

Optics for Biological Systems

Traction Force Microscopy

Analysis of cell displacement with Fiji software

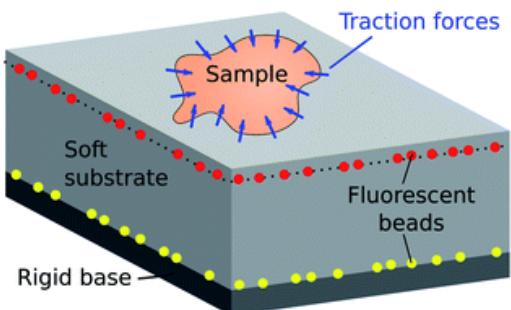
INTRODUCTION

INTRODUCTION

Study the forces between
the human and their
environment



Study the forces between
the cells and their
environment



INTRODUCTION

- Important to understand cancer cell invasion
- Development of new materials

The role of cell adhesion molecule in cancer progression and its application in cancer therapy

Takatsugu Okegawa¹, Rey-Chen Pong, Yingming Li, Jer-Tsong Hsieh

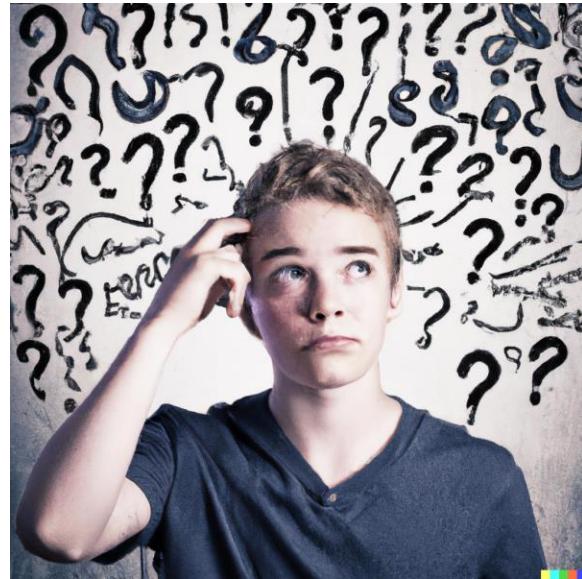


- Adhesion molecules play an important role in invasive metastasis



INTRODUCTION

fibronectin
cadherin
focal adhesion

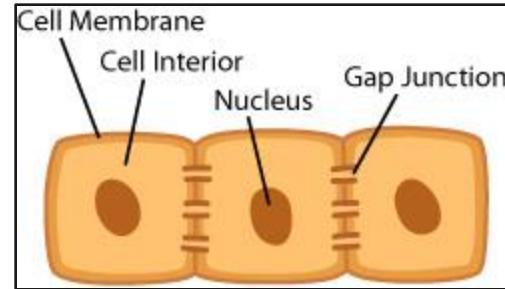


integrin
actin
TFM

Many terms must be defined

Epithelial cells → Definition

- Refers to the cells that line the **internal and external surfaces** of the body.
- They regulate the exchange of substances between the body and the environment.



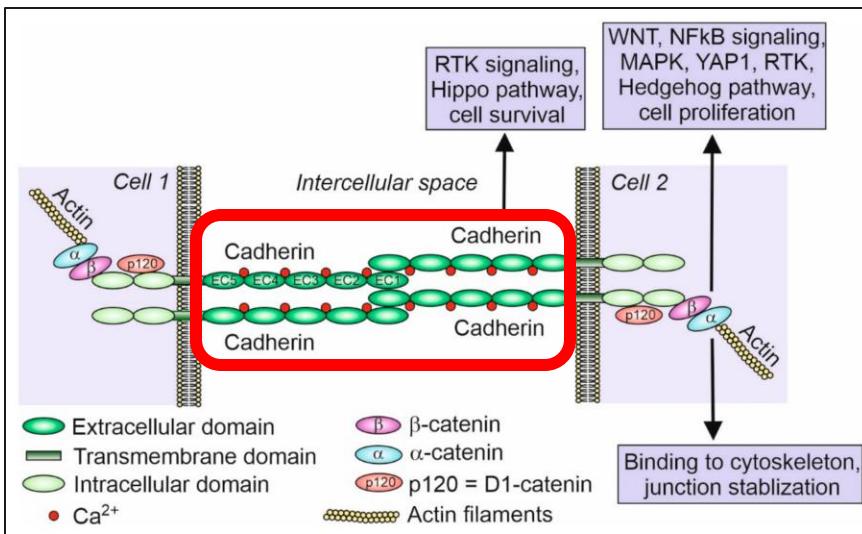
Simplified representation of epithelial cells

How do epithelial cells bind to each other ?

INTRODUCTION

Epithelial cells → Cell-cell adhesion via Cadherins

- In normal tissues, epithelial cells strongly adhere via cadherins, which are **calcium dependent cell-cell adhesion proteins**.



Curr Biol. 2013 Jul 22; 23(14): R626–R633.
doi: [10.1016/j.cub.2013.06.019](https://doi.org/10.1016/j.cub.2013.06.019)

PMCID: PMC3722483
PMID: [23885883](https://pubmed.ncbi.nlm.nih.gov/23885883/)

Three Functions of Cadherins in Cell Adhesion
Jean-Léon Maitre¹ and Carl-Philipp Heisenberg²

Langmuir. Author manuscript; available in PMC 2013 Jan 26.
Published in final edited form as:
Langmuir. 2009 Sep 1; 25(17): 10092–10099.
doi: [10.1021/la901109e](https://doi.org/10.1021/la901109e)

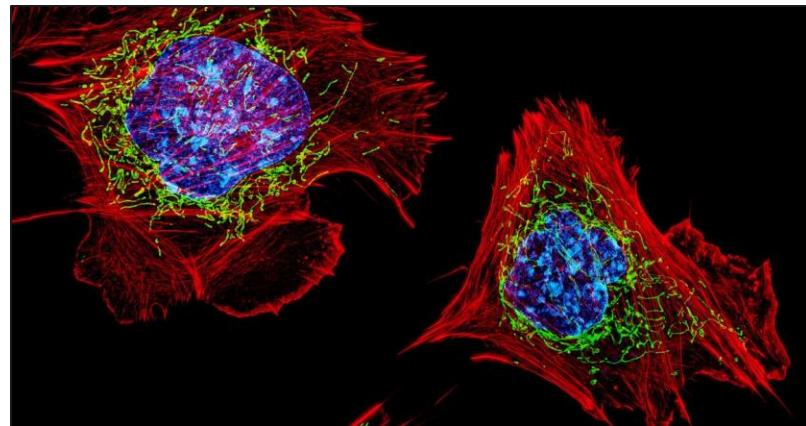
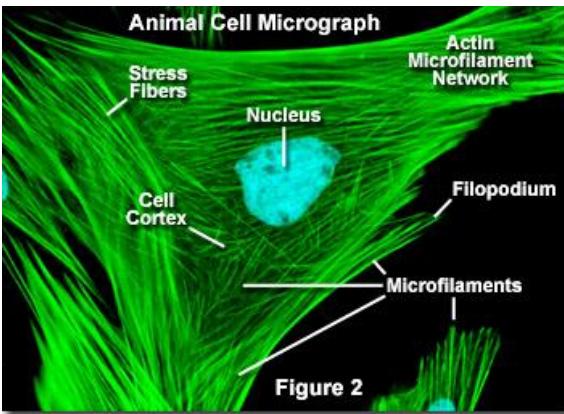
PMCID: PMC3556267
NIHMSID: NIHMS435250
PMID: [19583181](https://pubmed.ncbi.nlm.nih.gov/19583181/)

Cadherin and integrin regulation of epithelial cell migration
Jonathan Silvestre, Paul J.A. Kenis, and Deborah E. Leckband^a

What is the role of actin in cell displacement?

Epithelial cells → Actin Filaments

- Microfilaments, also called actin filaments, are protein filaments in the cytoplasm of eukaryotic cells that form part of the cytoskeleton.

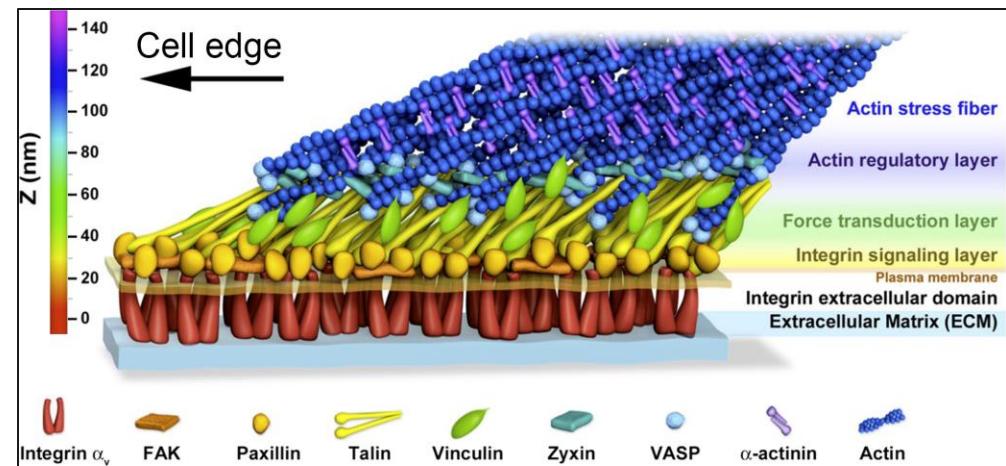
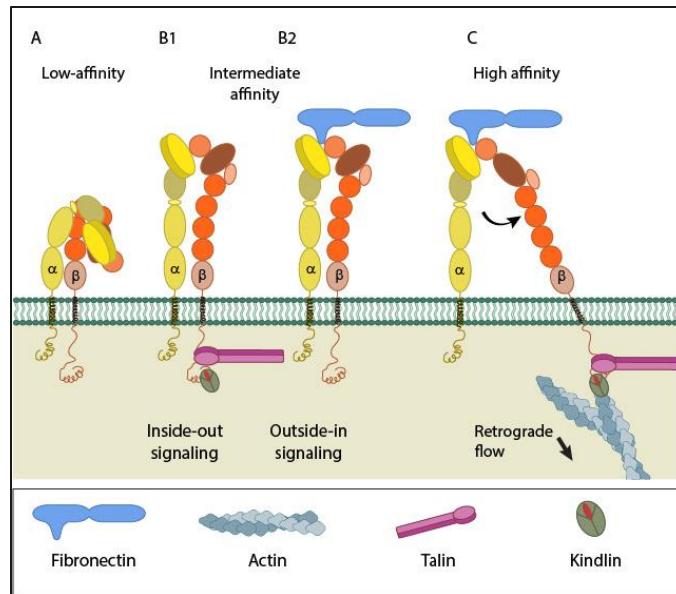


What are integrins ?

INTRODUCTION

Epithelial cells → Cell-substrate adhesion via Integrins

Integrins are transmembrane receptors that facilitate cell-cell and cell-extracellular matrix (ECM) adhesion.



What is fibronectin ?

Focal Adhesions

How to study cell displacement and adherence forces with the software program Fiji ?

MATERIALS & METHODS

Fluorescence Microscopy

Traction Force Microscopy

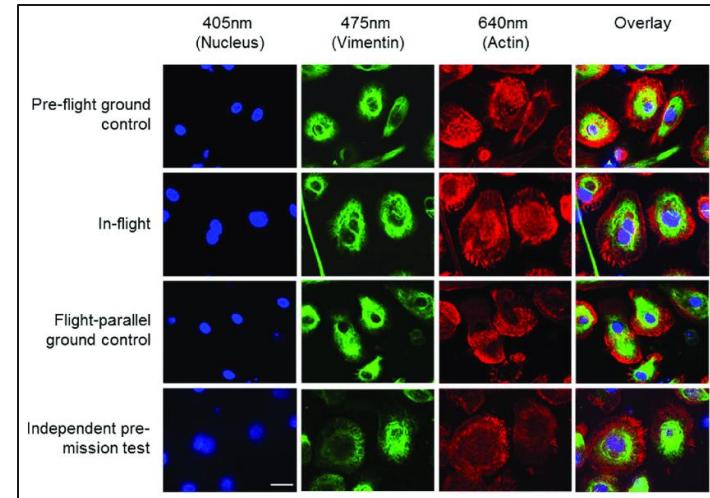
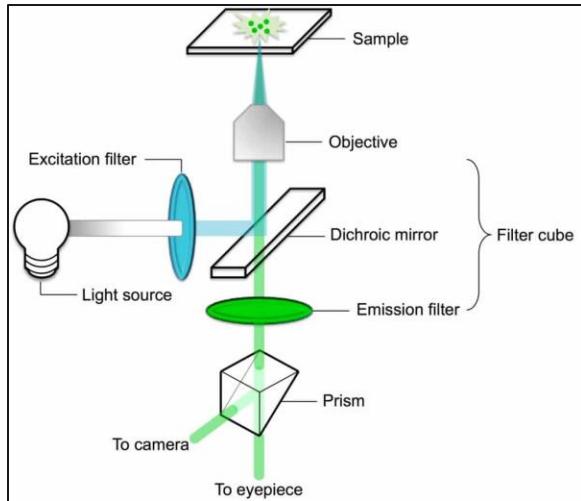
Protocol

Fluorescence Microscopy

Traction Force Microscopy

Protocol

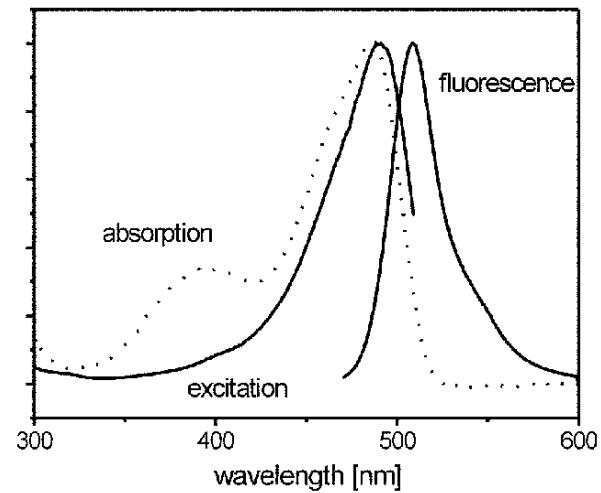
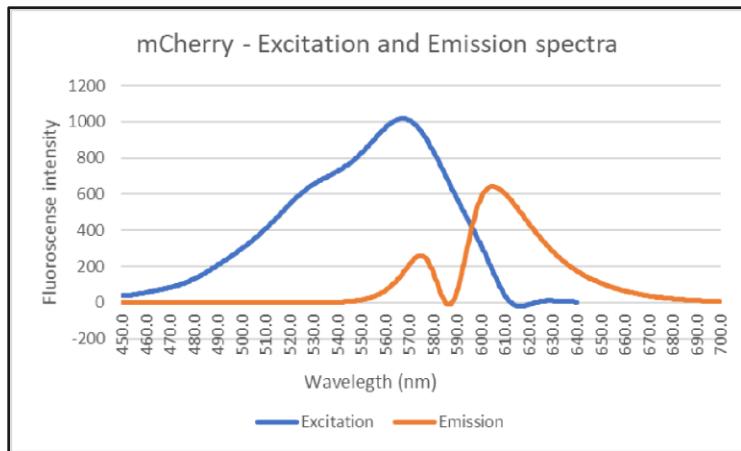
Fluorescence Microscopy



- Fluorescence microscopy is a type of microscopy that uses fluorescence to visualize the distribution of specific molecules in a sample by **exciting them with a specific wavelength of light**.

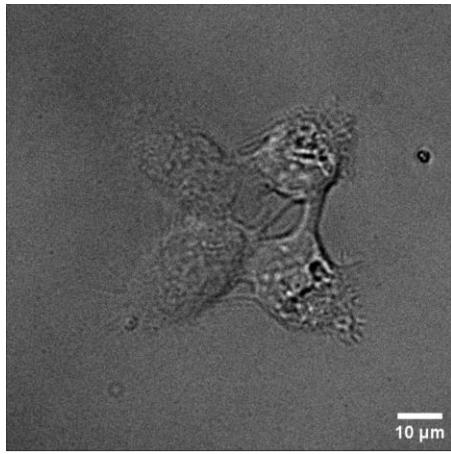
MATERIALS & METHODS

Fluorophores → mCherry & GFP

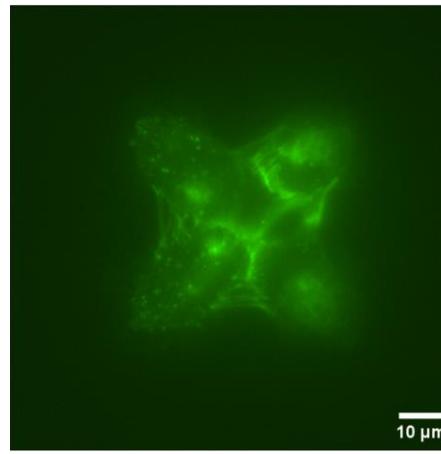
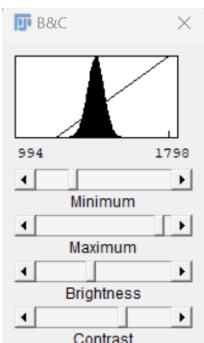


- mCherry and GFP are both fluorescent markers.
- mCherry is a **red-fluorescing protein**, while GFP is a **green-fluorescing protein**

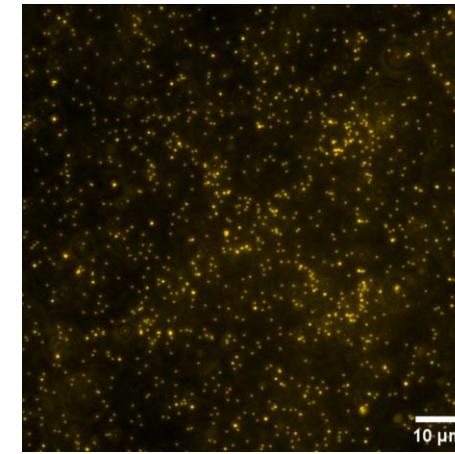
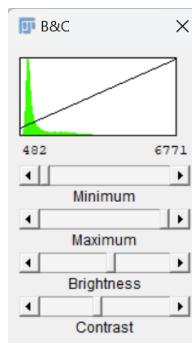
Cell imaging 3 different channels – Quad 1



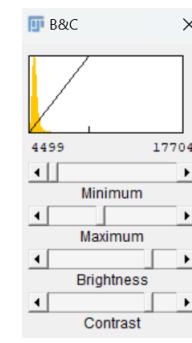
Brightfield



E-cadherin
GFP



Beads
mCherry



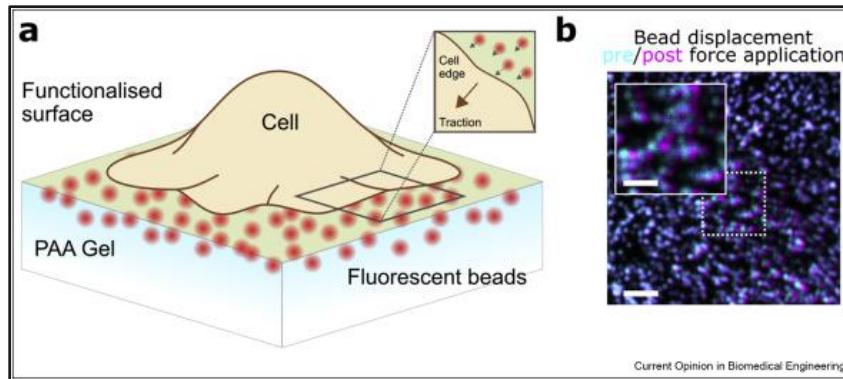
3 Channels - Stack 60 images

Fluorescence Microscopy

Traction Force Microscopy

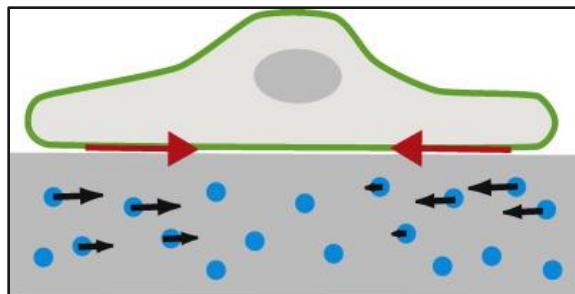
Protocol

Traction Force Microscopy : principle & set-up



The future of traction force microscopy

Huw Colin-York^a, Marco Fritzsche^{a,b}

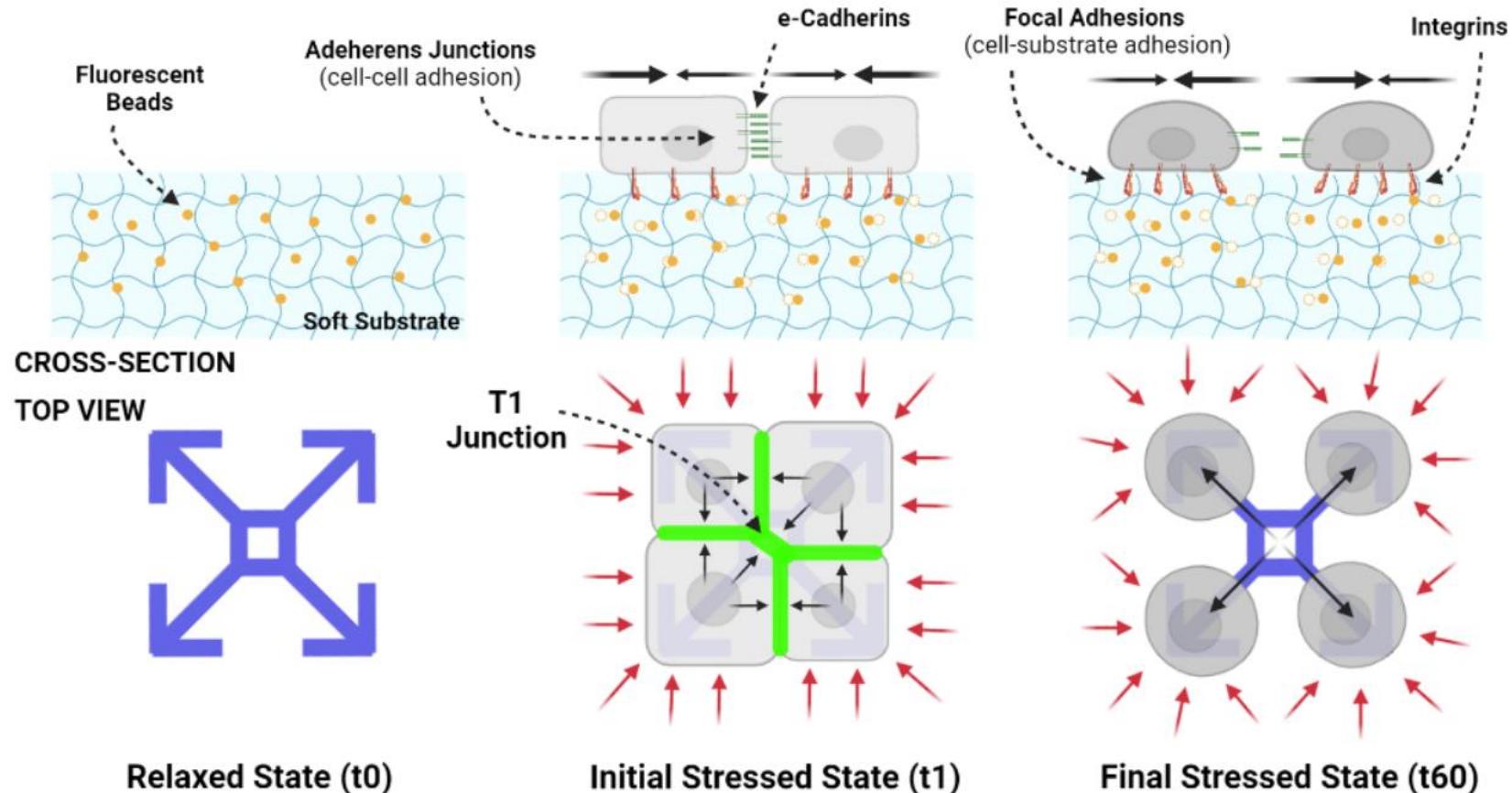


Traction force microscopy on soft elastic substrates: A guide to recent computational advances

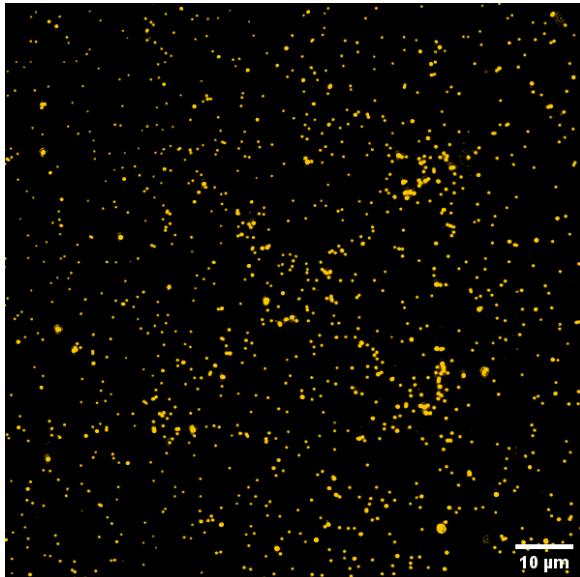
Ulrich S. Schwarz , Jérôme R.D. Soiné

MATERIALS & METHODS

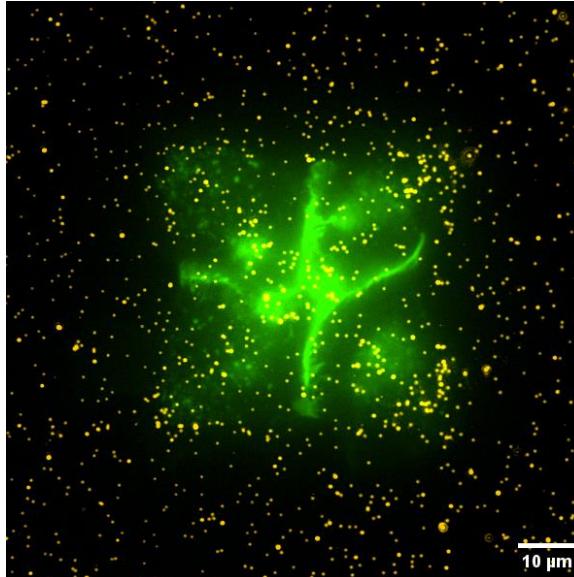
TFM workflow



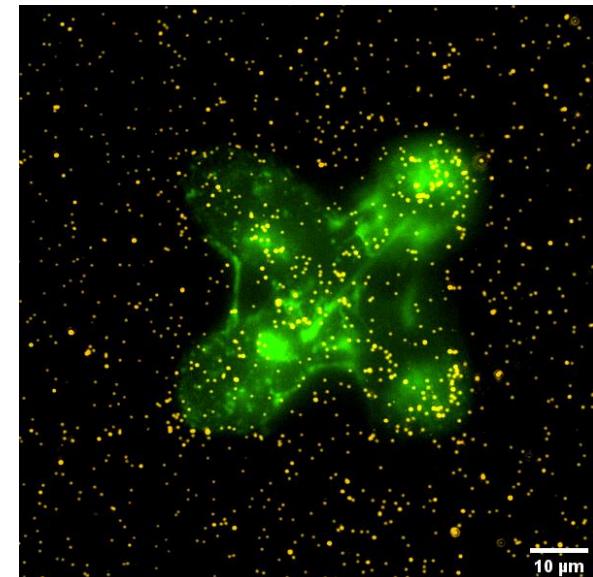
Cell imaging – Beads are displaced



Relaxed State (t0)



Initial Stressed State (t0)



Final Stressed State (t60)

Fluorescence Microscopy

Traction Force Microscopy

Protocol

Reference paper



Methods in Cell Biology

Volume 125, 2015, Pages 269-287



Chapter 15 - Measurement of cell traction forces with ImageJ

Jean-Louis Martiel * , Aldo Leal *, Laetitia Kurzawa *, Martial Balland §, Irene Wang §, Timothée Vignaud *
, Qingzong Tseng *, Manuel Théry *,  

<https://www.sciencedirect.com/science/article/abs/pii/S0091679X14000090?via%3Dihub>

Protocol Workflow

Two fluorescent bead images should be combined as a stack



Pre-Alignment to correct the experimental shift using the Align Slices in stack plugin



Displacement field is calculated by the Iterative PIV plugin

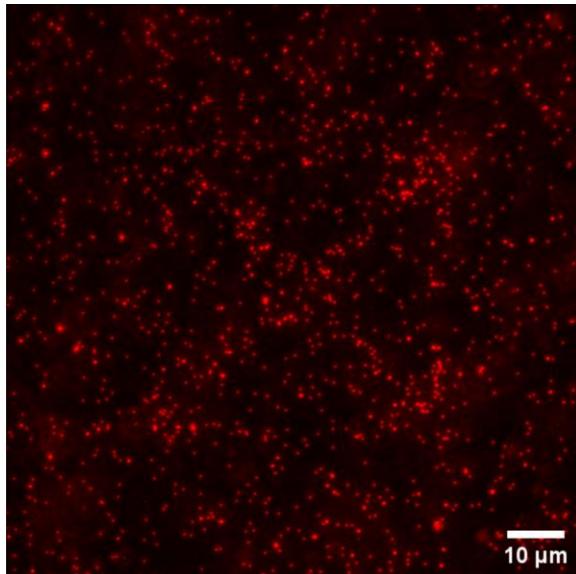


FTTC plugin to reconstruct the force field

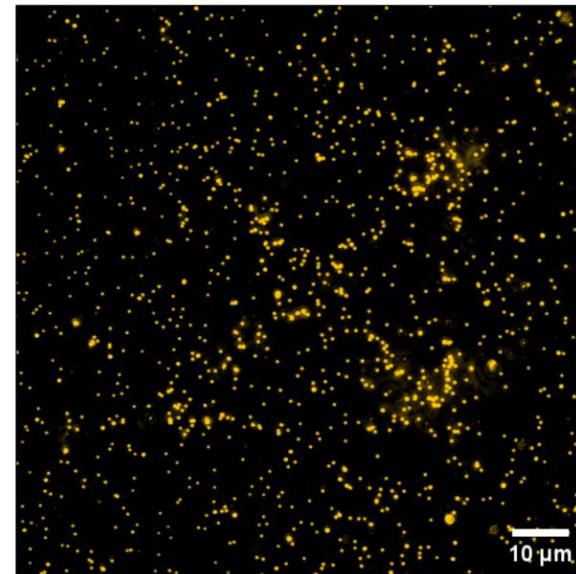


Retrieve data from the output force field

Step 1 : Images to Stack

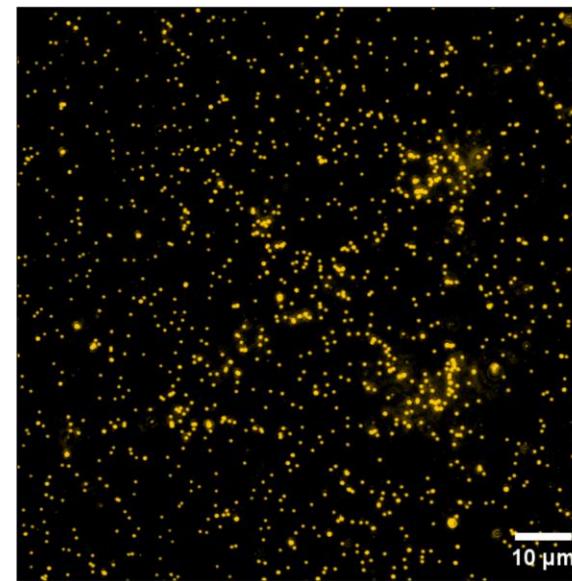
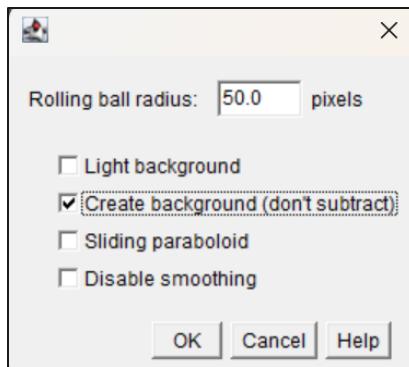


Ref QUAD 1 (Red Lut)



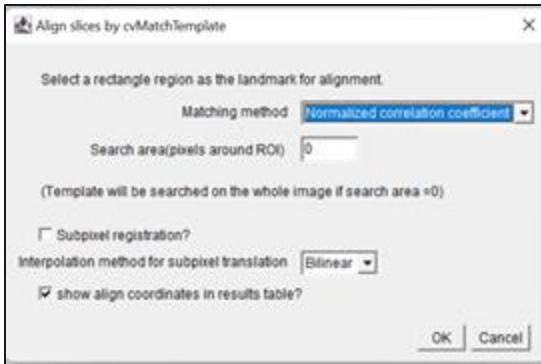
C3 Brightfield o6o

Step 2 : Subtract Background

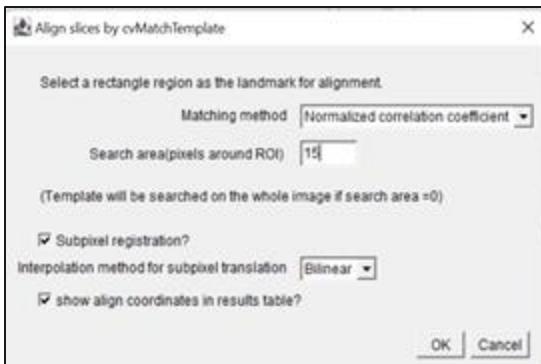


MATERIALS & METHODS

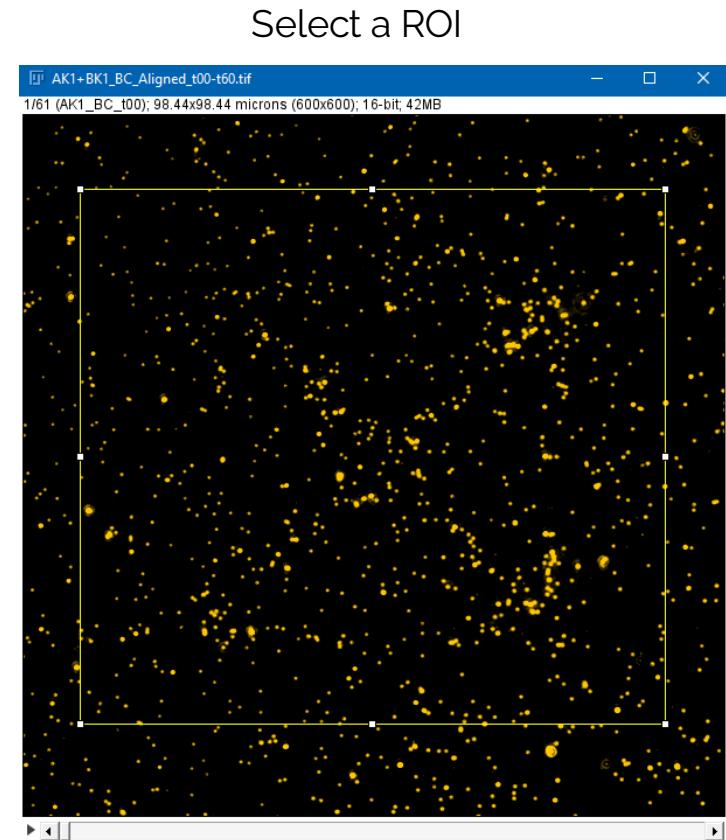
Step 3 : Align Slices



Default
Parameters

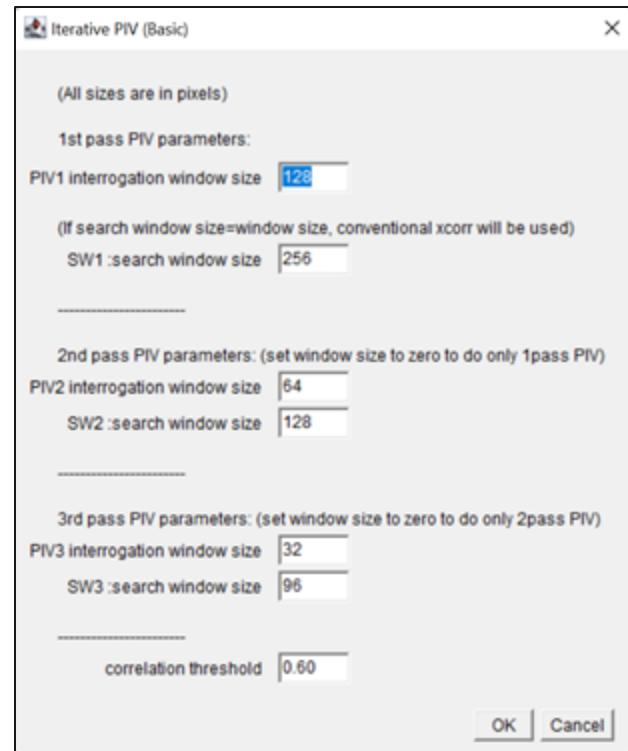
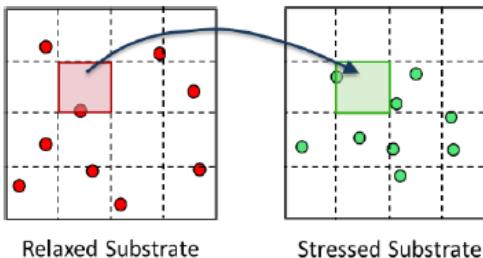


Optimised
Parameters

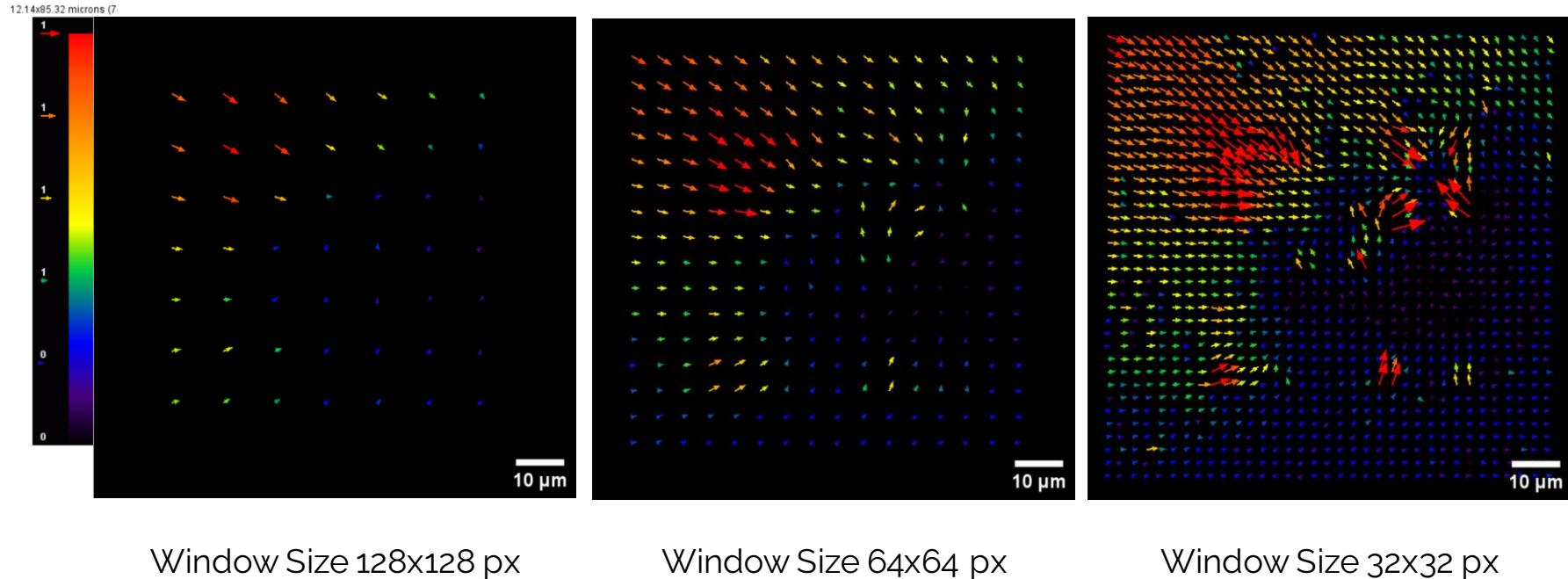


Step 4 : Iterative PIV Plugin – Window Size

- It is very complex to implement window sizes different to $2n$ in FIJI
 - Probably an intermediate between 16 and 32 is the best, such as 24



Step 4 : Iterative PIV Plugin



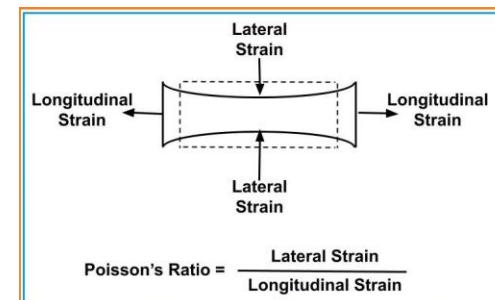
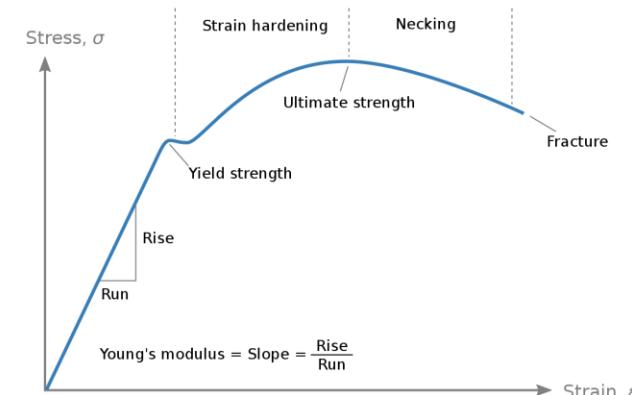
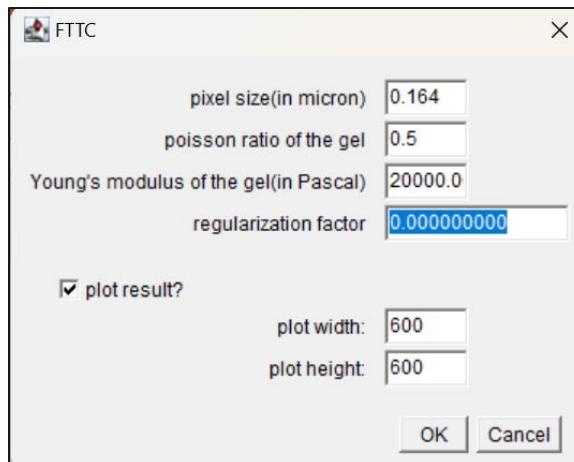
Window Size 128x128 px

Window Size 64x64 px

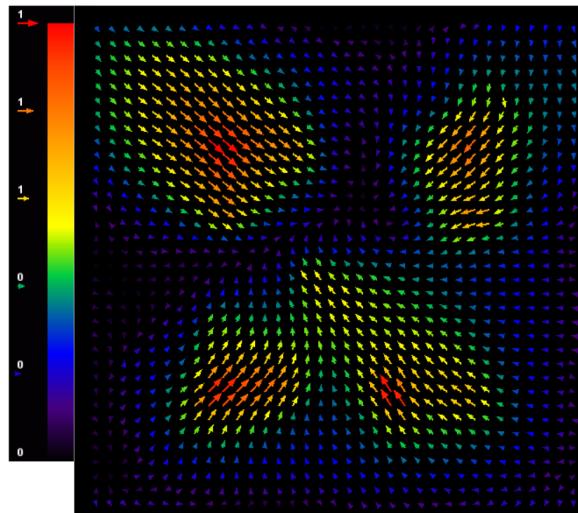
Window Size 32x32 px

Step 5 : FTTC Plugin - Mechanical properties

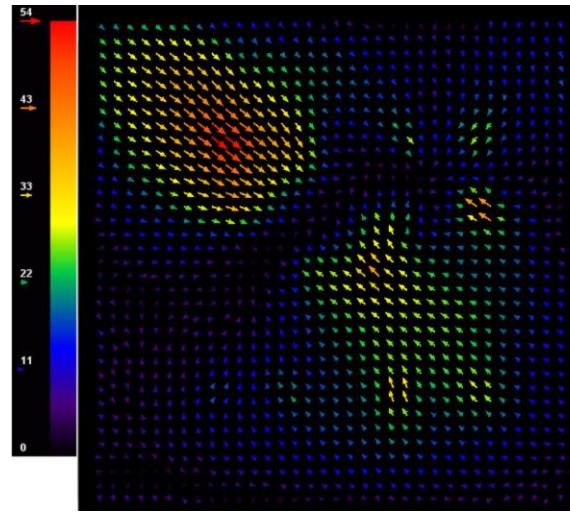
- Micron per pixel = $1/6.09 = 0.164$
- Young Modulus = 20 kPa
- Poisson Ratio = 0.5



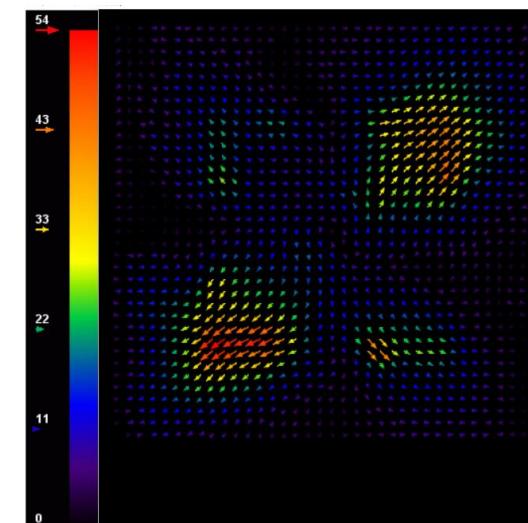
Step 5 : FTTC Plugin – Forces retrieval of Q1



FTTC_PIV_to-t1_Q1



FTTC_PIV_to-t60_Q1

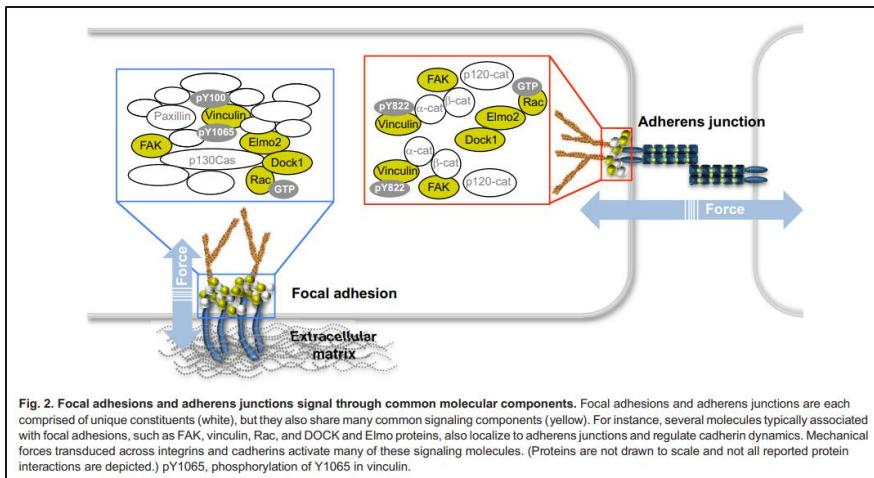


FTTC_PIV_t1-t60_Q1

RESULTS

RESULTS & DISCUSSION

Literature → Cadherin and Integrin cross-talk



COMMENTARY | 15 MARCH 2016

The mechanical regulation of integrin–cadherin crosstalk organizes cells, signalling and forces **FREE**

In collection: **Mechanobiology**

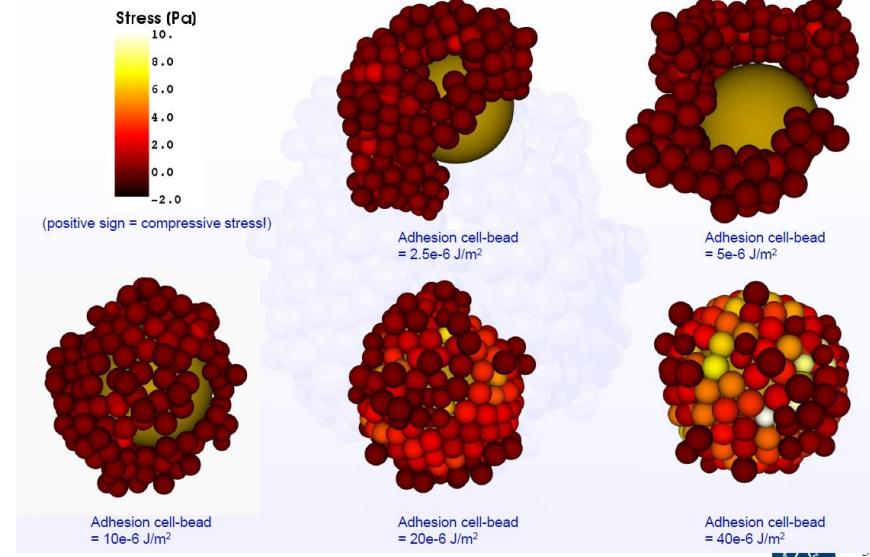
Keeley L. Mui , Christopher S. Chen, Richard K. Assoian

+ Author and article information

J Cell Sci (2016) 129 (6): 1093–1100.

<https://doi.org/10.1242/jcs.183699>

Cell expansion on microcarrier



(Bart Smeets & Herman Ramon, DEM research group, KU Leuven)



HYPOTHESIS

- There is cross-talk in cells that implies a Force balance between Integrins and Cadherins.

$$F_{Total} = F_{Integrins} + F_{Cadherins}$$

- At t1, Cadherins exhibit maximum Force contribution & Integrins exhibit minimum Force contribution.
- At t25, when Cadherins start to disrupt, there is a similar Force contribution of Integrins & Cadherins.
- At t60, when Cadherins are fully disrupted (no contribution), Integrins exhibit maximum Force contribution.
- Therefore, the total force can be expressed as a linear combination of both Integrin & Cadherin contributions

$$F_{Total} = a \cdot F_{Integrins} + b \cdot F_{Cadherins}$$

$$a \in [0,1] \quad \& \quad b = 1 - a$$

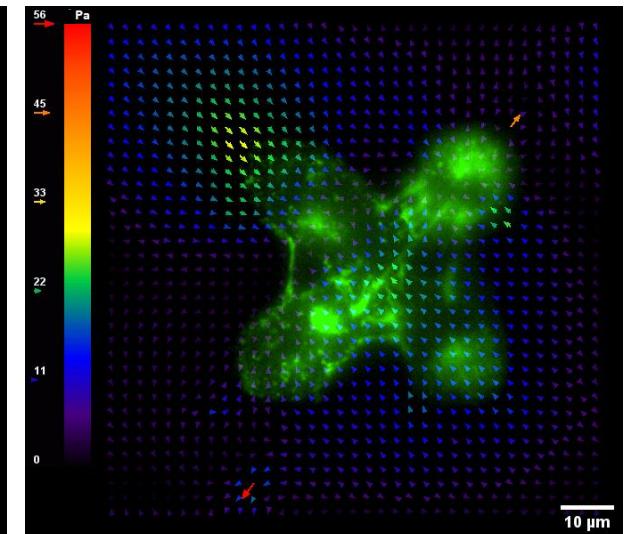
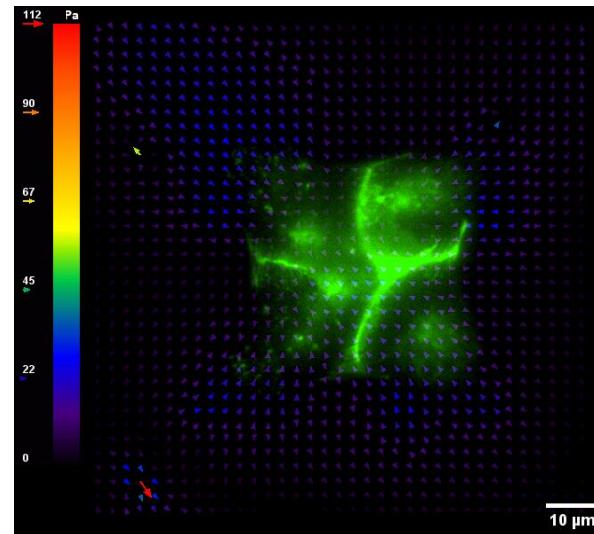
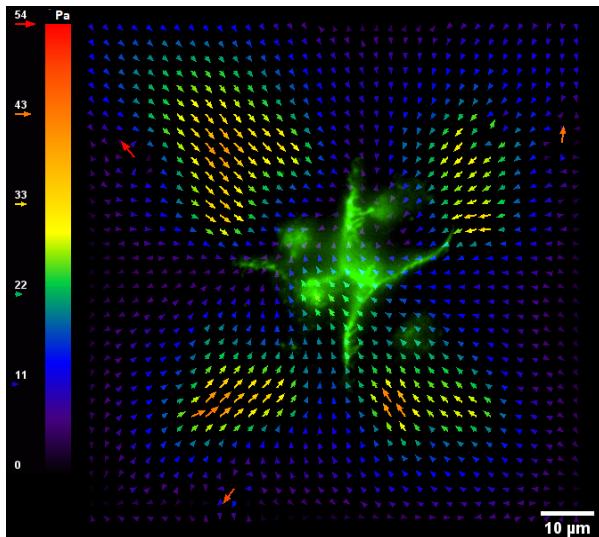
Force results

Automatization of the process

Force results

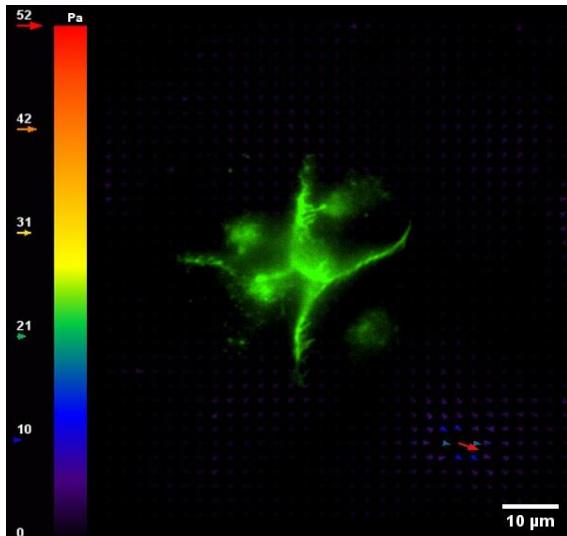
Automatization of the process

Overall Force Balance of Integrins & Cadherins in Q1

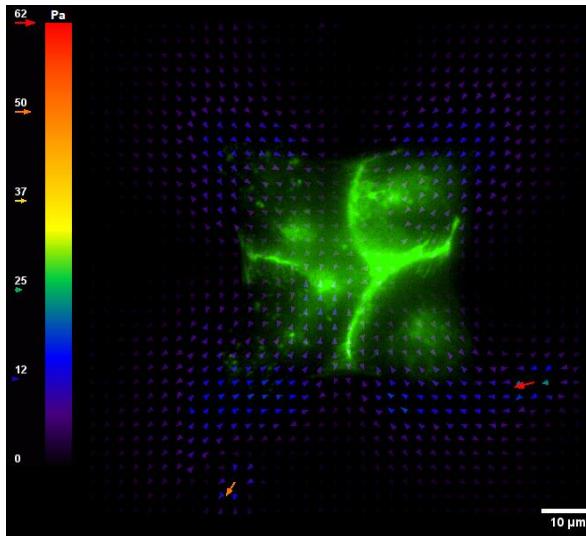


Force underestimation due to a processing mistake → Values are actually 40x larger!

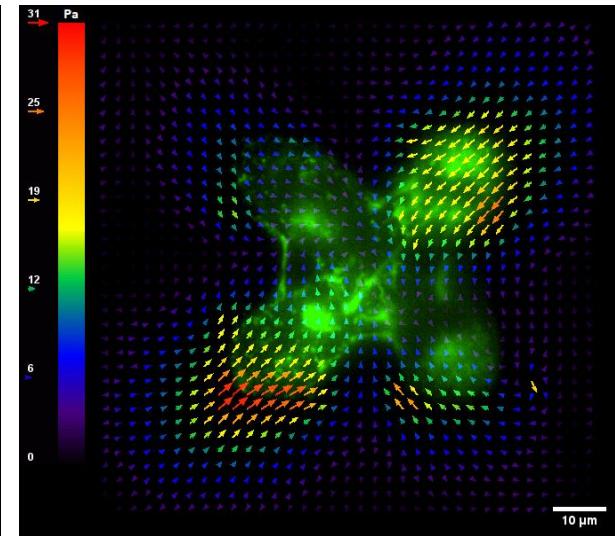
Relative Force Evolution of Integrins-Cadherins in Q1



Initial Stressed State (t1-t2)



E-Cadherin Rupture (t1-t25)



Final Stressed State (t1-t60)

Force underestimation due to a processing mistake → Values are actually 40x larger!

Summarised Qualitative Forces

Image of reference	Initial Stressed State t2 (Pa)	E-Cadherin Rupture t25 (Pa)	Final Stressed State t60 (Pa)
Relaxed State t0	30 (1.2 kPa)*	20 (0.8 kPa)*	10 (0.4 kPa)*
Initial Stressed State t1	0 (0 kPa)*	10 (0.4 kPa)*	20 (0.8 kPa)*

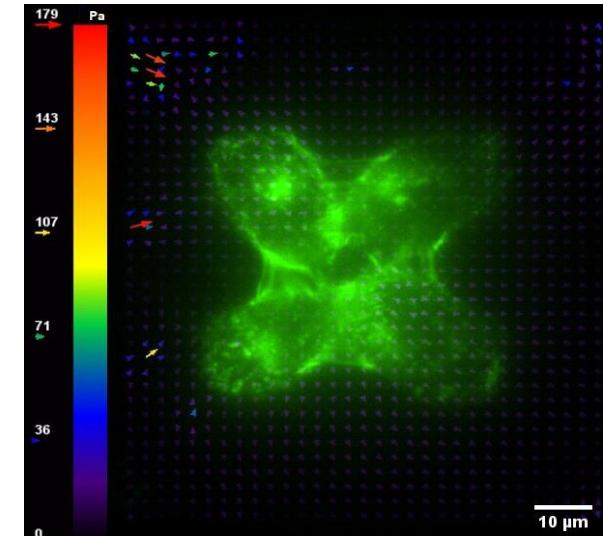
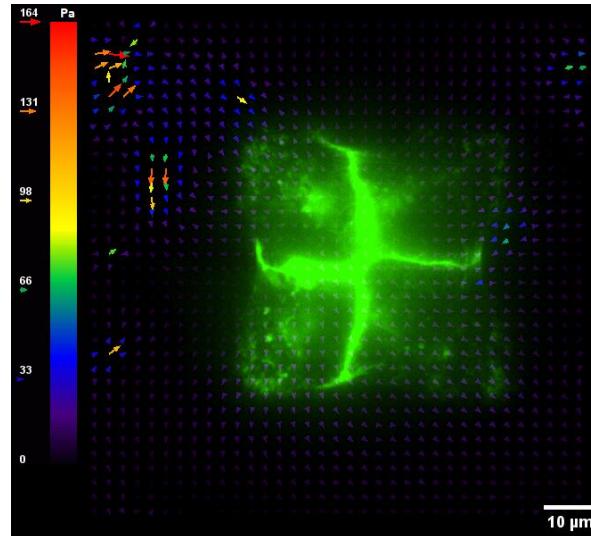
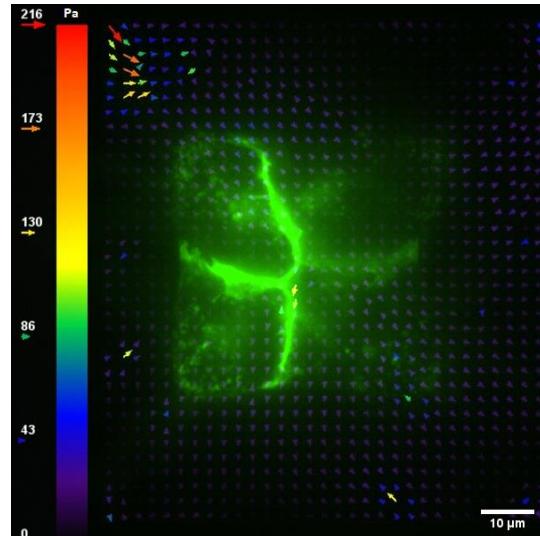
*Real approximated values, the other ones are underestimated.

Good indication that there might be a force balance!

Perhaps integrins need more time to make Focal Adhesions until saturation is reached for full force conservation.

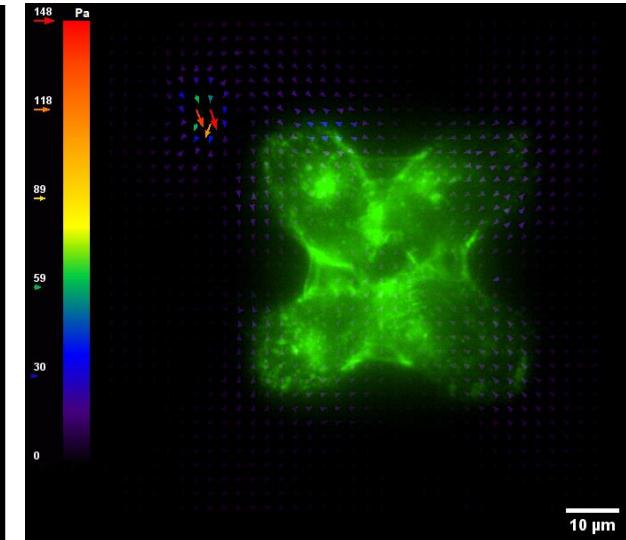
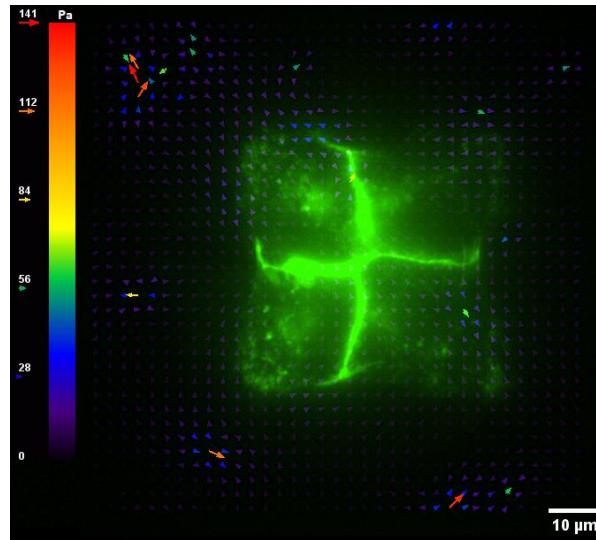
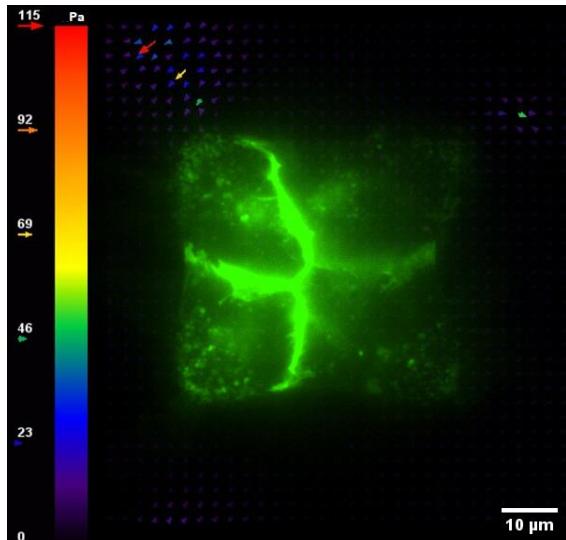
However, despite values are on the same order, these results are not quantitative and it's risky to obtain strong conclusions. Further experiments are required.

Overall Force Balance of Integrins & Cadherins in Q2



Force underestimation due to a processing mistake → Values are actually 40x larger!

Relative Force Evolution of Integrins-Cadherins in Q2



Force underestimation due to a processing mistake → Values are actually 40x larger!

Force results

Automatization of the process

RESULTS & DISCUSSION

Full movie -> Design of a macro

```

// CREATION OF THE STACK
for (i = 10; i < 60; i++) {
    name_file1 = "Stack" + i;
    name_file2 = "C3-Brightfield,-00"+i+".tif";
    j=i+1;
    name_file3 = "C3-Brightfield,-00"+j+".tif";

    namecon1 = "D:/OneDrive/Documents/PostCPGE/PHELMA 2020-2023/BIOMED 2022-2023/UGA/Optics for Bio-Systems/Data-TFM/QUAD1-IMAGES/" + name_file1;
    open(namecon1);
    namecon2 = "D:/OneDrive/Documents/PostCPGE/PHELMA 2020-2023/BIOMED 2022-2023/UGA/Optics for Bio-Systems/Data-TFM/QUAD1-IMAGES/" + name_file2;
    open(namecon2);
    namecon3 = "name"+name_file1+" use";
    run("Images to Stack", namecon3);
    namecon4 = "D:/OneDrive/Documents/PostCPGE/PHELMA 2020-2023/BIOMED 2022-2023/UGA/Optics for Bio-Systems/Data-TFM/QUAD1-STACKS/" + name_file1;
    saveAs(namecon4);
    close();

    open(namecon4);
    //setTool("rectangle");
    makeRectangle(138, 134, 351, 342);
    run("Align slices in stack...", "method=5 windowsizex=351 windowsizey=342 x0=138 y0=134 swindow=6 subpixel=false itpmethod=1 ref.sld");
    run("Iterative PIV(Cross-correlation)...", "piv1=128 piv2=64 piv3=32 what=[Accept this PIV and output] noise=0.20 threshold=5 c1=3");

    namecon6=name_file1+".tif_PIV3";
    selectWindow(namecon6);
    namecon7 = "D:/OneDrive/Documents/PostCPGE/PHELMA 2020-2023/BIOMED 2022-2023/UGA/Optics for Bio-Systems/Data-TFM/QUAD1-PIV-TRAIN-TIF/" + name_file1;
    saveAs("Tiff", namecon7);

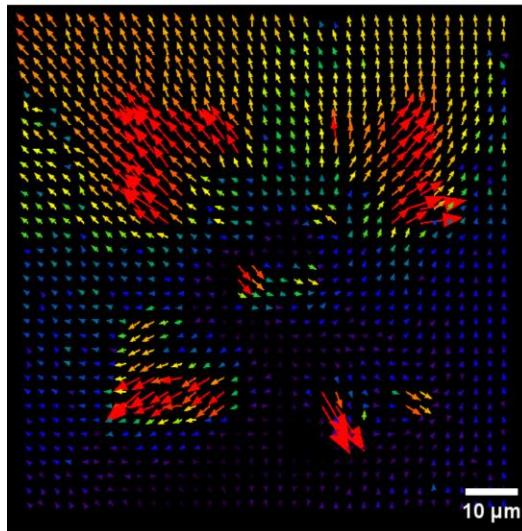
    namecon8=namecon6 +".tif";
    selectWindow(namecon8);
    close();
    namecon9 = name_file1+".tif_PIV2";

```

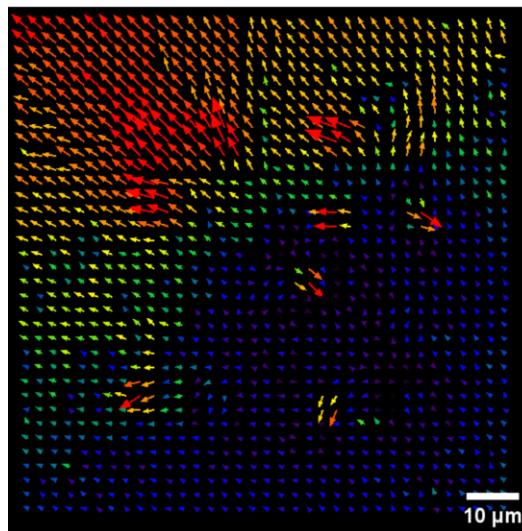
Automatize the process

RESULTS & DISCUSSION

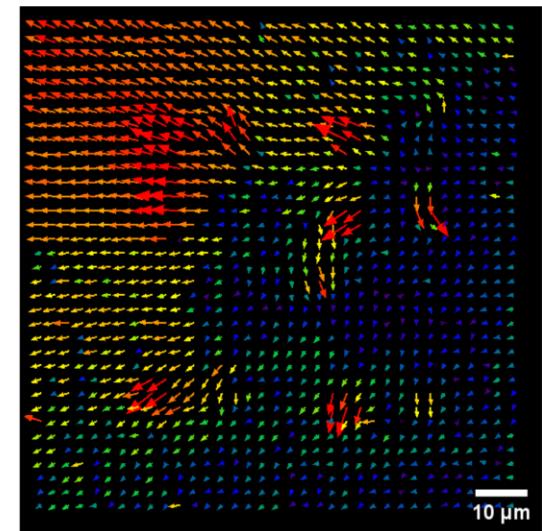
Full movie QUAD1 \rightarrow PIV $t_i/t_0, i \in [1-60]$



QUAD 1 16/REF



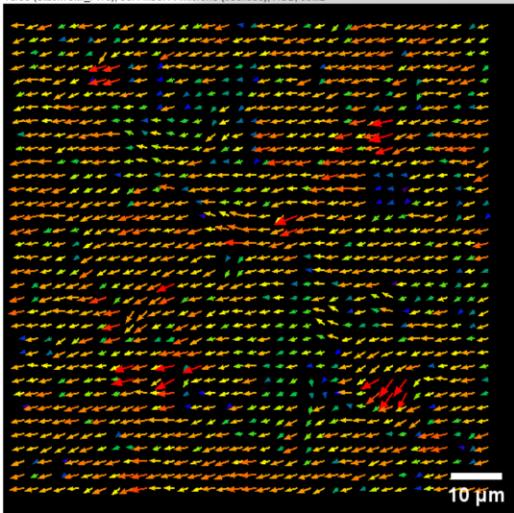
QUAD 1 37/REF



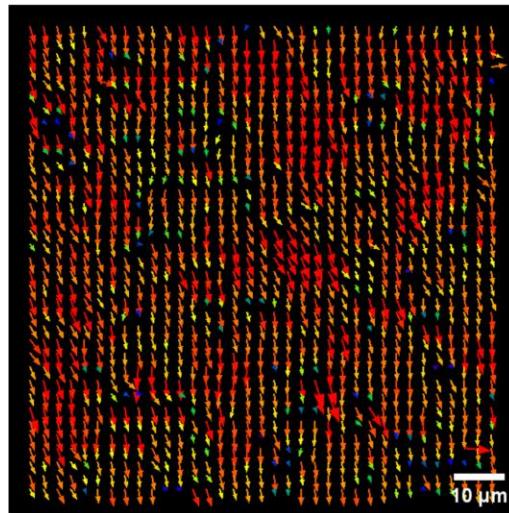
QUAD 1 56/REF

RESULTS & DISCUSSION

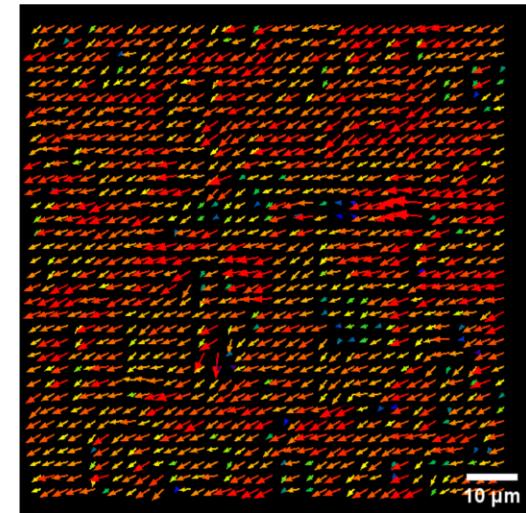
Full movie QUAD 1-> PIV n/n+1,



QUAD 1 17/18



QUAD 1 34/35



QUAD 1 49/50

CONCLUSION

CONCLUSION



Understand and read about the biology of cell adhesion from a mechanical POV.



Learn how to carry out the protocol for TFM and be able to retrieve cell tractions.



Implement our own automatization of the protocol.



Learn how to manage undesired and bad results and to be in a international team.

Thank You ! Merci ! Gracias !

LES
SHADOKS

