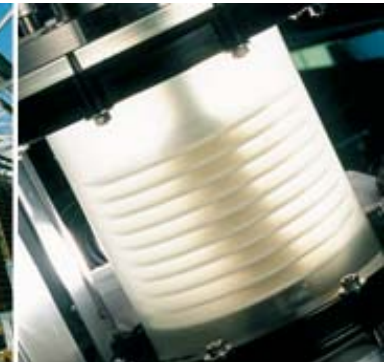
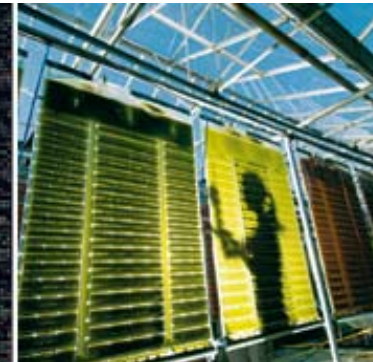
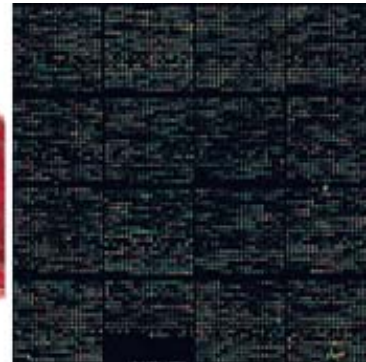
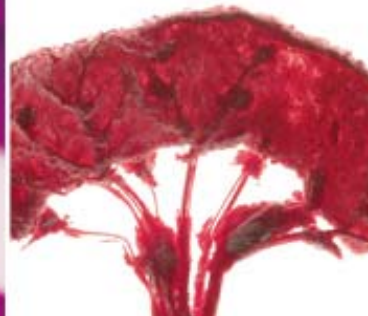
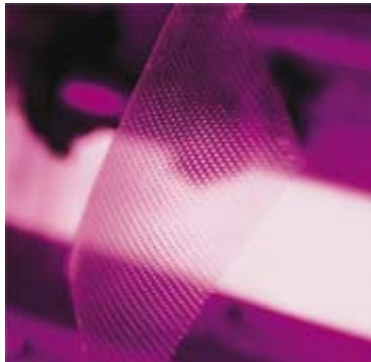


Label free cellular characterization by Micro-Raman spectroscopy

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Department: Cell systems



Outline

1. Introduction

- Micro-Raman spectroscopy
- Principal component analysis
- Concept of measurements

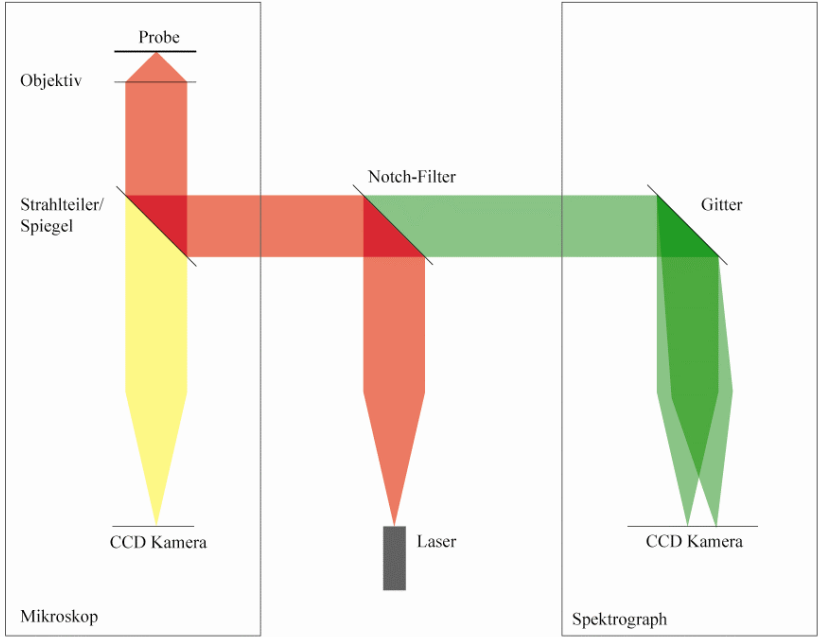
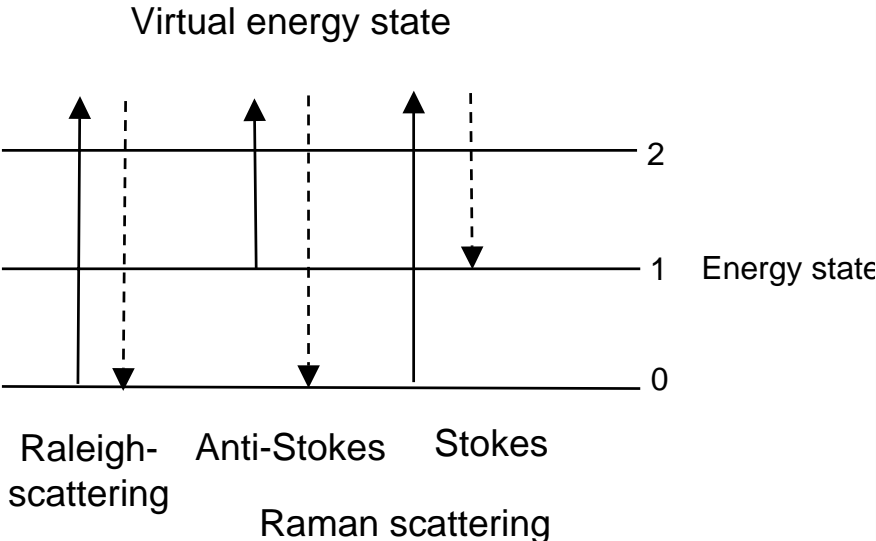
2. Results

- Sterility testing
- Vitality testing
- Cellular characterization (Discrimination / Differentiation)

3. Summary and Outlook



1. Introduction – Micro-Raman spectroscopy



Principle of the scattering process:

- Raman light is wavelength shifted

Laser – Notch/Edge filter (ex.) – Objective – Sample – Notch/edge filter (em.) – Grating - CCD

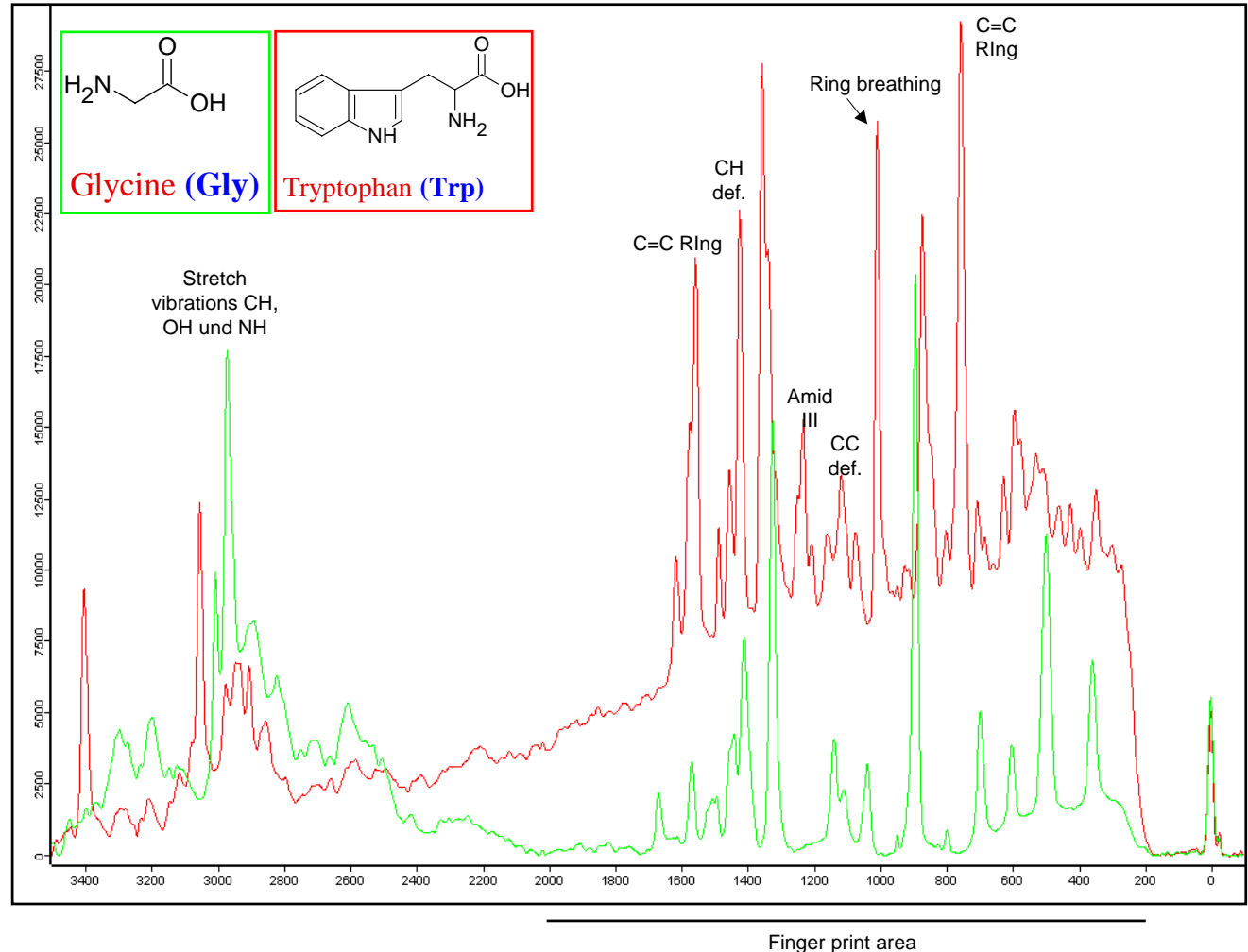


1. Introduction – Micro-Raman spectroscopy

Example of Raman spectra from different amino acids

Glycine and Tryptophan

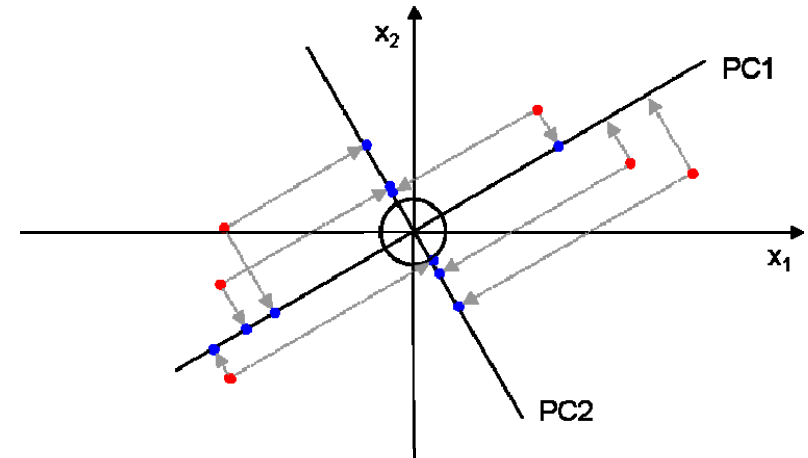
- Sample specific bands
- Discrimination of samples by comparison



1. Introduction – Principal component analysis (PCA)

PCA:

- Displays variances of different samples and within samples
- Reduces (multivariate) raw data to a few Principal components (PC)
- unsupervised method: no need of training samples

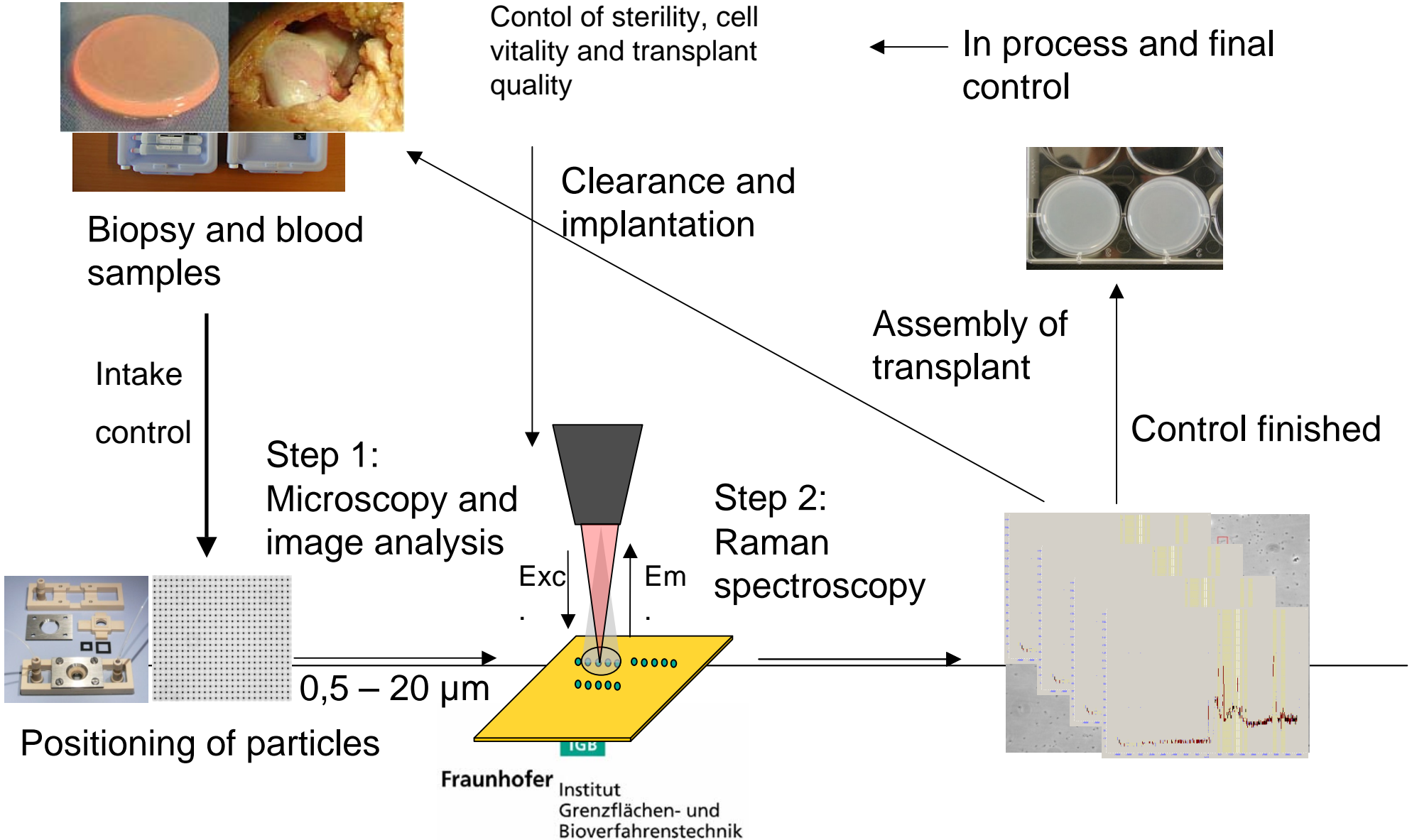


PC 1 describes the greatest variance within all samples.

PC 2 is placed orthogonal to the first PC.



1. Introduction - Concept of measurements

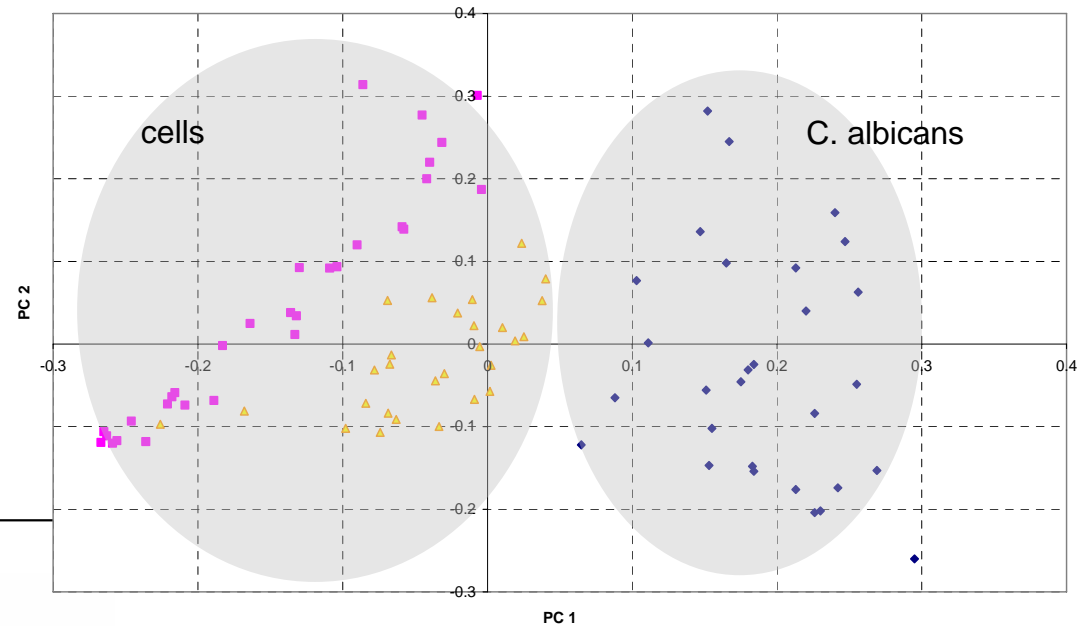
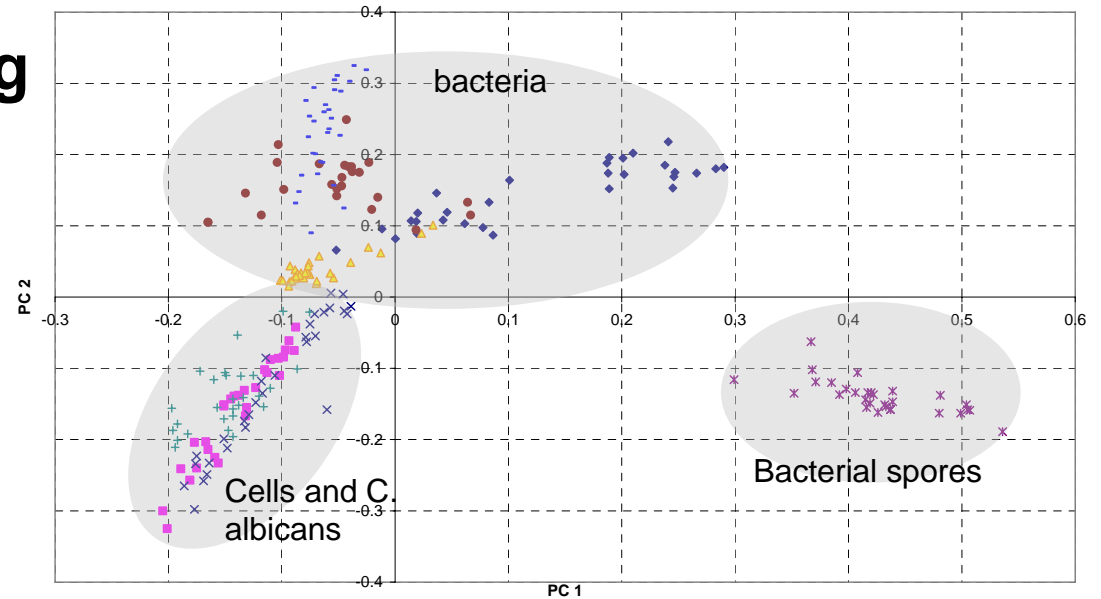


2. Results – Sterility testing

After particle positioning image analysis and Raman spectroscopy can be performed.

Discriminating between cells and microorganisms can be done with both techniques.

→ Complementary use of microscopy / image analysis and Raman spectroscopy



◆ C. albicans ◆ SAOS-2 ▲ SW-1353

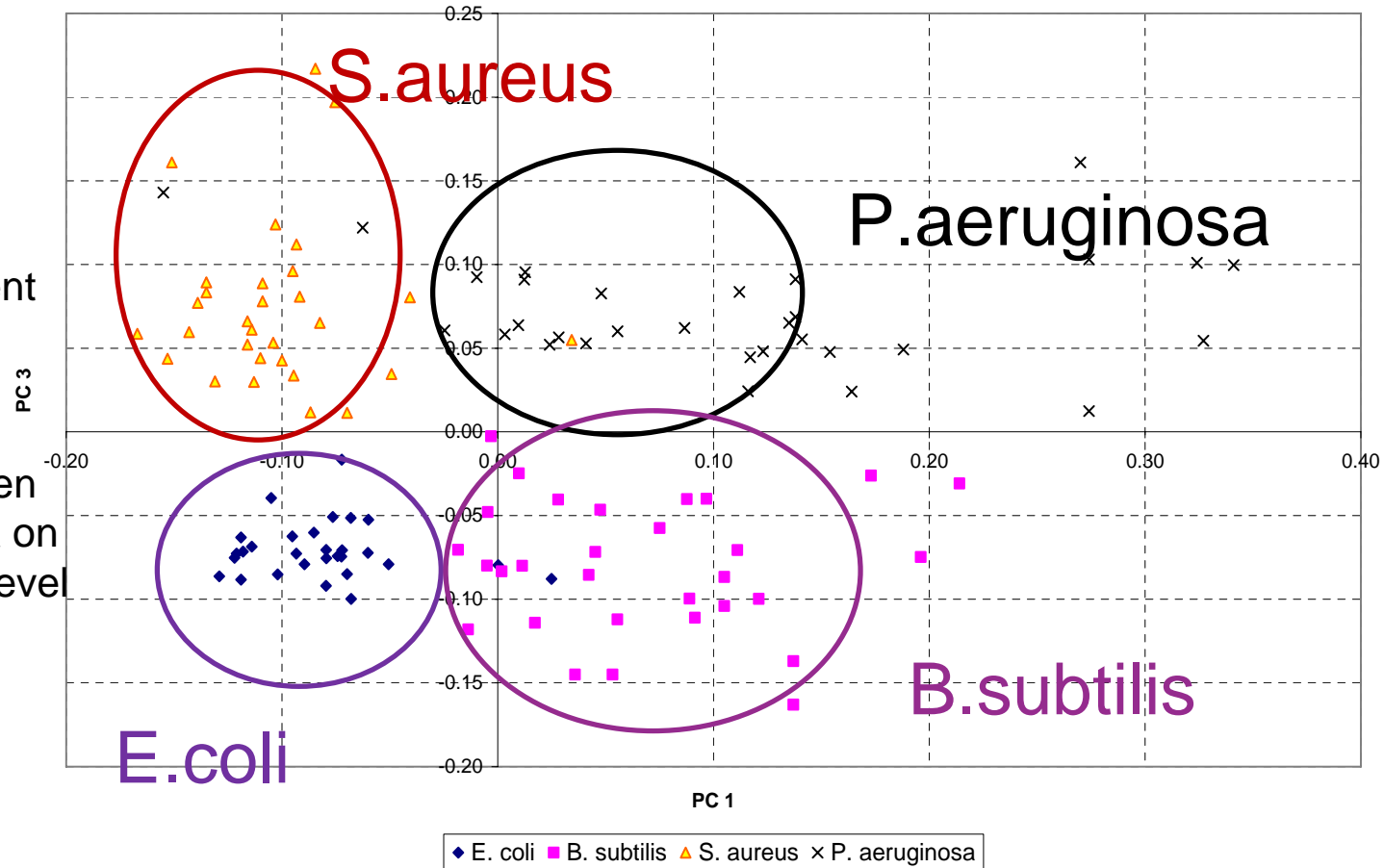


2. Results – Sterility testing

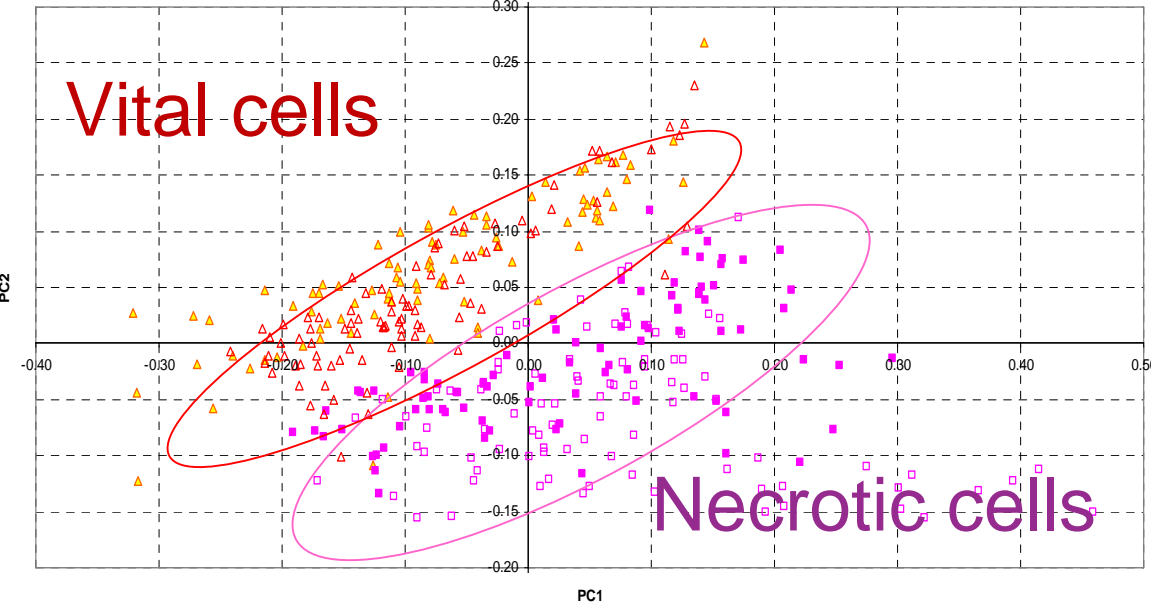
PCA of differentiate bacteria

- A clear clustering of the different kinds of bacteria is visible.

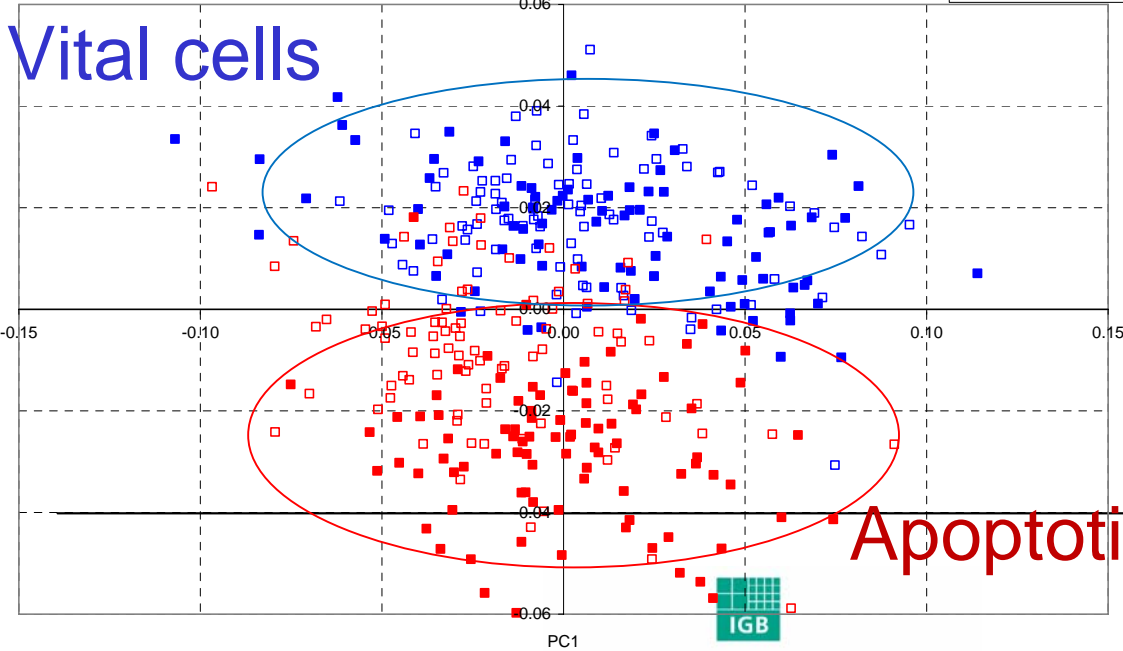
→ Raman spectroscopy can even be used to discriminate bacteria on a species level on a single cell level and in fluid.



2. Results – Vitality testing



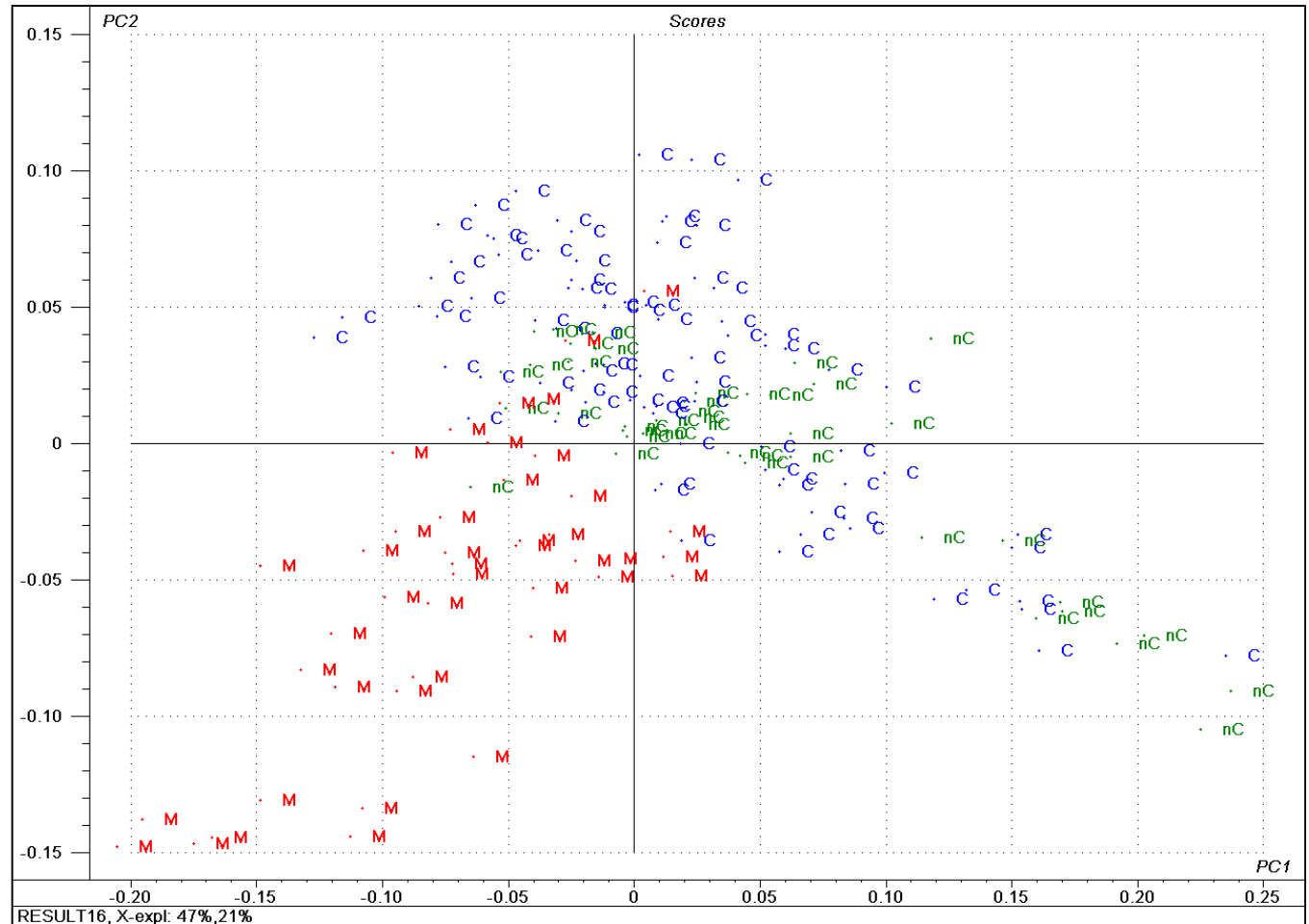
▲ SAOS-2 vital ■ SAOS-2 necrotic ▲ SW1353 vital ■ SW1353 necrotic



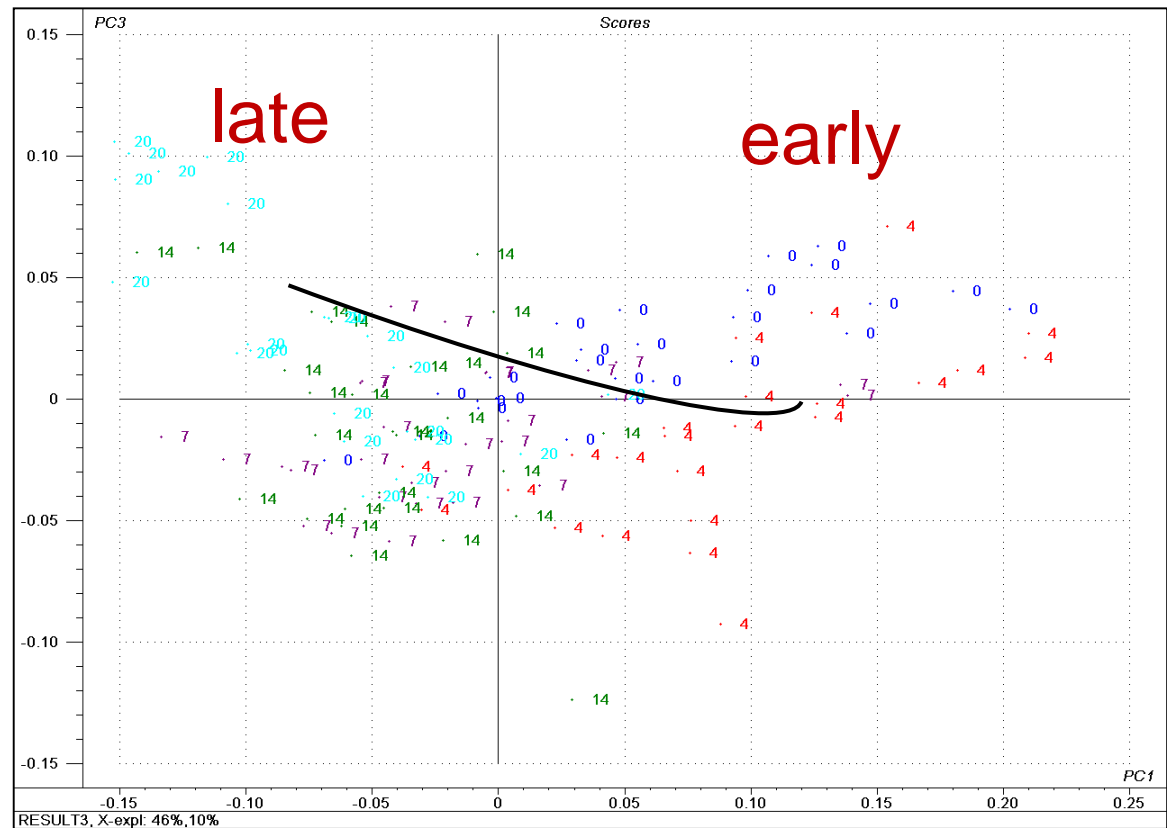
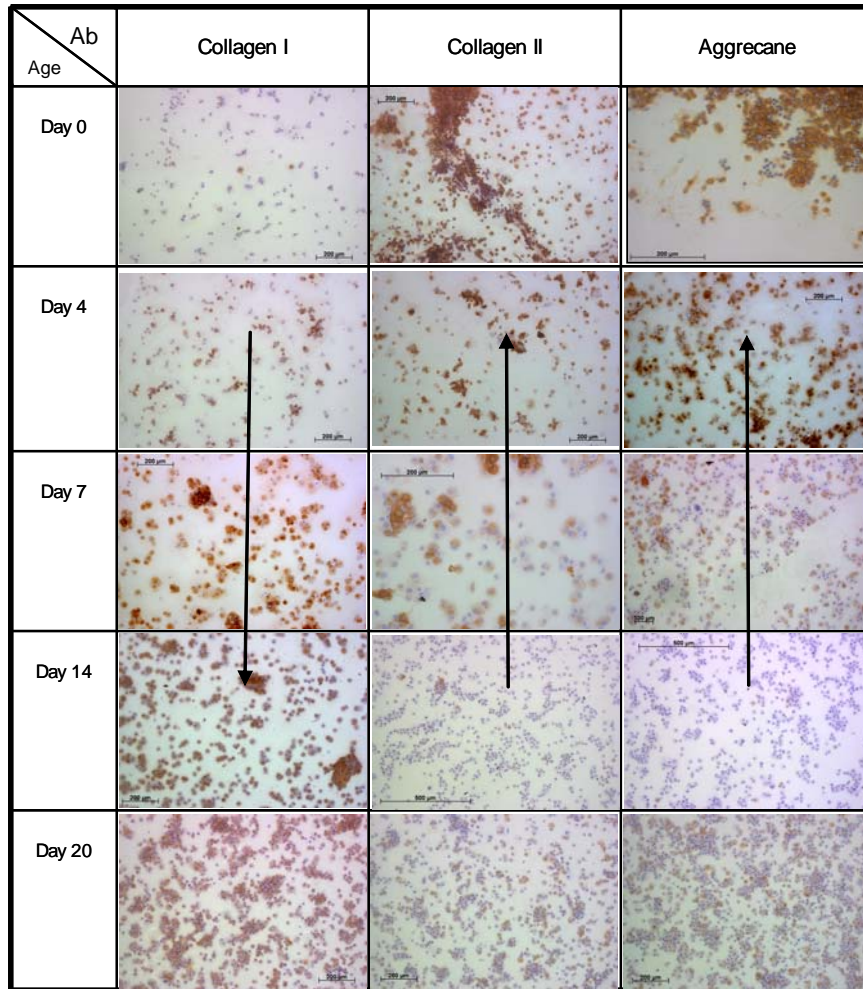
■ SAOS-2 vital ■ SAOS-2 apoptosis ■ SW1353 vital ■ SW1353 apoptosis

2. Results – Cellular characterization

PCA for cellular discrimination of porcine chondrocytes, neonatal, porcine chondrocytes and human MSC



2. Results – Cellular characterization



Cell dedifferentiation of chondrocytes in a 2D cell culture

3. Summary and Outlook

Summary:

- Raman based sterility testing and bacteria identification at the species level is possible
- Cell vitality can be detected with Raman spectroscopy
- Discrimination of cell types and of differentiation stages is possible

Outlook:

- Quantification of the promising results
 - Automization of spectral processing and interpretation
 - Validation of the system for use in the production of transplants in Tissue Engineering and GMP
-



Acknowledgments



Fraunhofer Institut
Grenzflächen- und
Bioverfahrenstechnik

2. Results – Vitality testing

Annexin-5 FITC and PI labeling for defining the status of cell vitality

Raman measurements of the labeled cells – FITC and PI are not detectable (784 nm laser)

