FRAUNHOFER INSTITUTE FOR LASER TECHNOLOGY ILT

MARKER-FREE CELL SELECTION FOR PRODUCTION OF BIOLOGICS

DQS certified by
DIN EN ISO 9001:2015
Reg. No. 069572 QM15

Fraunhofer Institute for Laser Technology ILT
Director
Prof. Constantin Häfner

Steinbachstraße 15
52074 Aachen, Germany
Telephone +49 241 8906-0
Fax +49 241 8906-121

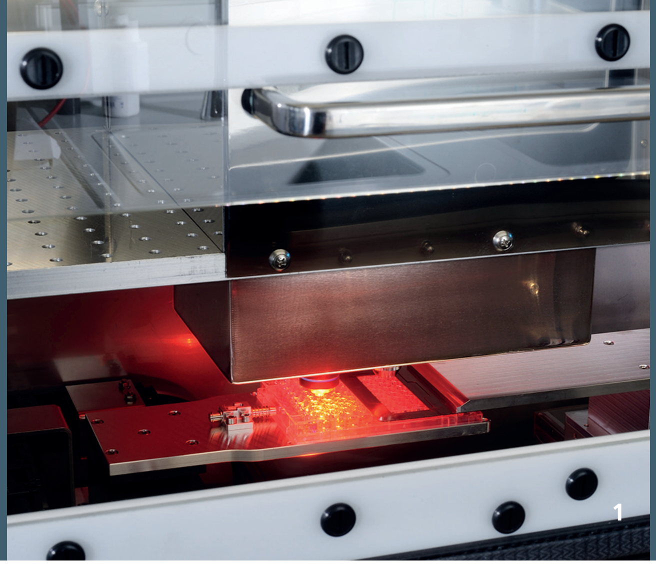
info@ilt.fraunhofer.de
www.ilt.fraunhofer.de

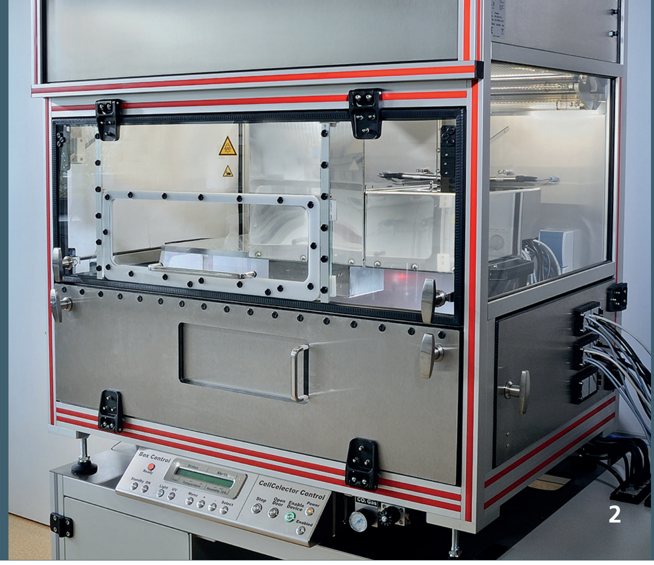
Fraunhofer Institute for Laser Technology ILT

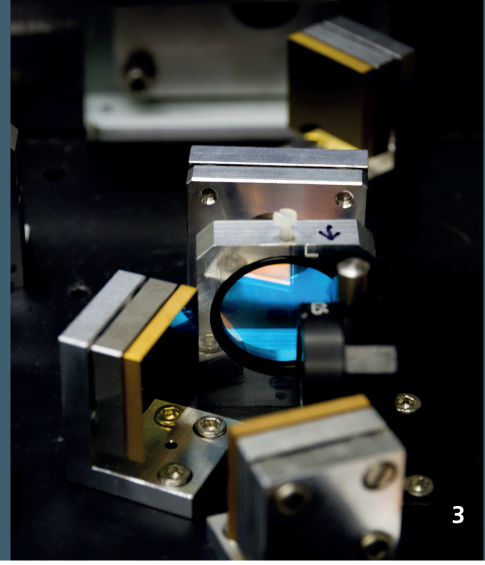
The Fraunhofer Institute for Laser Technology ILT is one of the most important development and contract research institutes in laser development and application worldwide. Its activities encompass a wide range of areas such as developing new laser beam sources and components, laser-based metrology, testing technology and industrial laser processes. This includes laser cutting, ablation, drilling, welding and soldering as well as surface treatment, micro processing and additive manufacturing. Furthermore, Fraunhofer ILT develops photonic components and beam sources for quantum technology.

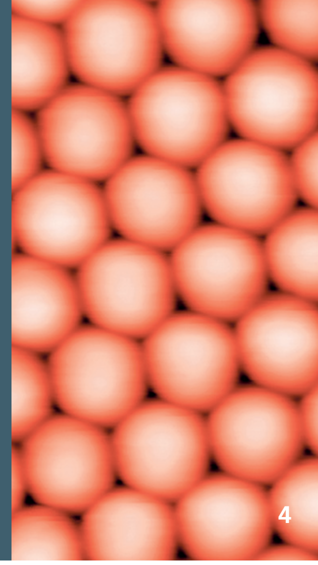
Overall, Fraunhofer ILT is active in the fields of laser plant technology, digitalization, process monitoring and control, simulation and modeling, AI in laser technology and in the entire system technology. We offer feasibility studies, process qualification and laser integration in customized manufacturing lines. The institute focuses on research and development for industrial and societal challenges in the areas of health, safety, communication, production, mobility, energy and environment. Fraunhofer ILT is integrated into the Fraunhofer Gesellschaft.

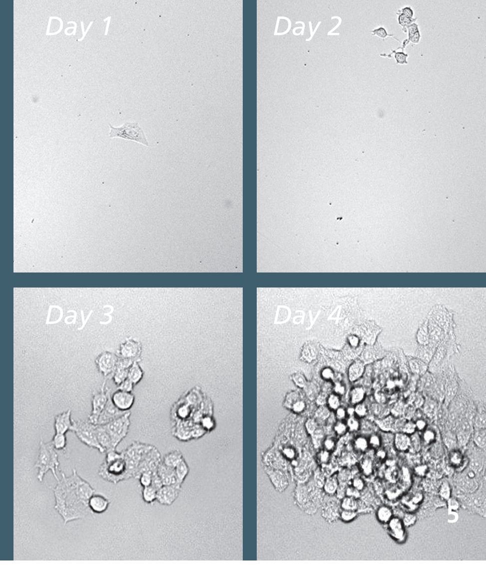
Subject to alterations in specifications and other technical information. 06/2022.











MARKER-FREE CELL SELECTION FOR PRODUCTION OF BIOLOGICS

For the development of biologics, genetically modified cell lines are often used to efficiently produce special molecules. The production of such cell lines is a very time-consuming and costly process, however. Three Fraunhofer Institutes – for Laser Technology ILT (Aachen), for Interfacial Engineering and Biotechnology IGB (Stuttgart) and for Applied Information Technology FIT (Sankt Augustin) – have developed a new procedure, "OptisCell", with which this process can be automated and thereby significantly accelerated.

Production of Biologics

In biotechnological production, so-called biologics are becoming increasingly important. They are considered to be particularly well tolerated by humans and are, therefore, being used in the production of medicines more and more. Unfortunately, developing a cell line for the production of biologics takes about twelve months and costs about 400 million euros. Particularly time-consuming is the functional identification, i.e. the recognition of whether a cell can produce the desired protein to a sufficiently high degree so that an industrial use is feasible. The isolation of so-called high-producer cells is also very time-consuming and cost-intensive.

Advantages of the OptisCell Process

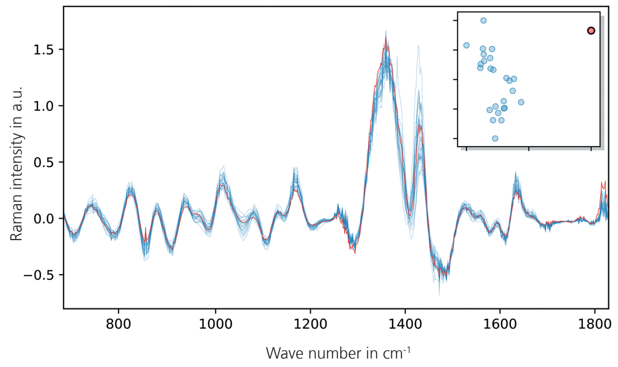
The OptisCell process reduces the time needed to develop these kinds of cell lines from twelve to three months. Marker-free Raman spectroscopy is used for the functional identification of the cells: Suitable cells are isolated with a laser-based single cell process. This eliminates the expansion and cultivation steps for cell clones that have insufficient production rates. Suitable cell candidates for biotechnological production can thus be selected much earlier than in the conventional process. The automated process cycle of OptisCell also makes it possible to screen for a large number of candidates and to accurately track the process for later quality control.

Process Chain

In a first step, the cells are examined for the production of a specific protein using Raman spectroscopy. The system uses machine learning to distinguish the spectra of producing and non-producing cells. In the second step, the producing cells are transferred to a commercially available microtiter plate with a laser pulse. Laser-induced Forward Transfer (LIFT) is the name of the innovative process, in which an absorber layer on the carrier is vaporized with a laser pulse; The small vapor bubble creates a jet in the medium (e.g. hydrogel), thus transferring the selected cell to the microtiter plate. Surface Enhanced Raman Scattering (SERS) spectroscopy is used to determine how efficiently the cell produces the desired protein. This makes it possible to quickly and automatically find high-producer cells and to select them for further use.

Characterization of Protein Production

For the detection of protein-producing cells, Raman spectra from potential high-producer cells are first subjected to pre-processing, which frees and normalizes the spectra of interfering signals – such as cosmic spikes, white noise and other background signals. After feature dimensions are reduced with PCA (Principle Component Analysis), four independent models use a weighting method to decide which spectra contain high-producer cells. The accuracy with which cells are recognized as true high-producers is up to 92 percent.



Raman spectra with result of the weighting procedure.

In addition to this process, proteins are detected using special chips that enhance the Raman effect due to their surface properties (with SERS spectroscopy). Even the smallest amounts of a target protein can be detected.

Cell Transfer and Handling

For the OptisCell process, the three partners have devised a combined system concept that enables them to analyze and transfer cells. The system provides UV and MIR beam sources for the LIFT process and features a three-objective and five-axis system for flexible handling. Thus, transfer and receiver carriers can be moved independently in the x and y directions with a repeat accuracy down to 100 nm and at speeds of up to 3 m/s relative to each other. The lenses are accessible via a third axis (z-axis). OptisCell will make it possible to transfer cells more quickly and without contact.

In several studies, CHO cells were used for Raman analysis and transfer. With both UV and MIR laser radiation, the LIFT process was successful here in up to 85 percent of the transfers. Transferred CHO cells survive and proliferate for up to seven days. The survival rate of the transferred cells is about 60 percent after the seven days. The plant has been designed to operate on a workbench, allowing treatment of the cells in a sterile and air-conditioned environment. This significantly increases the survival rate of the cells and thus the efficiency of the process.

Contact

Dr. Nadine Nottrodt
Telephone +49 241 8906-605
nadine.nottrodt@ilt.fraunhofer.de

Dr. Martin Wehner
Telephone +49 241 8906-202
martin.wehner@ilt.fraunhofer.de

1 Process chamber for Raman spectroscopy and LIFT process.

2 OptisCell module on a sterile and air-conditioned workbench.

3 Raman filters for the OptisCell process.

4 Surface of the detection chip for the SERS measurement.

5 CHO cells according to the LIFT procedure.