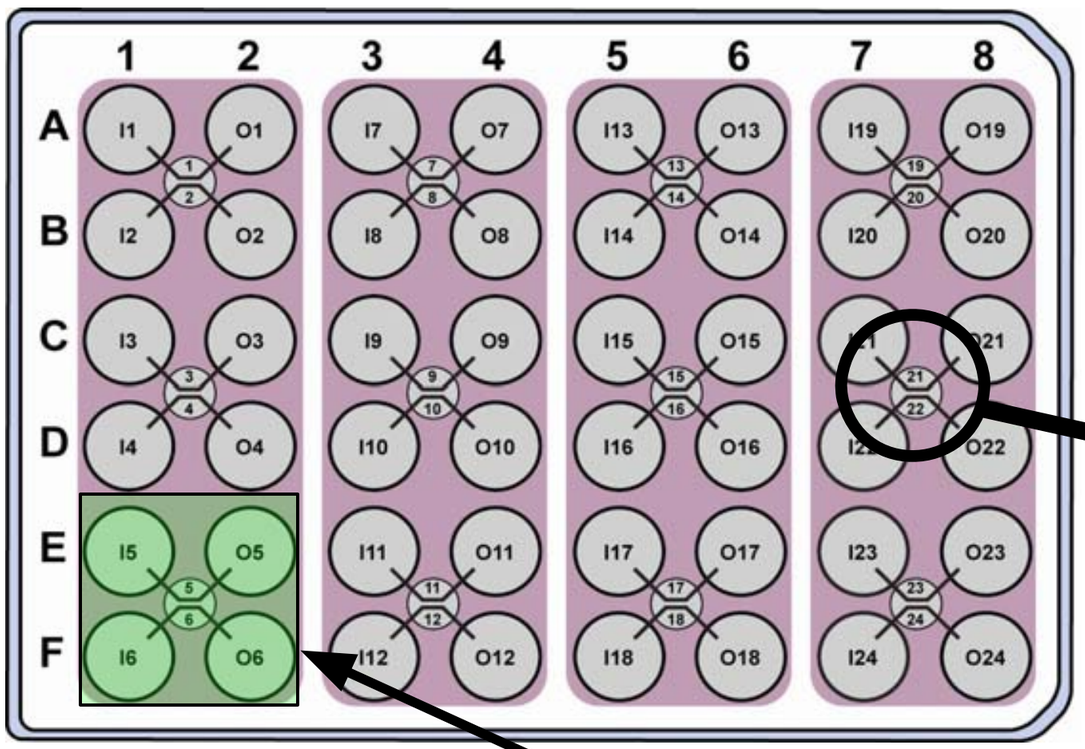
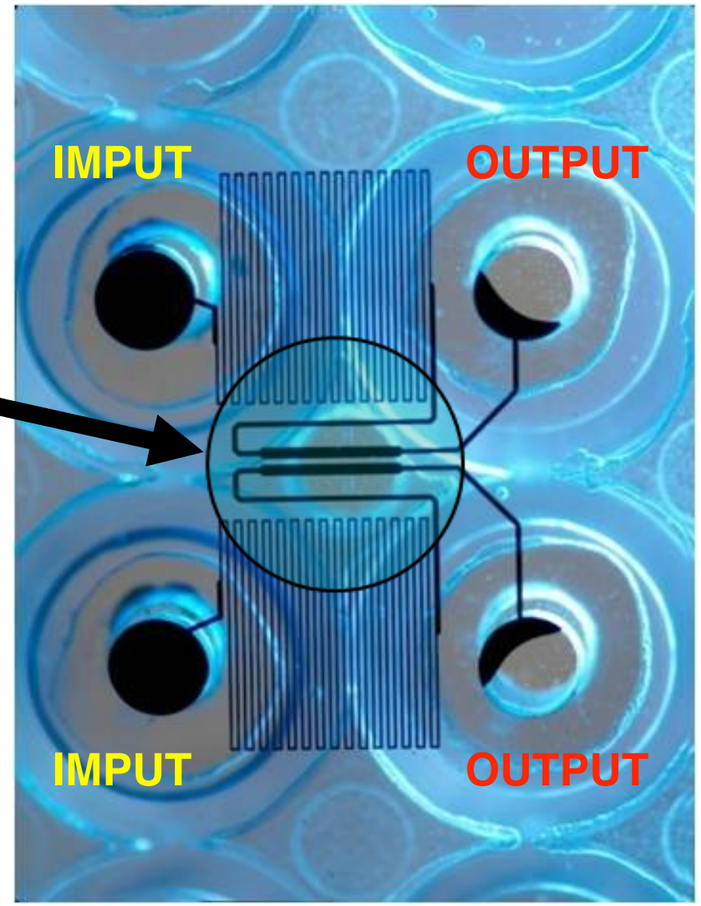
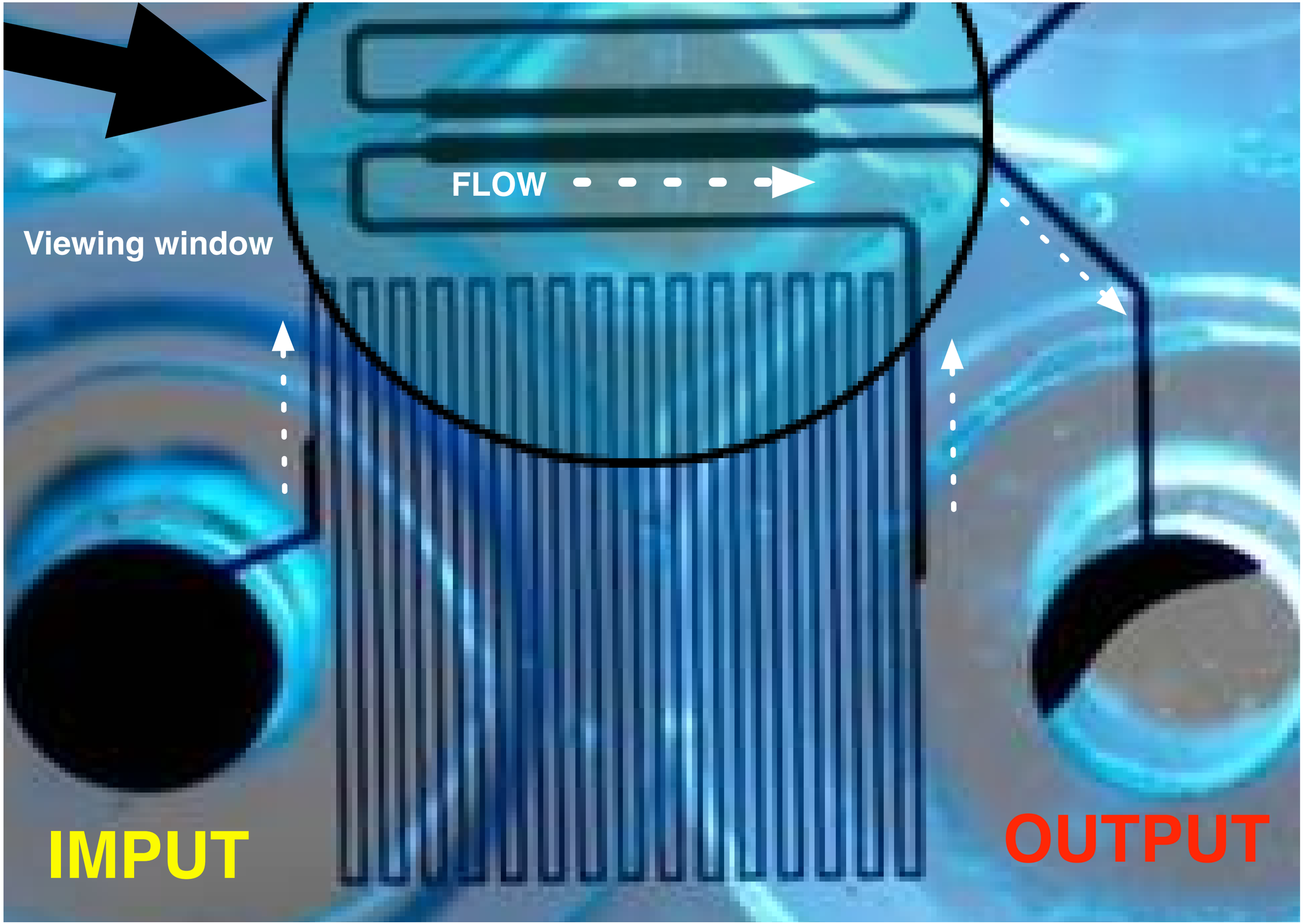
48-well Plate
24 flow channels
(one input well each)



Viewing window

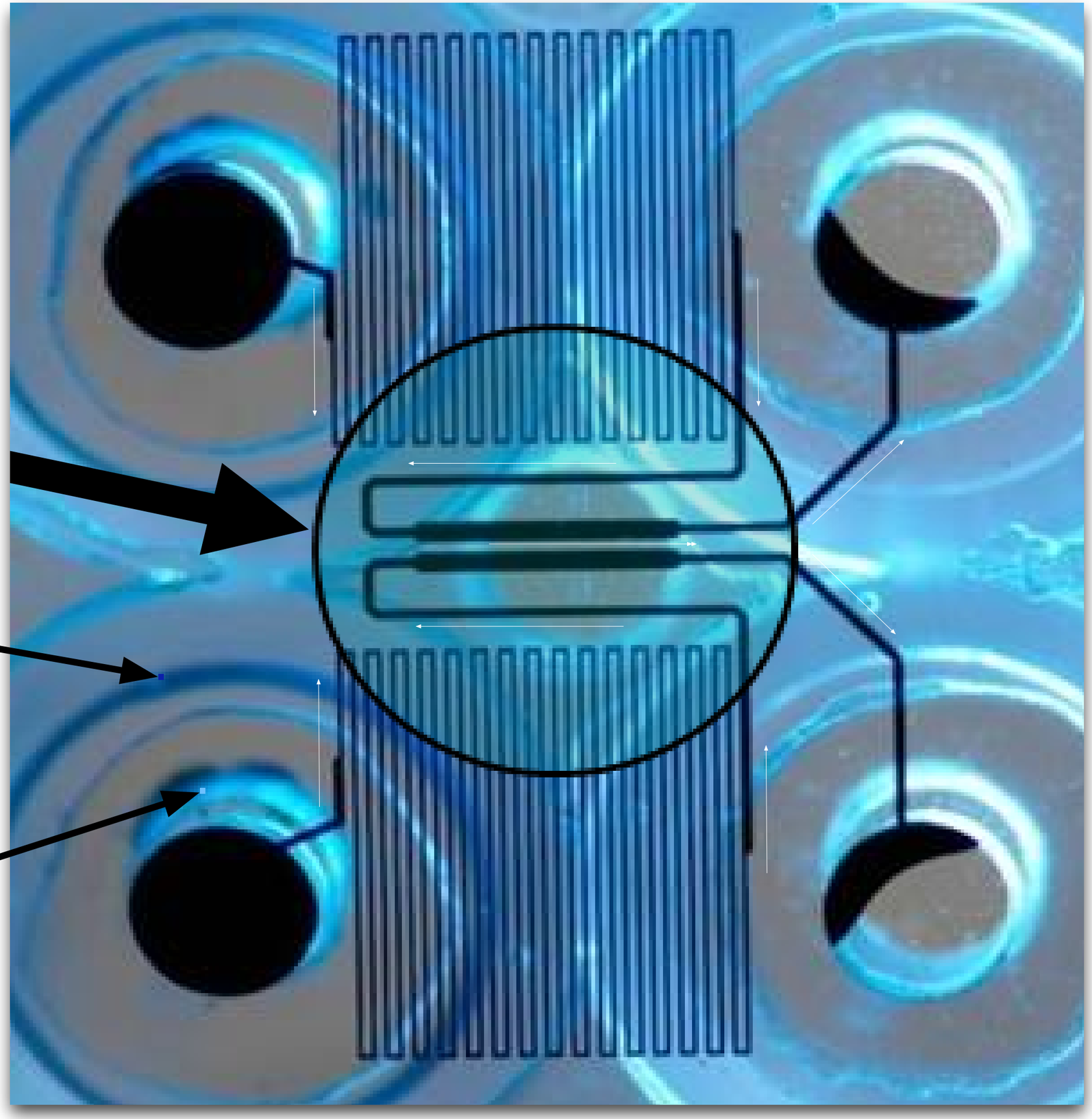


Gruppi di **4 pozzetti** (2 IMPUT e 2 OUTPUT)
per avere *due canali paralleli* visibili
nella stessa viewing window

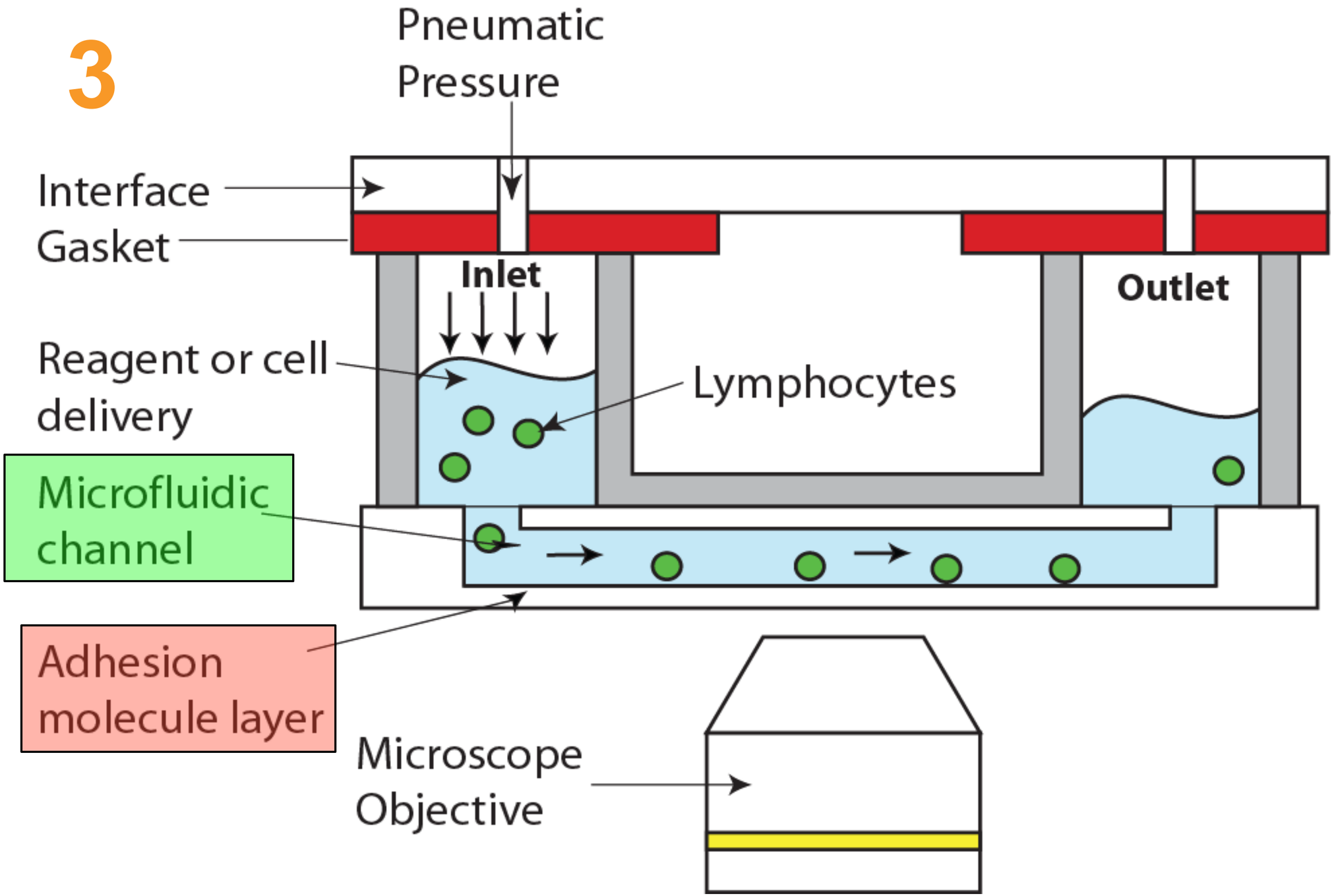

FLOW
Viewing window
IMPUT
OUTPUT

External well

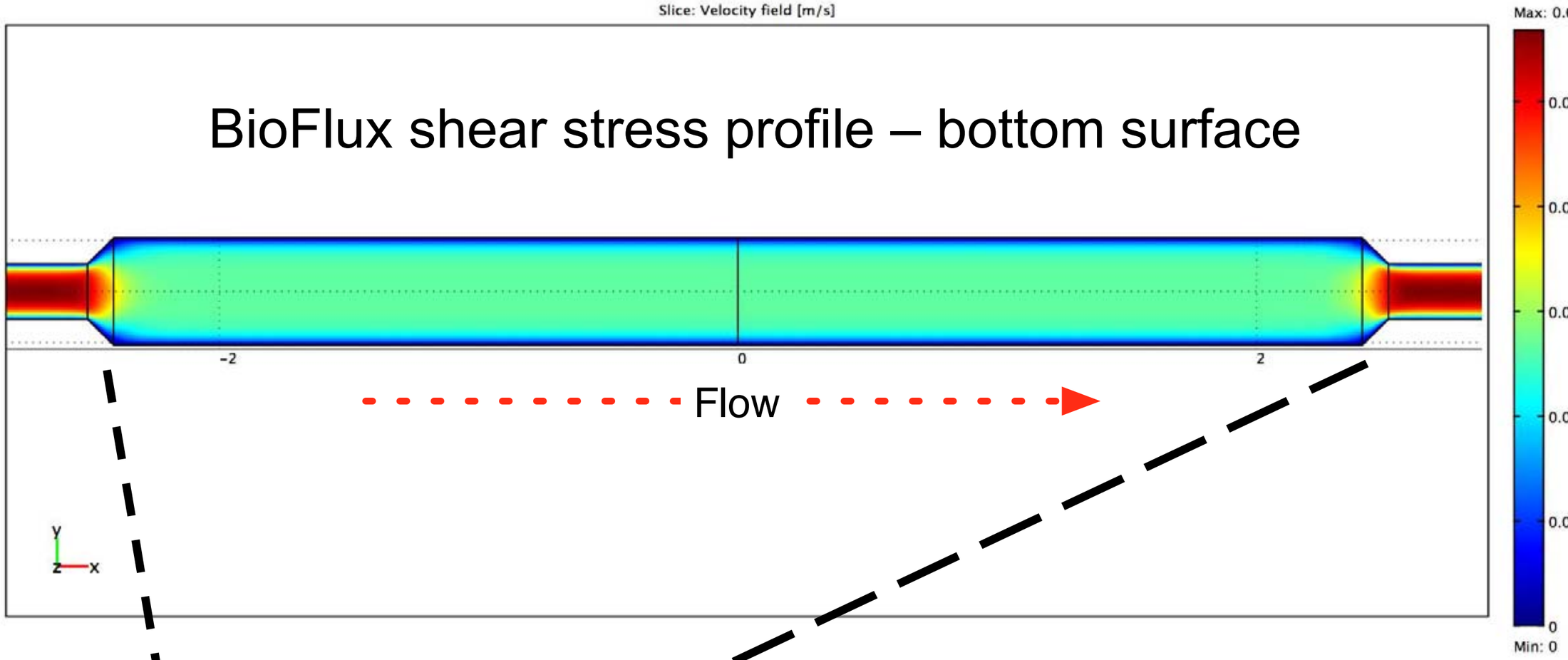
Internal well
(connected to microfluidics
and to be filled with cells)

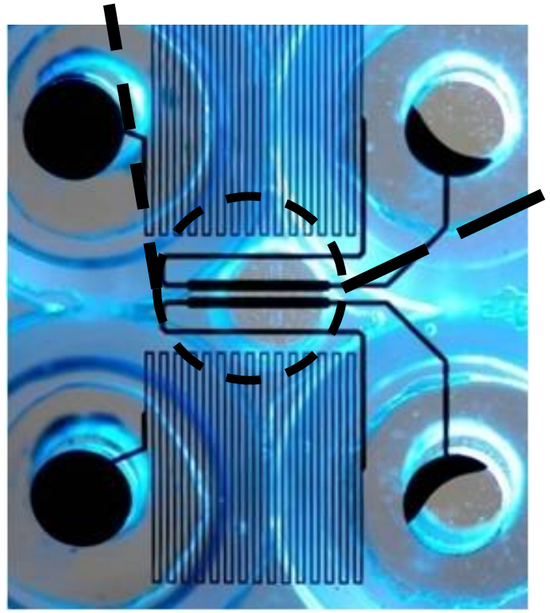


3


Pneumatic
Pressure
Interface
Gasket
Inlet
Outlet
Reagent or cell
delivery
Lymphocytes
Microfluidic
channel
Adhesion
molecule layer
Microscope
Objective

BioFlux shear stress profile – bottom surface





Uniform, laminar flow
within the region of interest

48 wells - 24 channels plate

IMPUT 1	OUTPUT 2	IMPUT 3	OUTPUT 4	IMPUT 5	OUTPUT 6	IMPUT 7	OUTPUT 8

A

B

Viewing windows 1-2

C

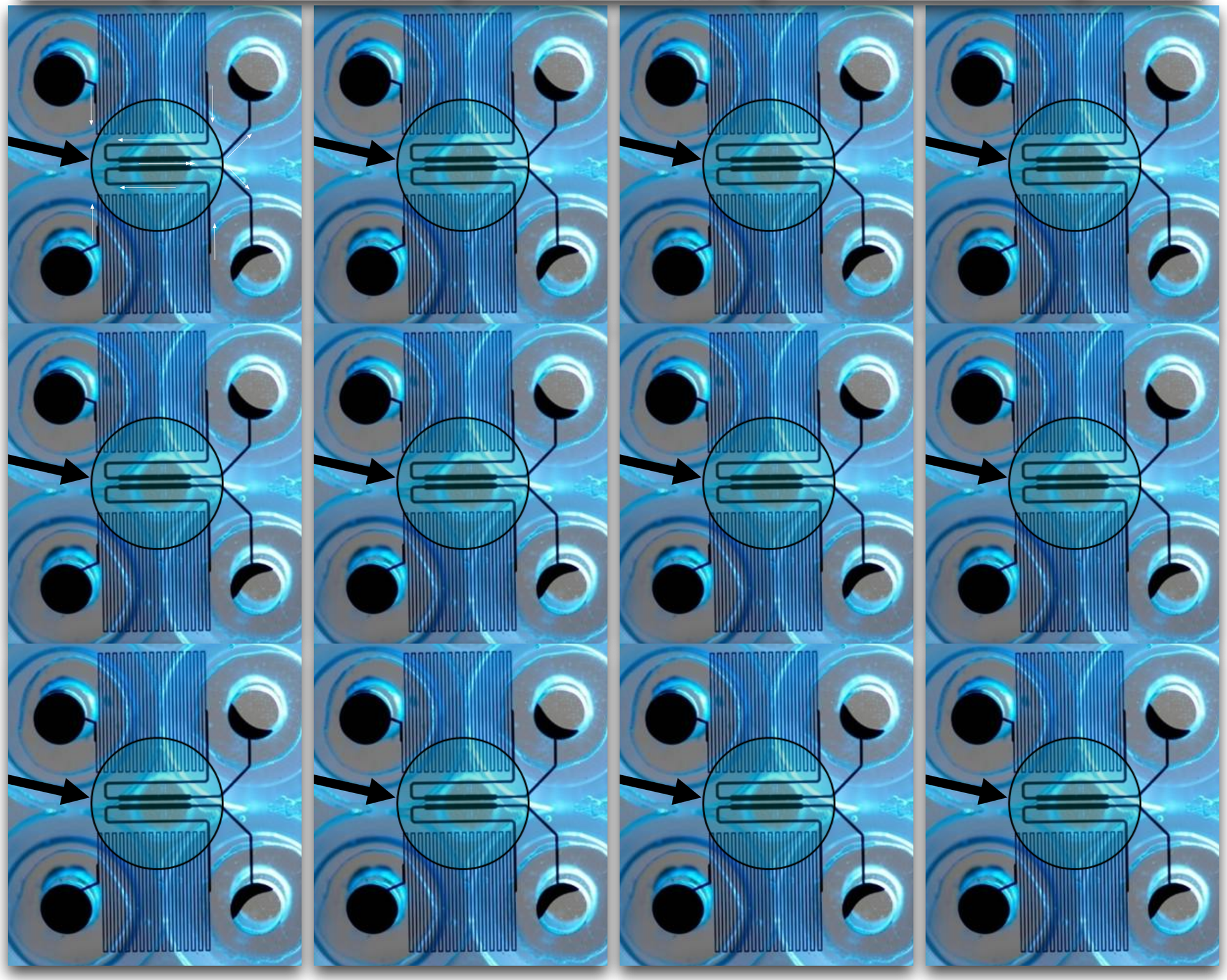
D

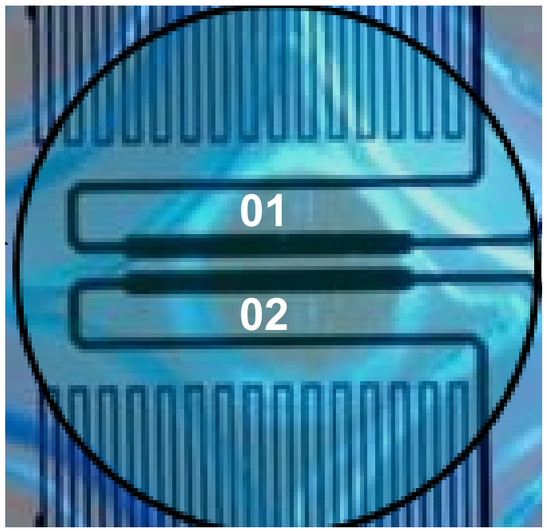
Viewing windows 3-4

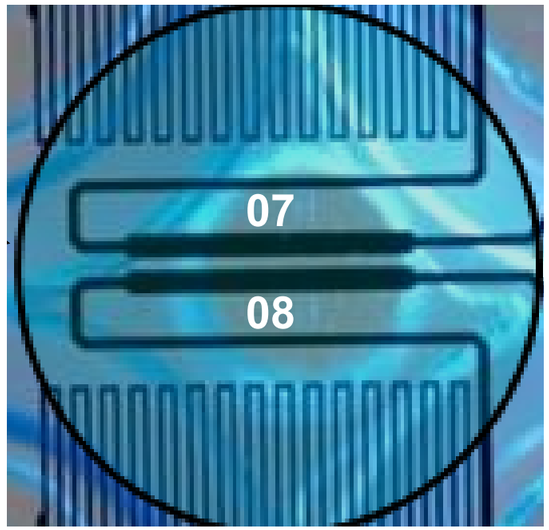
E

F

Viewing windows 5-6




01
02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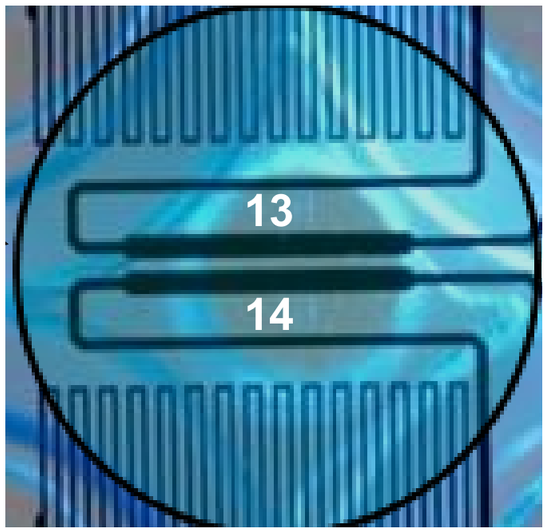
08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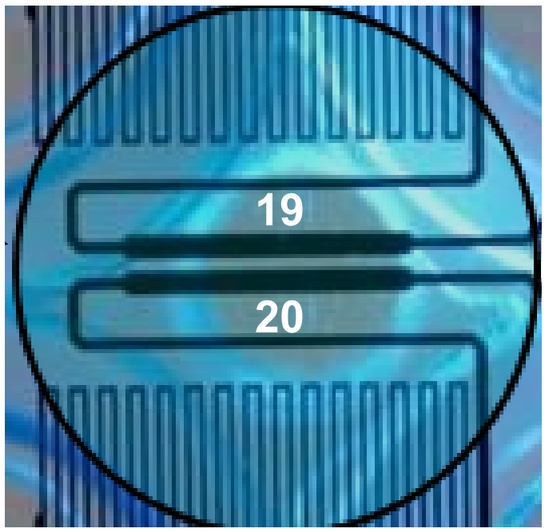
14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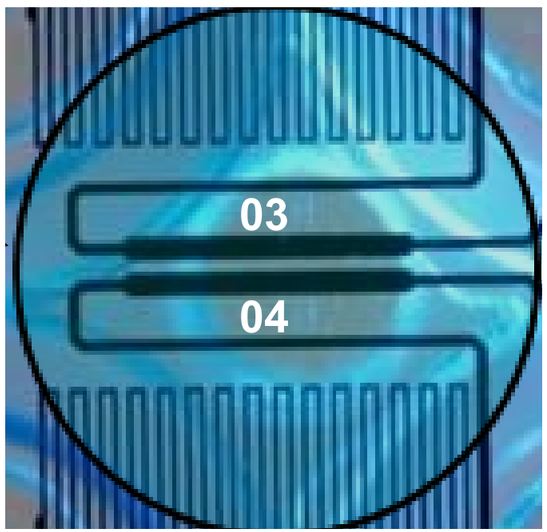
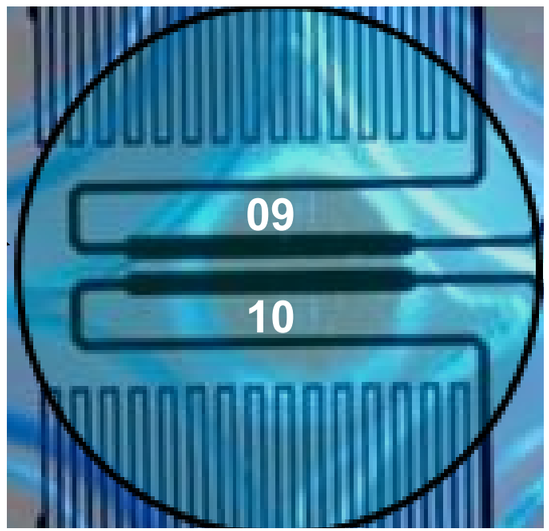

03
04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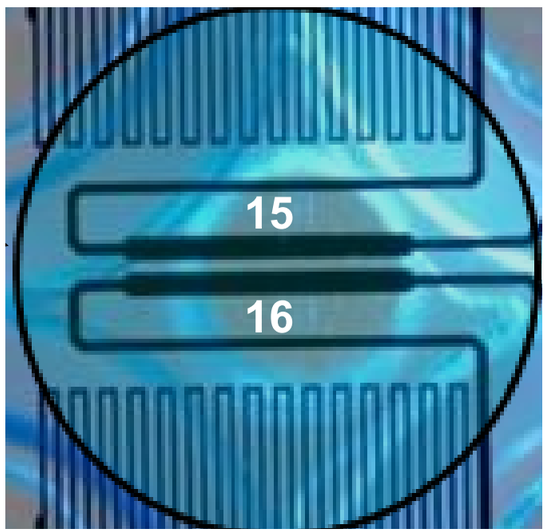
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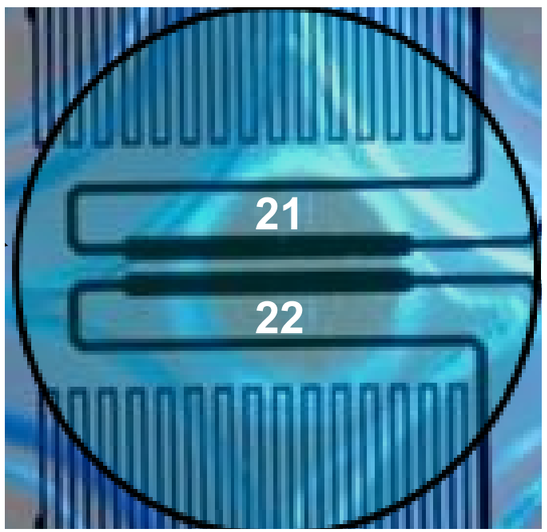
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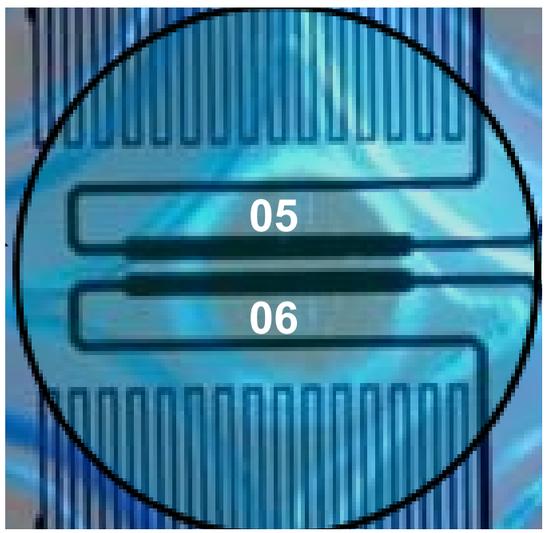
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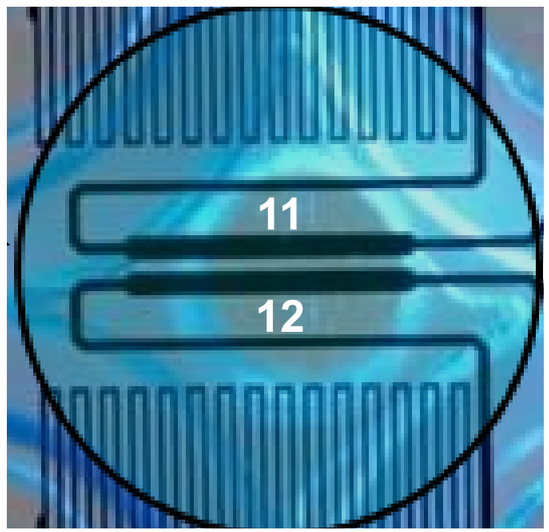
06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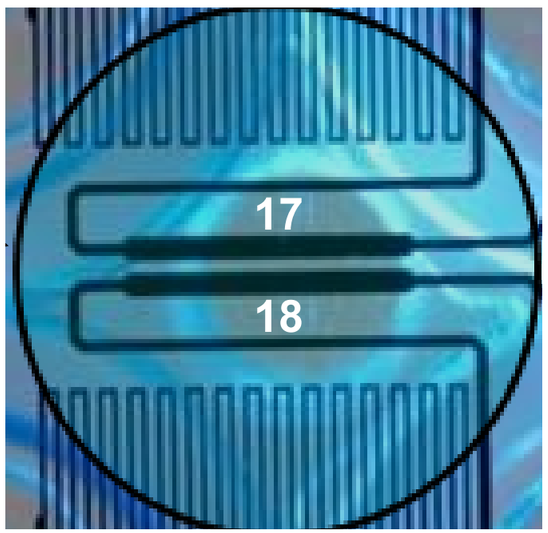
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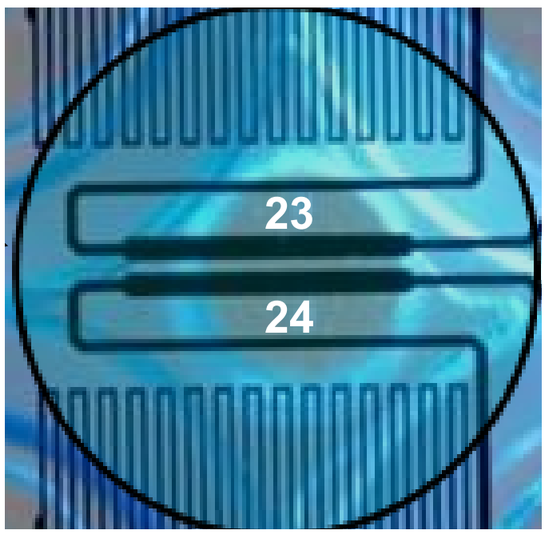
23
24

BioFlux experiment - standard setting

1- Channel coating molecules: E, P-Selectins or PNAD (1-2 microg/ml) in PBS; ICAM-1, VCAM-1, MadCAM1 (1-5 microg/ml) in PBS. Chemokines (1-5 microg/ml) in PBS.

2- Channel coating min. volumes: **10 microliters / channel**

3- Channel coating direction: E, P-Selectins, PNAD, ICAM-1, VCAM-1, MadCAM1 from **IMPUT to OUTPUT** channles at **0,75 dynes/cm^2**; chemokines from **OUTPUT to IMPUT** channles at **0,5 dynes/cm^2**,

or

E, P-Selectins, PNAD, ICAM-1, VCAM-1, MadCAM1, chemokines from **OUTPUT to IMPUT** channles at **0,5 dynes/cm^2;**

4- Channel coating time: E, P-Selectins, PNAD, ICAM-1, VCAM-1, MadCAM1 = 1 hour to overnight at RT; chemokines = 10 min. to 1 hour RT.

5- Channel washing direction: with PBS from **IMPUT to OUTPUT** or from **OUTPUT to IMPUT** (depending on the coating)

6- **Cells**: **10^6 / ml** in adhesion buffer; RT or 37^{o}C ∓ pre-treatments; 60000 cells / condition (in 60 microliters max);
10^6 cells = 16 experimental conditions max.

7- Wells: **internal well** filled with 10 microliters (min) to 60 microliters (max)

8- At **2 dynes/cm^2**: 10 microliters = 3 min. flux; 60 microliters = 18 min. flux (fluxing time is linear with volume)

9- Cells fluxing: **always** from **IMPUT to OUTPUT** wells (time is depending of the vol., see point (8))

10- Recording: HD-DV, 1 file / condition **or** 1 file / all conditions in parallel (record the ref. numbers printed on the channels)

BioFlux experiment - standard flow-chart