CellTool
live sorting

BioRam® –

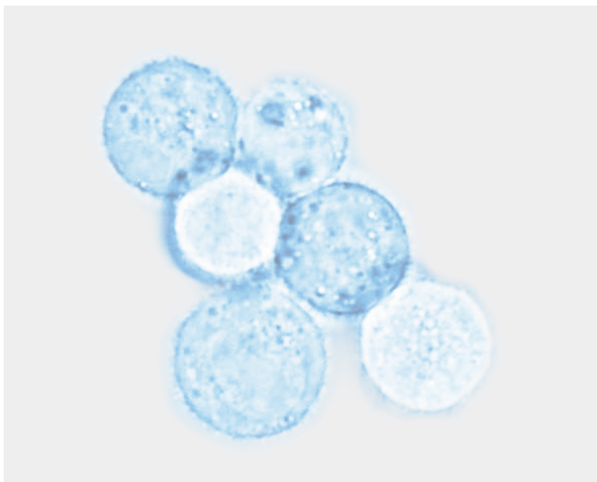
Photonic fingerprinting®

Raman spectroscopy for single cells

BioRam: Identifying and characterizing single cells without labelling

BioRam offers physicians, pharmacists and biologists uncomplicated access to the world of **Raman spectroscopy**. The innovative combination of Raman microscopy, optical trapping features and specially tailored software allows **single cells** to be identified and analysed simply and with a high degree of precision, **without** the need for biochemical markers, fluorescent labels, antibodies or beads – **absolutely safe** for living cells.

This opens up completely new possibilities.



The key to success: Pure cell populations – unaltered and viable

With BioRam you can now:

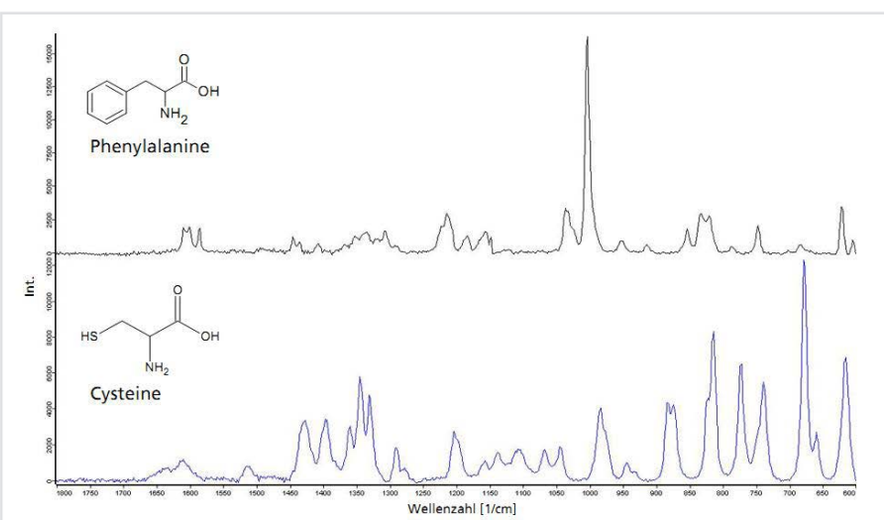
- Identify cell types and activity states **without labelling** and **under physiological conditions**, even in fluids
- Distinguish different stem cell types or determine their differentiation stage
- Identify cells to a high degree of specificity, even those that cannot clearly be defined by surface markers or where markers simply do not exist.
- Identify with high sensitivity extremely **rare cells** in human samples
- Work with **minimal sample volumes**
- Sort cells after analysis within specially developed fluidic chips
- Reuse the cells after analysis in their original condition – **without** interference from biochemical labelling, or due to **physical stress**, e.g. shear stress.
- Monitor and document cellular processes in cell and tissue cultures – **contact-free** and even during the production process
- Investigate the interactions between cells and active substances in cell cultures.

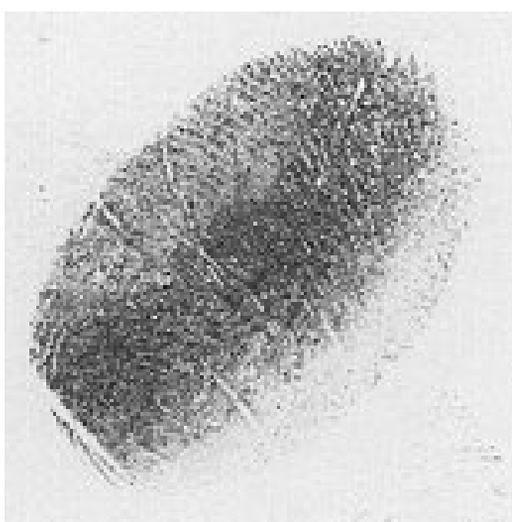
The Principle: Identifying cells from their biophotonic profile

BioRam is based on the Raman effect: when a molecule is excited by laser light, a small fraction of the light is scattered with a shift in frequency compared to the incident light. This **frequency shift** is highly specific for the molecule under observation – as characteristic as a **fingerprint**. In materials science this principle has been used successfully for the contact- and destruction-free characterization of materials.

In biology, the potential of Raman spectroscopy has also been confirmed: several research teams have independently shown that the Raman spectra of all the biopolymers in a cell form **clearly recognisable clusters** according to cell type, activity state and differentiation state. Cells can be identified on the basis of these clusters, using a software-based, automated database comparison. In contrast to other methods involving, for example, PCR or labelling techniques, cells may be found which were not explicitly being sought after using defined markers or sequences.

In order to apply Raman spectroscopy to the routine analysis of **biological samples**, it has been necessary to solve particular technical challenges. Biological samples often emit fluorescence which interferes with their weak **Raman signals**. Furthermore, a tolerable wavelength and optimal physiological conditions during measurements are also required to preserve cell viability. In cooperation with the University Würzburg, Department Tissue Engineering und Regenerative Medicine, CellTool has met these challenges. With BioRam CellTool presents the first **robust and reliable system** for Raman spectroscopy specially designed for the requirements of biologists, pharmacists and physicians and that can be operated intuitively.



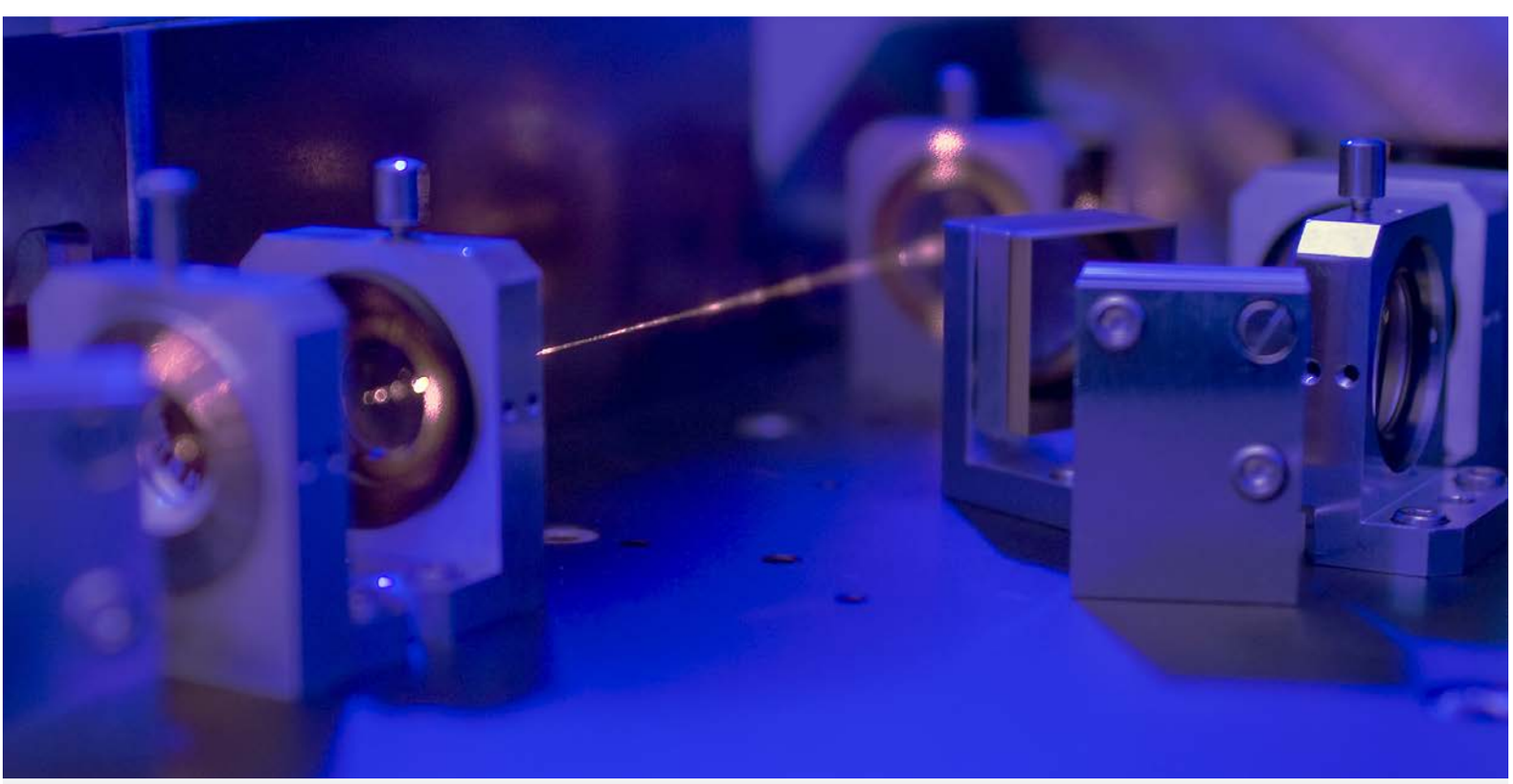


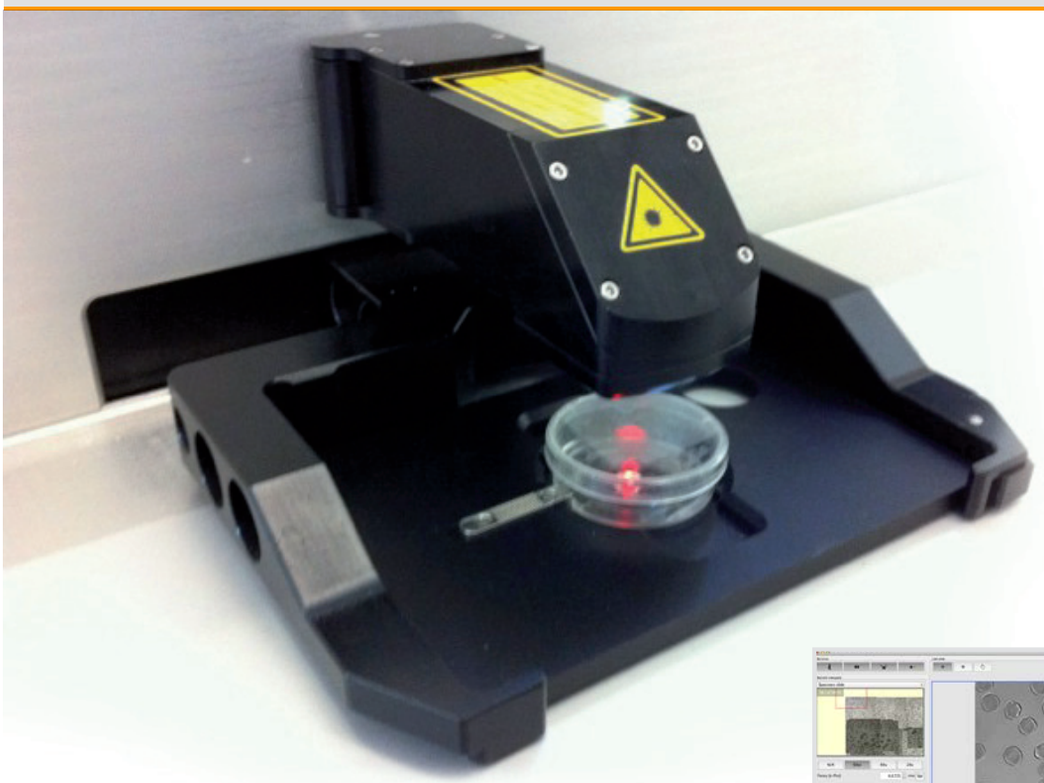
Raman spectra from amino acids (1)

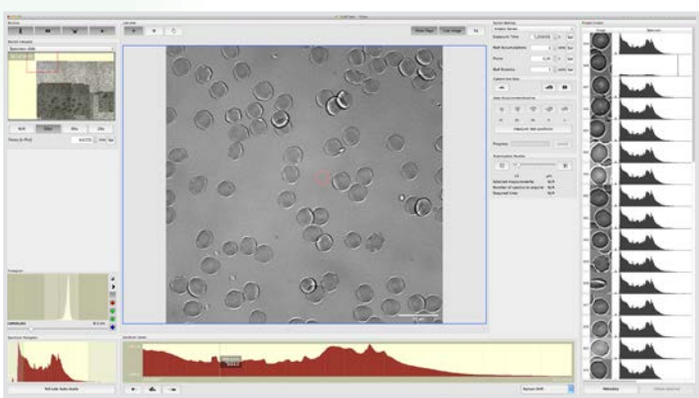
The system: Adapted to living cells

BioRam is an integrated platform.
The basic version comprises the following components:

- High-end microscope in a **creative design** that also enables all classical microscopic techniques: bright field, fluorescence, etc.
- Chamber for living biological samples – temperature-controlled and protected from contamination
- 785 nm Raman laser – the **optimal wavelength** for biological samples: not damaging to cells, minimally absorbed by aqueous solutions, minimal interference effects
- Elements for laser – beam guidance – **efficient**, safe and stable
- Spectrograph, detection range -100 to 3,400 cm^{-1} with ultra-sensitive CCD camera
- PC or Mac with **innovative software** for automated system control (including motorized microscope table, laser control, shutter, interlock system and individually configurable user interface)
- Spectrum editing tool and multivariate **data analysis** for the evaluation of results and for the identification of spectral differences in the data







The measurement technique: Just get going

Samples can be used without special preparation, either as living tissue or in aqueous solution on Raman-compatible slides. Alternatively, cells can be analysed **directly in culture medium** in Raman compatible petri dishes or microfluidic-chips. A 3D-mouse is used to home in on the cells to be investigated, and the **automatic generation** of the Raman spectra is started with a mouse click.

Coordinates for further measurements can be saved and returned to later. The generated spectra are analysed by special algorithms and the cells identified by **database comparison**. At the same time, live images, raw data and all parameters for the microscope and laser settings are also saved for subsequent checking of the measurements and for further analyses.

The potential: Rapid, exact analysis and new applications

BioRam introduces a paradigm shift in the identification and sorting of cells.

Raman spectroscopy has clear advantages over conventional methods and offers previously unimaginable opportunities.

No limit to cell viability and culturability

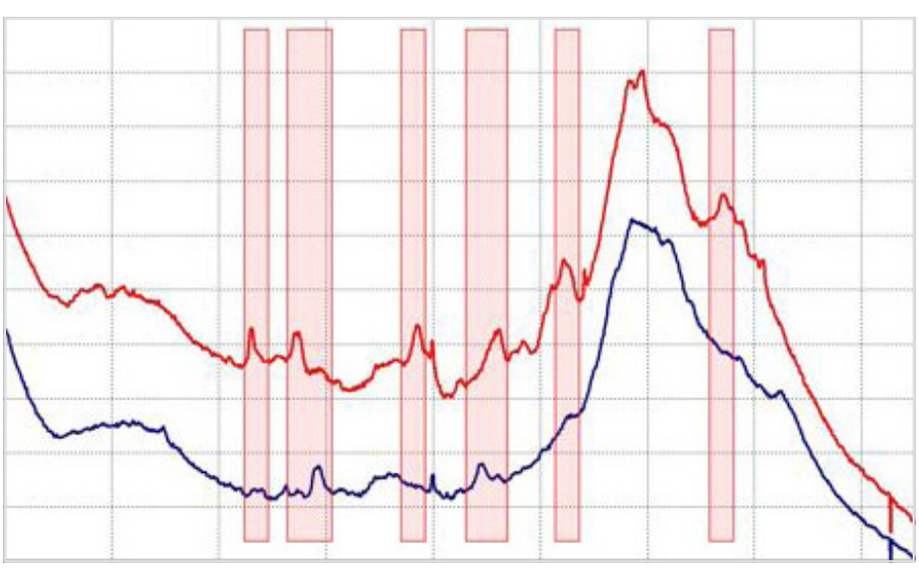
In contrast to cell identification with FACS or MACS, cells analysed by BioRam remain **viable** and are **unchanged** by the procedure – with no need for magnetic beads, biochemical markers or fluorescent labels. The cells are fully culturable and available for further experiments. In this way, cancer cells could be further characterized or their interactions with various active substances analysed directly [1].

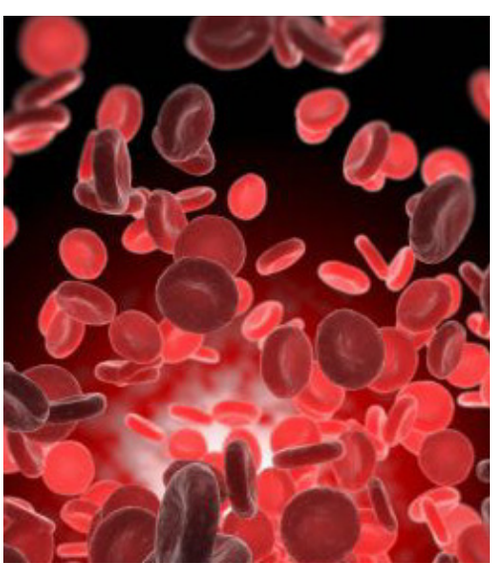
BioRam's applications range from biological research to clinical practice, e.g. for the support of patient-specific therapeutic decisions.

Clear results, even in liquids

Not all cells are clearly identifiable on the basis of surface markers. For the characterization of these cells – especially when comparing cancer cells with healthy cells, or stem cells with differentiated cells – **Raman spectroscopy** is superior to other methods.

The technique generates **unequivocal results**, e.g. for erythrocytes, leukocytes, acute myeloid leukaemia cells or breast cancer cells [2, 3]. This enables pathogenic blood cells to be identified quickly and reliably in unfixed **tissue** or **biopsy material** for medical diagnosis. Similarly, **stem cells** can be isolated from **peripheral or cord blood** and may be used in their native state for research or therapies.



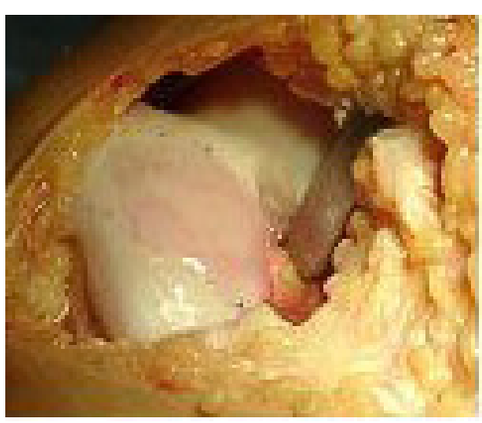


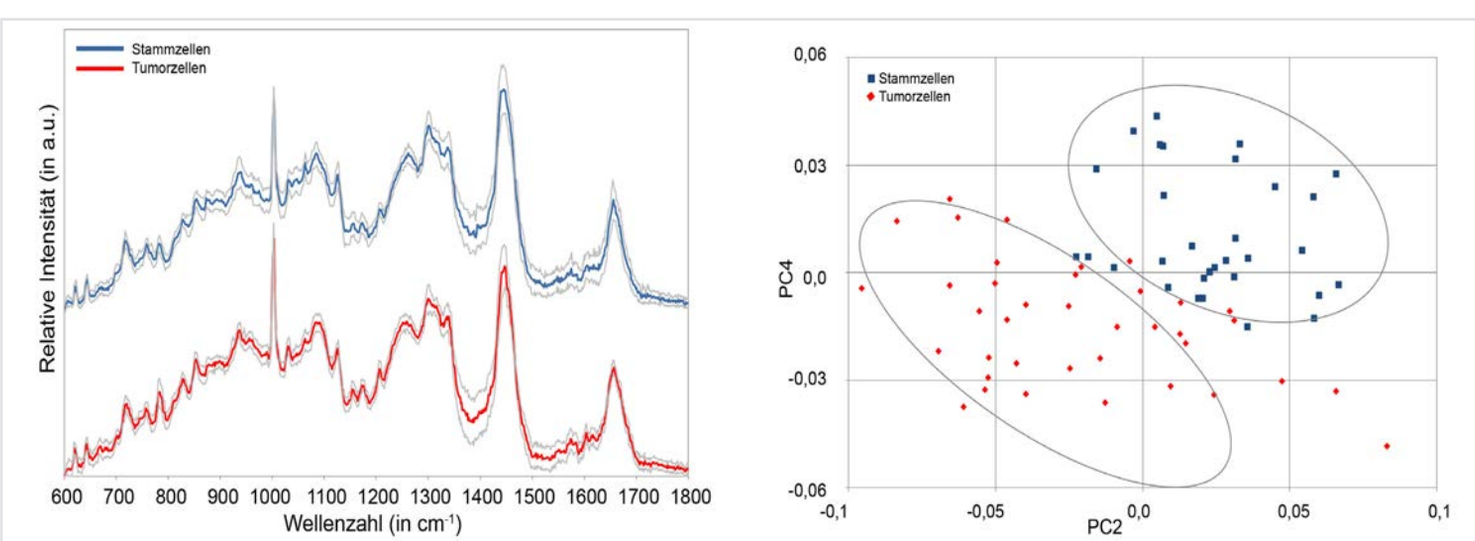
Raman spectra of different blood cells

The potential: Fast and non-destructive analysis, even in tissues

The BioRam system now allows cultured tissues to be analysed non-invasively – this represents a huge advantage over common histological, immunohistochemical and biochemical methods that all involve the destruction and processing of the tissue. For example, in autologous cartilage transplants the viability and **purity** of chondrocytes and their **differentiation state** were tested with the aid of Raman spectroscopy [4].
Living human skin fibroblasts, keratinocytes, melanocytes and immortalized keratinocytes have also been identified in a **rapid** contact-free procedure with no loss in cell viability [5].
In a culture of mesenchymal **stem cells**, a contamination with stromal fibroblasts has been demonstrated with a high degree of **specificity** [6]. This opens up many possible applications, for example in monitoring and quality control in **cell culture** and **tissue engineering**.

Graft from autologous cartilage biopsy material





Typical Raman spectra of human cells and illustration of cluster

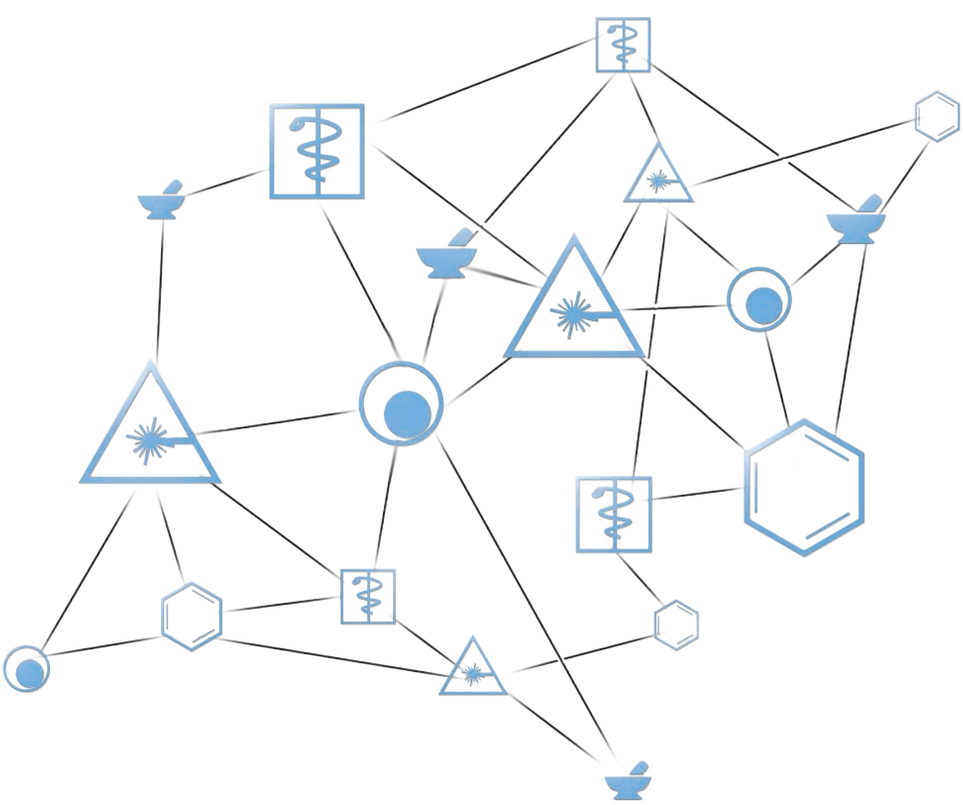
A network of experts: Sharing and developing knowledge

CellTool's BioRam is a targeted, **intuitive** system for biological, pharmaceutical and medical scientists and practitioners. To unlock the potential of Raman spectroscopy for **biomedical applications**, we work closely with physicists, programmers, biologists and physicians – internally and externally. We cooperate with **leading research teams** worldwide, and rely on an open dialogue with our customers. Not only do we offer **a thorough introduction** to the system and on-going support, but we also organize frequent seminars to discuss experiences, share knowledge and initiate **new developments**.

A modular structure: Greater possibilities

In cooperation with our partners, we develop additional modules and components for the BioRam system specifically designed for **diverse applications.**

These include a system for Raman-based pathological appraisals, a device for sterility testing in liquid samples, and a "workstation" device for combining Raman spectroscopy and laser-micromanipulation in order to differentiate cells, when this is not possible by Raman spectroscopy alone. In addition we offer **Raman-compatible supplies** such as slides, petri dishes and **fluidic chips.**



CellTool: Management

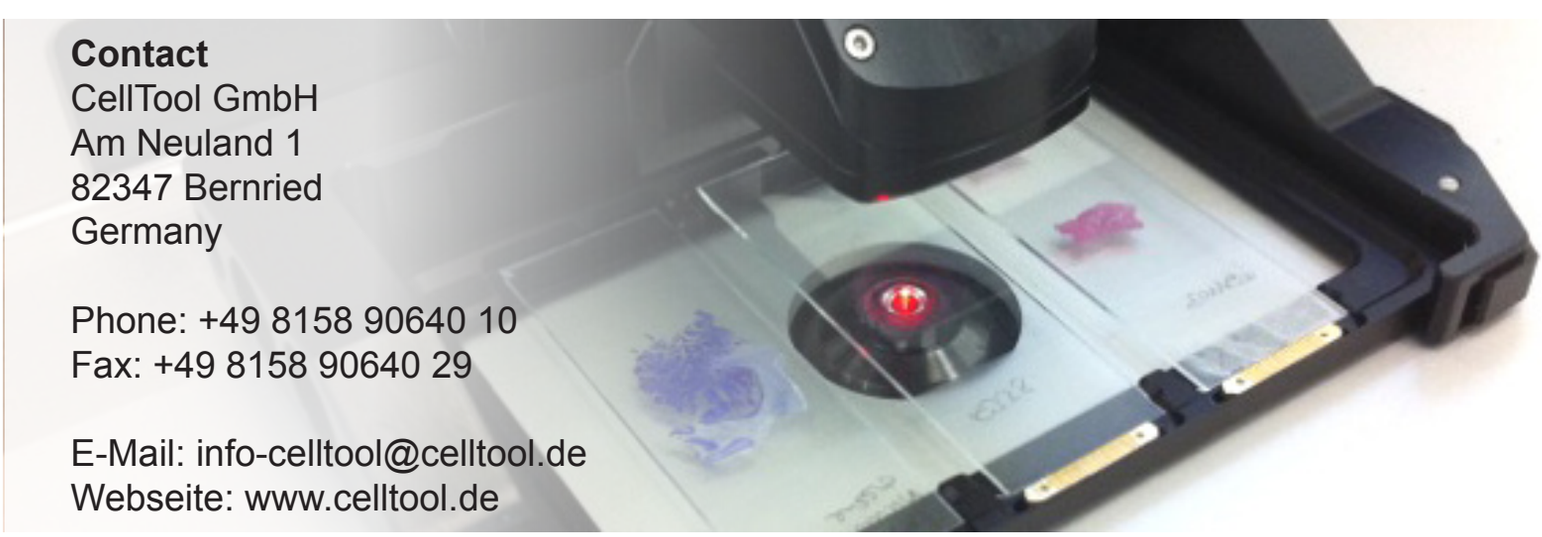
Dr. Karin Schütze *cofounder and general manager (CSO)* is a biologist and expert in non-contact cell handling and enrichment based on innovative photonic technologies. As cofounder and head of the former PALM company she has expertise in developing complex photonic systems into easy-to-handle tools. Her speciality is cell research using biophotonic methods and the development of dedicated application protocols.

Raimund Schütze is a master in goldsmith and studied several semesters in mathematics and physics as well as electrical engineering. He was cofounder and General Manager of the former PALM company. His operating experience and technical skills are fundamental to the precision and functioning of the CellTool systems.

CellTool: The Company

CellTool is a company that develops, manufactures and distributes innovative systems for non-contact and marker-free recognition, quantitative characterization and gentle sorting of living cells based on Raman spectroscopy.

CellTool systems are especially designed to meet the requirements of biologists and physicians. They are extremely user friendly. Cell handling and cell measurements are highly automated. In combination with innovative microfluidics cells can be characterized in a physiological environment keeping them viable for downstream applications. Capabilities are fast diagnosis, patient-specific drug screening or individual cell therapy.



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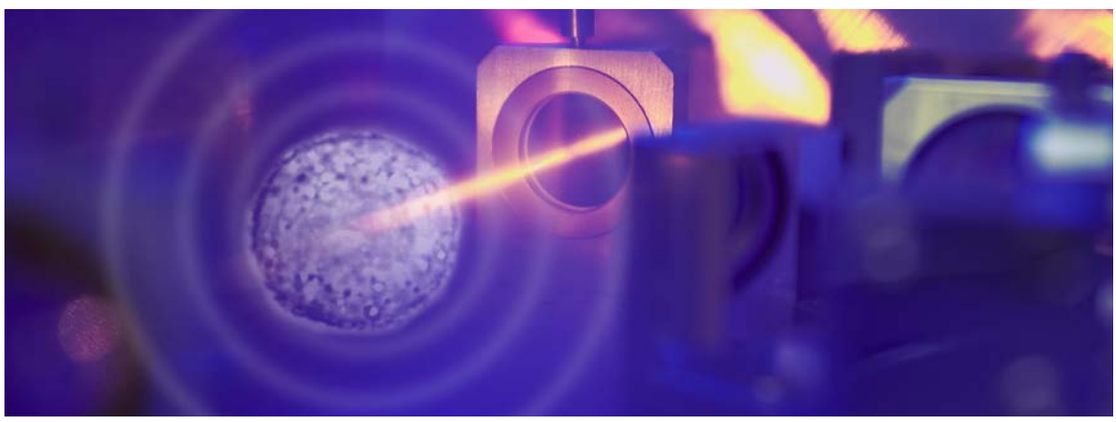

Selected Publications

(1) Evaluierung der Raman Spektroskopie für die marker- und zerstörungsfreie Qualitätskontrolle im Tissue Engineering. Steffen Koch (2010) Fraunhoferverlag ISBN: 978-3-8396-0112 – 9

(2) Nondestructive Identification of Individual Leukemia Cells by Laser Trapping Raman Spectroscopy. James W. Chan et al. Anal. Chem. (2008) 80: 2180 – 2187

(3) Tumour cell identification by means of Raman spectroscopy in combination with optical traps and microfluidic environments. Sebastian Dochow et al. Lab Chip (2011) 11: 1484

(4) Raman spectroscopy as a tool for quality and sterility analysis for tissue engineering applications like cartilage transplants. Marieke Pudlas et al. Int J Artif Organs (2010) 33,3: 228 – 237

(5) Raman Spectroscopy: A Noninvasive Analysis Tool For The Discrimination of Human Skin Cells. Marieke Pudlas et al. Tissue Engineering (2011) C 17,10: 1027 – 1040

(6) Non-contact discrimination of human bone marrow-derived mesenchymal stem cells and fibroblasts using Raman spectroscopy. Marieke Pudlas et al. Medical Laser Application (2011) 26: 119 – 125

Images

Page 2: Osteosarcoma cells – Steffen Koch, Fraunhofer IGB Stuttgart, Germany
Page 3: Raman spectra – Steffen Koch (2010) Fraunhoferverlag ISBN: 978-3-8396-0112-9, Germany
Page 4: Laser guidance for Raman spectroscopy – Carsten Bolwien, Fraunhofer IPM Freiburg, Germany
Page 6: Spectra of various blood cells – Steffen Koch, Fraunhofer IGB Stuttgart, Germany
Page 7: Graft from autologous cartilage biopsy material, before and after transplantation Heike Walles, Fraunhofer IGB Stuttgart, Germany
Page 7: Typical Raman spectra of mesenchymal stem cells and osteosarcoma cells with cluster plot after statistical analysis – Marieke Pudlas, Fraunhofer IGB Stuttgart, Germany
Page 9: Mesenchymal stem cells with laser beam – Steffen Koch und Carsten Bolwien, Germany

BioRam® -

Photonic fingerprinting®

Raman spectroscopy for single cells

- BioRam® is an innovative combination of microscopy, Raman spectroscopy and data analysis for biological and medical applications.
- With BioRam® you can investigate all kinds of micro-organisms, cell-lines, primary cells and tissues without labelling and under physiological conditions.
- BioRam® enables identification of rare cells in human specimens with high specificity.
- No shear forces, no physical stress, small sample volumes – the cells remain viable for downstream applications.
- Easy access to high information density of Raman spectra from biological specimen, optimized and adapted to the needs of biologists, medical scientists and physicians.

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