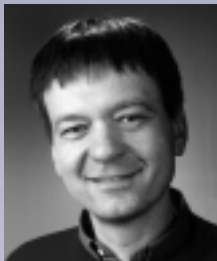


High Throughput Screening



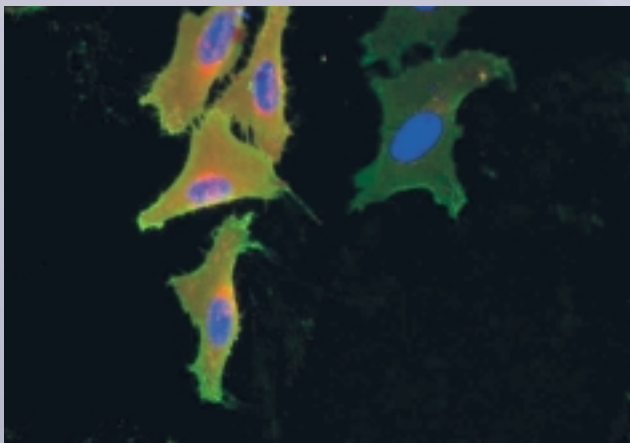
Christof Fattinger

The future of today's pharmaceutical research and drug discovery is based on millions of optical analyses of the molecular activity in biological microsamples.

Gaining know-how through automation

Enhancing knowledge through completely automated experiments in extremely small biological samples is one of the most exciting innovations of our times. The new findings are achieved by sophisticated, integrated hardware and software systems. These new tools permit biological, medical and pharmaceutical research to examine and characterize the biological activity of small, organic molecules in large chemical libraries comprising several million compounds. This and the pioneering progress in fluorescent

Fig. 1:
Cell nucleus (blue),
viral protein in endoplasmic reticulum (red),
viral protein on cell surface (green).
Photo: Urban Liebel,
EMBL Heidelberg.



sample technology offer the scientist the possibility of quantitatively and qualitatively examining the interaction of synthetic molecules with proteins identified as a target molecule for a potential drug – and all with pinpoint accuracy. The combination of molecular biology, medical chemistry and molecular analysis by automation with state-of-the-art process control and data capture essentially influence and change the way we work in all fields of the life sciences. More than anywhere else, this technology is proving to be a great success in pharmaceutical research and drug discovery.

The goal of drug research

One of the most impressive examples illustrating the efficiency of automated analyzing systems is the decoding of the human genome in the past decade. Today, the complete list of all genes and proteins making up the biology of man are available to medical research. It must be said, however, that this list gives little or no indication as to which protein needs to be modulated to be able to treat a disease. It is only the understanding of the functioning of a specific gene or protein and the explanation of the molecular interaction on which the disease is based or by which it is caused that leads to the identification of a potential drug. The protein associated with the disease is called the target molecule or drug target. The first critical step in researching into a new drug is the identification and evaluation of a specific, pharmaceutically relevant protein. Researchers are aiming at modulating this protein in order to treat the disease in question.

Modern chemistry in Medicine

In recent years, the methodology of medical chemistry has also changed.

Formerly, chemists synthesized relatively large quantities of pure substances which were then tested in complex and sophisticated biological systems. The combination of synthetic chemistry, automation and data processing has given rise to a new approach in medical chemistry: the diversity of synthesized compounds is more important than their quantity. New, parallel syntheses and the combinatorics provided by modern chemistry create new, multi-faceted substance libraries of potential drugs.

Analysis of microscopically small samples

Screening is the first stage in the discovery process for a new drug. It stands for the automatic investigation and testing of the activity of chemically synthesized molecules in biological microsamples. The examined samples may be of a totally different nature and origin. Normally they consist of protein solutions containing the target molecule. However, they may also be specific cell lines that produce the target molecule to be examined. The analysis of molecular interaction in the biological sample is performed by means of an optical signal whose properties depend on the molecular binding status of the examined sample. The analyzed parameters include, for example, absorption, intensity of fluorescence, polarization, or the time-resolved fluorescence in a test volume of a few microliters.

Parallel processing provides high throughput rates

As the chemical substance libraries to be tested consist of several hundred thousand or even millions of different compounds, the screening of the complete library can only be achieved within a few days or weeks by per-

forming the analyses in parallel steps. Present-day screening systems with high and ultra-high throughput rates, so-called High Throughput- and Ultra High Throughput Systems, analyze the biological samples fully automatically in microtiter plates with 384 or 1536 sample wells. Each sample well contains only a few microliters of the specimen to be examined. During the screening process, liquid handling stations add different reagents at precisely defined intervals in precisely defined microvolumes to the individual sample wells. The optical reader of the screening system measures and records the molecular interaction between the test substances and the target molecule. To make sure that every test sample is evaluated according to the same analyzing protocol, liquid handling and optical readout in all sample wells must be exactly synchronized. Powerful, state-of-the-art process software takes care of the entire process control – from the handling of microtiter plates to data acquisition.

For years, research teams of Hoffmann-La Roche and Carl Zeiss have closely cooperated in the development of an integrated hardware and software platform for Ultra High Throughput Screening. The result is the **plate::explorer®** – a real all-rounder in pharmaceutical drug research. With the high-precision analyzer **plate::vision®** integrated into the screening system, researchers have succeeded for the very first time in providing a fully automatic analysis of the time behavior of the molecular interaction between a target molecule and the synthesized substances at a throughput rate of 100,000 samples per day.

Secondary screening of molecular affinities

The active substances identified in a screening process are denoted as "hits". They are the basis for further,

more refined analyzing processes. The next step in the discovery process is called secondary screening and consists of the precise investigation and confirmation of the interaction of the target molecule with the hit identified in primary screening. Here, the molecular affinity between the target molecule and the synthesized, active substances is precisely analyzed and quantified in a series of tests under biological conditions. Qualitative and quantitative information on the interaction between the examined target molecule and the hit identified in screening is the basis for a refined chemical synthesis program that will culminate in a promising drug to be tested in clinical trials.

Screening of cells – the challenge of the future

In today's High Throughput Screening methods an average value from several hundred to thousand cells is obtained of the optical signals measured on the cells. In High Throughput Screening it is not yet possible to achieve analyses of cellular resolution. Optical measurement is performed by calculating a mean value for a large number of cells in the wells of the microtiter plate. If the molecular interaction in the cell fails to lead to a macroscopic optical signal, the interaction between the target and the examined molecule will remain undiscovered.

The future enhancement of High Content Screening methods based on individual cells for the detailed investigation of molecular activity at a cellular or subcellular level is the most fascinating technical and scientific challenge in modern microscopy today.

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Fig. 2: Screening – systematic analysis of molecular affinities in substance libraries, UHTS principle.

