



ibidi Application Guide

Transfection

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Selected Publications

W. Deng, et al. Tunable light and drug induced depletion of target proteins. *Nat Commun*, 2020, 10.1038/s41467-019-14160-8

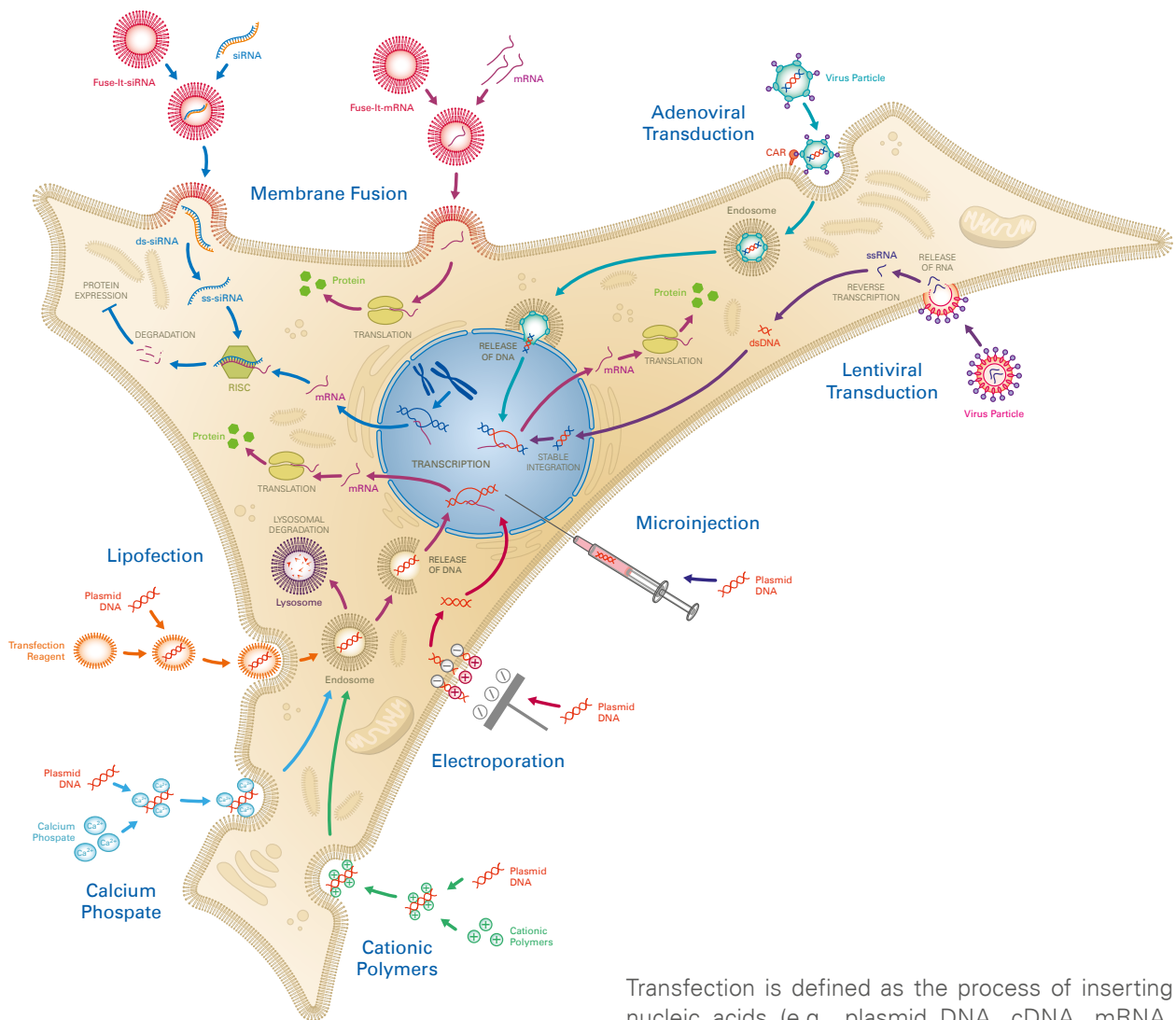
R. Žaja, et al. Comparative analysis of MACROD1, MACROD2 and TARG1 expression, localisation and interactome. *Sci Rep*, 2020, 10.1038/s41598-020-64623-y

S. Meier, et al. The molecular basis of chaperone-mediated interleukin 23 assembly control. *Nat Commun*, 2019, 10.1038/s41467-019-12006-x

A. Skowyra, L.A. Allan, A.T. Saurin, P.R. Clarke. USP9X Limits Mitotic Checkpoint Complex Turnover to Strengthen the Spindle Assembly Checkpoint and Guard against Chromosomal Instability. *Cell Rep*, 2018, 10.1016/j.celrep.2018.03.100

S. Salcher, M. Hermann, U. Kiechl-Kohlendorfer, M.J. Ausserlechner, P. Obexer. C10ORF10/DEPP-mediated ROS accumulation is a critical modulator of FOXO3-induced autophagy. *Mol Cancer*, 2017, 10.1186/s12943-017-0661-4

The Principle of Transfection



Transfection is defined as the process of inserting nucleic acids (e.g., plasmid DNA, cDNA, mRNA, miRNA, siRNA) into the cytoplasm of eukaryotic cells. In addition, proteins and nanoparticles such as beads or dyes can be transfected.

In the field of cell biology, transfection is an important and widely used tool for analyzing the function of various genes. This method can be used for gene silencing via RNAi, gene editing via CRISPR/Cas9, as well as overexpression studies by cellular integration of plasmid DNA, mRNA, or proteins. Many researchers conduct transfection experiments on a daily basis, which requires a concise understanding of the biological background, precise and detailed planning, and a robust transfection protocol.

Kim T.K., Eberwine J.H. Mammalian cell transfection: The present and the future. *Anal Bioanal Chem*, 2010, 10.1007/s00216-010-3821-6

Transfection Methods

Naturally, cells usually do not take up foreign nucleic acids without external stimulation. Thus, an efficient and biocompatible transfection method is needed, which has to be accurately adapted to the cell type and the material to be transferred.

Many transfection methods have been developed, which can be basically subdivided into three different groups: chemical transfection, physical transfection, and viral transduction. In this Application Guide, you can find a brief description of the most commonly applied methods, summarizing the characteristics, advantages, and pitfalls of each approach.

Chemical Transfection

- Lipofection
- Membrane Fusion
- Calcium Phosphate
- Cationic Polymers

Viral Transduction

- Adenoviral Transduction
- Lentiviral Transduction

Physical Transfection

- Microinjection
- Electroporation

Chemical Transfection

Lipofection

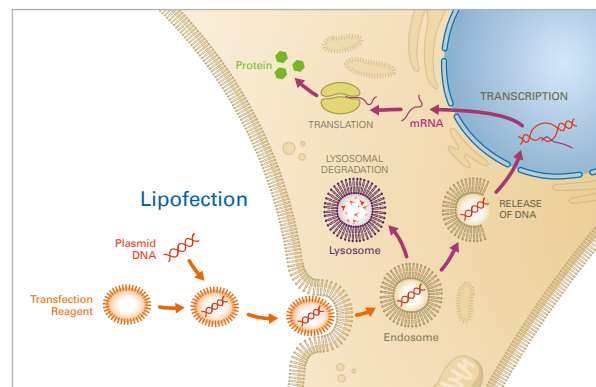
Applications:

Lipofection is commonly used to transfer nucleic acids such as RNA or DNA into eukaryotic cells. After protocol optimization, this method is easy to apply and yields highly reproducible results of transient and stable transfections. However, lipofection efficiency strongly depends on the cell type and has to be tested and optimized in advance. Especially for primary and non-dividing cells, the viability after the transfection process might be decreased due to the high cellular sensitivity. In contrast to membrane fusion, lipofection is based on endosomal molecule uptake, which might delay the time of analysis.

Principle:

Usually, a mixture of neutral and cationic liposomes forms complexes with the nucleotides of interest. These complexes pass the cell membrane and then release the nucleotides into the cytoplasm via endocytosis. Then, the nucleotides either successfully escape the endosome or undergo lysosomal degradation, of which the latter might lead to reduced transfection efficiency. After endosomal escape, the nucleotide is expressed in the target cells.

Felgner P.L., et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. 1987, Proc Natl Acad Sci U S A, 10.1073/pnas.84.21.7413



ibidi Solutions:

The [ibidi \$\mu\$ -Slides](#), [\$\mu\$ -Dishes](#), and [\$\mu\$ -Plates](#) are ideally suited for lipofection using various cell numbers and volumes.

Membrane Fusion

Applications:

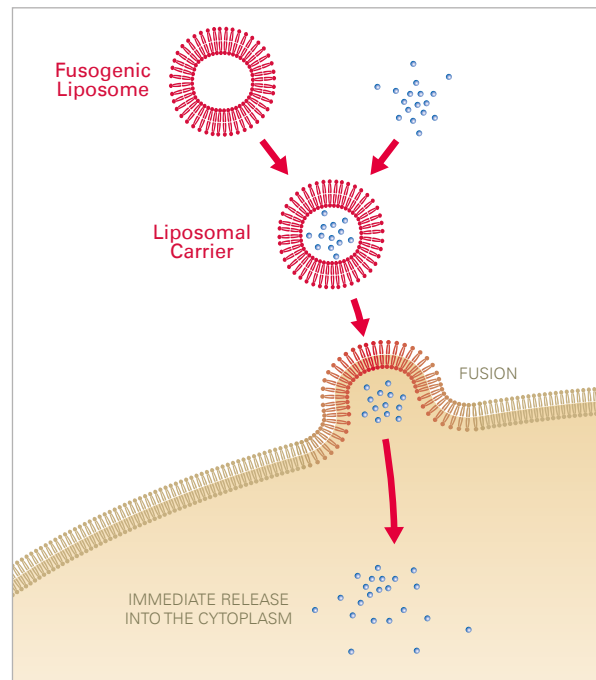
Membrane fusion is a novel and highly superior transfection method to incorporate various molecules and particles into mammalian cells. Not only cells that undergo cell division, but also primary and non-dividing cells can be transfected by using this innovative method. It allows for highly efficient transfer of mRNA, siRNA, proteins, beads, dyes, and other molecules. Since the reagents are non-toxic to sensitive and difficult-to-transfect cells, even primary neurons, keratinocytes, and stem cells retain high viability after the transfection. Additionally, the very short incubation times minimize cell stress and provide an almost unaffected cell behavior after the molecule transfer.

Principle:

Liposomal carriers, which consist of neutral and cationic lipids, are used to transfer the molecule of interest via membrane fusion into the cell. The molecules are first incorporated into the liposomal carriers, which upon contact, instantly fuse with the cell membrane. The included molecules are then directly released into the cytoplasm without processes such as endocytosis and lysosomal degradation. This results in fusion efficiencies up to 80–100% within only seconds to a few minutes, depending on the cell type.

M. Hoffmann et al. Changing the Way of Entrance: Highly Efficient Transfer of mRNA and siRNA via Fusogenic Nano-Carriers. J Biomed Nanotechnol, 2019, 10.1166/jbn.2019.2663.

Csiszár, A., et al. Novel Fusogenic Liposomes for Fluorescent Cell Labeling and Membrane Modification. Bioconjug Chem, 2010, 10.1021/bc900470y



ibidi Solutions:

The [ibidi \$\mu\$ -Slides](#), [\$\mu\$ -Dishes](#), and [\$\mu\$ -Plates](#) are ideally suited for membrane fusion using various cell numbers and volumes.



Find suitable products and more detailed information at www.beniag.com.

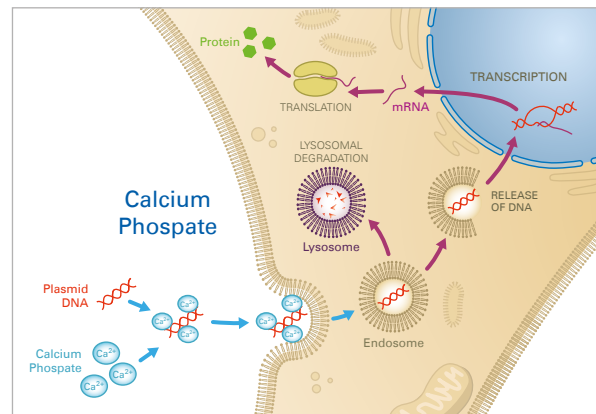
Calcium Phosphate

Applications:

The calcium phosphate transfection is an inexpensive and simple method for transient or stable nucleotide transfer into most cell lines. The transfection efficiency, however, strongly depends on the cell constitution, the pH, and the quality and the amount of the used nucleotides. Therefore, the optimal experimental conditions have to be tediously established in advance. Further, the calcium phosphate transfection is toxic and therefore not suitable for most sensitive primary cell lines.

Principle:

A mixture of the nucleotides, calcium, and phosphate buffer forms a precipitate that is taken up by the cells via endocytosis. Then, the nucleotides either escape the endosome or undergo lysosomal degradation, of which the latter might lead to reduced transfection efficiency. Successfully escaped nucleotides can then be expressed in the target cells.



ibidi Solutions:

The [ibidi \$\mu\$ -Slides](#), [\$\mu\$ -Dishes](#), and [\$\mu\$ -Plates](#) are ideally suited for calcium phosphate transfection with various cell numbers.



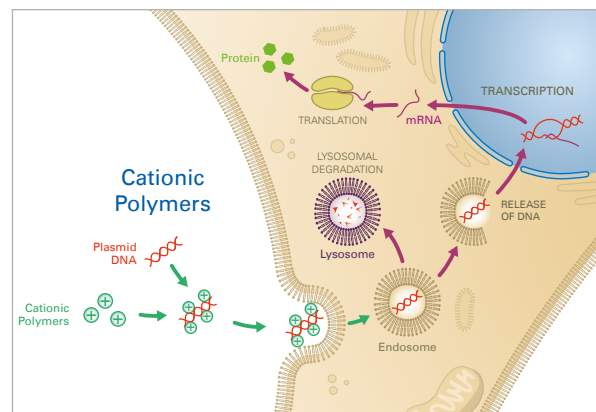
Cationic Polymers

Applications:

Using cationic polymers, nucleotides can be transiently transfected into eukaryotic cells in an inexpensive and simple manner. After protocol optimization, the results can be easily reproduced. As a drawback, transfection by cationic polymers has a very low efficiency (<10%) in a number of cell types, including primary cells. Further, cationic polymers are highly cytotoxic and therefore not suitable for transfection of sensitive cells and generation of stable cell lines.

Principle:

The negatively charged nucleotide backbones form complexes with cationic polymers, such as diethyl-aminoethyl (DEAE)-dextran. The complex is then taken up by the cells, mostly via endocytosis. If no lysosomal degradation occurs, the nucleotide can escape from the endosome into the cytosol of the host cells, subsequently resulting in transgene expression.



ibidi Solutions:

The [ibidi \$\mu\$ -Slides](#), [\$\mu\$ -Dishes](#), and [\$\mu\$ -Plates](#) are ideally suited for cationic polymer transfection using various cell numbers.



Viral Transduction

The term “transduction” is used to describe a virus-mediated transfer of nucleic acids into cells. In contrast to transfection of cells with foreign DNA or RNA, no transfection reagent is needed here. The viral vector, itself, also called virion, is able to infect cells and transport the DNA directly into the nucleus, independent of further actions. After the release of the DNA into the nucleus, the protein of interest is produced using the cells’ own machineries.

Adenoviral Transduction

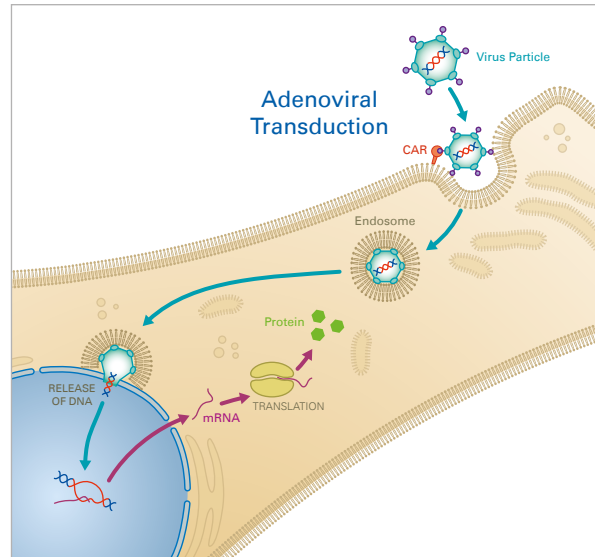
Applications:

Adenoviral vectors have proven to be a very successful transduction tool in many eukaryotic cell types, such as human and rodent cells. Besides dividing cell lines, this method gives access to difficult-to-transfect cells, such as primary cells. Adenovirus-mediated transduction is always transient, meaning that no nucleotide integration into the host genome occurs. Transduction efficiencies of up to 100% can easily be achieved. Importantly, biosafety level S2 is needed for adenoviral transduction.

Principle:

Adenoviruses are a class of double stranded DNA viruses that efficiently deliver nucleotides directly into target cells. After initial binding to the Coxsackievirus and adenovirus receptor (CAR), which is expressed on the cell membrane, the virus enters the host cell via endocytosis. Following endosomal escape, the viral genome is transported into the nucleus, where it is expressed by the replication machineries of the host cell. In contrast to other viruses, the adenoviral DNA remains episomal, i.e., it is not integrated into the host genome. Nowadays, replication-deficient adenoviruses are widely used for transduction and gene therapy, due to their high efficiency and low pathogenicity.

Lee C.S., et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. Genes Dis, 2017, 10.1016/J.GENDIS.2017.04.001



ibidi Solutions:

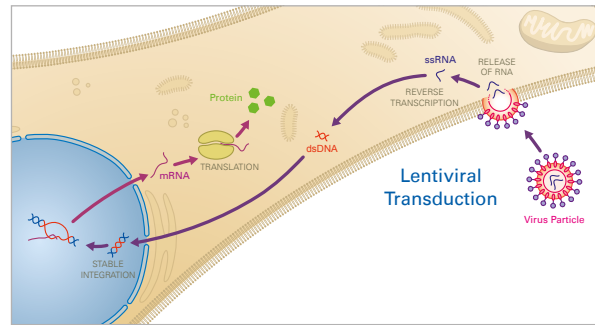
The [ibidi \$\mu\$ -Slides](#), [\$\mu\$ -Dishes](#), and [\$\mu\$ -Plates](#) are ideally suited for transduction using various cell numbers and volumes.



Lentiviral Transduction

Applications:

Recombinant lentiviral vectors are powerful tools for gene transfer with some advantages over other delivery vectors: Besides cells that undergo mitosis, they also have the ability to transduce non-dividing cells. Further, lentiviruses enable stable gene transfer *in vitro* and *in vivo*, as they integrate into the host cell genome and offer the possibility of positive cell selection. They have a broad host cell range that also includes cell types such as primary neurons, lymphocytes, and macrophages. Moreover, lentiviral vectors have also proven to be effective in transducing brain, liver, muscle, and retina *in vivo* without toxicity or immune responses. Biosafety level S2 is needed for lentiviral transduction.



Principle:

Lentiviruses—a subclass of retroviruses—have the ability to permanently integrate into the genome of the host cell. After the virus has entered the cell, the viral RNA is transcribed by the reverse transcriptase to produce double-stranded DNA that enters the nucleus. Finally, the transgene is integrated into the host genome via the lentiviral integrase enzymes. When using lentiviral transduction, the user has to take into account effects caused by genomic integration.

Sakuma T., Barry M.A., Ikeda Y. Lentiviral vectors: basic to translational. Biochem J, 2012, 10.1042/BJ20120146

ibidi Solutions:
The [ibidi μ-Slides](#), [μ-Dishes](#), and [μ-Plates](#) are ideally suited for transduction using various cell numbers and volumes.

Method Comparison

	Adenoviral transduction	Lentiviral transduction	Transfection
Viral genome material	dsDNA	RNA	-
Transgene expression	Transient	Stable	Transient or stable
Genome integration	Episomal	Integration into host genome	Dependent on transfection method
Cell types	Dividing and non-dividing cells	Dividing and non-dividing cells	Dividing and non-dividing cells, dependent on transfection method
Efficiency	Up to 100% transduction efficiency	Up to 100% transduction efficiency	Variable transfection efficiency
Biosafety level	2	2	1

Physical Transfection

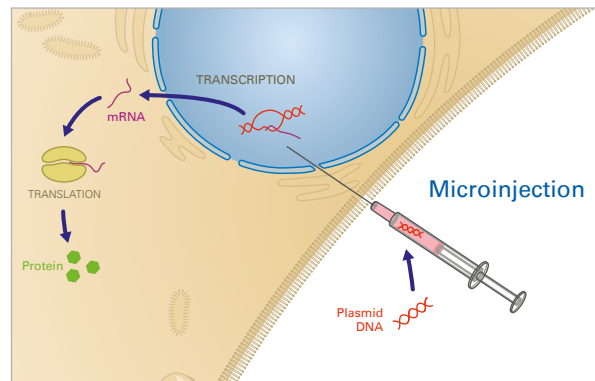
Microinjection

Applications:

Microinjection allows for the efficient transfer of controlled nucleotide amounts into the nucleus of a specific target cell. It is a very precise, but time-consuming and expensive transfection method with a very low throughput only. Microinjection is mostly used for special applications, such as single cell manipulation or the generation of transgenic animals, e.g., by pronuclear injection in mice.

Principle:

With this physical method, the target cell is positioned under a microscope and being fixed by a pipette. The nucleotide solution is then directly injected into the cytoplasm and/or the nucleus using a fine glass capillary needle. This precise procedure demands a rather expensive microinjection system and a lot of skill and practice. Once within the nucleus, the nucleotide can immediately integrate into the endogenous DNA. Given these prerequisites, microinjection is highly effective and offers the transfer of precisely controlled nucleotide amounts. However, as each single cell needs to be microinjected individually, this method is very time-consuming.



ibidi Solutions:

ibidi provides specialized μ -Dishes with low walls, which have been designed to give easy access to the target cells for microinjection. The [μ-Dish 35 mm, low](#) and the [μ-Dish 50 mm, low](#) enable exact positioning of micromanipulators for a precise injection process. Further, their excellent optical quality allows for high-quality visualization during microinjection.

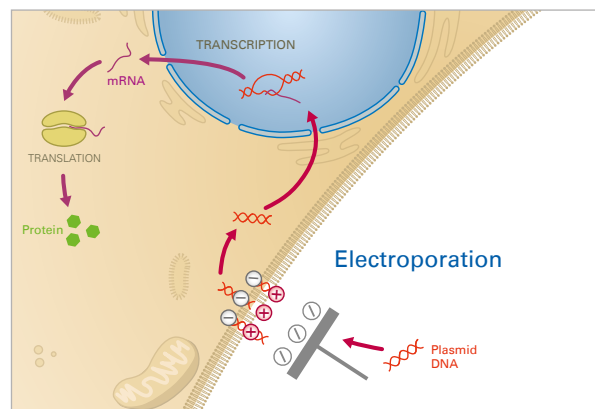
Electroporation

Applications:

Electroporation allows for the transient and stable transfection of any cell type. This method is easy and reliable, but it requires high cell numbers due to high rates of cell death during the procedure. Therefore, electroporation is not suitable for sensitive and difficult-to-culture cell types, such as primary cells. Further, a special, expensive electroporation device is required.

Principle:

During electroporation, a mixture of the cells and the nucleotide of interest is exposed to an intense electric field. This leads to transient cell membrane destabilization, making the cell membrane permeable to the nucleotides that are present in the surrounding solution. The cell membrane is transiently permeabilized by a short electric pulse, allowing the nucleotides, which are present in the surrounding solution, to enter the cytoplasm. After removing the electric field, the cell membrane stabilizes, enclosing the nucleotides in the cytoplasm, where they are expressed.



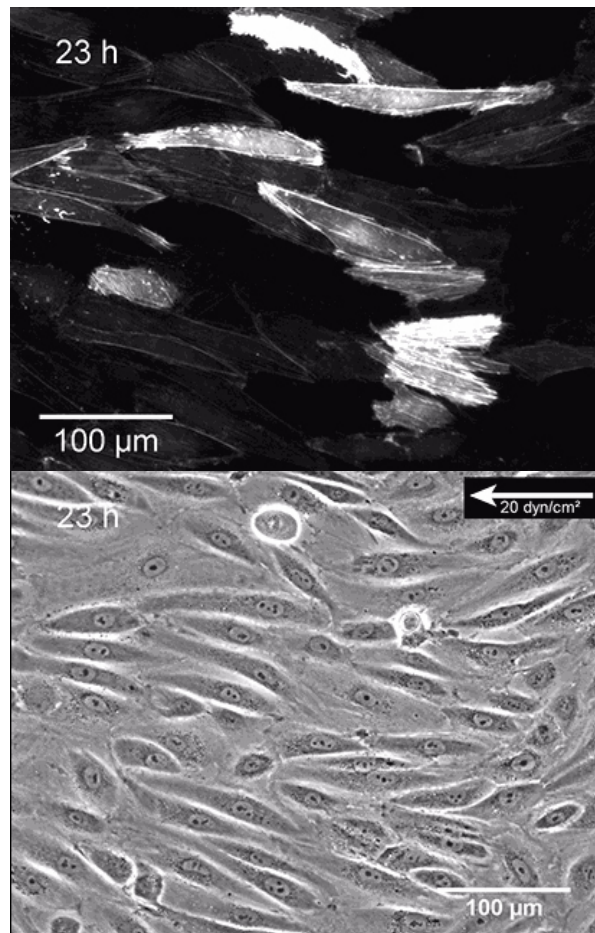
Actin Dynamics Under Flow

Several cell types in biofluidic vessels, such as endothelial cells and immune cells, are constantly exposed to shear stress *in vivo*. This mechanical stimulus has a great impact on the physiological behavior and adhesion properties of cells, and should be taken into account when performing respective studies.

With the help of the ibidi channel slides, [μ-Slide I Luer](#) or [μ-Slide VI^{0.4}](#), and the [ibidi Pump System](#), the F-actin cytoskeleton can be visualized in living cells under shear stress conditions. The ibidi Pump System is ideal for the long-term application of physiological shear stress to a cell layer and enables the adjustment of different flow rates. The system is fully compatible with live cell imaging and high resolution fluorescence microscopy. Optionally, the fixation and immunofluorescence staining of the cells can be directly performed in the μ-Slide I Luer.

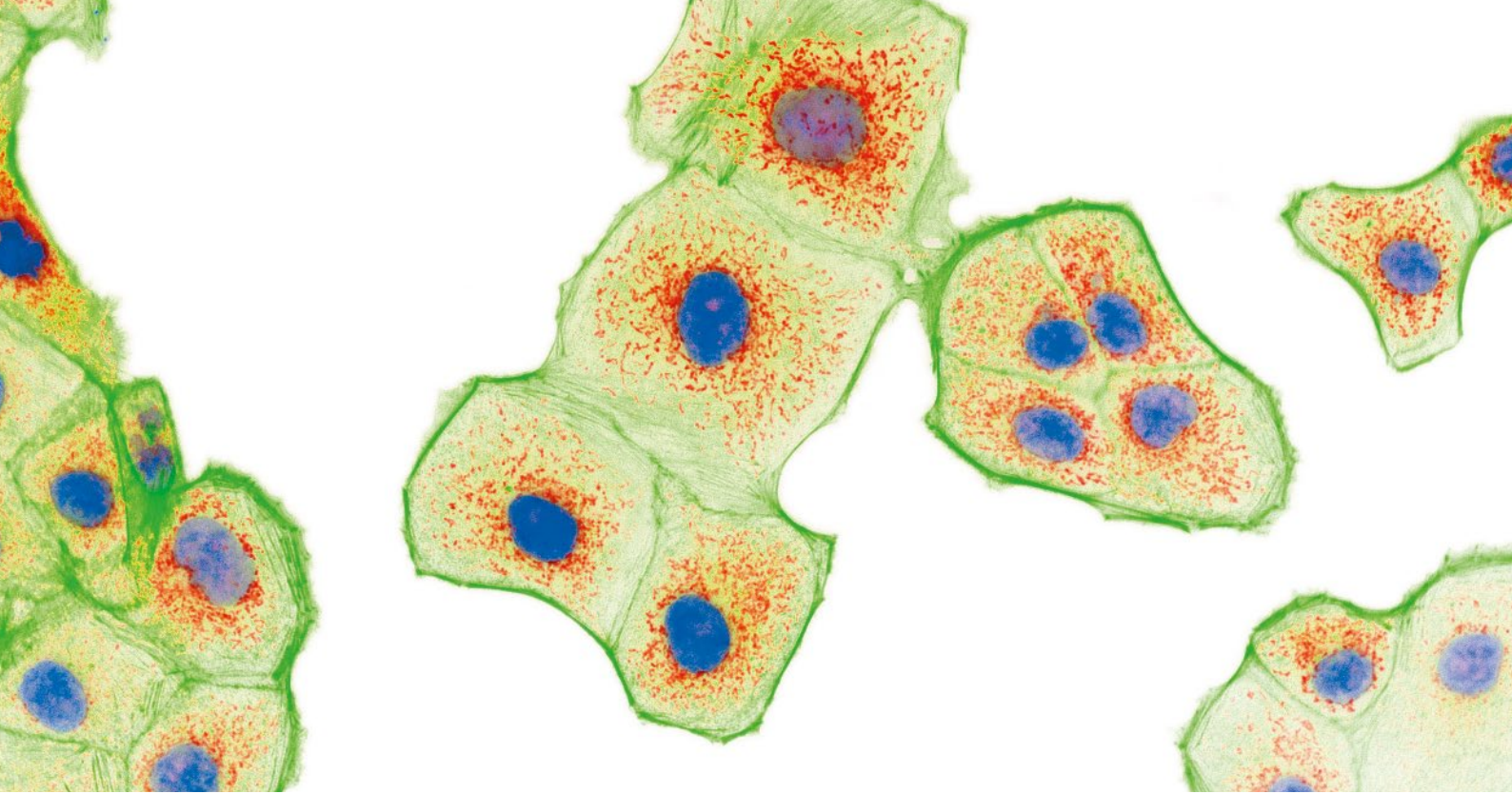
Experimental Setup:

- Device: [ibidi Pump System](#)
- Slide: [μ-Slide I^{0.4}Luer](#) (ibiTreat)
- Cells: LifeAct-expressing endothelial cells (HUVEC, P1)
- Shear stress parameters: 20 dyn/cm²



Live cell imaging under flow: actin cytoskeleton visualization in HUVEC after transduction with an adenoviral LifeAct vector and cultivation under 20 dyn/cm².

[Click here](#) to watch the movie on our website.



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