# Metabolomic investigation of human blood products using Raman-Trapping Microscopy

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## Introduction

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. The idea behind is to improve quality of blood concentrates and to increase patient safety. 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. This is significant when considering that platelet concentrates have a limited shelf life of 5 days, forcing patients to receive outdated preparations while waiting for the results. The aim of the present study is to use Raman microscopy to develop a minimally invasive, innovative point-of-care method for a faster, simpler and highly reliable quality control of blood products.
Raman spectroscopy is based on focusing a laser light onto cells and detecting photons inelastically scattered by absorbing molecules. Photons, which are shifted to the red (long-wave) range are subsequently detected by a spectrograph. All molecules within the laser focus contribute to the Raman spectrum and thus, the spectral sum is as characteristic as a fingerprint. In this project we generated Raman profiles of red blood cells and platelet concentrates during the normal storage process. The identified Raman parameters are a quality feature for blood products with regard to aging/functionality.

## The BioRam®: a confocal Raman Trapping Microscope

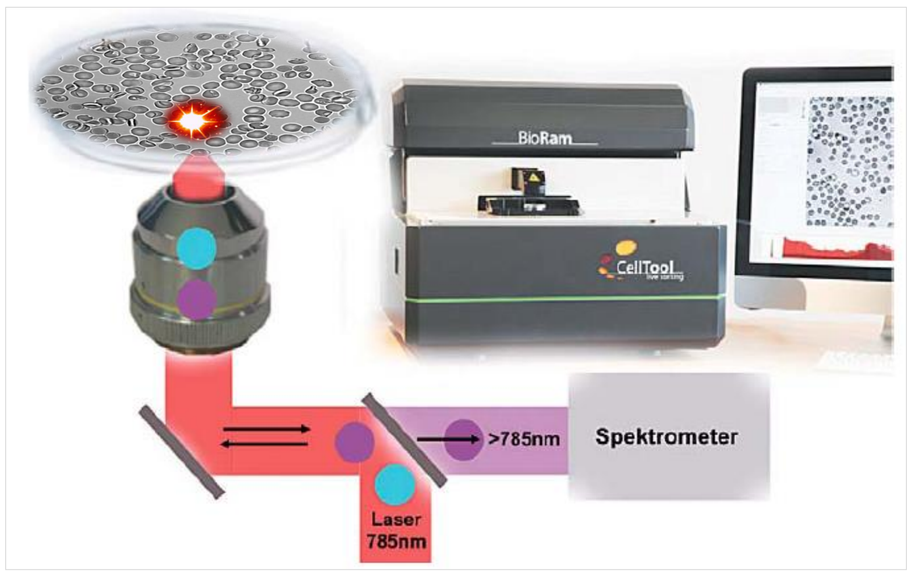



**Fig. 1:** The BioRam® is a confocal Raman Trapping Microscope which is specially designed for biological applications. It allows quick and easy examination of platelets or erythrocytes from blood concentrates without the risk of contamination. The laser is focused through the objective to a spot size of about 2 µm$^3$. The applied laser wavelength of 785 nm was shown to be safe for cells. Simultaneously induced optical trapping forces arrest cells in suspension during Raman spectra acquisition.

## The Principal Component Analysis (PCA)

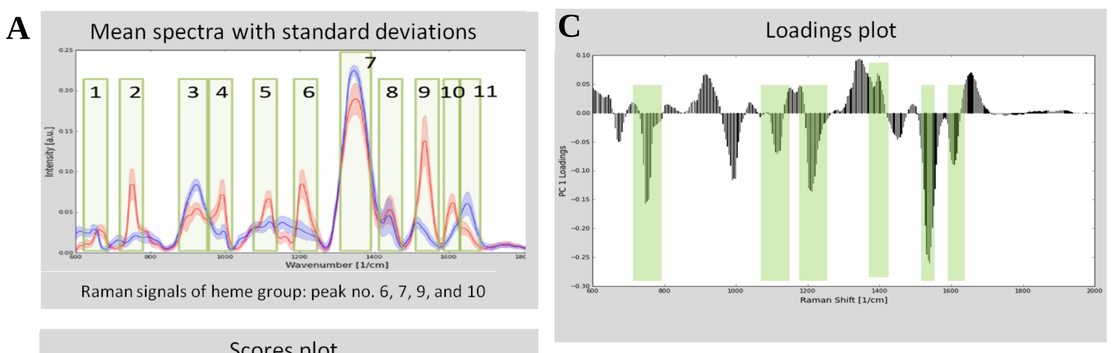



**Fig. 2: All molecules within a cell contribute to the Raman spectrum, thus the spectral sum is as characteristic as a fingerprint. A.** Raman spectra of erythrocytes (red) and thrombocytes (blue). **B.** Principal component analysis (PCA). The spectral data are transferred in a dot plot, the so called Scores plot, in which each dot represents the Raman spectrum of one cell. This plot depicts if and how the samples differ from each other. **C.** Loadings plot. This plot shows which parts of the Raman spectra are important for the distribution of the data in the Scores plot. The higher a peak, the more important is it. Thereby it makes no difference if the peak is positive or negative. As different wavenumbers are associated with different biomolecules (DNA, RNA, proteins, lipids, etc.) the location of the peaks also tells about which biomolecules are responsible for e.g. discrimination of the cell samples.

## Analysis of blood samples from erythrocyte concentrates using Raman trapping microscopy

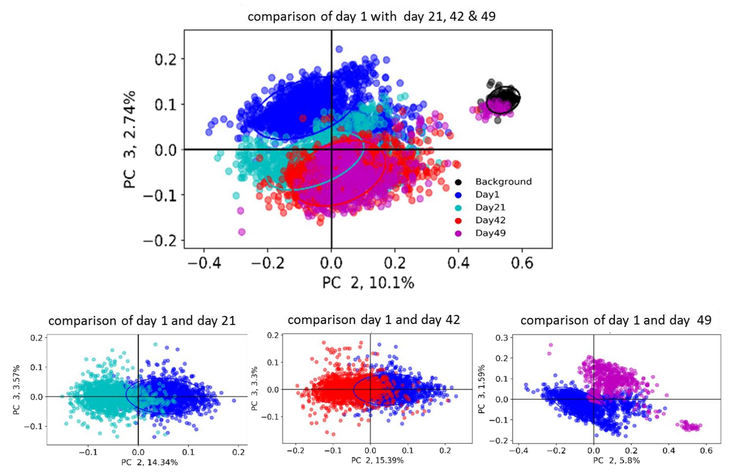



**Fig. 3: Principal component analysis of erythrocyte concentrates from healthy donors in relation to the storage period.** The PCA visualizes every measurement and its relative position to all other measurements. Blood samples from 15 independent donors were analyzed using Raman spectroscopy at different time points of the storage period. There is a significant shift of the measure points from day 1 until day 42 after donation.

## Correlation of Raman spectra with the functionality of erythrocytes and thrombocytes

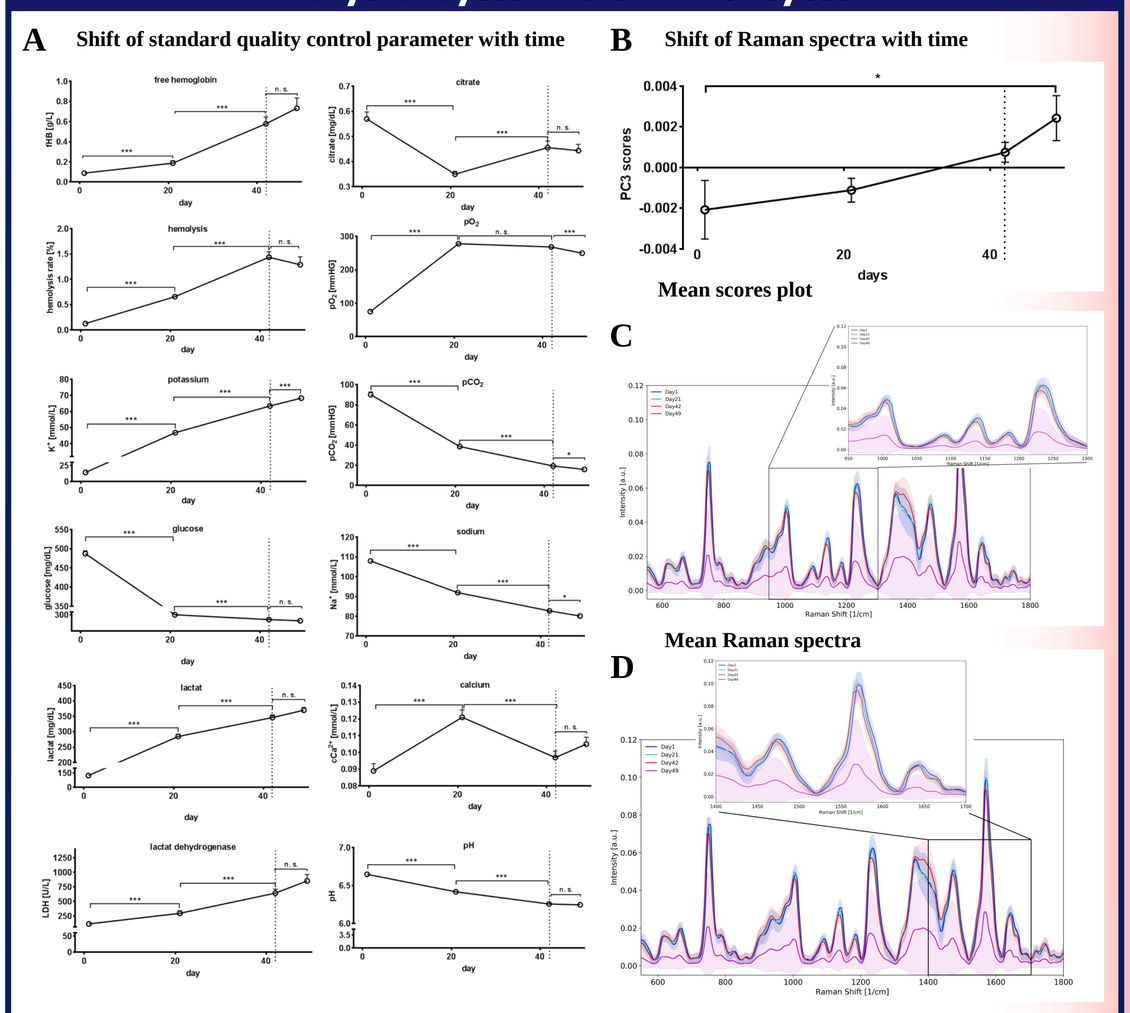



**Fig. 4**: **Correlation of Raman spectra with routine quality control data on red blood cell concentrates. A.** Mean values and SEM of different quality control parameter are shown. **B**. The mean scores and SEM of PC3 for each day are shown. * $P < 0.05$, *** $P < 0.0005$. Mean spectra of erythrocytes for each day are shown. Two regions of interest for the separation of 'young' and 'old' erythrocytes can be found in the range between 950 and 1300 cm$^{-1}$ **(C)** and between 1400 and 1700 cm$^{-1}$ **(D).** $n$ = 15 independent donors.

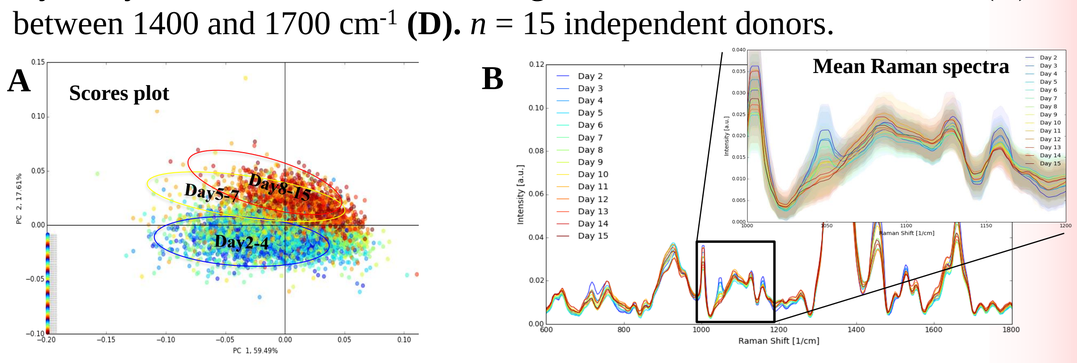
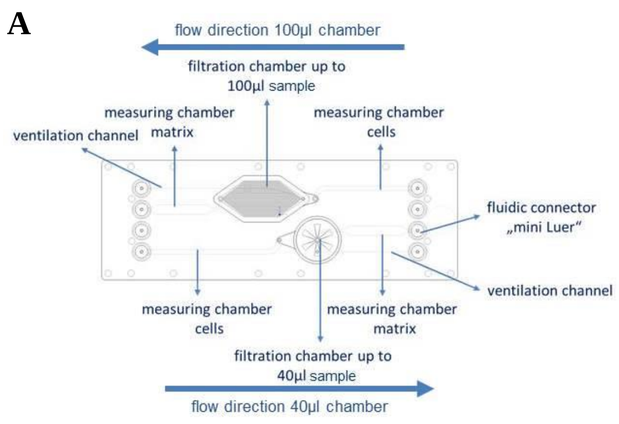



**Fig. 5**: **Correlation of Raman spectra with routine quality control data on thrombocyte concentrates.** PCA **(A)** and mean spectra **(B)** of 8 independent donors are shown. The Raman peak at 1050 cm$^{-1}$ is correlated with the age of the thrombocyte samples. **C**. Mean values and SEM of CD62 trap activation are shown.

## Results and Conclusions

We were able to show that **erythrocytes** and **thrombocytes** possess their own Raman profile. This will allow the identification of residual **leukocytes** in the preparation as the spectra of leukocytes should be different compared to erythrocytes and thrombocytes. Standard quality control studies of **erythrocyte concentrates** during storage showed the expected metabolic consumption by decrease of glucose and citrate and increase in lactate and lactate dehydrogenase (LDH) as well as an increase in free hemoglobin and hemolysis. This is in concordance with a significant shift of Raman spectra from day 1 until day 42. Standard quality control studies of **platelet concentrates** showed the expected decrease in platelet activation capacity. With Raman spectroscopy a wavenumber region of 1000-1100 cm$^{-1}$ was identified that correlates with aging of the cells. Thus, Raman spectroscopy may lead to essential improvements in the field of transfusion medicine. For the first time, the opportunity opens up to ensure a functionality and quality control analysis of blood products immediately before transfusion, thus minimizing risk of contamination and increasing patient safety.

## Outlook – Development of a detection unit based on Raman spectroscopy with a corresponding microfluidic chip as point-of-care analysis to assure quality and functionality of blood products



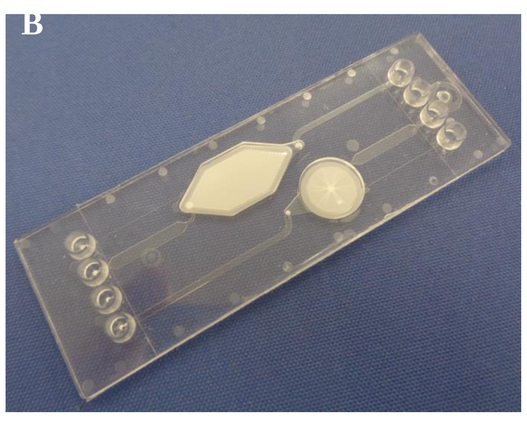




**Fig. 6**: **The Separation chip „Fluidic 783".** Filtration chip module for separation of blood cells and storage matrix with chambers for 100µl and 40µl sample volume. The sample is injected via a mini-Luer fluidic interface. Blood cells and storage matrix are separated from each other by a integrated separation membrane. The sample is analysed within the detection channels. The bottom of the chip which is made from a plastic polymer (mcs COC-02) that can be separated from the Raman signal using special algorithms developed in the "HämatoRam" project.
**A**. Schematic drawing of the chip. **B**. Injection moulded chip with integrated separations.

The project received funding from: German Federal Ministry of Education and Research "KMU-innovative: Medizintechnik: HämatoRam" 13GW0112A.