cells in focus

ibidi Application Guide

Actin Visualization

Selected Publications

A. A. Varlet, M. Fuchs, C. Luthold, H. Lambert, J. Landry and J. N. Lavoie. Fine-tuning of actin dynamics by the HSPB8-BAG3 chaperone complex facilitates cytokinesis and contributes to its impact on cell division. Cell Stress and Chaperones, 2017, 10.1007/s12192-017-0780-2
read abstract

J. Sroka et al. Lamellipodia and Membrane Blebs Drive Efficient Electrotactic Migration of Rat Walker Carcinosarcoma Cells WC 256. PLOS ONE, 2016, 10.1371/journal.pone.0149133
read abstract

P. J. Wen et al. Actin dynamics provides membrane tension to merge fusing vesicles into the plasma membrane. Nature Communications, 2016, 10.1038/ncomms12604
read abstract

About F-Actin

F-Actin—A Crucial Protein for Cellular Function and Motility

In most eukaryotic cells, actin is the most abundant protein. As an important part of the cytoskeleton, actin is essential for cell stability and morphogenesis. It is involved in many crucial processes, such as cell division, endocytosis, and cell migration.

Actin is present in two forms:

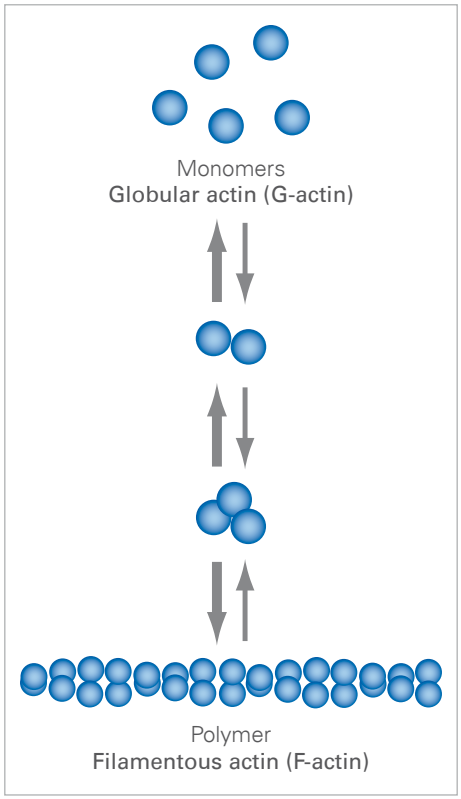
- Monomeric, globular G-actin
- Polymeric, filamentous F-actin

The monomeric G-actin has the ability to polymerize, thereby creating the F-actin polymer filaments (also named actin filaments or microfilaments). These microfilaments are an essential part of the cytoskeleton and build up many higher order structures in cells (e.g., stress fibers, lamellipodia, and filopodia).

Given these numerous and important functions in the cellular architecture, it is no surprise that the visualization of F-actin is indispensable in many research areas:

- Cell biology
- Cellular structure and morphology
- Function and regulation of actin binding proteins
- Chemotaxis and migration
- Muscle cell research
- Cytoskeleton biophysics
- Cellular adherence, cellular interactions, interactions with ECM
- Cancer research

LifeAct stains filamentous actin structures in living or fixed eukaryotic cells and tissues, having the lowest potential interference with actin dynamics *in vivo* and *in vitro*.



G-actin has the ability to polymerize, resulting in the F-actin polymer. This process is reversible, allowing the actin monomers to dissociate again from the ends of the actin filaments.

T. Oda, M. Iwasa, T. Aihara, Y. Maéda and A. Narita. The nature of the globular- to fibrous-actin transition. Nature, 2009, 10.1038/nature07685
read abstract

M. Melak, M. Plessner, and R. Grosse. Actin visualization at a glance. Journal of Cell Science, 2017, 10.1242/jcs.204487
read abstract

Actin Staining Techniques

F-actin visualization using fluorescent markers is an important tool for getting a deeper understanding of the structural cytoskeletal dynamics. For the observation of F-actin-related processes, non-invasive live cell imaging has become the state-of-the-art technique. Depending on the application and the investigated model organism and cell type, there are different F-actin staining techniques available—each of them with its own advantages and disadvantages.

For further details, please read this concise review, which summarizes the actin visualization techniques that are currently available:

M. Melak, M. Plessner, and R. Grosse. Actin visualization at a glance. Journal of Cell Science, 2017, 10.1242/jcs.204487
read abstract

At a Glance: Different Methods of Actin Visualization

This table refers to standard applications in mammalian expression systems.

	Phalloidin	Actin-Coupled Fluorescent Proteins	LifeAct
Suitability for fixed samples	+++	n.d.	+++ (LifeAct Protein)
Suitability for live cell imaging	-	++	+++
Maintenance of actin functionality	-	+	+++
Biocompatibility	-	++	+++
Quality of signal-to-noise ratio	+++	++	+++
Actin binding specificity	+++	+++	+++

LifeAct®

LifeAct is a short, 17-amino acid peptide that specifically binds to F-actin. It is derived from the budding yeast (*Saccharomyces cerevisiae*) protein Abp140, which has been successfully used to label actin cables in this model. Conjugated with GPF, LifeAct-GFP can easily be introduced into living and fixed eukaryotic cells to visualize F-actin, while retaining highest actin functionality.

In contrast to other actin labeling agents, such as phalloidin and actin-coupled fluorescent proteins, LifeAct can visualize actin kinetics with the lowest potential interference. It is non-toxic and can thereby be used in both living and fixed cells and tissues.

The biocompatibility of LifeAct has been further proven in a transgenic mouse model *in vivo*. In this model, either LifeAct-EGFP or LifeAct-mRFPruby—both driven by a chicken actin promoter with a CMV enhancer—were introduced into the murine germline. The resulting LifeAct mice were viable, fertile, and showed highly specific and clear LifeAct staining in nearly all cell types. Importantly, actin was evenly distributed and no changes in the cytoskeletal organization were observed.

B. J. Belin, L. M. Goins, and R. D. Mullins. Comparative analysis of tools for live cell imaging of actin network architecture. Bioarchitecture, 2014, 10.1080/19490992.2014.1047714
read abstract

J. Riedl, A. H. Crevenna, K. Kessenbrock, J. H. Yu, D. Neukirchen, M. Bista, … R. Wedlich-Soldner. Lifeact: a versatile marker to visualize F-actin. Nature Methods, 2008, 10.1038/nmeth.1220
read abstract

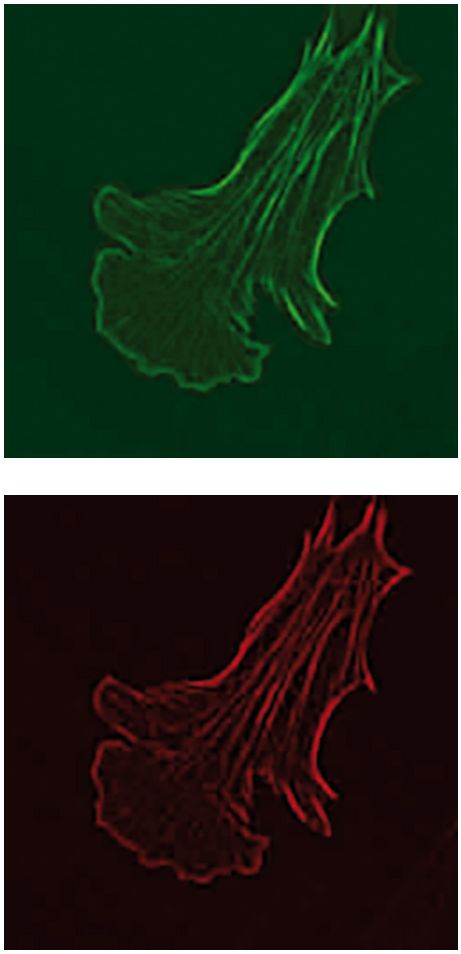
J. Riedl, K. C. Flynn, A. Raducanu, F. Gärtner, G. Beck, M. Bösl, … R. Wedlich-Söldner. Lifeact mice for studying F-actin dynamics. Nature Methods, 2010, 10.1038/nmeth0310-168
read abstract

Since 2008, LifeAct is regarded as the gold standard for live cell imaging of F-actin. LifeAct constructs are widely used and published. Compared to other genetically encoded actin markers, such as fluorescent protein-coupled actin monomers, antibodies, and small molecules, LifeAct markers show the least interference with cytoskeleton dynamics and artefacts due to overexpression. This interference generally depends on the transfection system, the expression level, and the cell type. As a precaution, ibidi recommends our customers to perform suitable control experiments. Other potential trouble-causing parameters, such as transfection and transduction toxicity as well as imaging phototoxicity should be considered as well.

Sliogeryte K, et al. (2016) Differential effects of LifeAct-GFP and actin-GFP on cell mechanics assessed using micropipette aspiration. J Biomech 49(2):310–317. 10.1016/j.jbiomech.2015.12.034.
read abstract

Courtemanche N, Pollard TD, Chen Q (2016) Avoiding artefacts when counting polymerized actin in live cells with LifeAct fused to fluorescent proteins. Nat Cell Biol 18(6):676–683. 10.1038/ncb3351.
read abstract

Flores LR, Keeling MC, Zhang X, Sliogeryte K, Gavara N (2019) Lifeact-GFP alters F-actin organization, cellular morphology and biophysical behaviour. Sci Rep 9(1):3241. 10.1038/s41598-019-40092-w.
read abstract



LifeAct specifically stains F-actin in fibroblasts and co-localizes with phalloidin.

Phalloidin

Phalloidin is a toxin that originates from the death cap mushroom (Amanita phalloides). It binds to F-actin, thereby preventing its depolymerization—ultimately leading to cell death by the paralysis of the cytoskeleton.

The binding of phalloidin to F-actin is irreversible and highly specific, making it a standardly-applied tool for F-actin visualization in fixed cells. Typically, it is conjugated to a fluorophore such as Rhodamine or FITC. After the staining procedure, the endogenous actin filaments with the bound phalloidin can be imaged by fluorescence microscopy. One major drawback of using phalloidin for F-actin imaging is its high toxicity. As it disturbs actin functionality and even leads to cell death, it is unsuitable for live cell imaging applications and should only be used in fixed cells.

Scheme of the death cap mushroom (Amanita phalloides), which contains the toxin phalloidin.

J. Wehland, M. Osborn, and K. Weber. Phalloidin-induced actin polymerization in the cytoplasm of cultured cells interferes with cell locomotion and growth. Proc. Natl. Acad. Sci. USA, 1977, 74, 5613-5617
read abstract

E. Wulf, A. Deboben, F. A. Bautz, H. Faulstich, and T. Wieland. Fluorescent phallotoxin, a tool for the visualization of cellular actin. Proc. Natl. Acad. Sci. USA, 1979, 76, 4498-4502
read abstract

Actin-Coupled Fluorescent Proteins (Actin-GFP)

Actin-coupled fluorescent proteins, such as actin-GFP, are widely used for F-actin visualization in living cells. Here, the fusion construct of actin and the fluorescent protein is introduced into the cells of interest (e.g., via plasmid transfection or viral transduction) and can be imaged by fluorescent live cell microscopy afterwards. The application of actin-coupled fluorescent proteins is relatively simple, non-toxic, and proven to be useful for actin visualization in living cells using diverse experimental approaches.

However, one clear disadvantage of this method is the inevitable expression of ectopic actin, which can alter the behavior of the cell. In addition, the relatively large size of the GFP (~27 kDa), can cause unwanted effects such as reduced F-actin functionality. This technique requires the precise establishment and accurate control of the actin-GFP expression for each separate cellular model, in order to prevent artificial effects that might alter the experimental outcome.

K. Sliogeryte, S. D. Thorpe, Z. Wang, C. L. Thompson, N. Gavara, and M. M. Knight. Differential effects of LifeAct-GFP and actin-GFP on cell mechanics assessed using micropipette aspiration. J. Biomech. 2016, 10.1016/j.jbiomech.2015.12.034
read abstract

The LifeAct Guide: Using LifeAct in Living and Fixed Cells

To optimize the F-actin visualization in your experimental setup, ibidi has developed a broad LifeAct portfolio:

LifeAct Plasmids

A range of plasmids for transient or stable transfections of various cell types; useful for brilliant visualization of F-actin.

LifeAct Adenoviral Vectors

Ready-to-use adenoviral vectors for efficient F-actin transduction, especially suitable for studies in difficult-to-transfect cells.

LifeAct Lentiviral Vectors

Lentiviral vectors for easy generation of stable LifeAct-expressing cell lines.

mRNA LifeAct

A ready-to-use LifeAct-encoding mRNA with a GFP2 tag for the brilliant visualization of F-actin in living cells.

LifeAct-TagGFP2 Protein

A recombinant protein for remarkably fast staining and immediate functional analysis of F-actin in living and fixed cells.

LifeAct Stable HT-1080 Cell Line

A stable, LifeAct-expressing human fibrosarcoma cell line for direct use in cell-based assays.

Please note:

To readily achieve high imaging performance, transfection or transduction of LifeAct can be easily done in ibidi's µ-Slides and µ-Dishes. Discover more in our detailed Application Notes.

For more detailed information:

Listen to ibidi's recorded webinar "LifeAct Actin Marker: A New Tool for the Visualization of Dynamic Cellular Processes" or watch our movie, "LifeAct: Actin Marker for Live Cell Imaging (MV19)".

Find Your LifeAct Solution for F-Actin Visualization in Living Cells

	LifeAct-Plasmid	LifeAct-mRNA With Fuse-It-mRNA	LifeAct-Protein With Fuse-It-P	LifeAct-Adenovirus	LifeAct-Lentivirus
Method Suitable for [1]					
Cell lines	+++	+++	+++	+++	+++
Primary cells	+	+++	++	+++	+++
Non-mammalian cells	-	+	+	-	-
Transfection Stability					
Transient	Yes	Yes	Yes	Yes	No
Stable	Yes	No	No	No	Yes
Protocol and Analysis					
Protocol simplicity	+++	++	++	+++	+++
LifeAct readout start	1–2 days	few hours	few minutes	1–2 days	1–2 days
LIfeAct readout duration	several days (infinity if stable)	several days	up to 1 day	several days	several days (infinity if stable)
Signal intensity	+++	+++	+	+++	+++
Extra Benefits					
Prevention of over-expression, artefacts	-	+	+++	+	+
Prevention of viral response	+++	+++	+++	-	-
Biocompatibility of transfer method	+	+++	+++	++	++
Actin functionality	+++	+++	+++	+++	+++
Safety Consideration					
Biosafety Level	BSL1	-	-	BSL2	BSL2

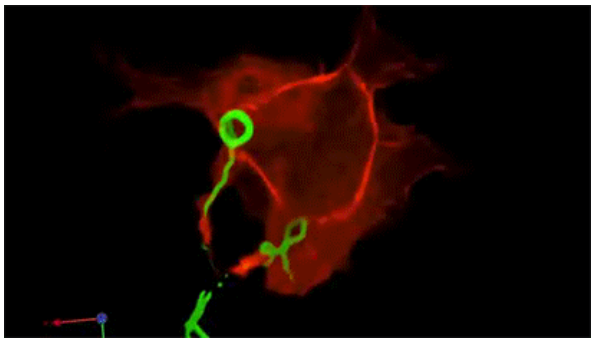
[1] *Not all of these combinations have been tested in all indicated cell types. We encourage you to use this table as a guideline to understand how the LifeAct system can be optimally applied.*

LifeAct Applied: Experimental Examples

Restructuring of the Human Macrophage Cytoskeleton During Borreliae Uptake

Borrelia bacteria are the cause of the Lyme disease, also known as Lyme borreliosis. To prevent the dissemination of borreliae, their uptake and elimination by macrophages has been shown to be necessary. This process involves dynamic restructuring of the macrophage cytoskeleton, particularly of the actin microfilaments.

In this experiment, the LifeAct Plasmid was used to visualize actin cytoskeleton reorganization in human macrophages during phagocytosis of borreliae.

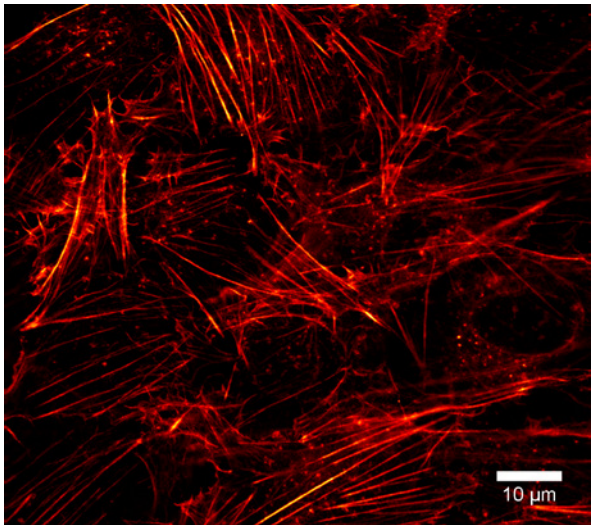


Phagocytosis of borreliae by a primary human macrophage. Time-lapse movie of a confocal z-stack showing a primary human macrophage expressing RFP-LifeAct (red) internalizing several GFP-expressing spirochetes (green) with actin-rich cell protrusions. Sequence 41 min. Data by Dr. Mirko Himmel and Prof. Stefan Linder, PhD, Universitätsklinikum Hamburg-Eppendorf, Germany, http://www.linderlab.de/.

Click here to watch the movie on our website.

Super-Resolution Microscopy (STED) of the Actin Cytoskeleton

Using LifeAct-TagGFP2 Protein, the actin cytoskeleton can be visualized in detail. In this experiment, fixed Rat1 fibroblasts were incubated with LifeAct-TagGFP2 Protein in a µ-Slide VI$^{0.4}$, ibiTreat. Simulated emission depletion (STED) microscopy was performed to create a super-resolution image.

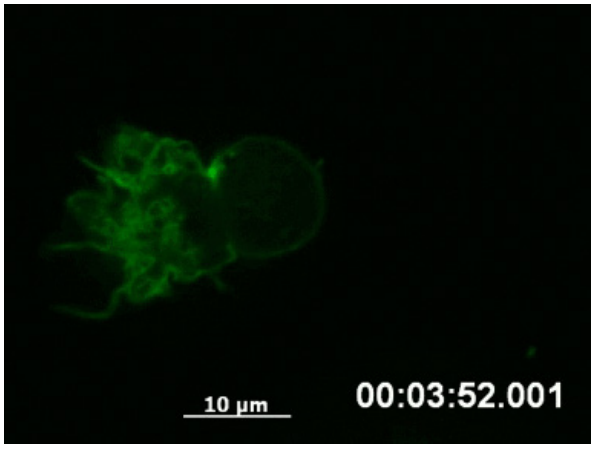


Super-resolution microscopy of the actin cytoskeleton in Rat1 fibroblasts using LifeAct-TagGFP2 Protein. Microscopy was performed on the STEDYCON super-resolution STED nanoscopy system (Abberior Instruments GmbH, Göttingen, Germany) with a Plan-Neofluar 100x/1.4 objective lens.

Live Cell Imaging of Actin Dynamics in a Chemotactic Gradient

F-actin networks play an important role during cell migration, which can be investigated in detail using chemotactic gradients. Primary dendritic cells were isolated from mice and transfected with the LifeAct Plasmid.

For the chemotaxis assay, cells were seeded on the µ-Slide Chemotaxis and a chemotactic gradient (CCL19) was applied. One day after the transfection, F-actin dynamics in the migrating cells were visualized using live cell imaging.



Live cell imaging of actin dynamics in a LifeAct-expressing primary dendritic mouse cell after the application of a chemotactic gradient.

Click here to watch the movie on our website.

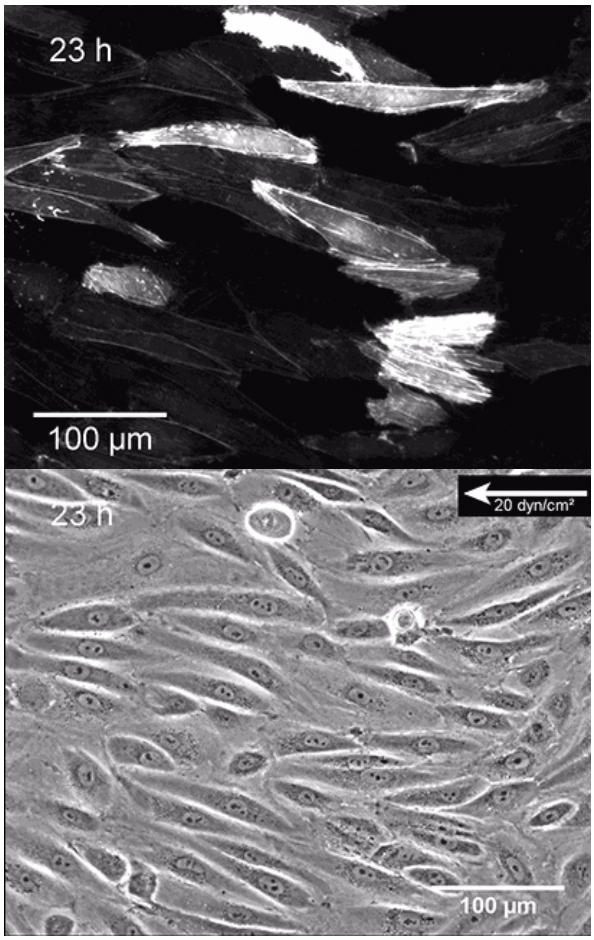
Actin Dynamics Under Flow

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.

By combining the ibidi channel slides, µ-Slide I Luer or µ-Slide VI$^{0.4}$, and the ibidi Pump System with ibidi's LifeAct technology, 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), transduced with the LifeAct Adenoviral Vector rAV-LifeAct-TagGFP2
- Reagents: LifeAct Adenoviral Vector rAV-LifeAct-TagGFP2
- Shear stress parameters: 20 dyn/cm^2

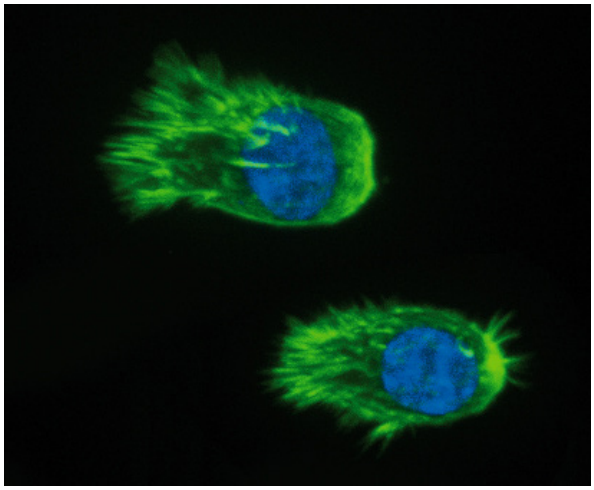


Live cell imaging under flow: actin cytoskeleton visualization in HUVEC after transduction with the LifeAct Adenoviral Vector rAV-LifeAct-TagGFP2 and cultivation under 20 dyn/cm^2.

Click here to watch the movie on our website.

F-Actin Visualization in a 3D Hydrogel Matrix

It is well known that cells behave differently in a 3D environment than in the conventional 2D cell culture. For F-actin visualization in migrating cells in a 3D culture system, stably LifeAct-expressing HT-1080 cells were embedded in a synthetic hydrogel. The polymerized cell-hydrogel mixture was immobilized on a µ-Slide Angiogenesis. After 20 hours, Z-stacks of the whole cell body were collected using high resolution confocal microscopy. The Z-stacks were projected to merged images, accurately showing the F-actin dynamics of each single cell in a 3D matrix.

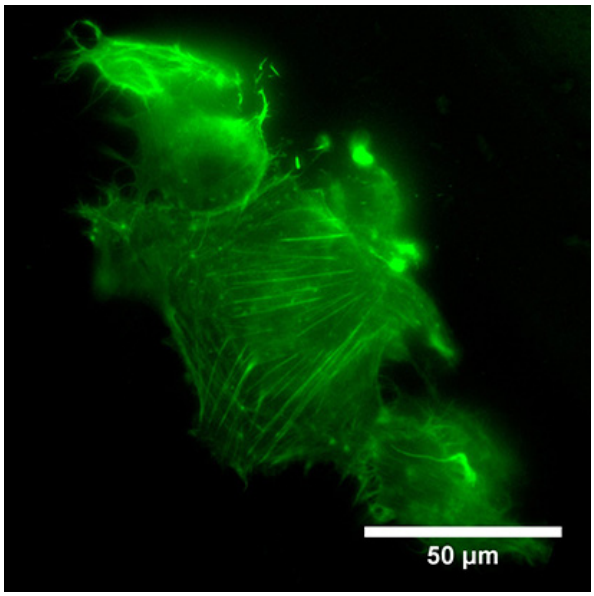


Z-stack of HT-1080 LifeAct-TagGFP2 cells in a 3D hydrogel environment.

F-Actin Visualization in Living Cells Using the LifeAct-TagGFP2 Protein

The LifeAct-TagGFP2 Protein is ideally suited for the quick and efficient visualization of the actin cytoskeleton in living cells. For the staining procedure, you can use any method for protein transfer that works for your cells of interest.

Rat1 fibroblasts were grown until confluency and washed with PBS before adding LifeAct-TagGFP2 Protein solution (30 µg/ml). Cells were scraped several times with a sterile pipette tip and incubated at 37°C for 5 minutes, leading to mechanical perturbation of the cell membrane and protein incorporation along the scrape. After a further washing step with PBS, medium was replaced and cells were imaged immediately.



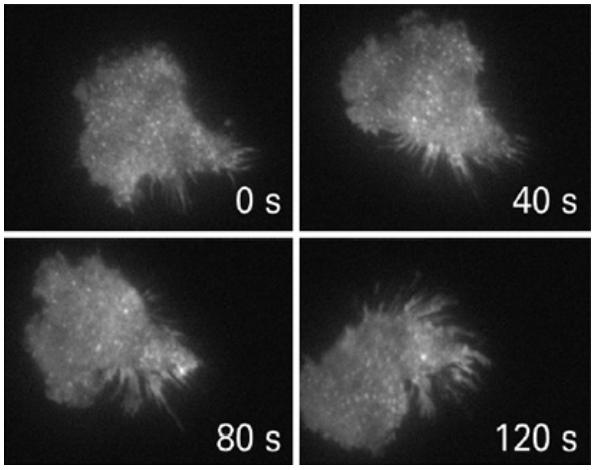
Live cell imaging of F-actin in Rat1 fibroblasts after LifeAct-TagGFP2 Protein transfer (30 µg/ml, 3 minutes).

The Actin Cytoskeleton During Chemotaxis of Primary Murine T Cells

Primary T cells were isolated from the spleen of a LifeAct mouse. An under-agarose assay (UA-assay) was performed to analyze chemotaxis and chemokinesis. Fluorescent live cell images illustrate the movement of the LifeAct-stained actin cytoskeleton.

J. Riedl, K. C. Flynn, A. Raducanu, F. Gärtner, G. Beck, M. Bösl, … R. Wedlich-Söldner. Lifeact mice for studying F-actin dynamics. Nature Methods, 2010, 10.1038/nmeth0310-168
read abstract

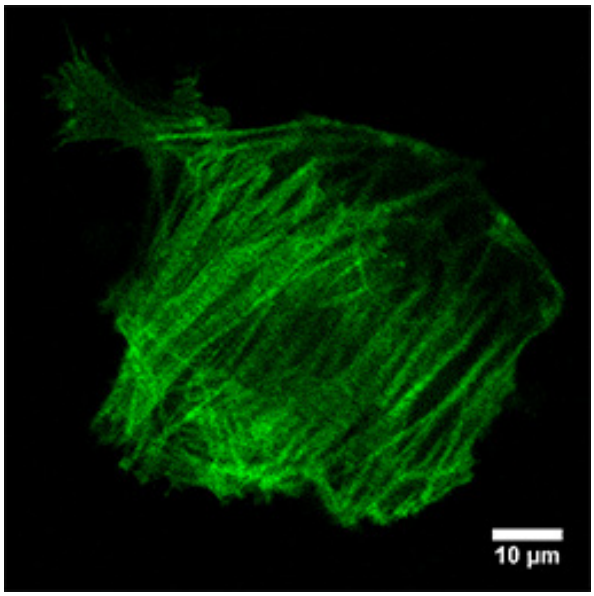
B. Heit and P. Kubes. Measuring Chemotaxis and Chemokinesis: The Under-Agarose Cell Migration Assay. Science Signaling, 2003, 10.1126/stke.2003.170.pl5
read abstract



Live cell imaging of the actin cytoskeleton in migrating primary T cells.

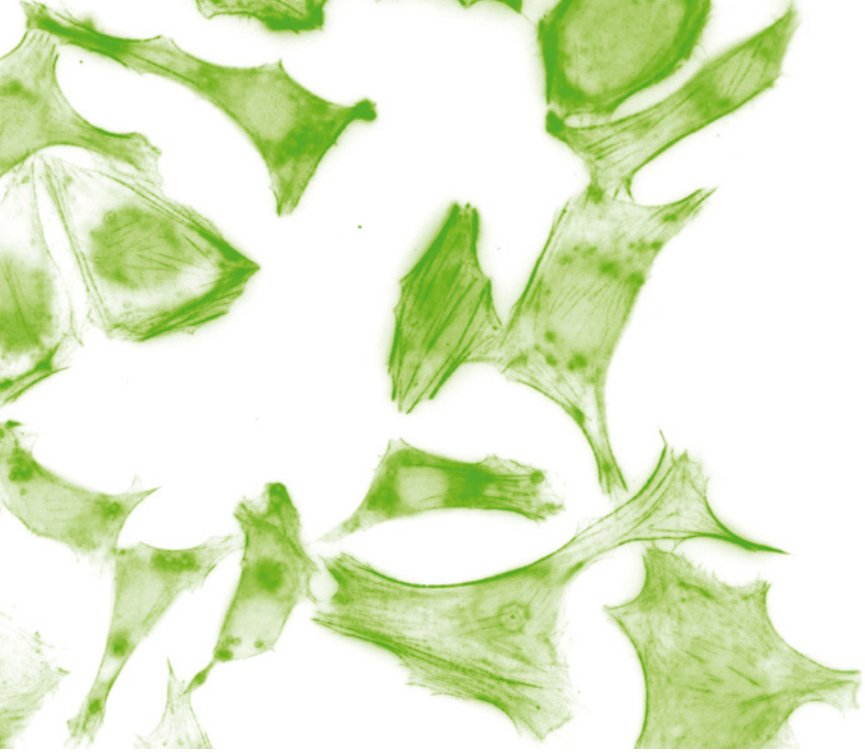
Visualization of the Contraction of Cardiomyocytes

For the visualization of the contraction rates of cardiomyocytes, Fuse-It-mRNA vesicles were filled with mRNA LifeAct-TagGFP2 and fused with human iPSC-derived cardiomyocytes. 16 hours after mRNA LifeAct-TagGFP2 transfer, the contractions per minute were measured. The myocytes showed contraction rates of about 70 beats per minute. This value is in the normal range of unmodified myocytes which show 50 to 80 contractions per minute.



Live cell imaging for the visualization of the contraction rates of cardiomyocytes using mRNA LifeAct-TagGFP2.

Click here to watch the movie on our website.



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