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Label-free Cell Analysis and Fast Bacteria Detection Using Raman-Trapping-Microscopy

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Abstract: *Raman-Trapping Microscopy integrates Nobel prize technologies – Raman & Optical Trapping - into a microscope making it available for biomedical use. Cells, bacteria, exosomes or viruses are analyzed reflecting their natural features and behavior.*

The huge advantages of Raman spectroscopy for label-free and gentle cell analyses increasingly gains attention in the field of biological research, biomedical applications as well as in medical diagnosis. Well-established in chemistry, physics and pharmacology, vibrational spectroscopic methods such as Raman spectroscopy only recently started to make its way into the biomedical laboratories.

The subtle combination of Raman microscopy with optical trapping features enable the biologists to get easy access to Raman spectral data about cells and tissues providing non-invasively, non-destructive, yet highly sensitive and specific information about the features and status of the cell.

Raman spectroscopy analyses the entire metabolom of a cell whilst most of the current cell analysis methods are based on surface labelling with antibodies, combined with fluorescent stains or magnetic beads. It is well known, that antibodies, fluorescent stains, or the exposure to fluorescence excitation light induces phototoxicity, changing their features and harming the cells drastically. Furthermore, those techniques require a huge amount of cells upfront.
In contrast, Raman spectroscopy is a purely physical method based on the interaction of light with matter. Raman spectra solely depict photons that interact with the biomolecules of the cell and are emitted at different wavelengths as the incident laser light. Raman photons carry the specific information of the involved biomolecules. They are recorded with a highly sensitive detector yielding a sum-spectra that is as unique as a "fingerprint". Raman pattern dependent on the cell's metabolic state, and changes due to disease, interaction with other cells or compounds but also due to reaction on environmental impact are immediately detected.

We have developed a Raman Trapping Microscope system (RTM) dedicated for biomedical applications. For instance, to detect diseased or infected cells, to determine tumor aggressiveness, discover biomarkers, observe stem cell differentiation or to monitor the quality of cell-based products during production and of the final product. Due to especial laser coupling, RTM possesses integrated trapping features that arrest cells within the laser focus during Raman analysis. In addition, the spectral intensity is multiplied which enables the acquisition of high-quality Raman spectra also from small sample concentrations and even from the depth of tissue samples, depicting biochemical information of individual cells in 3D. Moreover, optical trapping allows the measurements of non-adherent cells within their physiological environment, eliminating the need to attach cells before analysis or using cytospin techniques. Thus, single cells but also individual bacteria and even nanometer sized exosomes or viruses can be analyzed. We could prove that bacteria or virus infected cells show different Raman spectra pattern as compared to healthy cells and their reaction on drugs can be determined in a fast manner.

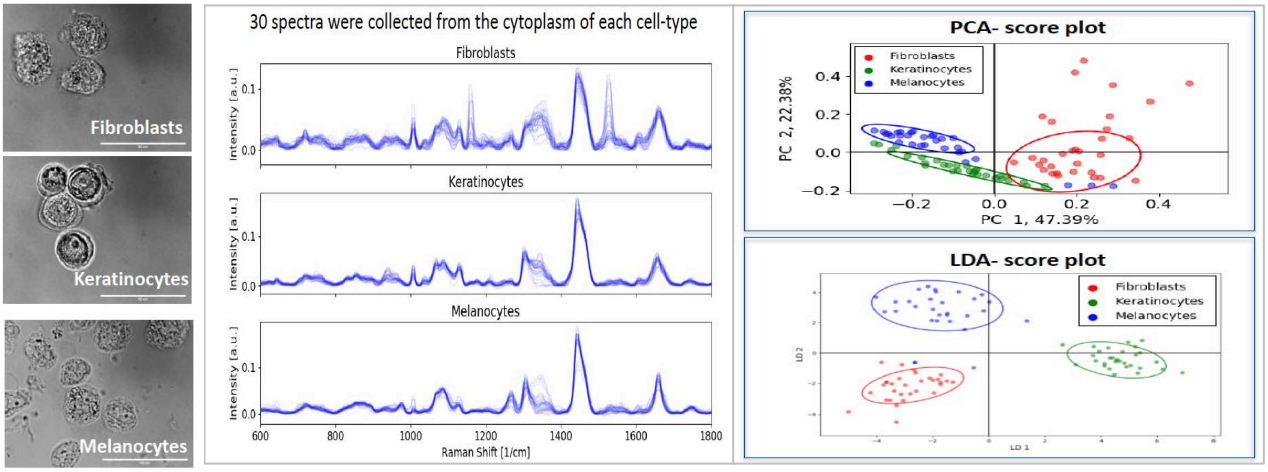
The RTM system is coupled with an automated data analysis platform dedicated to biomedical needs. In a push-of-a-button, this platform facilitates the extraction of biomedical relevant information from the acquired Raman spectra. It provides all necessary statistical procedures to compare samples, to detect subpopulation, and to identify the biochemical cellular changes based on statistical evaluations such as principal components analysis (PCA) and hierarchical cluster analysis (HCA). Raman spectra provide large number of digital data points. For a single spectrum standard acquisition collects around 973 pairs of data points at the spectral range of 350-3300 cm^{-1}. The complexity and dimensions of the data will be increased collecting thousands of spectra.

Here, we will demonstrate the potential of Raman trapping microscopy to characterize different skin cell types. We will show how Raman spectra of tumor cells differ from T-cells and how their spectral pattern changes due to interaction with T-cells. Furthermore, we will demonstrate automated analysis of blood cells monitoring their loss of functionality, e.g. due to storage time of a blood product or caused by bacterial contamination. Last but not least we will demonstrate how easy Raman can detect different types of bacteria directly within their native environment.

Raman spectra of skin cells acquired in 2D cell culture and within 3D-grafts

Skin grafts are used as a replacement after surgical interventions or acute skin trauma such as burns, giant Nevus or scars. They are produced by extracting healthy cells from the donor which are expanded using different separation media.
After quality check keratinocyte and fibroblasts are mingled within in hydrogel to construct epidermis and dermis layers, respectively. FACS sorting after cell expansion to define keratinocyte and fibroblast purity as well as DNA count of final product is expensive and time consuming. In addition, specific antibodies to identify fibroblasts are missing.
In contrast Raman spectra can easily discriminates fibroblasts from other skin cells. Raman measurements of pure cultures of keratinocytes, fibroblasts, and melanocytes were performed trapping and keeping individual skin cells and keeping them within the Raman laser focus during Raman spectra acquisition. The collected Raman spectra show differences due to different compositions of biomolecules within cell types, such as the differences in cellular expressions of glycogens, DNA/RNA, proteins and lipids as depicted in figure 1.

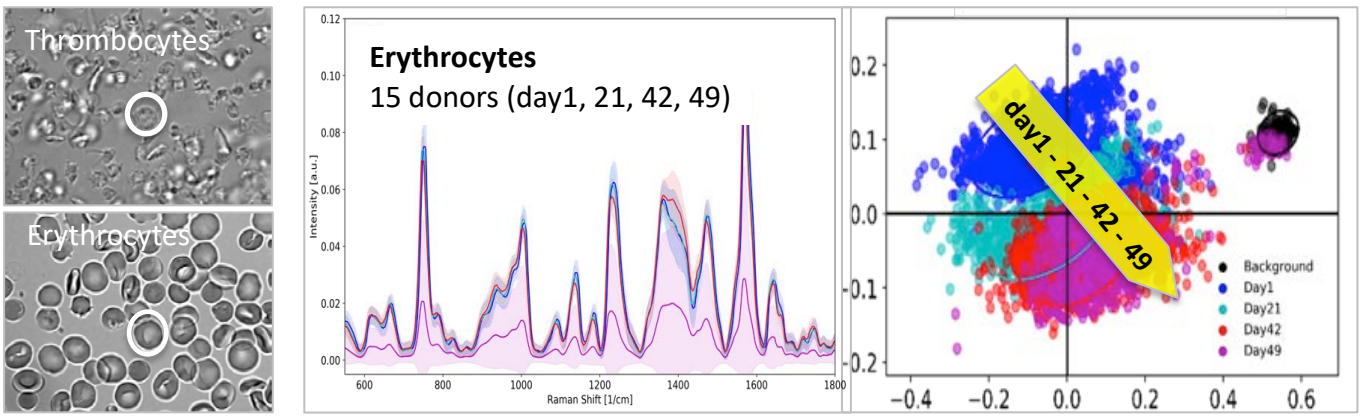
Figure 1: Bright field image, mean spectra and score plots of skin cells within 2D-cell culture



Metabolomic investigation of human blood products using Raman-Trapping Microscopy

Quality control of blood products has shifted focus towards detection of bacterial contamination as well as towards monitoring stability and functionality of blood concentrates during storage and prior to transplantation. Currently, it is not possible to test all blood products for sterility or integrity of the cellular components as for example commercial microbial detection systems require an incubation time for up to seven days. We could show that Raman spectra change with aging of the blood products. Changes of the Raman pattern correlate with structural changes of the hemoglobin, reducing oxygen transportation. Thus, Raman provides a fast and highly reliable quality measure that could easily be performed immediately prior to transplantation to increase patient safety (Figure 2).

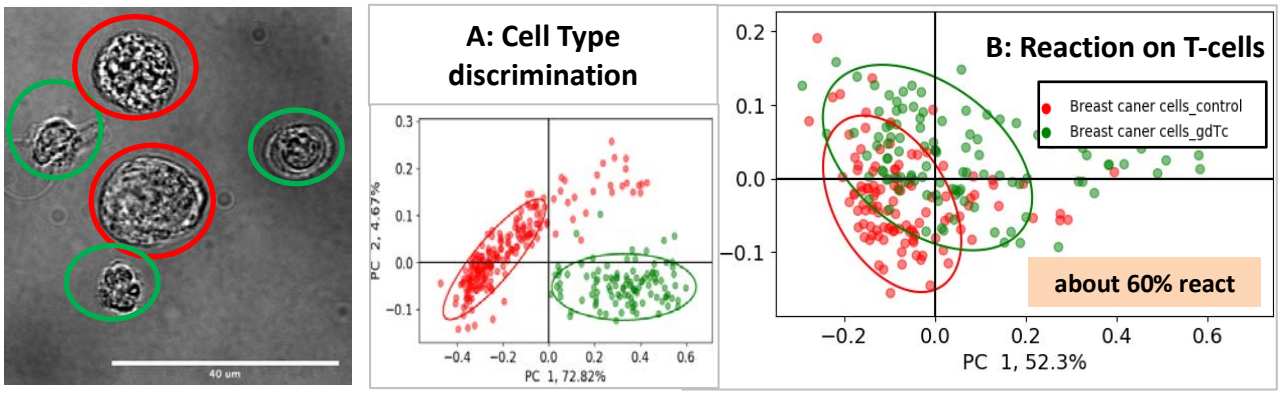
Figure 2: Bright field image of thrombocytes and erythrocytes. Raman mean spectra and score plots of Erythrocyte concentrates taken at different time points after donation



Raman spectra identify tumor cells and depict their reaction upon T-cells

T cells engineered to express a defined Gamma delta T cell receptor (TEGs) demonstrated strong and selective anti-tumor activity *in vitro* and *in vivo* against multiple tumors. TEGs recognize tumor cells by their metabolic dysregulation, providing a highly specific approach applicable to the majority of cancers and without unwanted targeting of healthy tissues. The ability to generate a T-cell immunotherapy (TCI) for any cancer type would be an important step towards patient specific cancer therapy. We could show that Raman spectra of breast cancer cells clearly differ from those of T-cells (Figure 3A). Furthermore, Raman spectroscopy depicts the change of Raman spectra from breast cancer cells when getting in contact with γδ T-cells. Spectral changes of about 60% of the cells are due to apoptosis (Figure 3B**).**

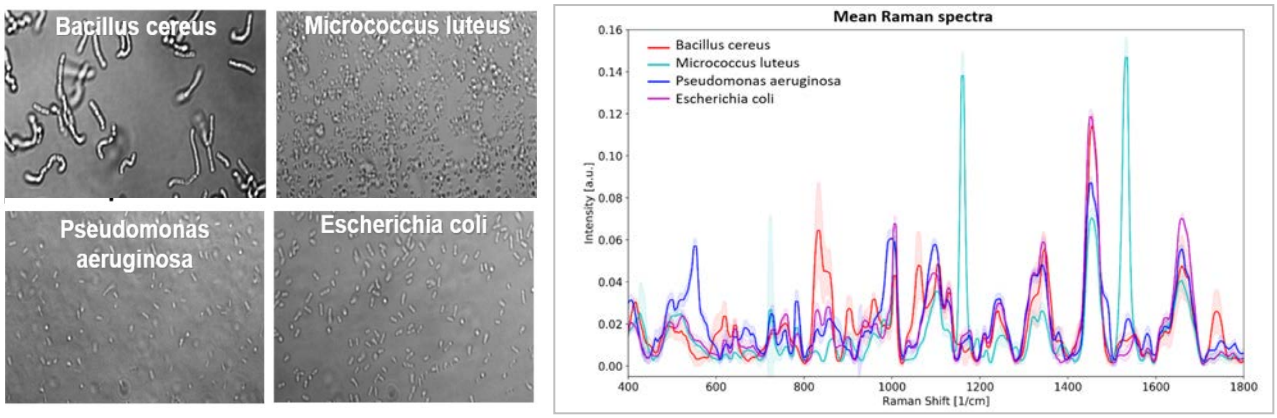
Figure 3: Bright field image of breast cancer cells (red) and T-cells (green) with corresponding scores plots depicting cell type discrimination (A) and cell-cell interaction (B).



Raman trapping microscopy for fast bacteria identification

Many approaches were developed to enhance Raman signals such as using Plasmon/resonance effects in Surface-Enhanced Raman Scattering (SERS), to enable Raman measurements of small cells. However, it requires chemical modification of the sample (applying nanostructures) and special coatings of the substrate surface. Thus, it is a sample destructive and time intense analysis. In contrast, due to raise of spectral intensity (>10 fold) within trapped samples, **RTM** analysis individual cells but also floating bacteria or exosomes in minutes - direct within their native environment and in a highly automated manner.
Raman trapping of a few single bacteria can provide enough spectral information to differentiate bacterial species. Here, four species - *Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Escherichia coli* - have been clearly discriminated using trapping & Raman measurements of 10 cells of each species. The Raman mean spectra were calculated, displaying characteristic spectral patterns of each species (Figure 4).

Figure 4: Bright field images of different bacteria with corresponding mean Raman spectra



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