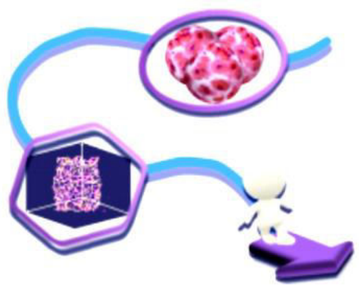

PhD in Automation, Robotics and Bioengineering

INTEGRATION OF A FLUIDIC SYSTEM WITH AN IMAGING PLATFORM: REAL TIME MONITORING OF FLUID DYNAMICS AND CELL MORPHOLOGY

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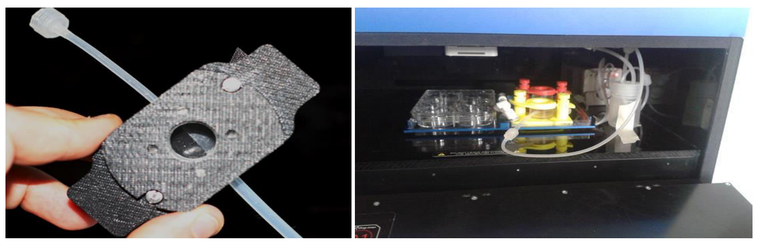
Introduction

The ability to **image real time** cell/biomaterial interactions in a **millimeter-sized culture environment** in a non-destructive manner is considered one of the main challenges in regenerative medicine[1]. The objective of this thesis is to develop new integrate methods for 3D live cell imaging in in-vitro bio-mimetic systems.
The aim of the first year was to: assess the feasibility to **integrate a fluidic system** (LiveBox1) **with an imaging platform** (Cell-IQ® system, developed by CM-Technologies, Tampere, Finland), and **investigate the correlation of cell morphology and function in a non invasive manner** to measure the effects of environmental changing (e.g. toxicity, shear stress, biochemical stimuli).

First step for integration in Cell-IQ system

An *ad-hoc* sample holder was designed, in order to allocate two Livebox1 units in Cell-IQ® incubator.



1. Static and Dynamic tests

Goal: evaluation of light and fluorescence microscopy image quality in LiveBox1 using static and dynamic flow conditions with a 4X objective.

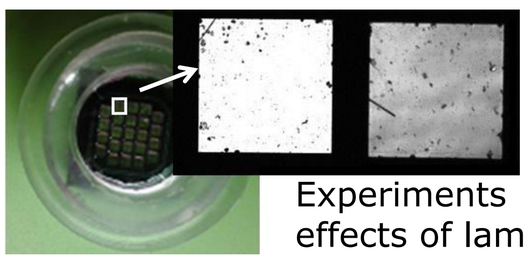
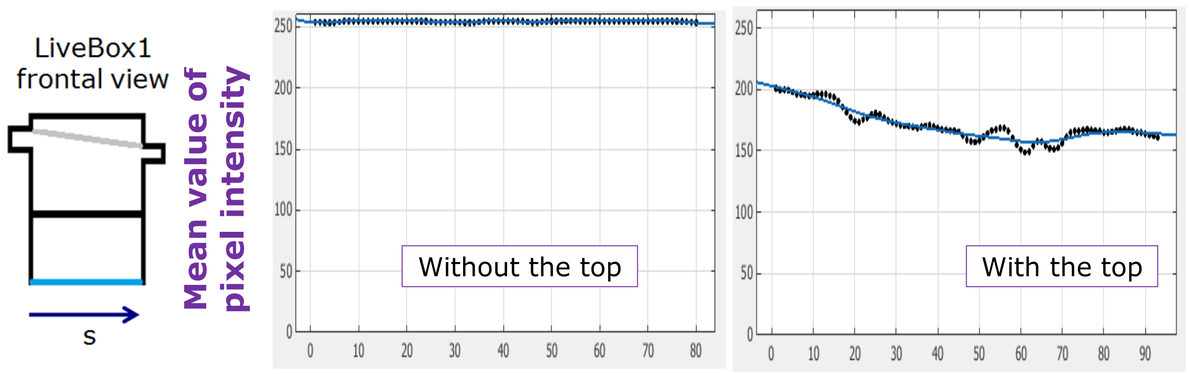


Image size allows the detection of a single unit of a CSEM membrane (as shown in figure).

Experiments were performed to analyze the effects of laminar flow rates.

✓ LiveBox1 design **doesn't influence sample's morphology**;

✓ **Pixel intensity is influenced** by the design of the upper part of LiveBox1.

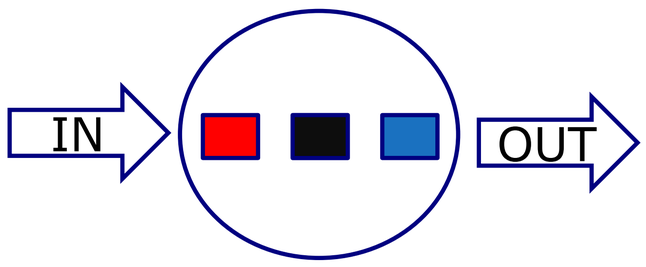


Note: LiveBox1 material (i.e. **PDMS**) **is not auto-fluorescent**.

2. Fluid Dynamic in LiveBox1

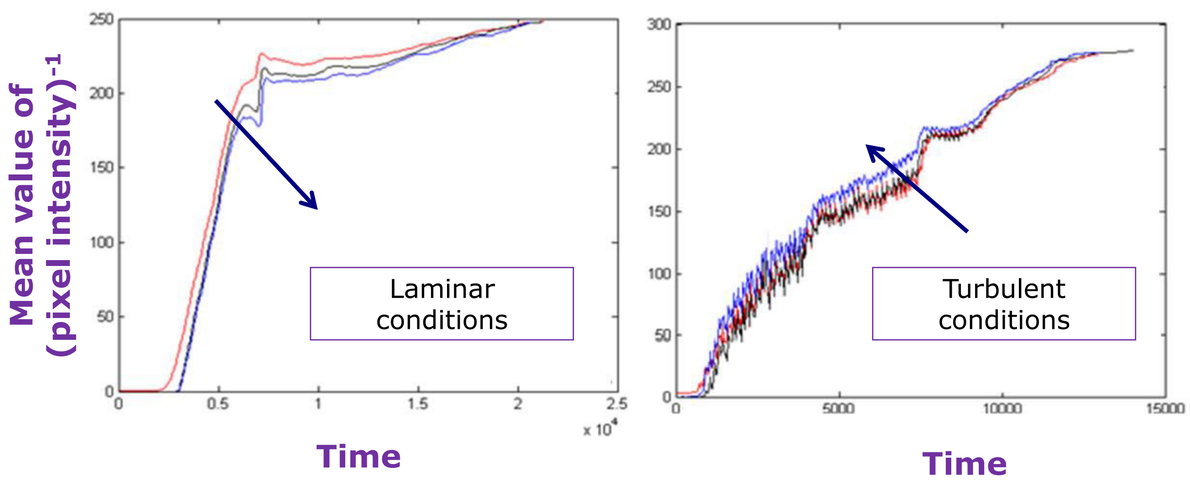
Goal: feasibility of using optical systems to investigate the dynamics of solutions in LiveBox1 imposing different flow rates (e.g. laminar (200÷500 µL/min) and turbulent (>500 µL/min) conditions).

In order to follow the filling phase of a dye solution in the LiveBox1 chamber, images at more than 2 frame/second were taken. Analysis was performed on three specific regions of the glass slide (using a 10X objective).



Regions (red, black and blue) were chosen accordingly to the distance from the inlet.

For each acquired frame, the inverse of pixel intensity is calculated: low pixel values are related to initial steps of mixing.



✓ Preliminary tests in laminar conditions confirm the results obtained from CFD models[2].

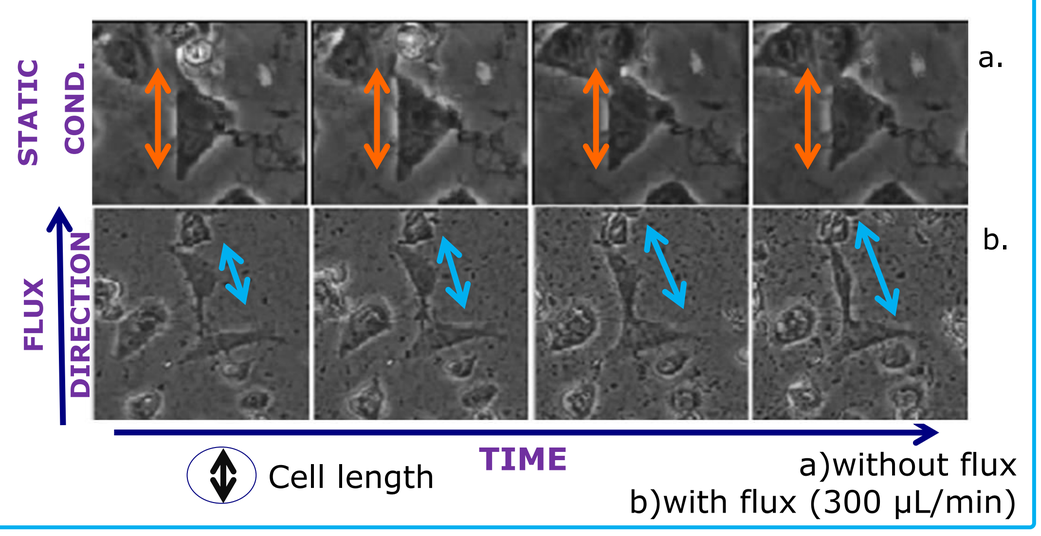
3. Time lapse cell imaging

Goal: feasibility to analyze cell morphology and its modification in LiveBox1.

2D cultures of endometrial epithelial cells (Ishikawa® cells) pre-coated with collagen and seeded in LiveBox1 were monitored in time lapse with the Cell-IQ® system. Three different laminar flow rates were evaluated. The control was represented by cells cultured in LiveBox1 with static conditions (no flow rate).

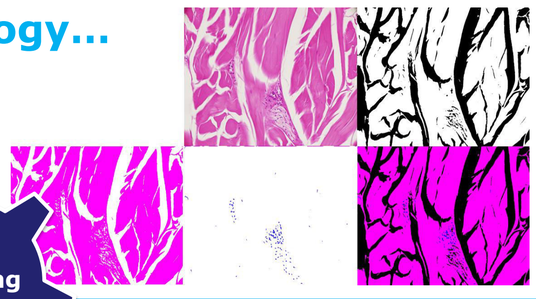
✓ As shown in figure, after 9 hours of perfusion **cells change the morphology** according to the flow direction (and compared with static controls).

Note: Cell culture phase was performed at FICAM (Medical School, University of Tampere).



Open-source and user-friendly GUI:

hisTOOLogy…

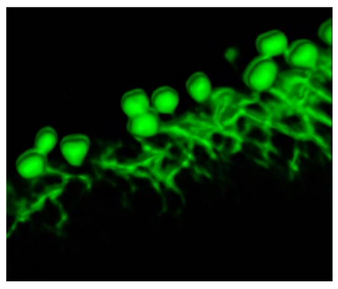


•…to **detect** and separate different histo-chemical **stainings**.

NEMO[3]…

• …to handle and process large number of optical microscopy images of neurons.

• …rendering and morphometrics analysis of **3D meshes** from 2D stack images (obtained using ITK routines)



Future developments:

- time-lapse multimodal imaging of 3D constructs in fluidic systems;
- integration of a system for environmental control (i.e. SUITE) to perform long term tests;
- use of a new bioreactor module for real time imaging tests.

1. J. S. Stephens, J. A. Cooper, F. R. Phelan Jr, J. P. Dunkers, "Perfusion Flow Bioreactor for 3D In Situ Imaging: Investigating Cell/Biomaterials Interactions", Biotech. and Bioeng., 97:4 2007;
2. D. Mazzei, M. A. Guzzardi, S. Giusti, A. Ahluwalia, "A low shear stress modular bioreactor for connected cell culture under high flow rates", Biotech. and Bioeng., 106:1, 2010;
3. L. Billeci, C. Magliaro, G. Pioggia, A. Ahluwalia, "NEuron MOrphological analysis tool: open-source software for quantitative morphometrics", Front.in Neuroinf., 7:2, 2013.

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