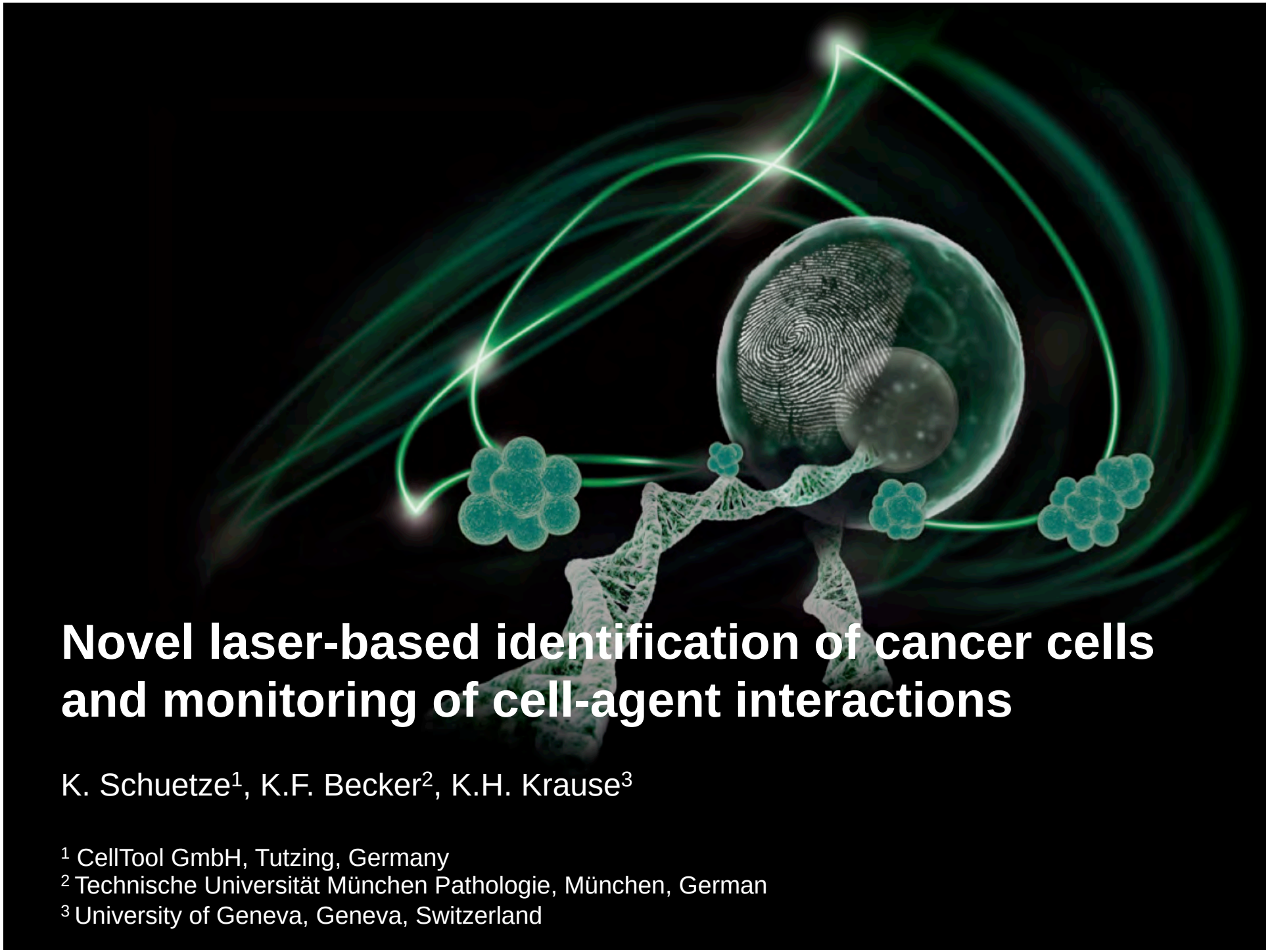


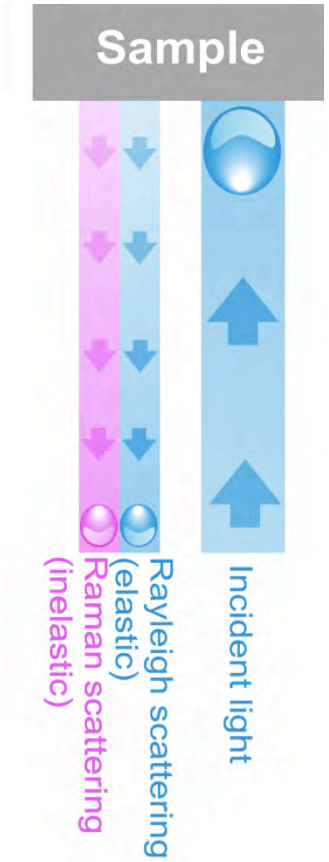
Novel laser-based identification of cancer cells and monitoring of cell-agent interactions

K. Schuetze[1], K.F. Becker[2], K.H. Krause[3]

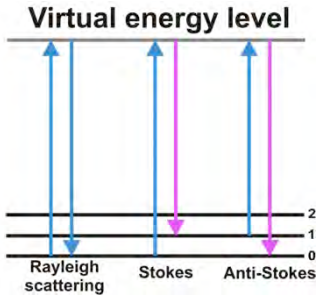
[1] CellTool GmbH, Tutzing, Germany
[2] Technische Universität München Pathologie, München, German
[3] University of Geneva, Geneva, Switzerland

Raman effect

Raman spectroscopy (RS) is molecular spectroscopic technique, based on the detection of light that has been **inelastically scattered** by a sample (“Raman effect”).



It occurs when photons of an incident light interact with molecules. The difference in the photon energy between scattered and incident light results from the change in the vibrational or rotational state of the molecules.



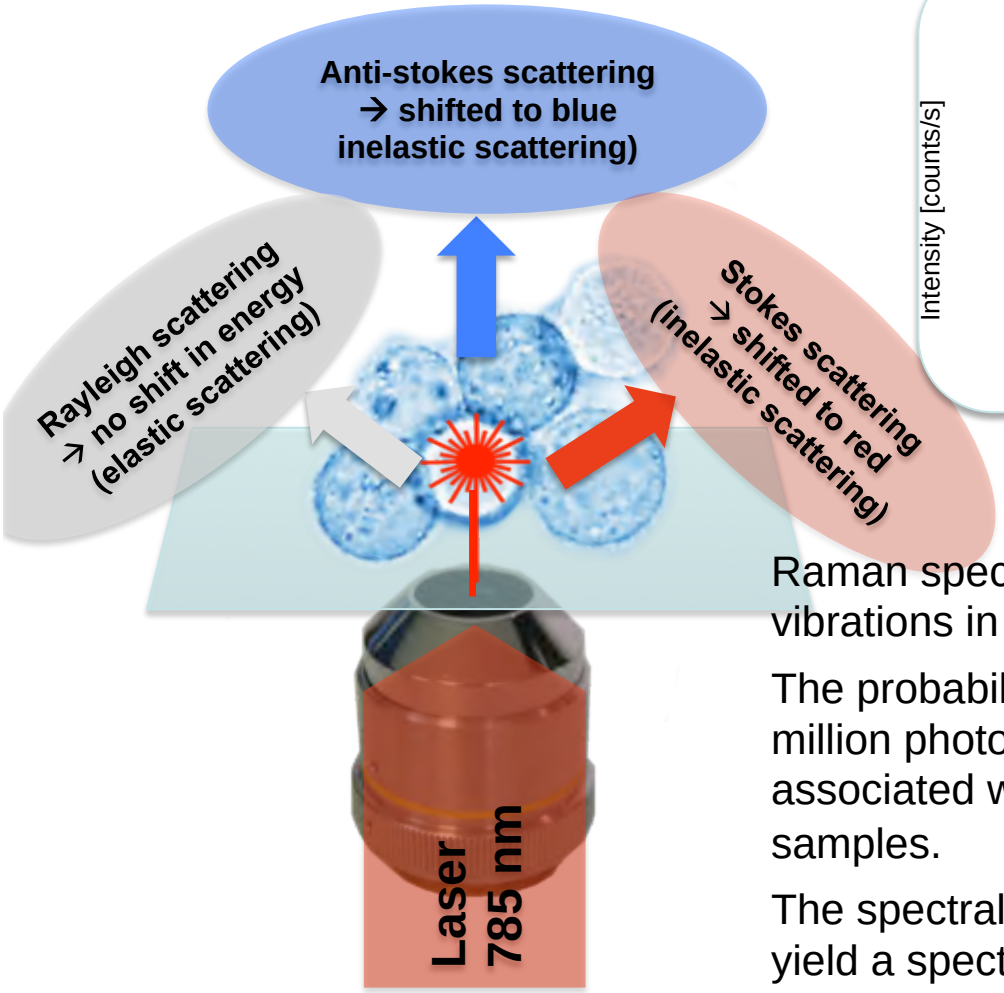
The scattered light can have a **lower** (Stokes Raman scattering) or a **higher** (anti-Stokes Raman scattering) frequency than the incident light.

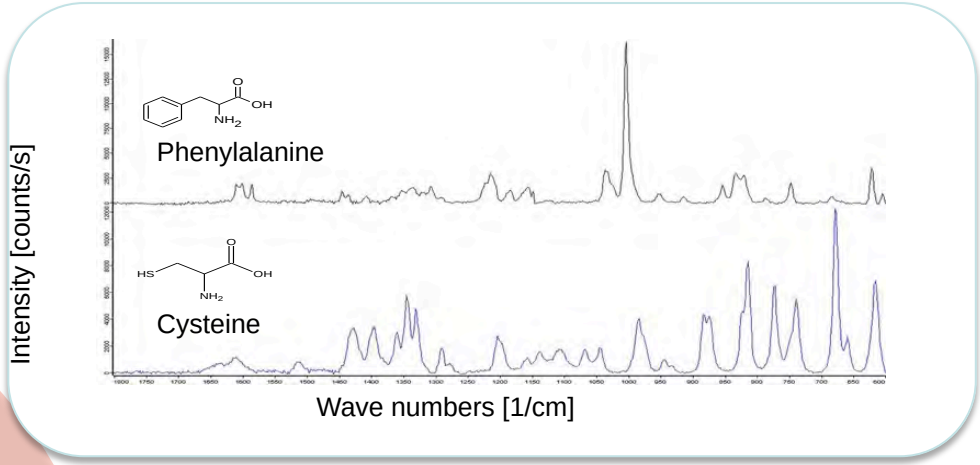
The Raman effect was first observed by Indian physicist C.V. Raman in 1928.

Chandra Venkata Raman
(1887 -1970)

Raman Spectroscopy is sensitive to the structural changes of complex biomolecules such as proteins, lipids, and nucleic acids, and therefore, provides information from the entire organism.

Raman Spectroscopy – a non-destructive method to characterize cells



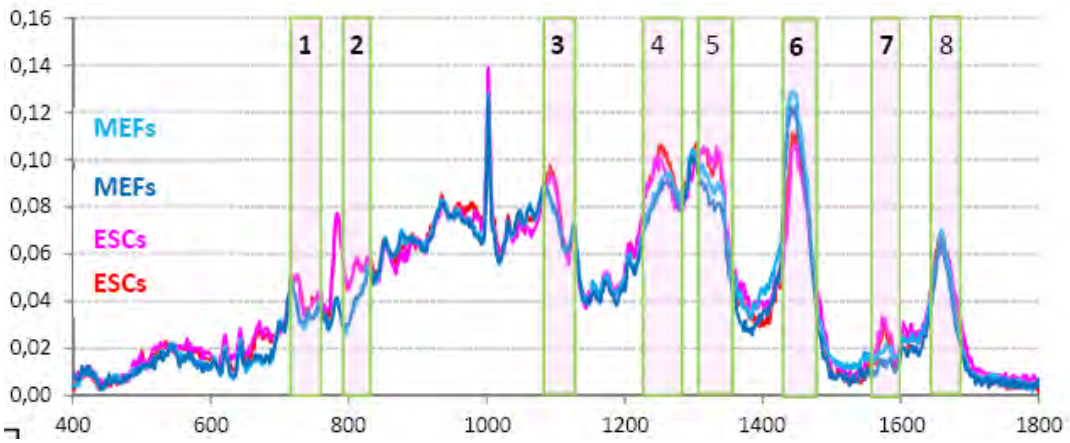


Raman spectroscopy uses laser light to induce molecular vibrations in cells.

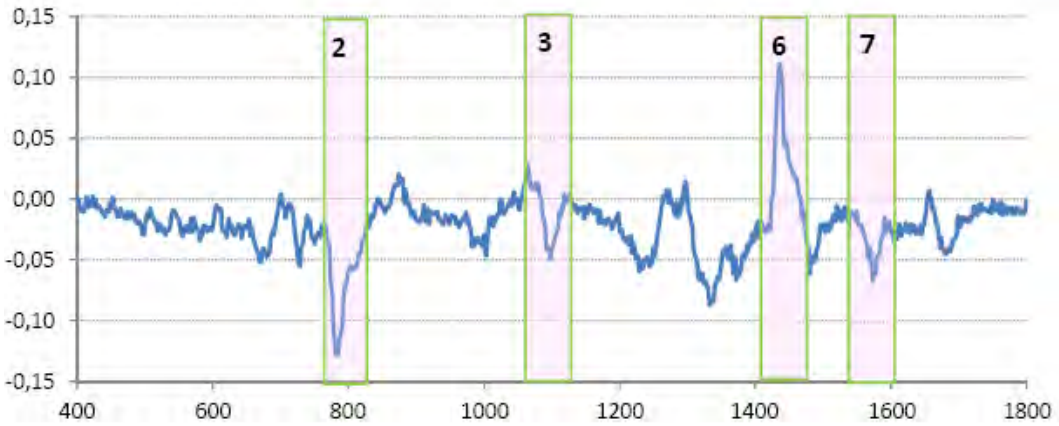
The probability of Raman scattering is weak (about 1 per 10 million photons) but results in well-resolved peaks, uniquely associated with different biochemical properties of the samples.

The spectral contributions from **all molecules** within the cell yield a spectral sum - as characteristic as a “fingerprint”.

Raman spectra – Statistical Analyses

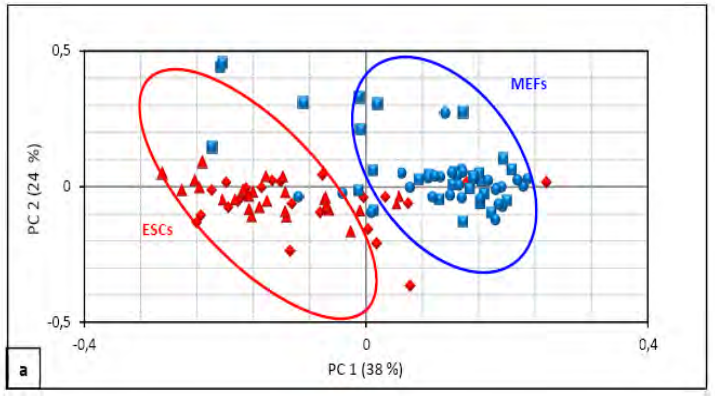


DNA proteins CH_2 proteins



Qualitative analysis by Principal component analysis:

- Data reduction
- Pattern recognition in spectral data
- Identification of chemical differences



Work by Anne Knopf and Katja Schenke-Layland, Fraunhofer IGB, Stuttgart

Raman Microscope for biomedical applications

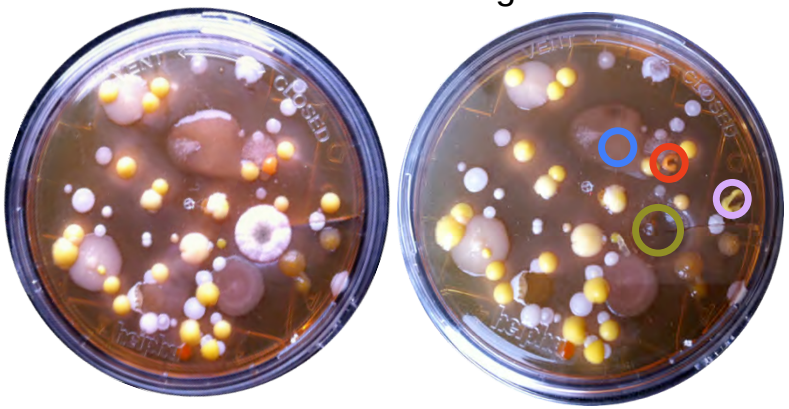
BioRam® unique features

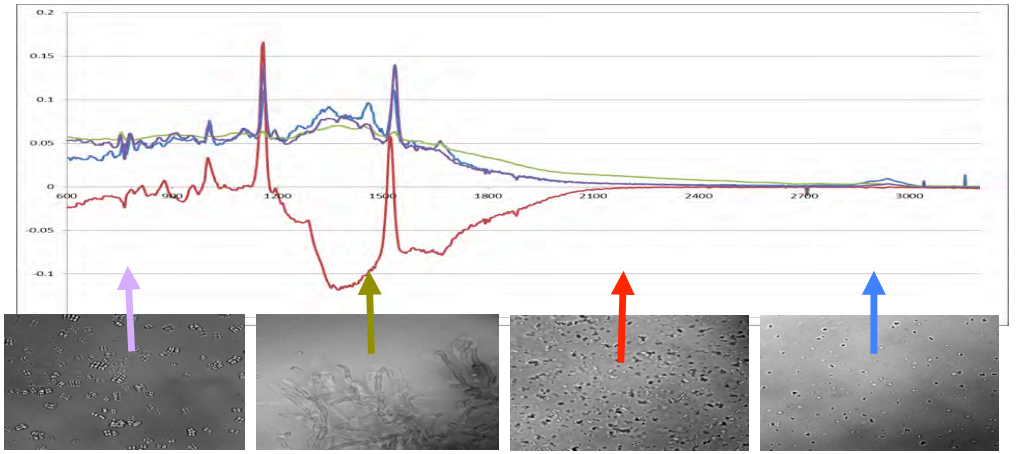
Inverted digital microscope platform with laser **trapping** configuration for live cell Raman spectroscopy with integrated data processing

- identify cell types and activity states **without labeling** and **under physiological conditions**, even in fluids
- distinguish different stem cell types or determine their **differentiation stage**
- **characterize cells** to a high degree of **specificity**, even those that cannot clearly be defined by surface markers or where markers simply do not exist.
- identify with high sensitivity extremely **rare cells** in human samples
- work with **minimal sample volumes**

NIR Raman Spectra of microorganism

Airborne microorganism





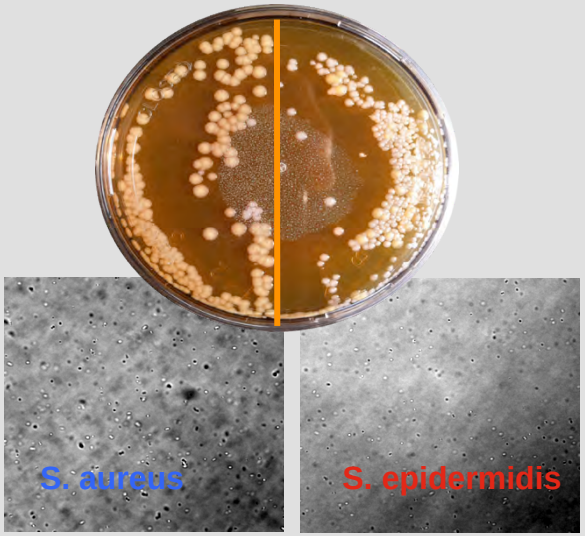
Bacteria from yellow colony

Fungi

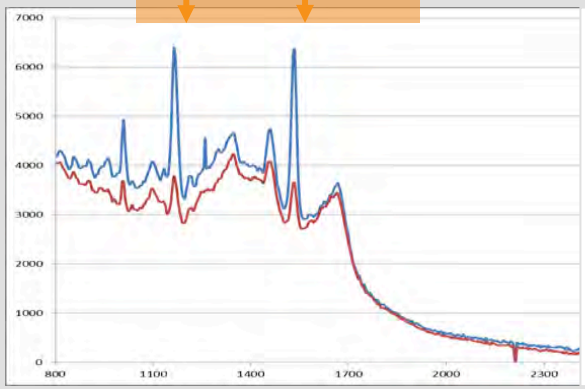
Bacteria from red colony

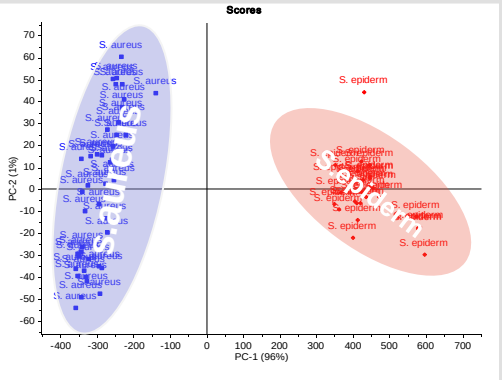
Bacteria from colorless colony

Staphylococcus aureus and S.epidermidis



Carotenoids

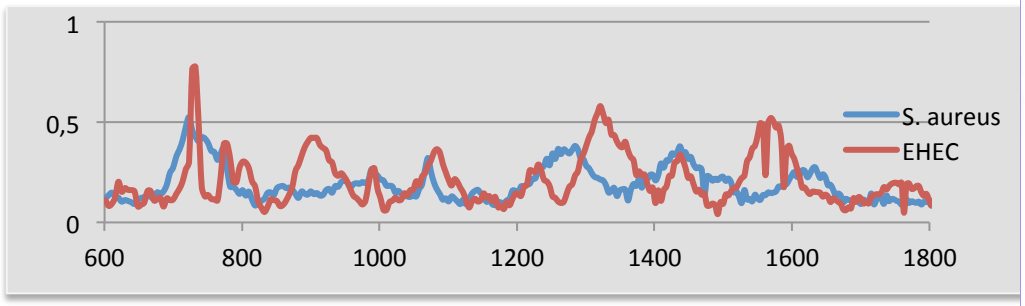


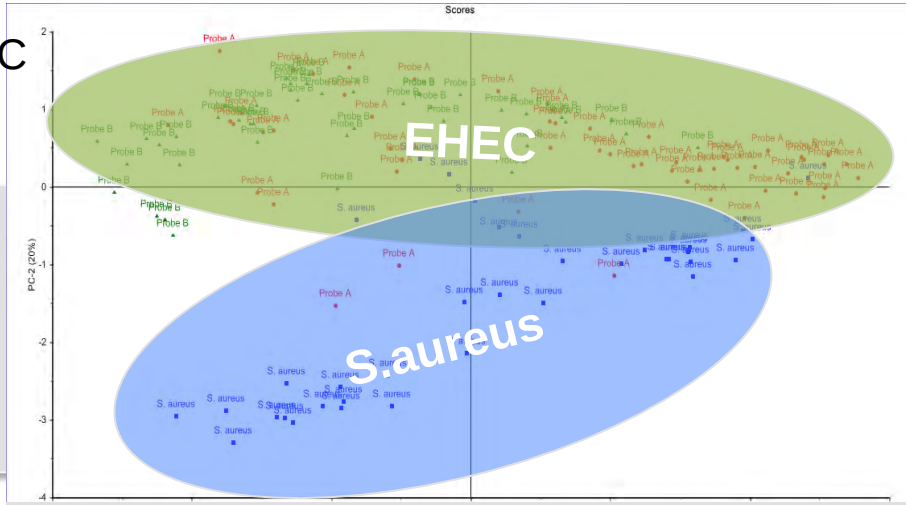


Spectra of S.aureus (blue) and S.epidermis (red)

Discrimination of S. aureus and EHEC bacteria

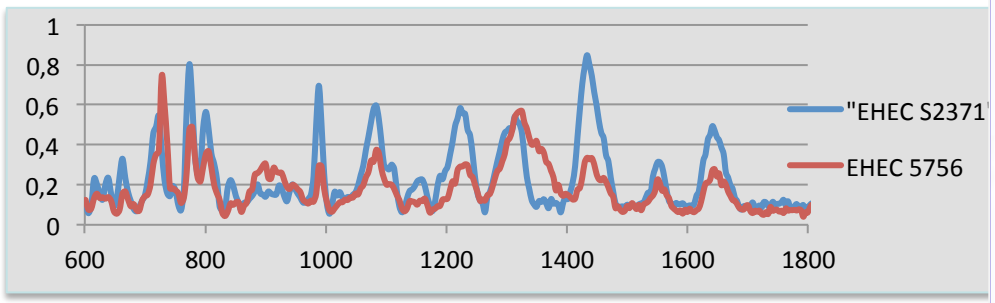
Mean Spectra and Staphylococcus aureus and EHEC
- **differences are clearly visible.**

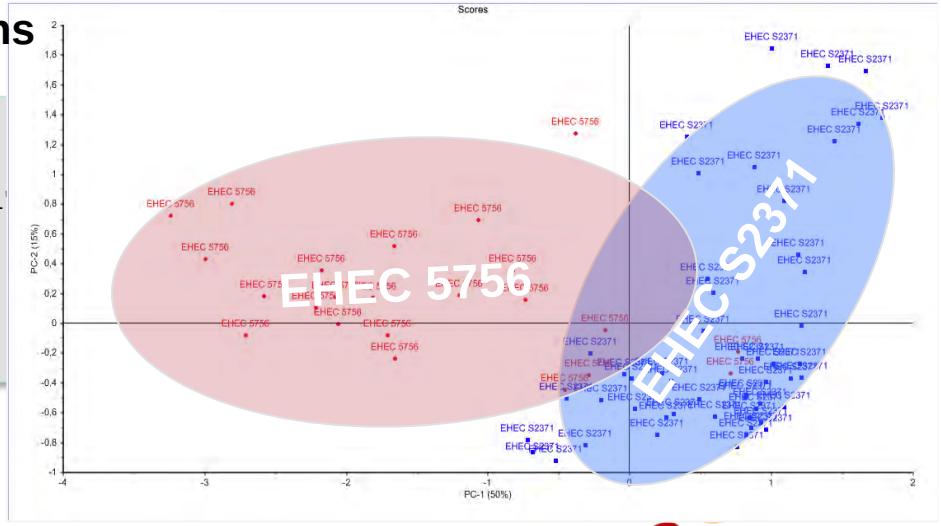




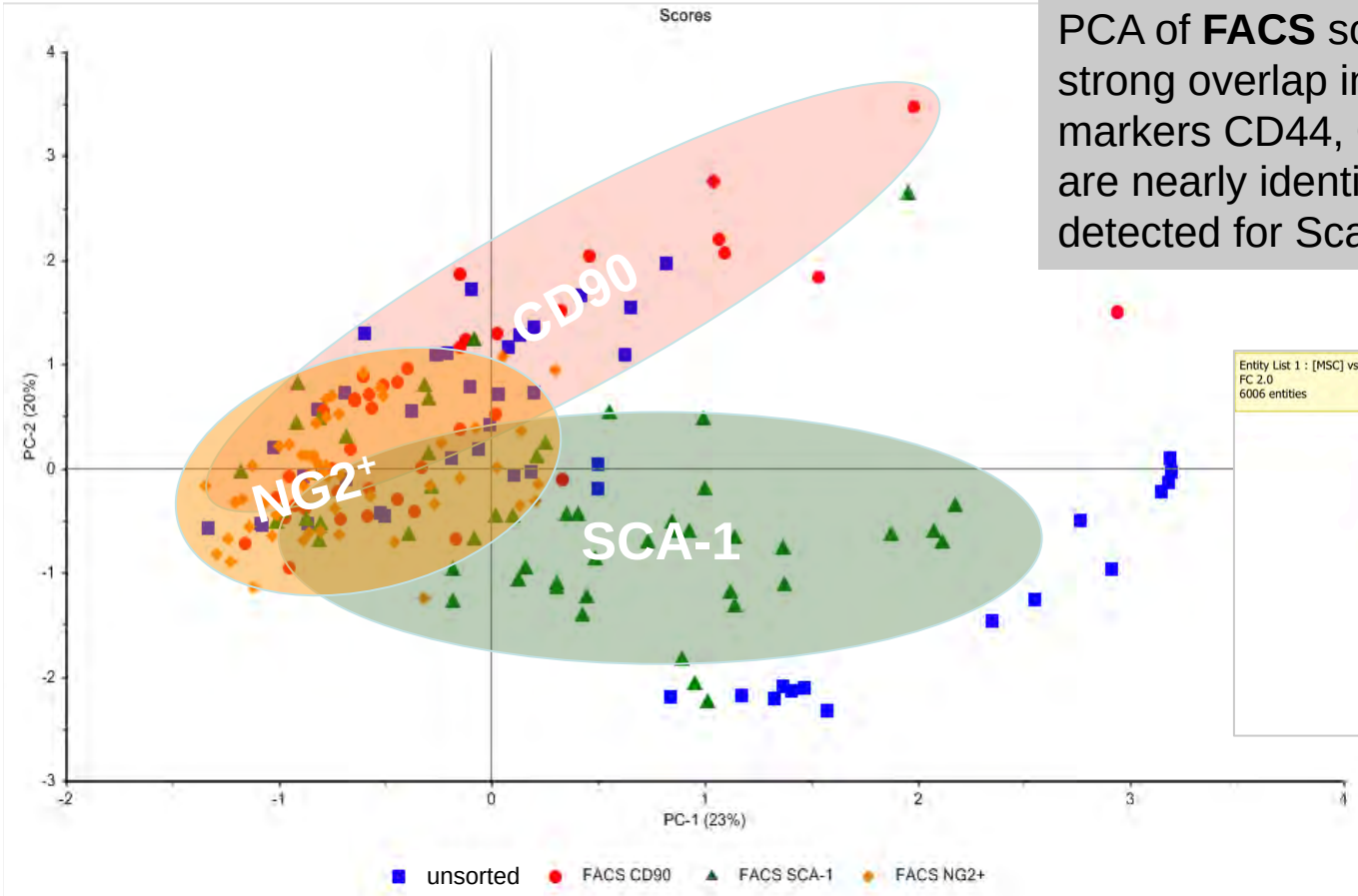
Principal component analysis

Mean Spectra of EHEC S2371 and EHEC 5756
- **it is possible to even discriminate EHEC strains**

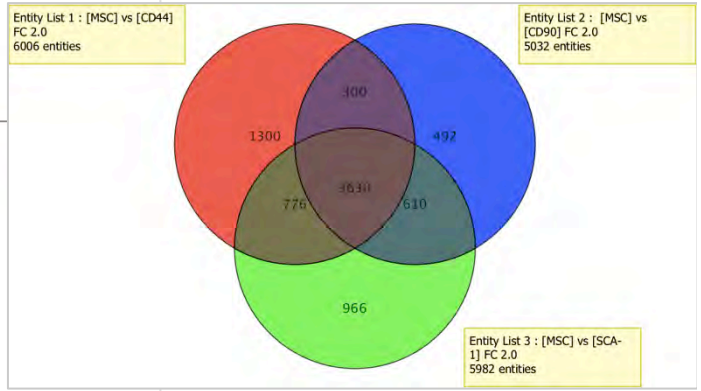




Comparison of mesenchymal stem cells (MSC) sorted by FACS



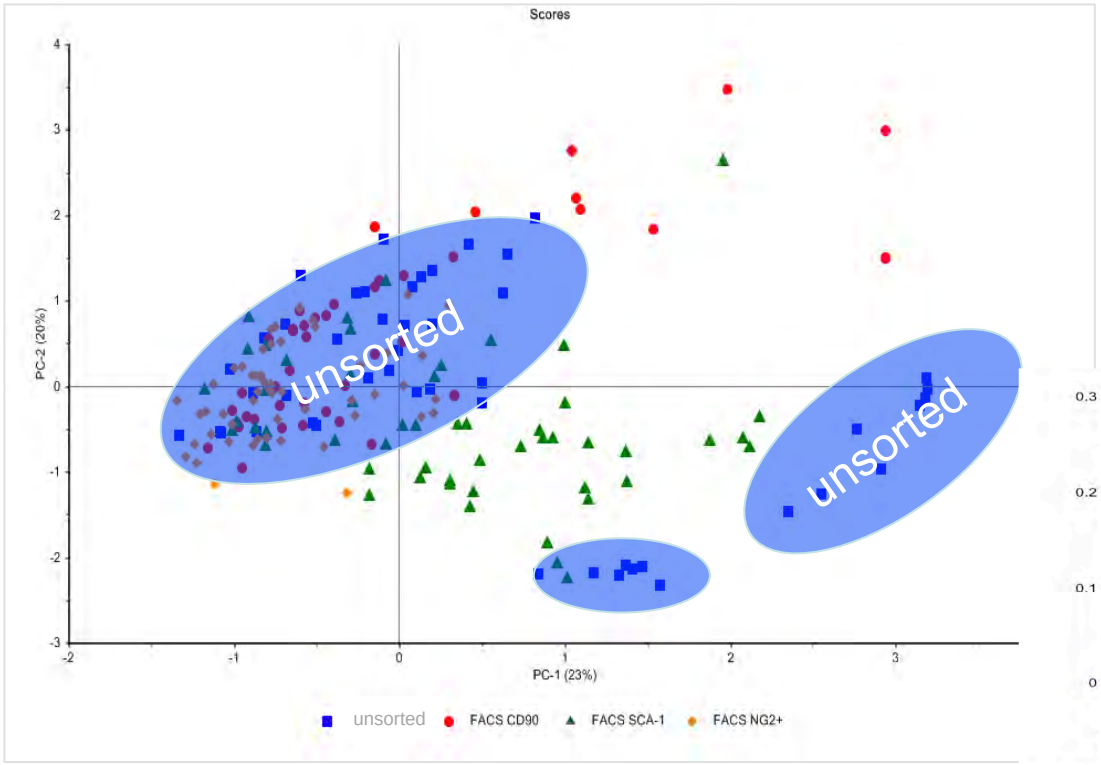
PCA of **FACS** sorted cells:
strong overlap indicates, that sorting by the markers CD44, CD90 and CD90 and NG-2 are nearly identical, differences can be detected for Sca-1 positive cells.



Confirmed by gene expression analysis – Venn diagram

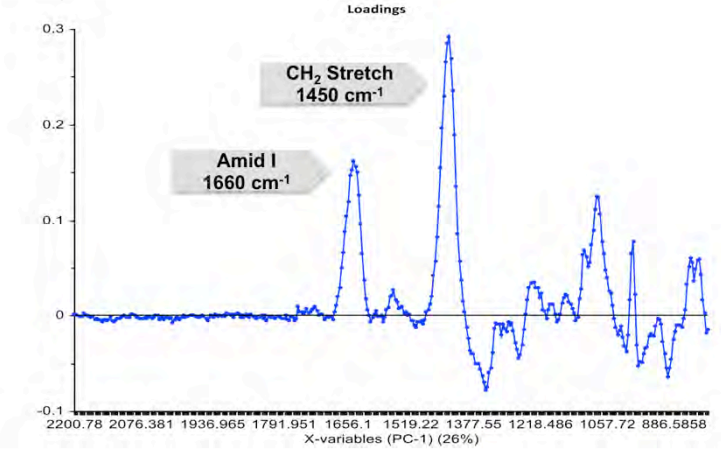
Sample: Bone marrow derived MSC cells (Gibco) characterized by their co-expression of markers CD90, NG2 and Sca1 in mice

Comparison of mesenchymal stem cells (MSC) sorted by FACS and MACS



Raman spectroscopy of control cells uncovers at least 3 clearly separated clusters.

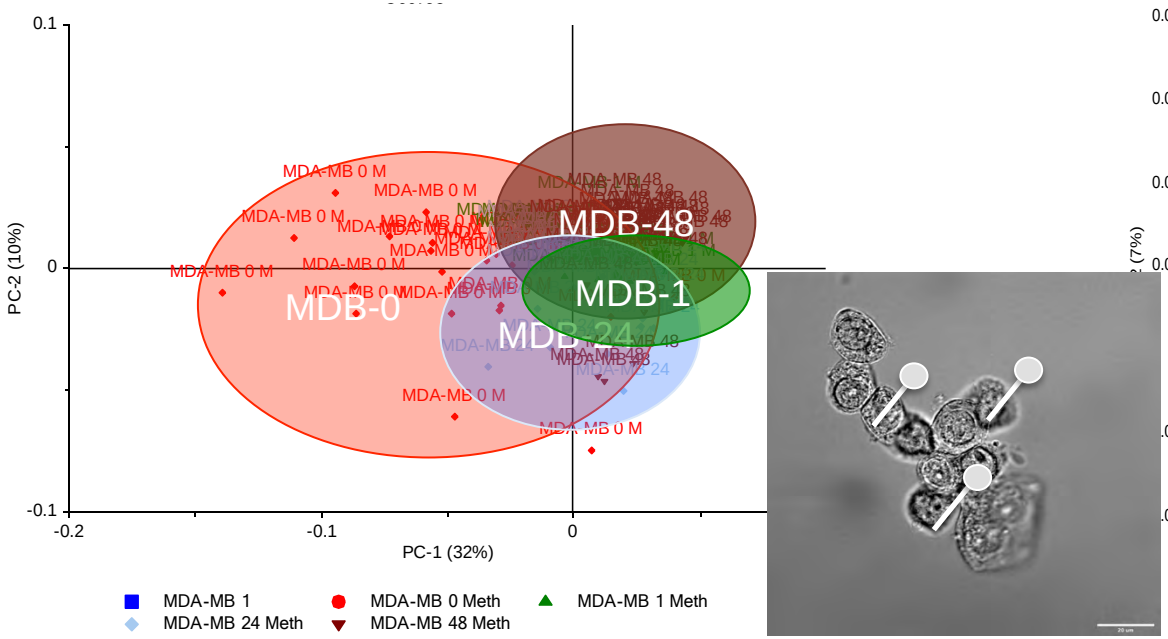
Without any antibody ☺



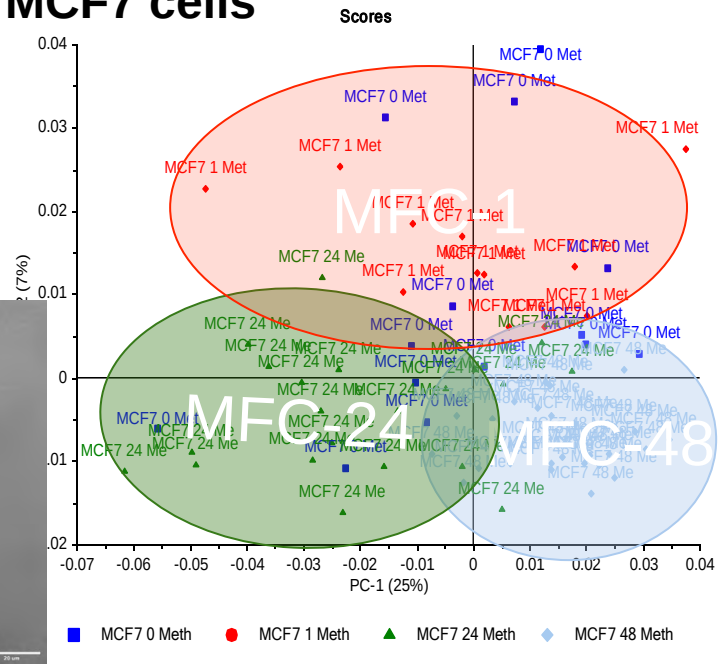
Differences are found in protein content along first principal component (PC1) and indicated by changes in the areas 1660 cm^{-1} (Amid I) and 1450 cm^{-1} (CH_2 stretch, in lipids, proteins).

Drug-Uptake Breast Cancer Cells & Herceptin

MDA-MB 468 cells



MCF7 cells



Human breast cancer cells with low expression of HER2 (IHC-Score 1+ Patients with that score would not be treated by Herceptin). In a viability assay (WST) cells do not react on antibody treatment with Herceptin. MDA-MB 468 cells express an activated receptor „phosphoHER2“, but also did not respond to a viability test (XTT-Assay).

Cells clusters with respect to different time points, but with overlap.

Technische Universität München

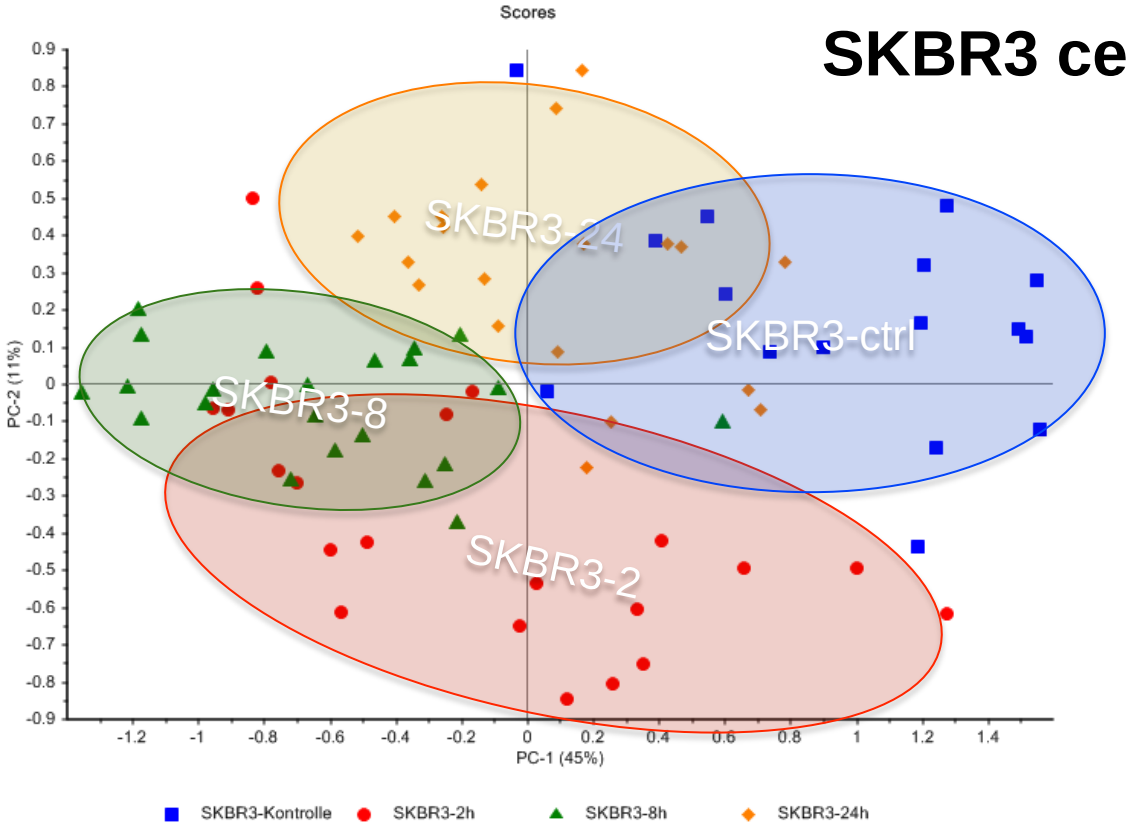
K.-F: Becker, K. Malinowsky
Institute for Pathology; TUM
Munich www.path.med.tum.de

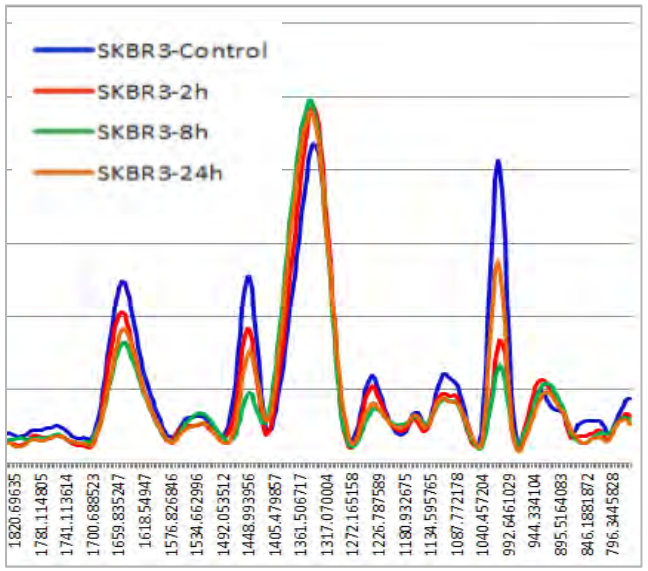
CellTool live sorting

Drug-Uptake Breast Cancer Cells & Herceptin

Technische Universität München

SKBR3 cells





SKBR3 cells are human breast cancer cells with high expression of HER2 (IHC-Score 3+ ; Patients with that score would be treated with Herceptin). In the viability assay (WST) cells do react on treatment with antibody Herceptin.

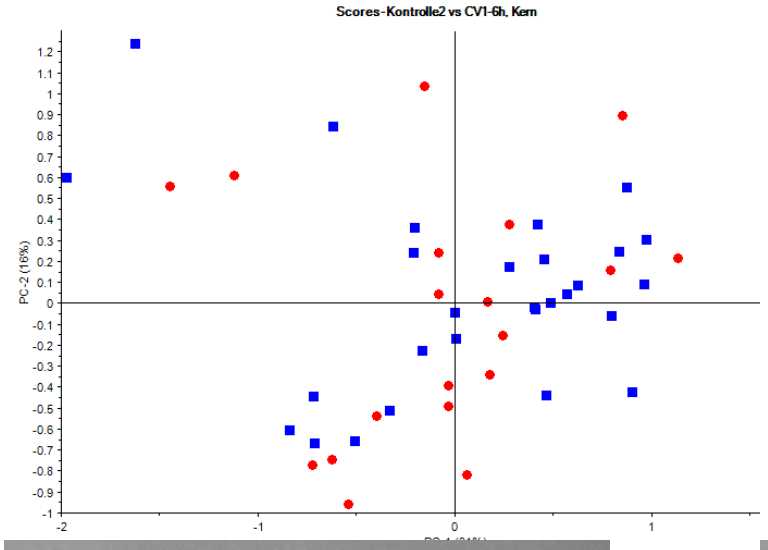
Raman spectra yield distinct clusters with minimal overlap.

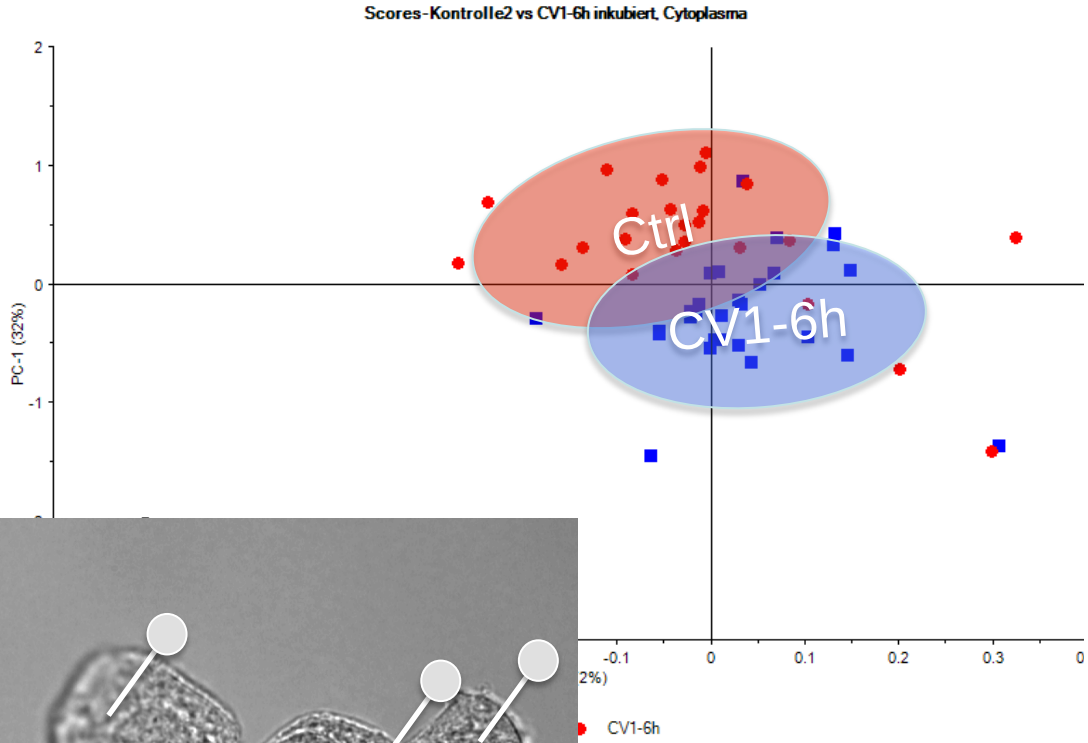
K.-F: Becker, K. Malinowsky
Institute for Pathology; TUM
Munich www.path.med.tum.de

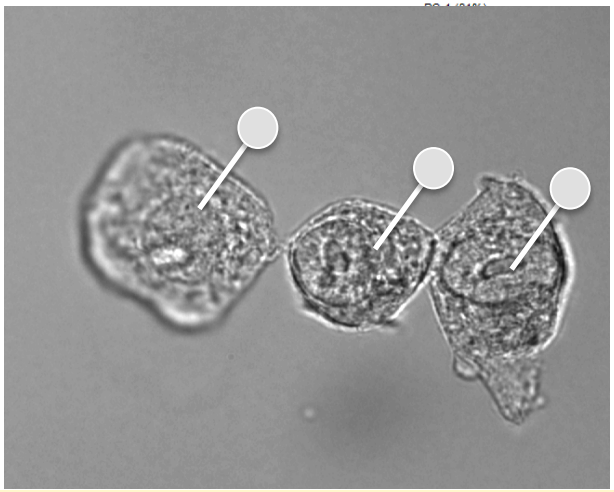
CellTool live sorting

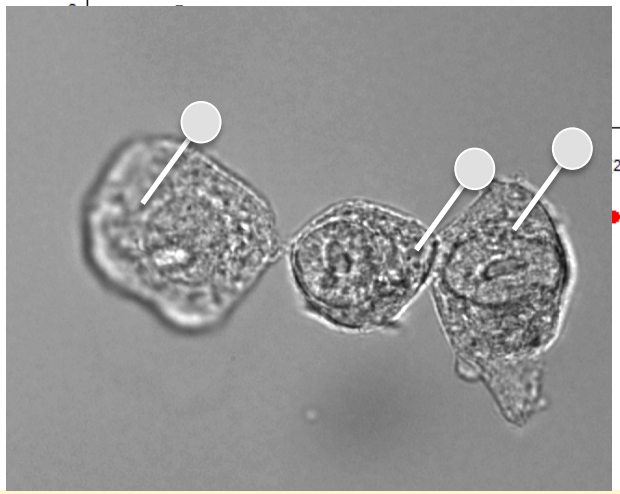
Viral Infection – or how the BioRam® teaches us biology

Monkey kidney CV1 cells incubated for 6 hrs with virus

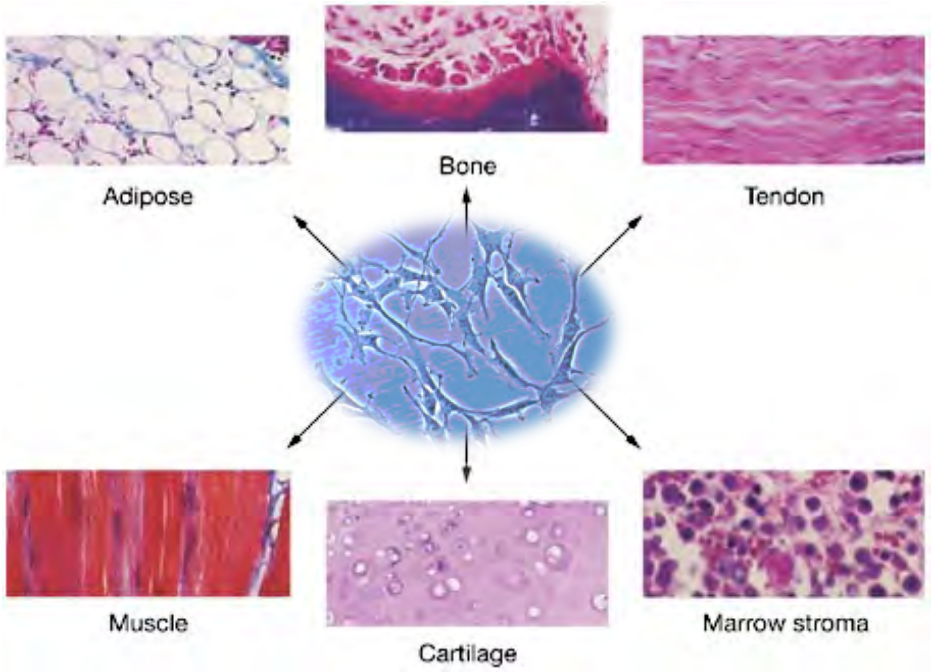








Potential of Humane Mesenchymal Stem Cells



BulletKit™

Lonza

Poietics® Human MSC were isolated from bone marrow.
After 14 days expansion 3000 cells/ well were plated in a 96 well MatriPlate with or without collagen coating.
For osteogenic differentiation cells were incubated in osteogenic differentiation BulletKit™.
Cells were fixed with 4% PFA. Spectra were taken and analyzed using principal component analysis (PCA).

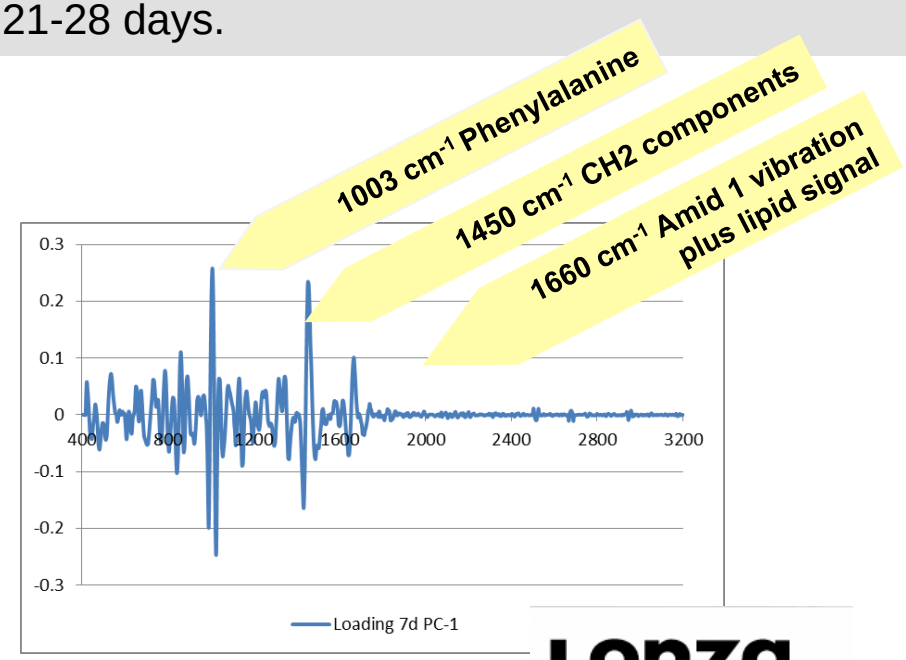
CellTool live sorting

Humane MSCs with osteogenic differentiation

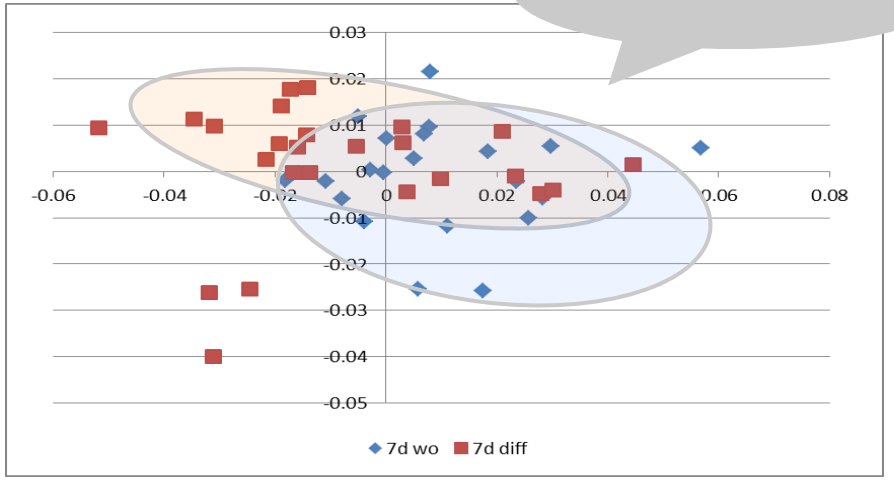
On day 7 osteogenic differentiation was visible in cells plated on glass but clearly demonstrated in cells plated on collagen.

➔ Collagen coating seems to accelerate differentiation

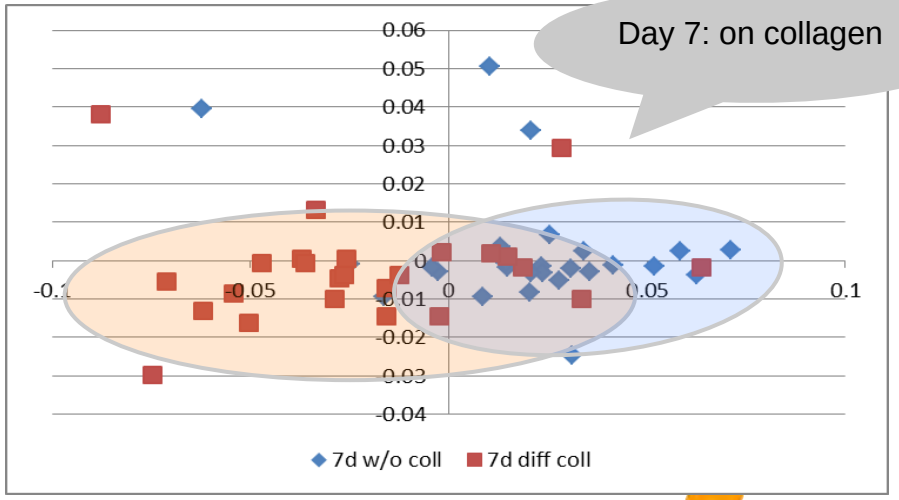
Common methods for analyzing osteogenic differentiation require long-term cultivation of 21-28 days.



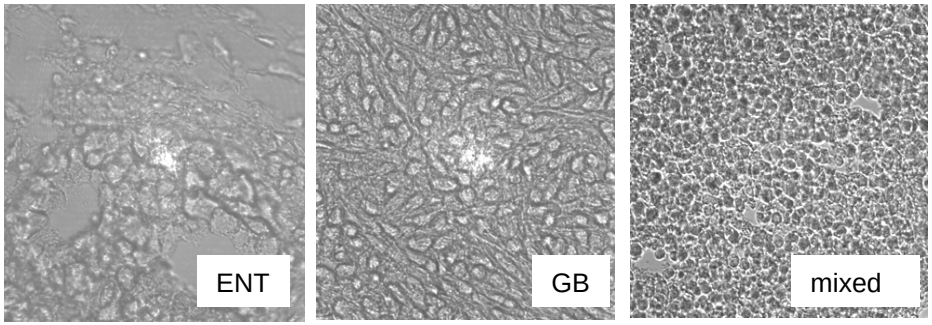
Day 7: on glass



Day 7: on collagen



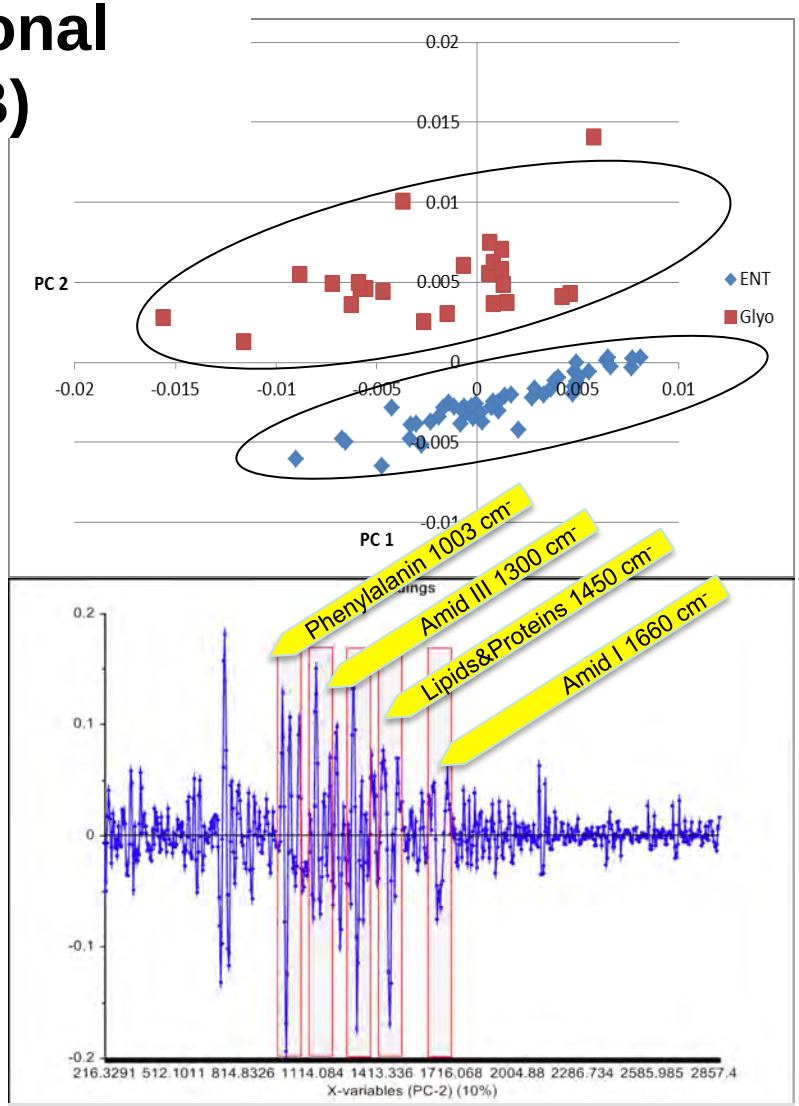
Identification of engineered neuronal tissue (ENT) and glioma cells (GB)



Microscope image of neuronal (ENT), glioblastoma (GB) cells, and a mixture of both (mixed). The bright spot is the focus of the Raman laser.

ENTs were established from human pluripotent stem cells and grown on a semi-permeable membrane. To generate the disease model, patient-derived glioblastoma cells were co-cultured on top of the ENTs to allow a gradual invasion of the tissue. .

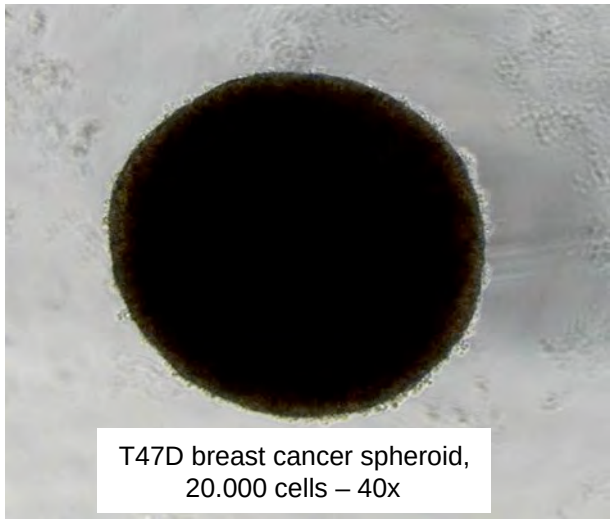
After training a support vector machine (SVM) model it was possible to check the unknown mixture. We found 35 ENT and 8 GB cells - i.e. 82% ENT and 18% GB cells in the mixture.



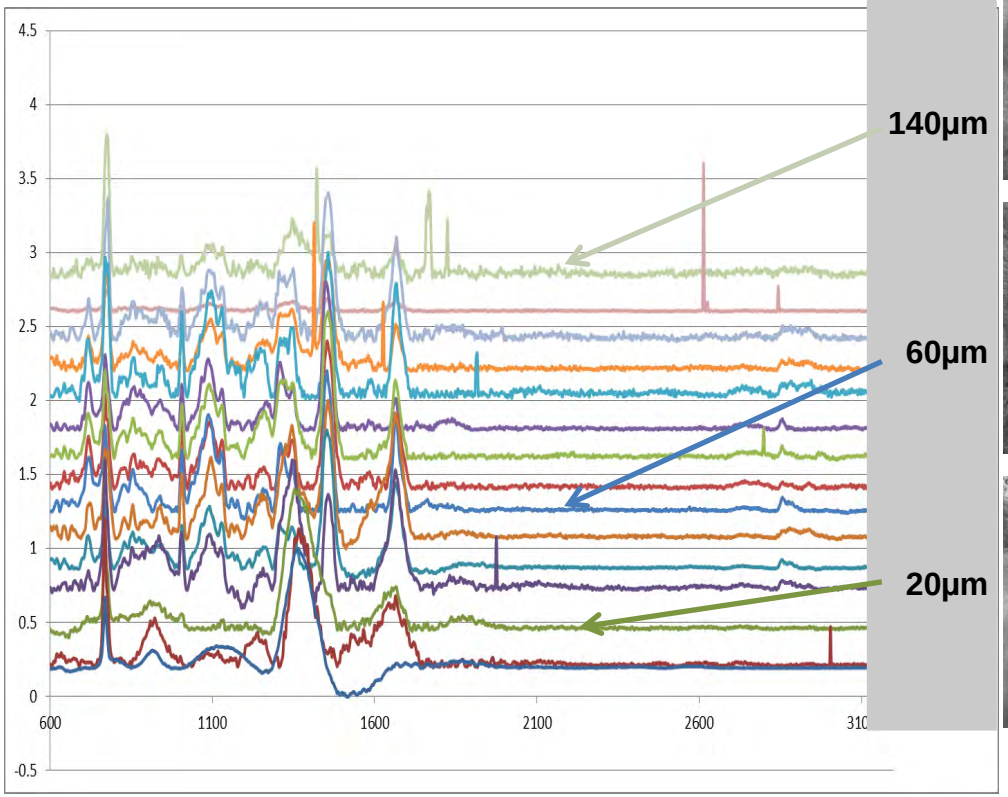
Loading of PC2. The main differences can be assigned to changes in the cellular protein content

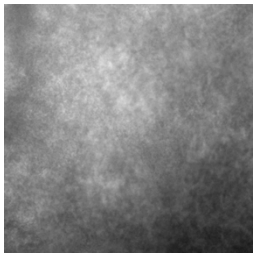
Raman-Spectroscopy in 3D cell cultures

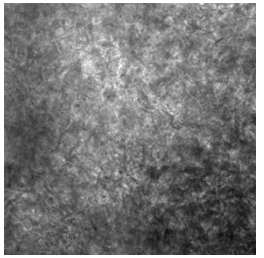
Penetration depth in in minitumors - spheroids

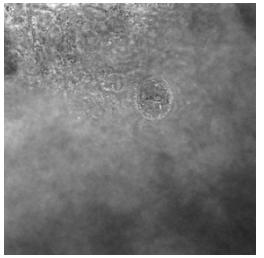


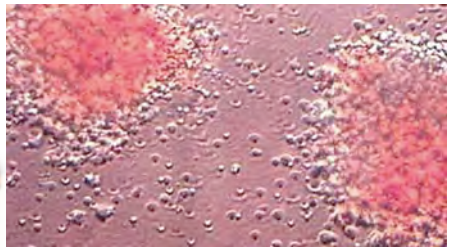
T47D breast cancer spheroid,
20.000 cells – 40x











spherotec®
spheroids for anti-cancer drug selection

In 20 µm depth within the spheroid cells are clearly visible under the microscope. At 60 µm cell contours are still recognizable. At 140 µm individual cells cannot anymore be resolved, however, clear Raman spectra can still be generated.

Shift of Paradigm – from biochemistry to biophotonics

Antibody based markers :

- limited to cell surface
- not available for all kinds of cells
- not always specific
- time intensive preparation
- markers are expensive

Markers are stressful for living cell

- cells change their characteristics
- stem cells may differentiate uncon
- marked cells are not allowed for th

Protein, DNA /RNA or Lipid analysis

- require large amount of cells
- a separate batch is required for eac

Raman spectroscopy provides rapid, non-invasive information about the metabolome keeping the cells entirely preserved.

Acknowledgements

- Heike Walles (University Würzburg)
- Eva Brauchle (Fraunhofer IGB Stuttgart)
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- Elke Lorbach (Lonza, Pharma, Cologne)
- Pjotr Religa (Karolinska Institutet, Stockholm, Sweden)
- Karl-Friedrich Becker (TUM, München)

Universitätsklinikum Würzburg

Universitätsklinikum Würzburg
Lehrstuhl für Tissue Engineering und Regenerative Medizin

Lonza

hyglos
creative bioscience solutions

neurix

SEVENTH FRAMEWORK PROGRAMME
Health

IDEA - **Id**entify **E**nrich, **A**ccelerate: Identification, homing and monitoring of therapeutic cells for regenerative medicine.

IDEA
cell technologies for health

Karolinska Institutet

photonics 4 life
EUROPEAN NETWORK OF EXCELLENCE FOR BIOPHOTONICS

From Infection to Detection

freshX
Entwicklung und Beratung