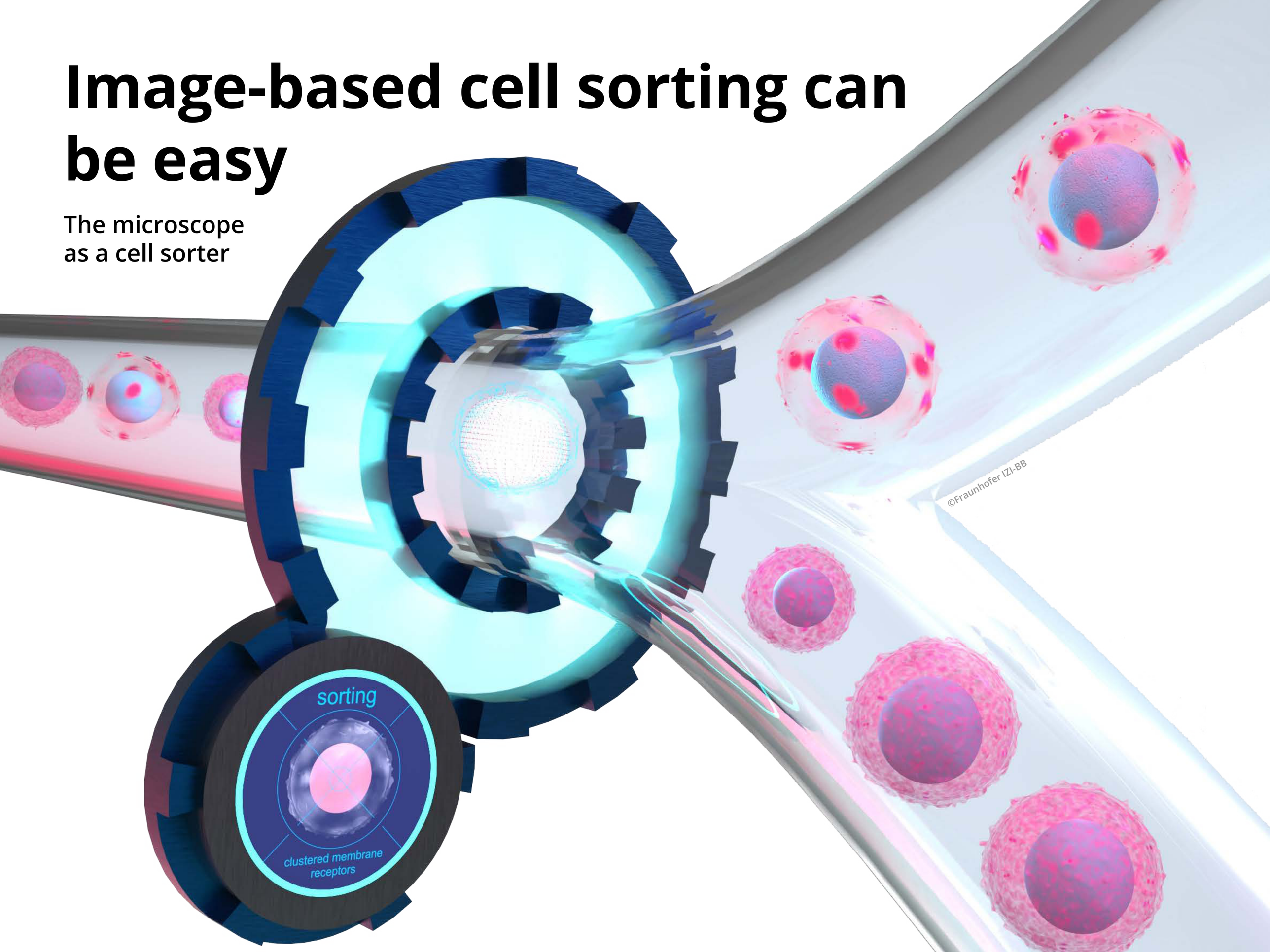
Image-based cell sorting can be easy

The microscope as a cell sorter



Although microscopy is one of the most important tools for studying cells and their diversity of phenotypes, current cell sorting methods do not record spatially resolved characteristics. Instead, cells are only isolated based on one-dimensional features, such as the presence/absence of surface markers. Sorting based on size, shape, or co-localization of cells, organelles, and proteins is therefore barely possible. Using a combination of microfluidic cell handling, microscopy, and automated image analysis, the group has now succeeded in sorting cells based on their microscopic image or the subcellular distribution of fluorescent markers. The method is particularly simple and gentle on cells and can be combined with almost any microscope for image acquisition. The high flexibility in the choice of imaging method is just one of the unique features that will open up numerous application possibilities.

Introduction

The separation of heterogeneous cell samples is a key process in modern biomedicine [1]. Conventional sorting methods use one-dimensional sorting criteria such as integral fluorescence intensity for sorting, thus leaving spatially-resolved features in cells unutilized [2]. To utilize features such as morphology or subcellular distribution of cell organelles and proteins for cell sorting, imaging methods must be combined with a high-precision sorting function.

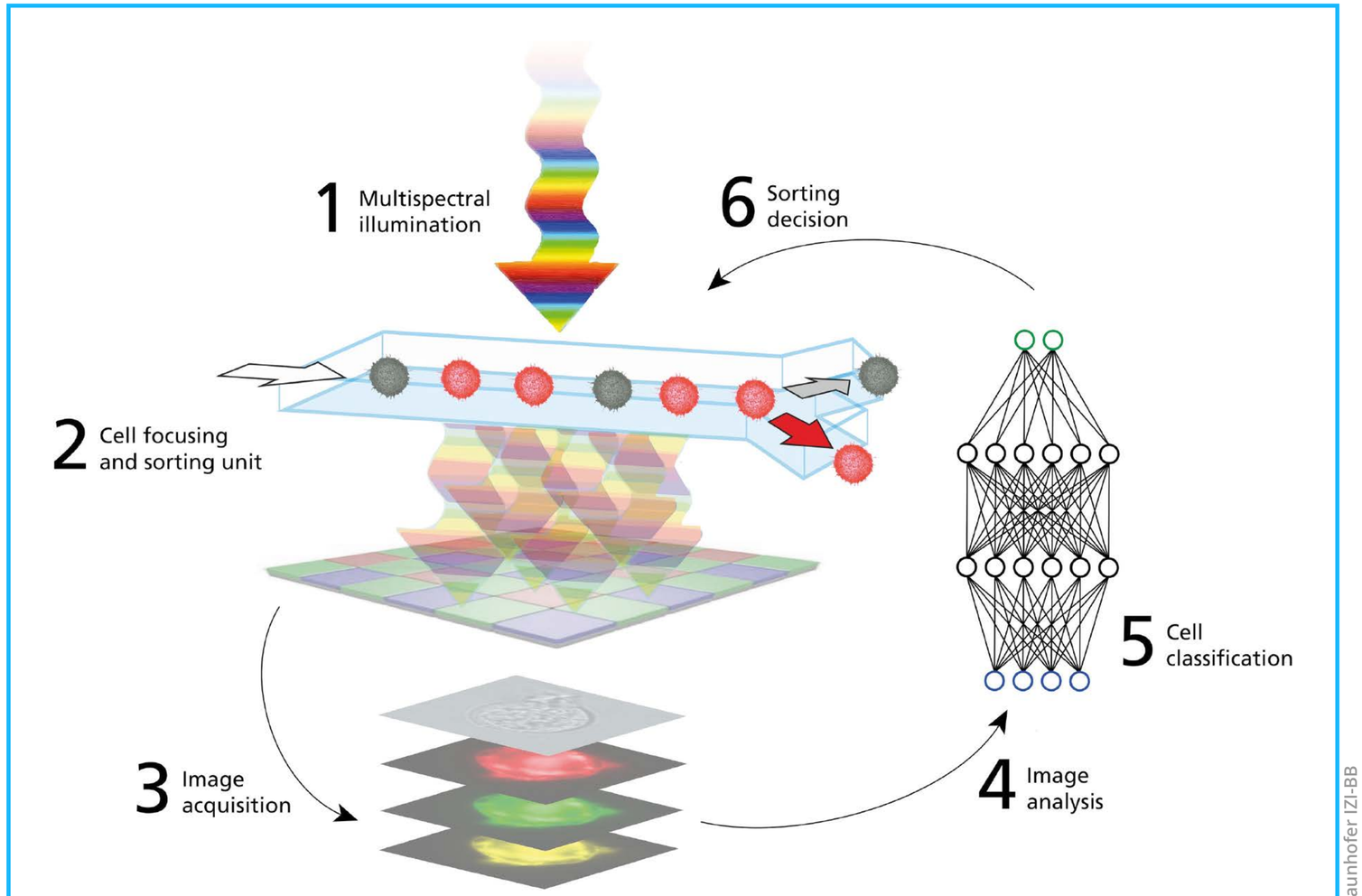


Fig. 1: Concept of an image-activated cell sorting. A flow cell is used to confine the cells in the focus of the imaging device before acquiring microscopic images in multiple imaging modalities. The images are then processed in real time, the cells are classified and a sorting decision is derived. On this basis, downstream actuator structures separate the corresponding target cells from the remaining cells.

Next-generation cell sorting

The use of cells as biomarkers in diagnostics, as target structures in drug development, or as a basis for cell therapy and tissue engineering often requires the isolation of specific cell types from heterogeneous cell mixtures. The target cells must first be identified before they can be isolated from the sample. Although microscopy is one of the most important methods used in many cell biology laboratories to identify and study cells and their various states, microscopic image data plays little role in most common sorting methods.

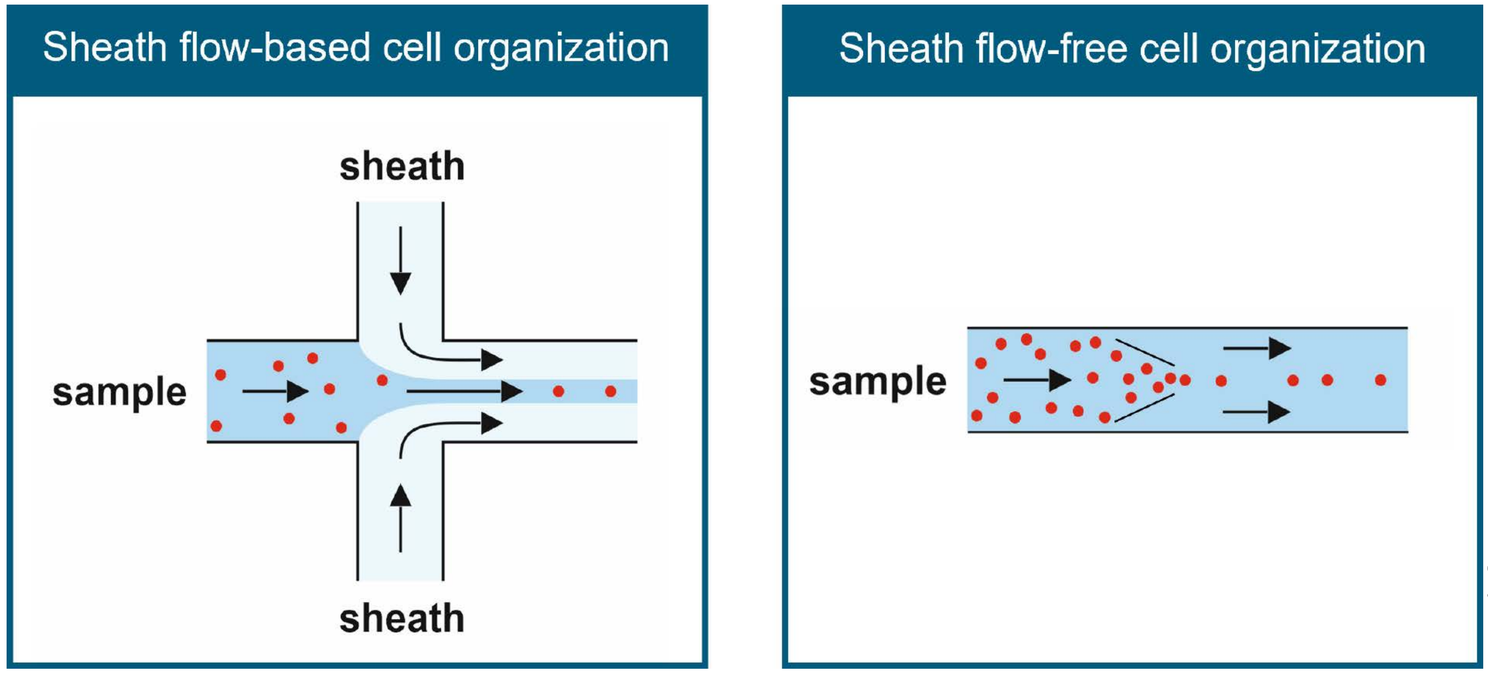


Fig. 2: Various options for focusing the cell sample in the detection area of an imaging device. Left: Normally, so-called "sheath flows" are used to restrict the sample flow in a narrow area. Under these conditions, high flow velocities are required to process a certain sample volume. Right: Sheath flow-free handling of sample objects, e.g., by electrokinetic forces, allows achieving the same volume throughput at significantly lower flow velocities, which greatly simplifies imaging.

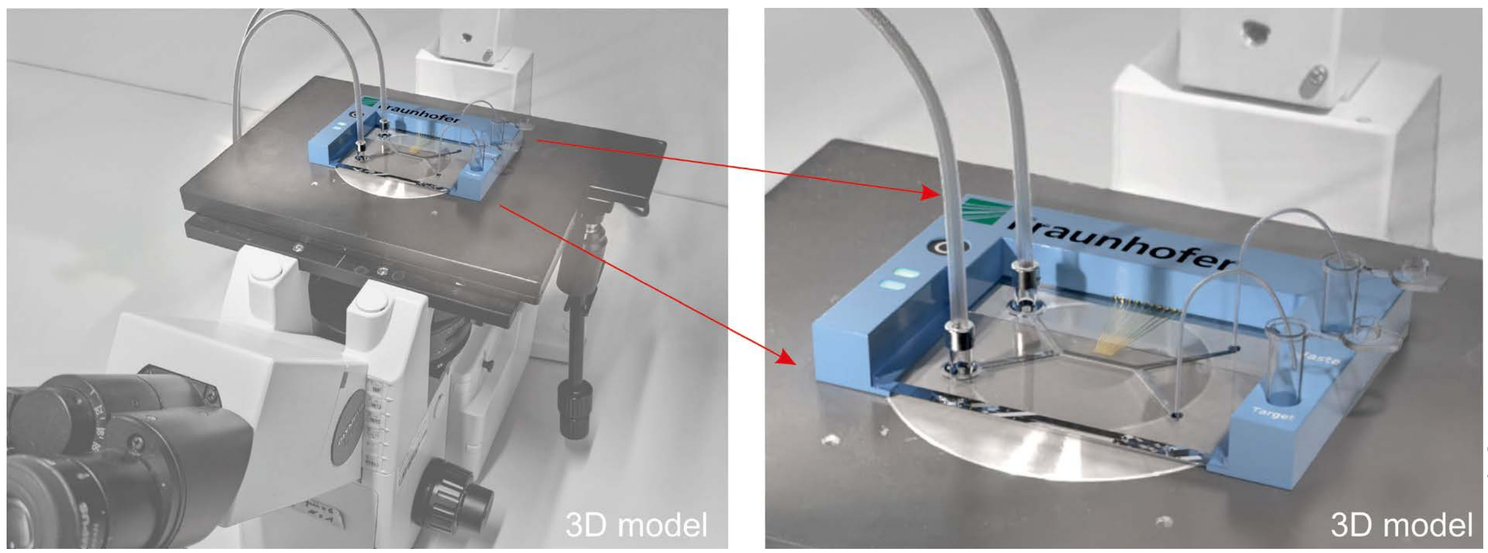


Fig. 3: A microfluidic cartridge serves as a microscope add-on. The cartridge can be mounted on almost any type of microscope and transforms it into an image-activated cell sorter.

Instead, cells are usually classified and sorted based on the presence or absence of certain surface markers. Essential characteristics such as size, shape, arrangement, number, or interaction of organelles and proteins (Table 1) can therefore not be recorded accurately or not at all using these methods. Similarly, the use of chemical or structural features, which can be detected label-free by certain microscopy techniques such as Raman or quantitative phase imaging, is challenging or impossible with conventional methods.

In recent years, new concepts for flow-through sorting that have been developed can use microscopic image information for cell sorting [3-6]. Here, the cells of a sample are guided into the focal area of an imaging optic employing a microfluidic system, where their image is captured in several brightfield or fluorescence channels (Fig. 1). The image information is then compared in real time with previously defined sorting criteria. If a cell meets these criteria, it is separated from the other cells by actuator structures located downstream.

Precise cell handling

The precise handling and positioning of the sample stream in the flow cell is of crucial importance and is usually realized by so-called sheath flows, which confine the sample stream in the flow cell and align it precisely at the same time (Fig. 2). However, this confinement is accompanied by an acceleration of the sample stream and high propulsion speeds, which is an enormous challenge for imaging of weak (fluorescence) signals with high spatial resolution and minimal motion blur. In this case, only the shortest integration times are permitted for capturing and processing the image information, which requires sophisticated optical systems and a complex IT infrastructure. Corresponding systems are therefore often complex, expensive, inflexible, or do not provide the necessary image quality for unambiguous identification of the target cells.

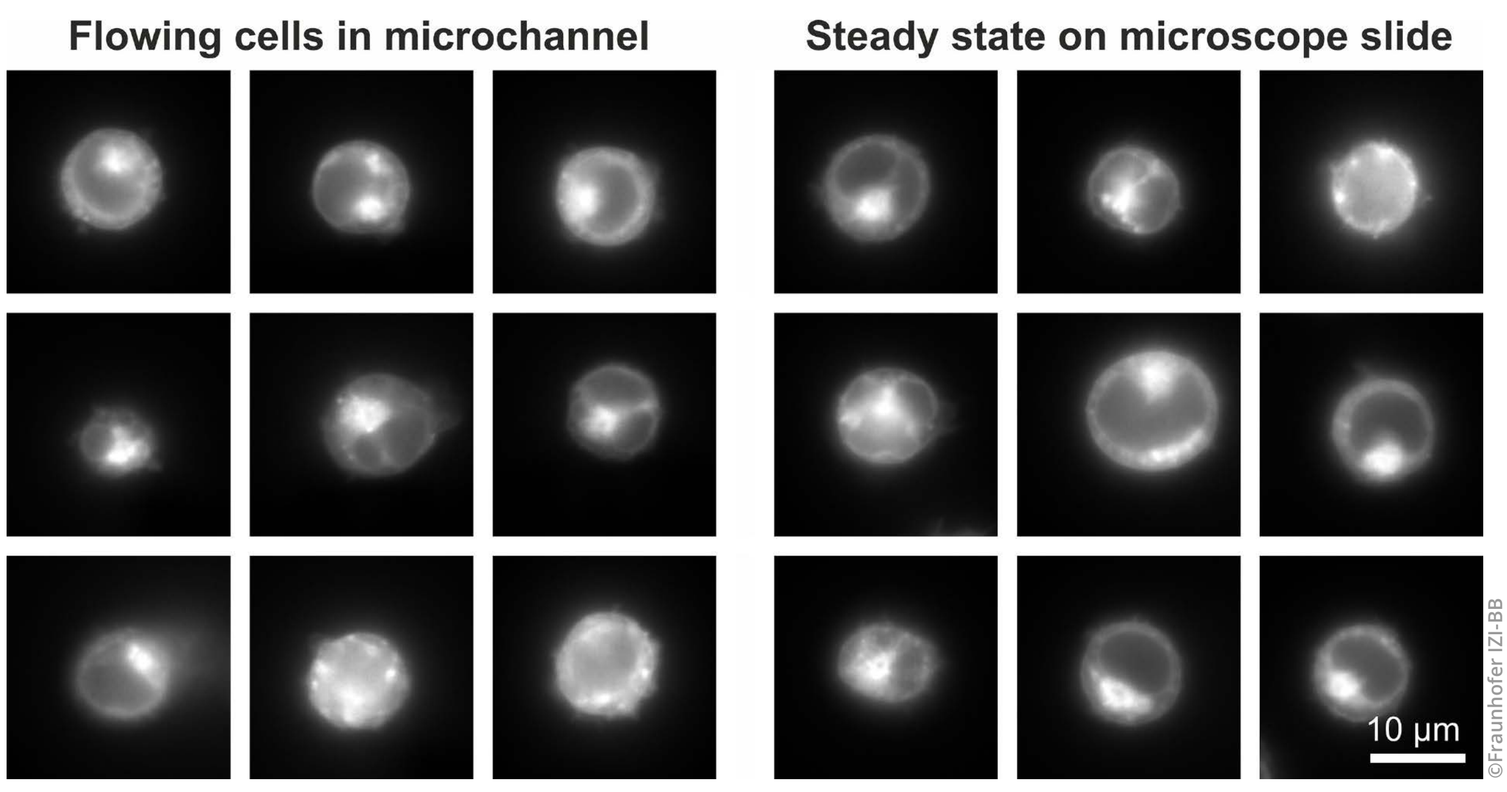


Fig. 4: High-quality imaging of cells flowing in the microchannel. The sharpness and image quality of the fluorescence images of cells flowing in the channel can hardly be distinguished from images taken under steady-state conditions (40x oil immersion objective, 500 µs exposure time, flow velocity in the channel: 500 µm s^{-1}).

A microfluidics module for microscopes

To overcome the challenges described above, a microfluidic cartridge was developed, which enables flow-through sorting without the need for sheath flows for cell focusing, and therefore operates at significantly lower propulsion speeds [7]. This allows very long integration times for image acquisition and processing, which means that the system can be connected to a simple microscope and operated with commercially available PCs for data analysis (Fig. 3). The quality of the image information is comparable to images under static conditions (Fig. 4). The possibility of sorting based on standard imaging of cells using microscopy also allows easy transfer of the characteristics of the target cells identified in the laboratory to the sorting process.

A special concept for handling the cells in the flow cell makes the approach so elegant: instead of positioning the sample fluid, as usual, using sheath flows, the objects under investigation are gently guided into the focus of the imaging optics with the highest local precision using alternating electrical fields in the radio frequency range. As there is no need to confine the sample flow, the sample volume can be processed in a reasonable amount of time even at significantly lower advance speeds (Fig. 2).

Table 1: Examples of spatial features in cells and possible use cases that can be addressed with image-activated cell sorting.

	Spatial feature	Example of application
	Nuclear-cytoplasmic ratio	Isolation of malignant cells for personalized medicine
	Morphology of cells or organelles	Isolation of special yeast strains for the food industry
	(Co-)localization of proteins or organelles	Isolation of activated immune cells
	Cell aggregation	Isolation of tumor antigen-specific T cells
	Chromosome number / FISH analysis	Isolation of malignant cells
	Membrane-attached extracellular vesicles	Isolation of virus-infected cells

The same principles are also used to divert the captured target cells from their original path through the microchannel: utilizing precisely controllable electric fields, the different cells can be handled individually and separated from the other cells, even at high cell densities.

As described in the latest publication, the concept enabled thousands of T cells to be separated from each other based on the subcellular distribution of fluorescence signals, without having to integrate elaborate optical systems or complex data processing architectures [7]. Since the image information on which the sorting process is based is determined only by the microscope, different imaging modes and even unconventional imaging techniques such as quantitative phase microscopy [8, 9] can be used relatively easily and flexibly for cell sorting.

The future will be even more colorful

As part of a research collaboration funded by the Fraunhofer Society with researchers from the Fraunhofer Institute for Applied Optics and Precision Engineering (IOF), the Fraunhofer Institute for Integrated Circuits (IIS) and the Berlin Institute of Health (BIH) at Charité – Universitätsmedizin Berlin, Germany, scientists are currently working on the simultaneous integration of transmitted light and fluorescence imaging in several color channels (www.cellsorting.fraunhofer.de). In addition, the method is to be combined with intelligent image analysis. The simplicity of the approach again becomes crucial here: Due to the relatively slow propulsion speed, sufficient time is available for image analysis, which can also be adjusted according to the computing time requirements by varying the distance between the cell inspection area and the downstream sorting function by simply moving the cartridge on the microscope stage. In this way, sufficient processing time is available in every scenario to analyze high-quality images from powerful AI in great depth and use them for sorting. In the future, it may be possible to correlate the physiological properties of a cell with its morphology and identify new markers for label-free sorting of certain cell types.

Conclusion

The microscope upgrade presented here enables gentle and precise flow-through sorting of cells based on their microscopic image. This provides access to spatially resolved information that cannot be captured with conventional sorting devices. By avoiding sheath flows and using gentle electrokinetic forces to handle the cells, the system is also very compact and can be easily placed on a microscope. In contrast to complex large devices, it is therefore suitable for almost any type of laboratory.

The use of different microscope variants also enables high-resolution imaging and offers maximum flexibility in terms of imaging technique: transmitted light, phase contrast or fluorescence imaging of different excitation and emission wavelengths can be easily combined as well as less common techniques such as quantitative phase imaging [8, 9] and many more. The particular features of the method enable the system to be used for every day sorting tasks as well as for addressing extraordinary questions or the low-loss processing of small, valuable cell samples.

Authors

Felix Pfisterer, Neus Godino,
Michael Kirschbaum

Affiliation:

Branch Bioanalytics and Bioprocesses IZI-BB,
Fraunhofer Institute for Cell Therapy and Immunology, Potsdam, Germany

Contact

Dr. Michael Kirschbaum
Fraunhofer Institute for Cell Therapy and Immunology, Branch Bioanalytics and Bioprocesses IZI-BB, Potsdam, Germany
michael.kirschbaum@izi-bb.fraunhofer.de

References

[1] *Iv, C.W.S. et al. (2015). Microfluidic cell sorting: a review of the advances in the separation of cells from debulking to rare cell isolation. Lab on a Chip. DOI: 10.1039/C4LC01246A.*

[2] *Holzner, G. et al. (2021). High-throughput multiparametric imaging flow cytometry: toward diffraction-limited sub-cellular detection and monitoring of sub-cellular processes. Cell Reports. DOI: 10.1016/j.celrep.2021.108824.*

[3] *Nitta, N. et al. (2018). Intelligent Image-Activated Cell Sorting. Cell. DOI: 10.1016/j.cell.2018.08.028.*

[4] *Nawaz, A. A. et al. Image-based cell sorting using focused travelling surface acoustic waves. Lab on a Chip. DOI: 10.1039/d2lc00636g.*

[5] *Nawaz, A. A. et al. (2020). Intelligent image-based deformation-assisted cell sorting with molecular specificity. Nature Methods. DOI: 10.1038/s41592-020-0831-y.*

[6] *Schraivogel, D. et al. (2022). High-speed fluorescence image-enabled cell sorting. Science. DOI: 10.1126/science.abj3013.*

[7] *Gerling, T. et al. (2023). High-precision, low-complexity, high-resolution microscopy-based cell sorting. Lab on a Chip. DOI: 10.1039/D3LC00242J.*

[8] *Habaza, M. et al. (2017). Rapid 3D Refractive-Index Imaging of Live Cells in Suspension without Labeling Using Dielectrophoretic Cell Rotation. Advanced Science. DOI: 10.1002/advs.201600205.*

[9] *Dudaie, M. et al. (2020). Label-free discrimination and selection of cancer cells from blood during flow using holography-induced dielectrophoresis. Journal of Biophotonics. DOI: 10.1002/jbio.202000151.*

Dr. Felix Pfisterer

Felix Pfisterer studied biotechnology at the Technical University of Berlin, Germany. After graduating in 2017, he completed his doctorate in 2024 on electric field-induced membrane permeabilization in the Microfluidic Cell Processing and Cell Analytics group at Fraunhofer IZI-BB, Potsdam, Germany. Since then, he has been working as a Senior Scientist with a focus on technical implementation and automation of microfluidic cell handling and cell analysis protocols.

Dr. Neus Godino

Neus Godino has held a Senior Scientist position at Fraunhofer IZI-BB Potsdam, Germany since 2012. She studied Physics and Electronic Engineering at the University of Barcelona, Spain, and received her Ph.D. at IMB-CNM (CSIC) in 2010 within the BioMEMS group. After years in the field of Lab-On-Chip and with experience in microfluidics, microfabrication, biosensors, and programming, she is currently working as a scientific technical coordinator of research projects regarding dielectrophoretic single-cell analysis and cell sorting.

Dr. Michael Kirschbaum

Michael Kirschbaum studied biology with a focus on neurobiology, physics, and biochemistry at the University of Tübingen, Germany. After graduating in 2005, he joined the Lab-On-Chip Technologies group at the Fraunhofer Institute in Potsdam, Germany, where he received his Ph.D. in 2009 in the field of bioanalytics. Since 2011, he has been leading the Microfluidic Cell Processing and Cell Analytics group at Fraunhofer IZI-BB in Potsdam, Germany, with a strong focus on the development of dielectrophoretic cell handling technologies.