 DOI 10.1515/plm-2013-0043 — Photon Lasers Med 2013; 2(4): 361–369

Congress report

LASER World of PHOTONICS – DGLM Application Panel

Unmet Needs in Photonics and Medicine

14 May 2013, ICM International Congress Center Munich, Germany

As part of the LASER World of PHOTONICS Congress and Exhibition, which was held from 13–16 May 2013 at the International Congress Center Munich, the *Deutsche Gesellschaft für Lasermedizin* (DGLM) e.V. organized an application panel on the topic "Unmet Needs in Biophotonics and Laser Medicine".

The basic theme "unmet needs" describes unsatisfied demands in biophotonics and laser medicine and underlines the objective of this application panel, namely to consider conceptual and technical gaps in the application of photonics and lasers in medicine – not least from a health-economical point of view.

Starting out from state-of-the-art techniques, the given lectures gave a description of the "ideal situation" and possible steps which are necessary to achieve this situation. In this way the lectures and discussions made the "blank spaces" apparent, i.e., missing links in the entire solution chain, to identify possible concepts and means of resolution and bring together researchers, engineers and manufacturers for future projects.

Conference Chairs
Carsten M. Philipp, Berlin, Germany
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Abstracts

Novel cell analysis based on Raman spectroscopy

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Background: In biomedical research, medical diagnosis and patient specific therapy, there is an increasing demand for non-invasive and highly sensitive cell recognition methods. Emerging technologies to monitor the cell state or cell fate are vibrational spectroscopic methods such as Raman spectroscopy. Well-established for decades in chemistry, physics and pharmacology, it was only recently that Raman systems adapted for biological and medical research and application became available. Meanwhile, several research teams have independently shown that Raman spectroscopy of microorganisms and cells yield distinct information about their metabolism and allow highly specific discrimination with regard to their types, cell activity or differentiation state.

State-of-the-art research: Raman spectroscopy enables simultaneous detection of almost any cellular component and gives insight into the cell metabolism in a completely non-destructive way. With this so-called "photonic fingerprinting", living cells can be identified and analyzed with a high degree of precision and specificity – without the need for biochemical markers, fluorescent labels, antibodies or beads – and the cells remain intact for further use. The advantage of Raman spectroscopy is its sensitivity as well as the possibility of working with small sample volumes and also within fluids. This provides a wide range of application possibilities especially for biological research and medical applications.

For example, Raman spectroscopy discriminates fibroblasts from mesenchymal stem cells (MSC), which cannot be distinguished by standard techniques such as antibody-based fluorescence labeling because common markers are not specific. Classical differentiation assays usually require 2–3 weeks. In comparison, Raman spectra are taken in minutes [1]. Osteogenic differentiation in MSC was detected at day 7 whilst common methods require long-term cultivation periods of 21–28 days. Thus, Raman spectroscopy has good potential for fast and efficient cell characterization and online monitoring of differentiation or cell-agent reaction.

Unmet needs: Cancer is still one of the most common causes of death and only little is known about its origin. Meanwhile strong effort is spend on early diagnosis of diseased cells to avoid tumor development. The most promising approaches are optical spectroscopic techniques such as Raman spectroscopy which has received considerable attention within the last years. Raman spectroscopy is a promising analytical technique for rapid and non-destructive diagnosis of human diseases [2]. It has the potential not only for early and improved diagnosis of cancer but also to advance its treatment [3]. "Raman fingerprinting" of cells may become a valuable tool for tumor diagnosis [4, 5].

Clinical requirements: In spite of the encouraging results that researchers in collaboration with clinicians have achieved, Raman spectroscopy is not yet established as a standard clinical tool. This is mainly due to the intense expertise that is required to operate the complex hardware as well as to the expert knowledge necessary to process and interpret the spectra for meaningful conclusions.

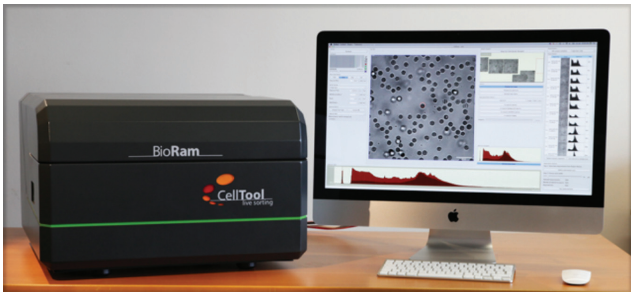

Figure 1 BioRam® – a compact, fully encased, user-friendly Raman system (CellTool GmbH Bernried) adapted for cell research and biomedical applications.

Our approach: Therefore, we developed and introduced the BioRam® system (Figure 1) – a compact Raman microscope specially designed for routine biological and medical work "from biologists for biologists and physicians".

The straightforward and easy to use device could now bridge the gap between laboratory bench-top studies and clinical diagnosis and could greatly improve patient specific care. The inverted microscope platform with the applied laser wavelength of 785 nm as well as the application-oriented operation and data processing software have been especially devised for non-contact gentle and caring cell analysis. Thus, it is for example possible to characterize living microorganisms and cells directly within their physiological environment and without the danger of contamination, unaffected by the procedure.

For routine data evaluation, spectra are processed applying customized data processing software. Data analysis is performed with the Unscrambler® statistical software (Camo, Norway) using "principal component analyses" (PCA), the most common tool for the analysis of spectral data.

The BioRam® system was used, inter alia, to discriminate Hodgkin cells from non-Hodgkin Burkitt cells as well as B-cell from T-cell derived lymphomas which demonstrates its feasible use in supporting cancer diagnosis. So far, no common methods could discriminate between these cells. Knowing the origin of Hodgkin's disease will greatly improve patient treatment modalities. Furthermore, it has been possible to monitor invading glioblastoma cells within an engineered neuronal tissue differentiated from human pluripotent stem cells [6].

To check the potential of Raman spectroscopy for pathogen identification and fast bacterial typing, two different strains from the genus *Staphylococci* were analyzed [7]. *Staphylococcus aureus* is a highly-adapted organism that is found in around 20% of the human population

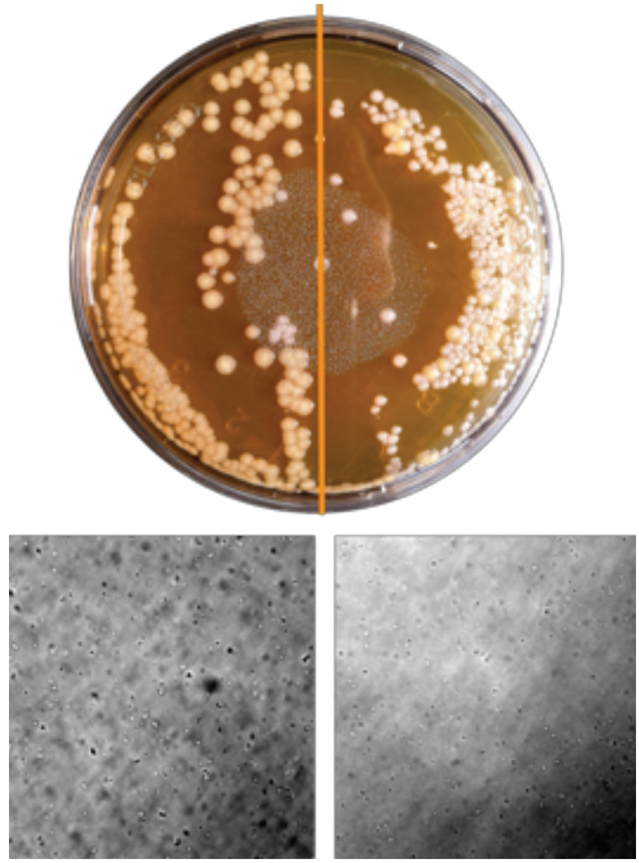

Figure 2 Culture plate and microscopic images of *S. aureus* (left) and *S. epidermidis* (right).

without causing infection. However, under certain conditions *S. aureus* can cause serious illnesses and is one of the most common pathogens causing nosocomial infections.

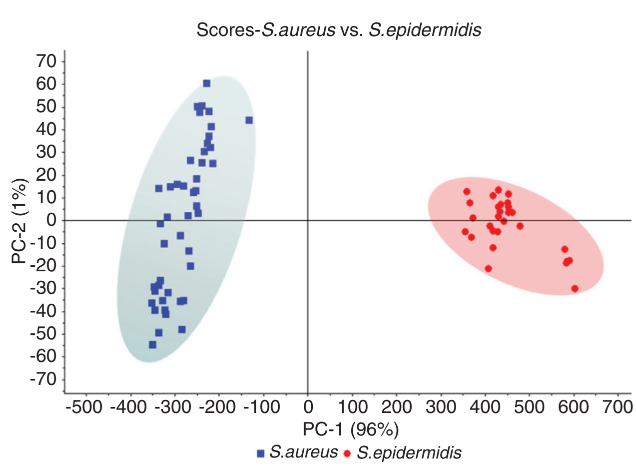

Figure 3 Score plot of the first two principal components demonstrates clear separation of *S. aureus* (blue squares) and *S. epidermidis* (red dots) along the first principal component (PC1) [7].

Raman measurements were performed on suspended bacteria (Figure 2) simultaneously trapped within the laser focus during spectra retrieval. Applying PCA yielded two very well separated clusters indicating the high potential of even further sub-classification (Figure 3).

In summary, Raman is a molecular spectroscopic technique that is highly sensitive to structural changes in complex biomolecules such as proteins, lipids, and nucleic acids. Biochemical changes within cells may either initiate disease or occur as a result of the development of the disease [2]. Thus, spectral changes within cells provide the clinician with diagnostic information and could serve as a fast and efficient "photonic marker". Raman systems adapted for non-destructive cell detection and characterization will become an important device for biologists and physicians to facilitate cancer and stem cell research but also to support clinical diagnosis, patient specific drug screening or to monitor the healing process. In regenerative medicine and tissue engineering, Raman spectroscopy may be applied to control the quality of engineered products.

Acknowledgement: This research received funding from the European Union's Seventh Framework Program (FP7/2007-2013) under grant agreement n HEALTH-F2-2011-279288 (IDEA) as well as from "Photonics4Life" (P4L grant agreement no.: 224014m).

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