FORCYTE biotechnologies

FLECS TECHNOLOGY

Unlocking access to cellular force generation

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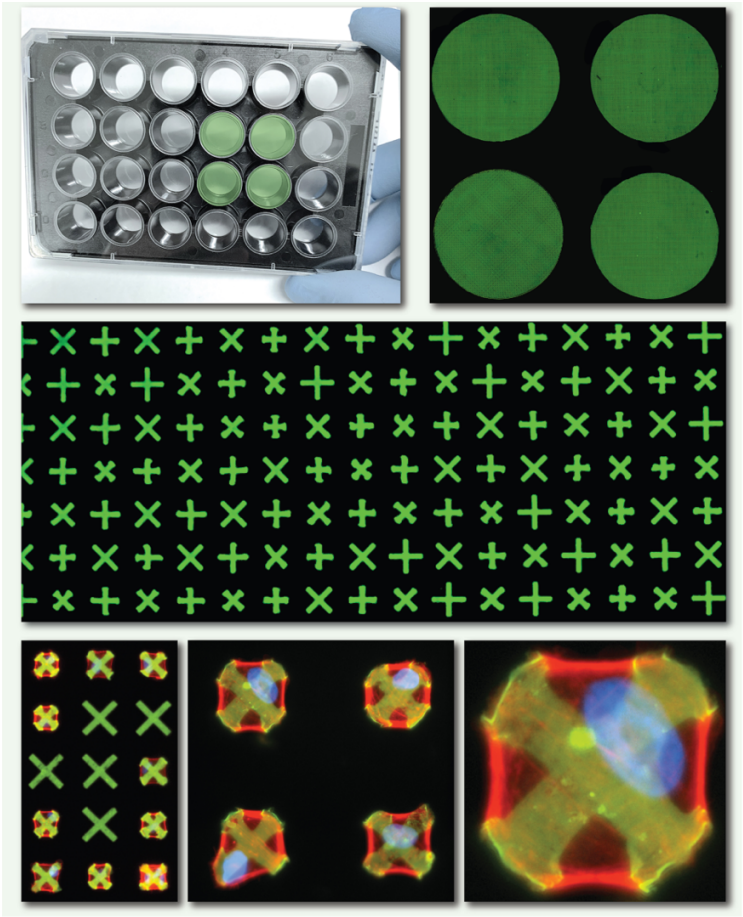
FLECS TECHNOLOGY:

24-well, single-cell contractility assay



24-well FLECS plate

Catalog Family: F2-X

User Manual

V1.01

Research Use Only

Author:

Ivan Pushkarsky, PhD

Purpose:

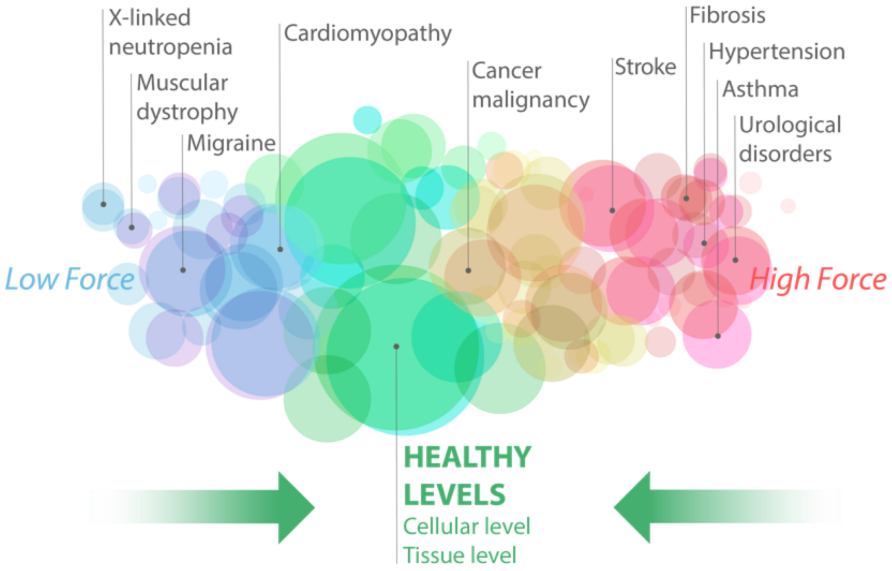
This user manual provides step-by-step instructions on how to utilize FLECS Technology (24-well-plate format) for performing functional measurements of cellular force generation.

INTRODUCTION

Cell force in health and disease

In human health, **cellular force generation** follows the "Goldilocks principle", where for a given cell type, tissue, state and function, there is an ideal amount force that sustains health, and where too much or inadequate force generation leads to disease.

Cell-generated mechanical forces



Too much force underlies conditions such as *asthma* and *hypertension* where hyper-contracted smooth muscle results in narrowed passages for airflow or blood flow, *fibrosis* where extreme force generation by myofibroblasts leads to stiffened and nonfunctional tissue, *preterm labor* where spontaneously uterine contractions prior to full term initiate early childbirth, and *overactive bladder* or *incontinence* where excessive force leads to a loss of voluntary control over urination.

Too little force has clear implications in the skeletal muscle systems where it can severely limit mobility, and in the cardiac system where it can lead to death. Furthermore, uncontrolled vasodilation can lead to systemic *hypotension* as well as trigger *migraine* headaches. Insufficient force generation by immune cells such as phagocytes can prevent efficient clearing of cell debris and pathogens, ultimately leading to *chronic inflammation* and potentially *autoimmune disease.*

The need to measure cellular force

In order to better understand the molecular mechanisms and genetic regulation behind cellular force generation and to identify new drug targets or candidates, it is critical to **measure force generation itself**, and not a non-specific molecular surrogate such as calcium flux.

FLECS TECHNOLOGY:

Overview

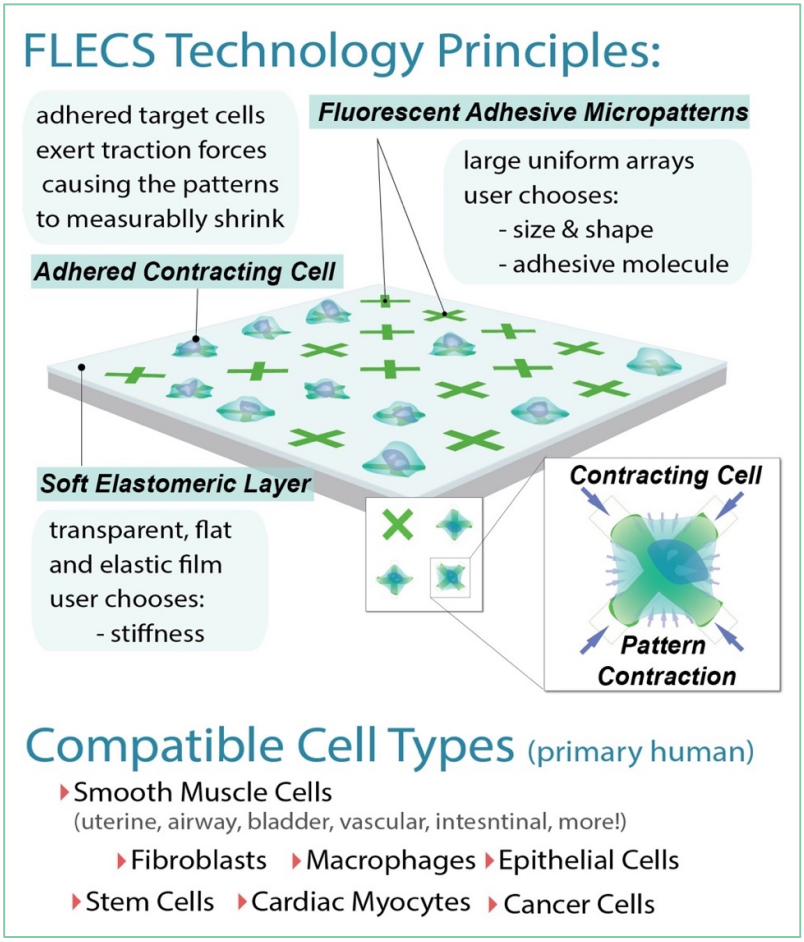
Customization

FLECS is a platform on which a variety of functional cell-based assays can be constructed. The following key parameters may be chosen

1. The adhesive molecule comprising the micro-patterns;
- Fibronectin
- Collagen I
- Collagen IV
- Other – inquire

2. The micropattern shape guiding cell morphology and force generation:
- 50 μm 'X'
- 70 μm 'X'
- Other – inquire

3. The bulk rigidity of the underlying film
- ~1kPa to 30 kPa



Forcyte's **FLECS 24-Well Single-Cell Contractility Assay Kit** provides a simple, automation-friendly *in vitro* system to assess cell contractility in potentially 100,000s of single-cells simultaneously and to screen cell contraction mediators. Any fluorescent microscope can be used to image the micropatterns and stained cell nuclei. Micropattern deformations correspond to cellular force. **Product is supplied as sealed 24-wellplate with sterile PBS. Optionally, live cell nuclear stain is supplied (Cat: 010D)**

Storage

FLECSplates should be **stored at 4C** whenever possible and hidden from light. Shelf-life under these conditions is at least 6 months from data of manufacture. ***DO NOT FREEZE.*** Individual wells can be opened and used one-at-a-time and the plate (with un-used wells) may be returned to storage.

INSTRUCTIONS FOR USE

Research use only

3-STEP FLECS ASSAYS

Research use only

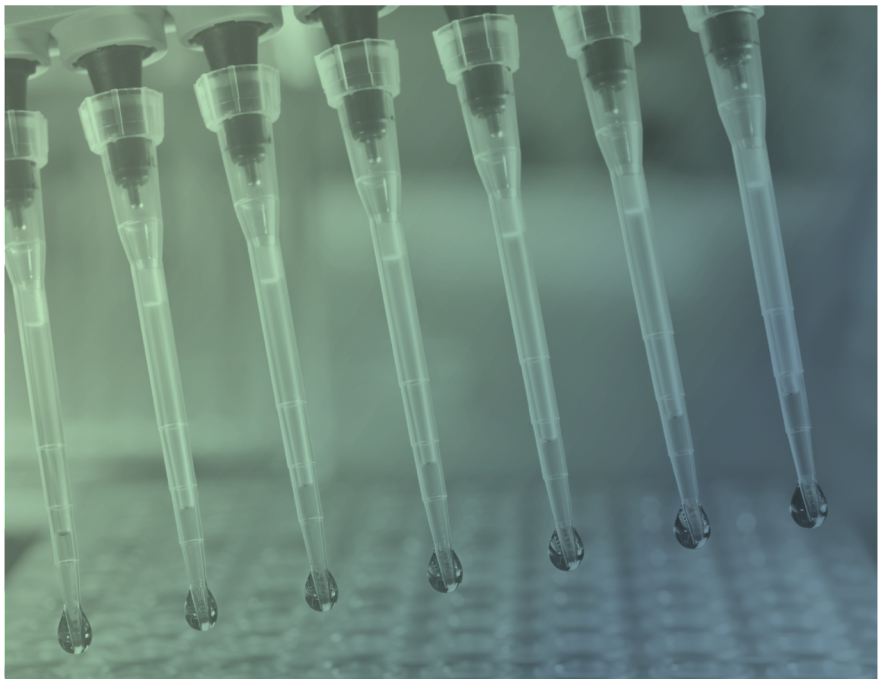
Important aspects of cell behavior that should be characterized prior to running large scale experiments are: best micropattern size, best adhesion molecule, time required for cells to adhere and spread, and best cell seeding density. Several experiments testing these various parameters are recommended to optimize your experimental protocol.

FLECS assays are executed in 3 general steps:

1 Assay preparation and cell seeding

2 Cell treatment and imaging

3 Image analysis



ASSAY PREPARATION AND CELL SEEDING

Step **1:** Remove the FLECS plate from its packaging, then, cut and peel the plastic cover from the wells that will be used. (These can be used one-at-a-time).

Step **2:** Carefully aspirate the sterile PBS from the wells that will be used without touching the soft bottom surface of the well.

Step **3:** Add **1-2 mL of the complete cell culture medium** that will be used to seed cells and incubate the plate at **37C** until the medium and plate equilibrate.

Step **4**: While waiting on Step # 3, prepare your cells by dissociating them, straining them through a 40 µm cell strainer to achieve a single-cell suspension, and counting them to achieve a final concentration **of 50K to 70K cells per mL**. (The exact concentration should be determined empirically).

Step **5:** After Step # 3 is complete, aspirate the culture media from the wells until only enough volume to cover the bottom remains.

Step **6:** Pipette 500 to 750 µL of cell solution into each well drop-wise, making sure to add a drop to different locations above the well surface to achieve uniform distribution.

Step **7:** Let the plate containing cells sit on a very flat surface at room temperature for 1 hour. At this stage, the cells will be rounded and evenly distributed as shown in the Figure 1.

Step **8:** Following the 1 hour room temperature incubation, transfer the plate into a 37C incubator.

Step **9:** After 30-60 minutes in the incubator, most cells will be adhered. At this point, you may optionally wash the wells to remove excessive cells. The goal is to maximize the number of micropatterns adhering exactly one cell. Washing may be done by serially adding and aspirating medium into the wells containing cells.

Step **10:** Incubate cells for the appropriate amount of time to perform your experiment. Typically, cells will require between 3-6 hours to fully spread. Imaging is recommended at any time between 6 to 48 hours following cell seeding.

CELL ADHESION & SPREADING

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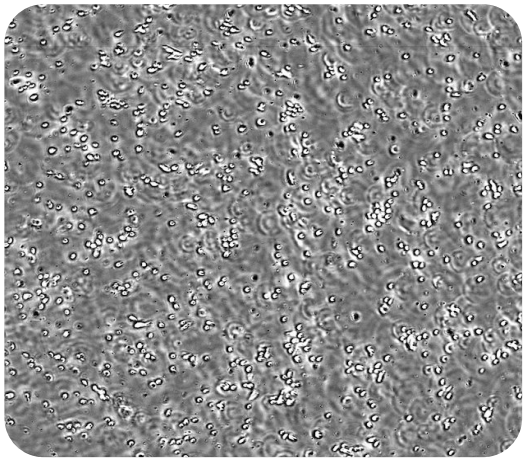
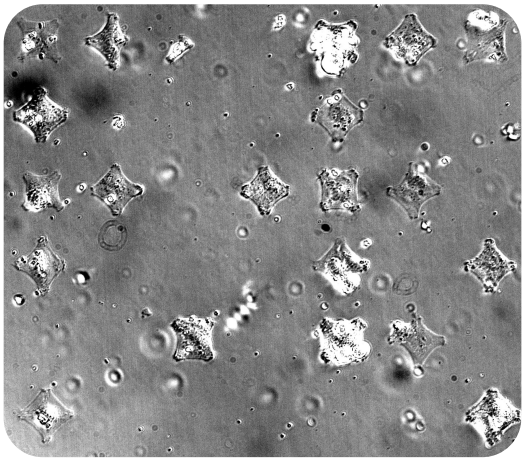
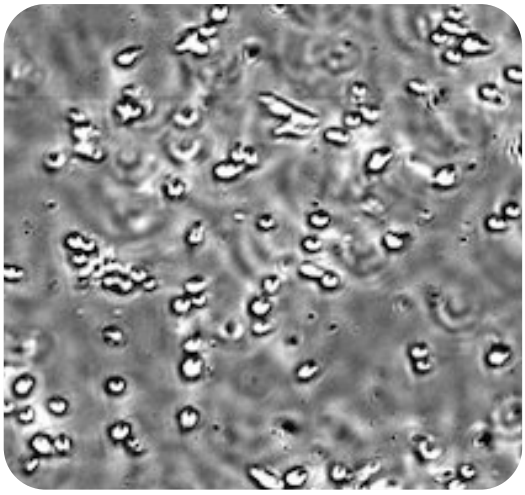
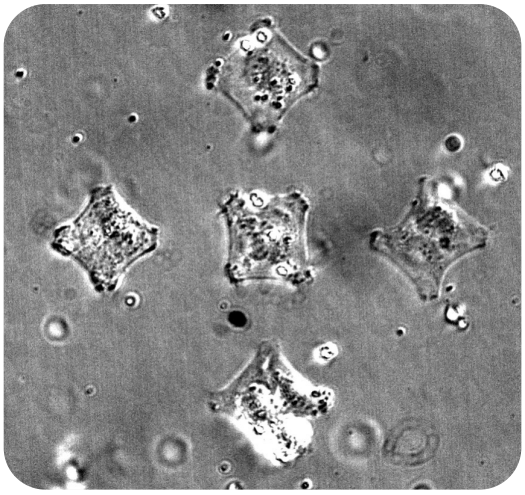
Cell morphologies to look for





2-8 hrs





Rounded cells prior to adhesion and spreading *(Image taken at 4x mag)*

Cells spread over micropatterns. At this stage, micropatterns may be imaged *(Image taken at 10x mag)*

CELL TREATMENT AND **IMAGING**

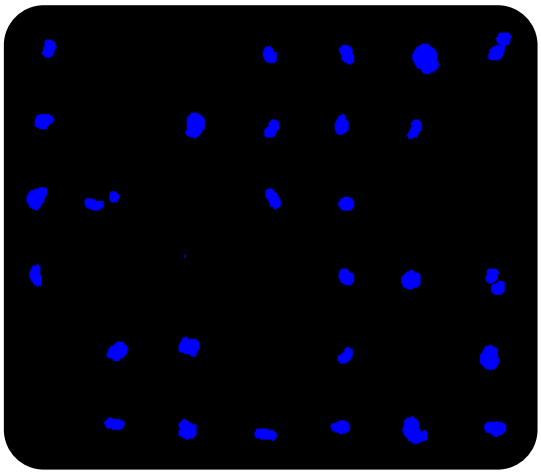
2-channel imaging to enable analysis

1. The precise cell treatment will depend on the investigator's experimental design. Drug may be added at early timepoints or just before imaging. Regardless, it is recommended that during solution exchange, some culture medium always covers the well bottom.

2. Immediately prior to imaging, it is recommended that live nuclear stain is added to the wells to aid in identifying the locations of single-cells. Forcyte can supply live cell staining solution (CAT: 010D) which should be added at a 1:10,000 dilution at least 10 minutes prior to imaging.

3. Imaging maybe done using any capable fluorescent microscope. It is recommended that temperature is held at ~37C to prevent unintended effects on cellular mechanical activity. **Imaging of both the micropatterns and of the cell stained nuclei, on a per-field-of-view basis, must be done to enable simple image analysis.** (Imaging with dedicated high-content imagers will enable alignment of images taken at different timepoints)

Micropattern channel



Nuclear channel

→ Both channels must be captured in the same field-of-view to enable image analysis

QUANTIFYING CONTRACTILITY

Research use only

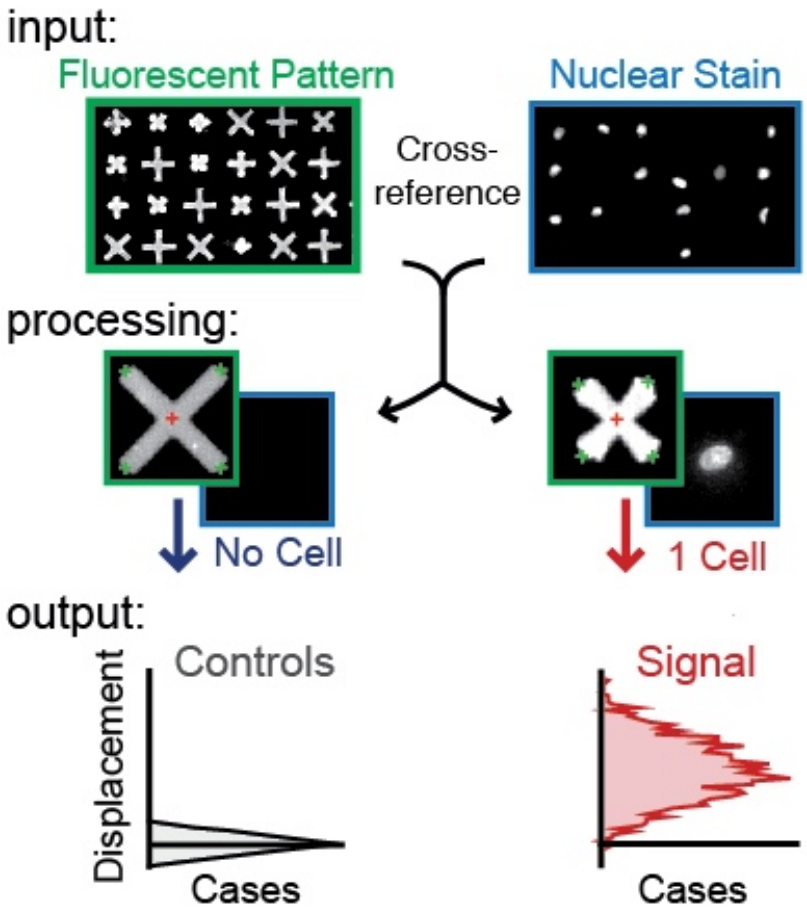
Image analysis

The strategy for analyzing image data is to group all of the image micropatterns into one of three cases:

i. **Case 1: No cells adhered** – these serve as "no contraction" controls
ii. **Case 2: Exactly one cell adhered** – these serve as data points
iii. **Case 3: More than one cell adhered** – this data may be used or rejected

Cell count is determined based on the number of cell nuclei co-localized to the pattern.

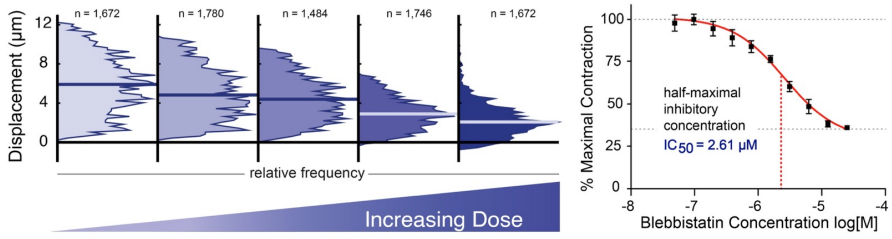
Once cell count is complete, the micropattern must be measured in size using an image analysis software such as ImageJ. Users may also contact Forcyte at info@forcytebio.com and we will provide fee-for-service analysis of your images. Total inward displacement of the micropatterns is the recommended metric. Alternatively, area of the micropatterns may also be used. The set of micropatterms without cells should be used as the reference for zero contraction. Refer to the figure below:



ADDITIONAL INFORMATION

Regarding FLECS technology

Example experiment



Increasing doses of myosin inhibitor, blebbistatin, is added to contracting smooth muscle cells. Increasing dosage leads to a decrease in observed population-wide contractility.

Publications

- Elastomeric sensor surfaces for high-throughput single-cell force cytometry | Nature Biomedical Engineering.
- Pushkarsky, I. FLECS Technology for High-Throughput Single-Cell Force Biology and Screening. *Assay Drug Dev. Technol.* **16**, 7–11 (2018).
- Koziol-White, C. J. *et al.* Inhibition of PI3K promotes dilation of human small airways in a rho kinase-dependent manner. *Br. J. Pharmacol.* **173**, 2726–2738 (2016).
- Yoo, E. J. *et al.* Gα12 facilitates shortening in human airway smooth muscle by modulating phosphoinositide 3-kinase-mediated activation in a RhoA-dependent manner. *Br. J. Pharmacol.* **174**, 4383–4395 (2017).
- Neuronal PAS Domain 2 (Npas2)-Deficient Fibroblasts Accelerate Skin Wound Healing and Dermal Collagen Reconstruction - Sasaki - - The Anatomical Record - Wiley Online Library.

Questions?

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