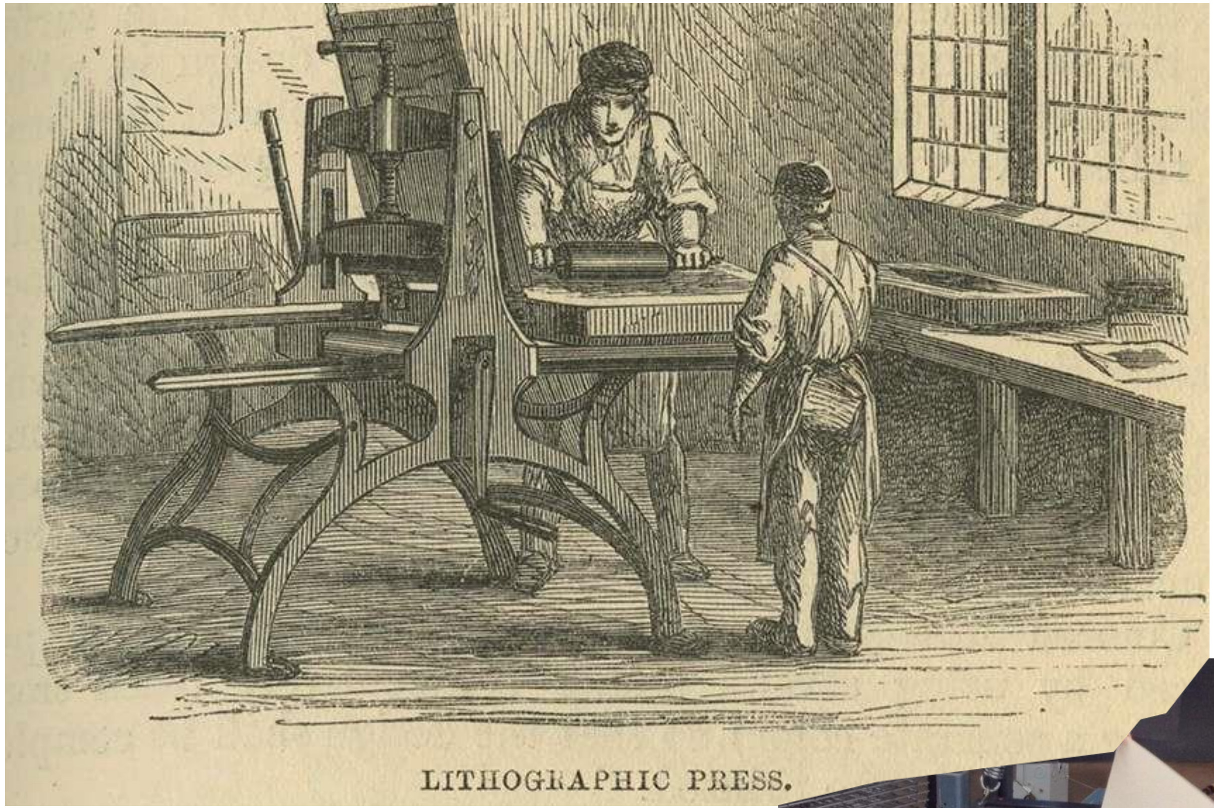
IFN
Istituto di Fotonica e Nanotecnologie - Institute for Photonics and Nanotechnologies
Consiglio Nazionale delle Ricerche
National Research Council

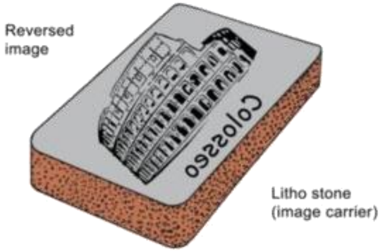
Immune system on chip: Modelling and measuring the IS under the microscope.

Francesca Romana Bertani, Luca Businaro,
Adele De Ninno, Annamaria Gerardino

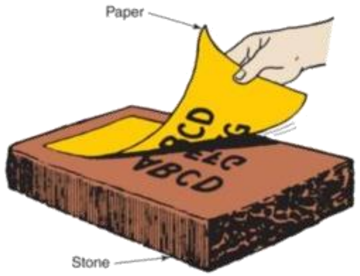
IFN Institute for Photonics and Nanotechnologies
National Research Council

In the beginning we were Lithographers





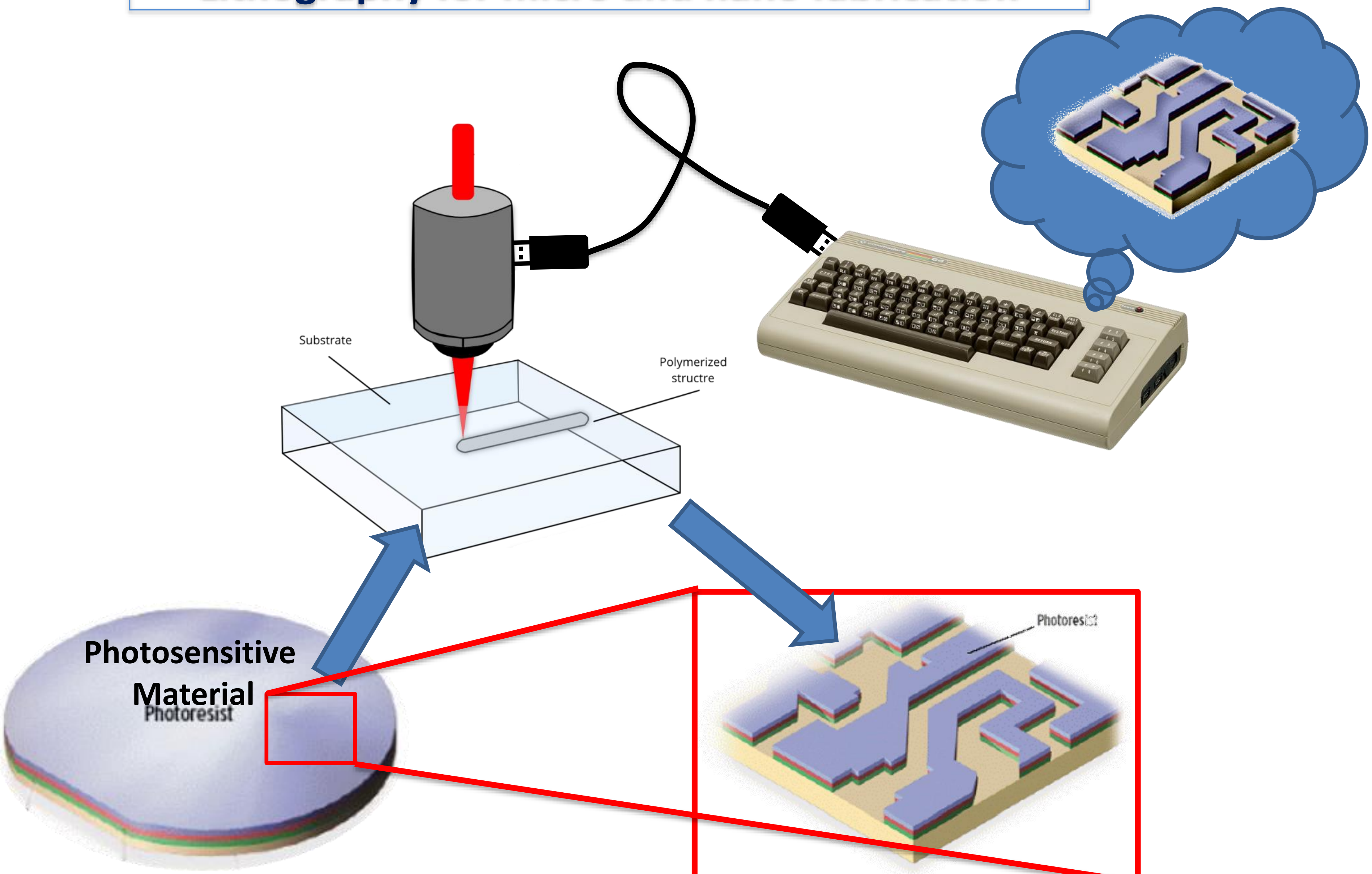
The image is drawn on slab of porous limestone with a greasy crayon.



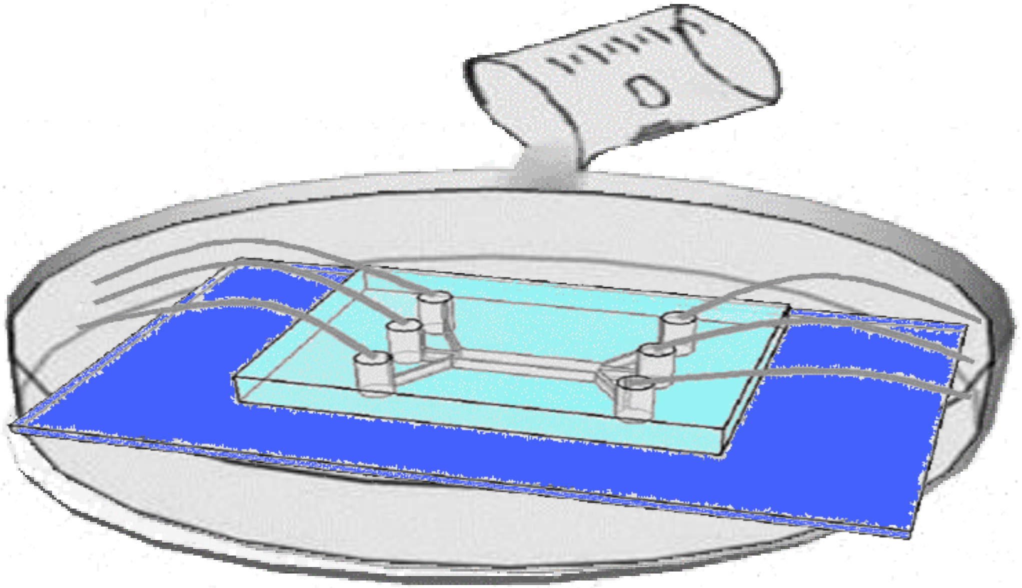
Words lettered in reverse on lithographic stones are readable on the printed sheets

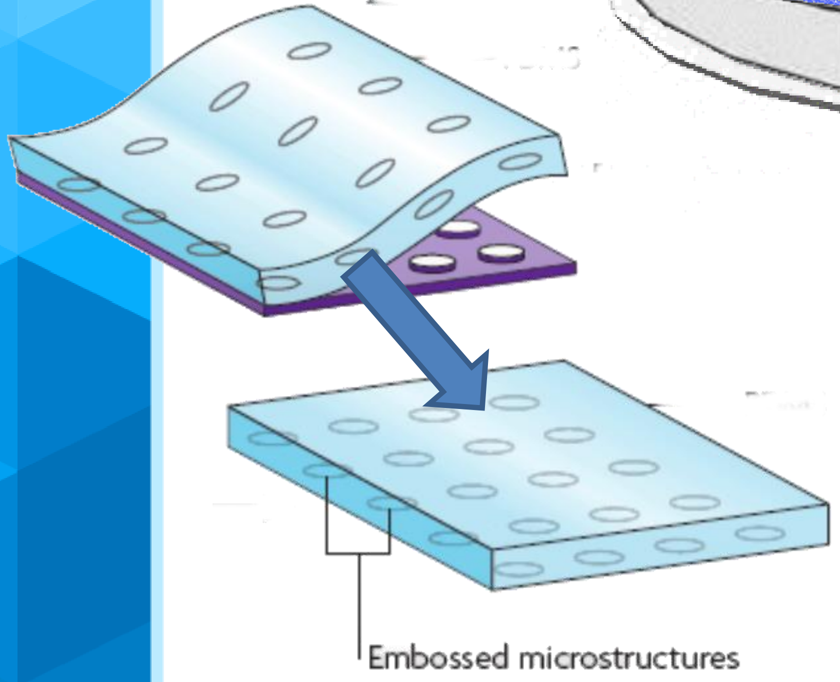
IFN Institute for Photonics and Nanotechnologies
National Research Council

Lithography for micro and nano fabrication



Lithography for Microfluidics

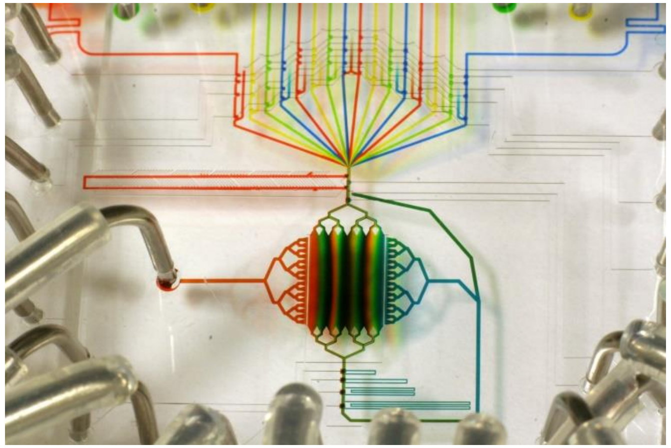


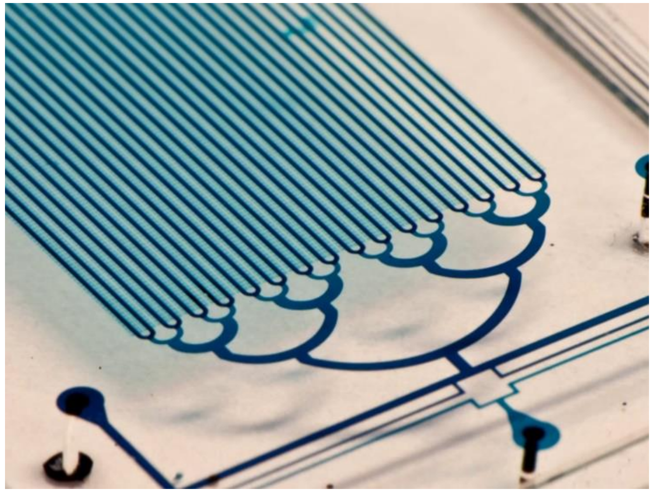


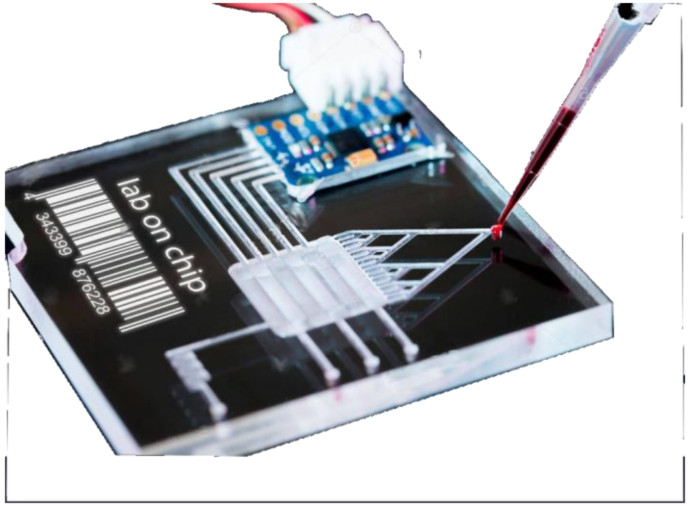
Lithography for Microfluidics

Microfluidics deals with the **behaviour**, **precise control** and **manipulation** of fluids that are geometrically constrained to a small, typically **sub-millimeter, scale**.
Typically, micro means one of the following features:

- small volumes (μL, nL, pL, fL)
- small size
- low energy consumption
- effects of the microdomain

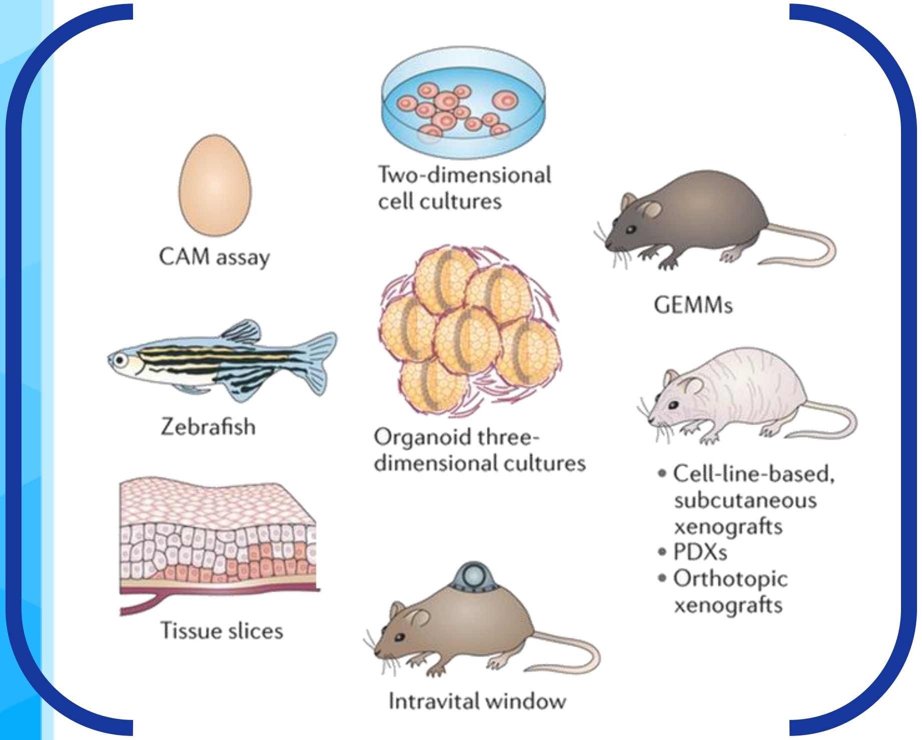




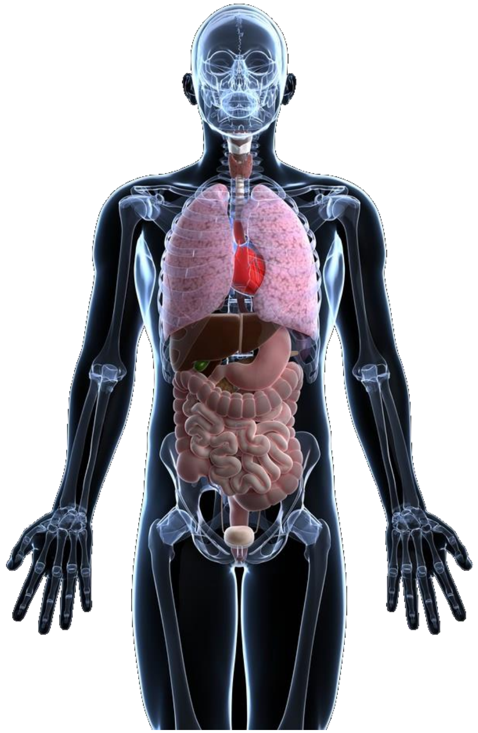


IFN Institute for Photonics and Nanotechnologies
National Research Council

OOC: a way to include human IS specificity



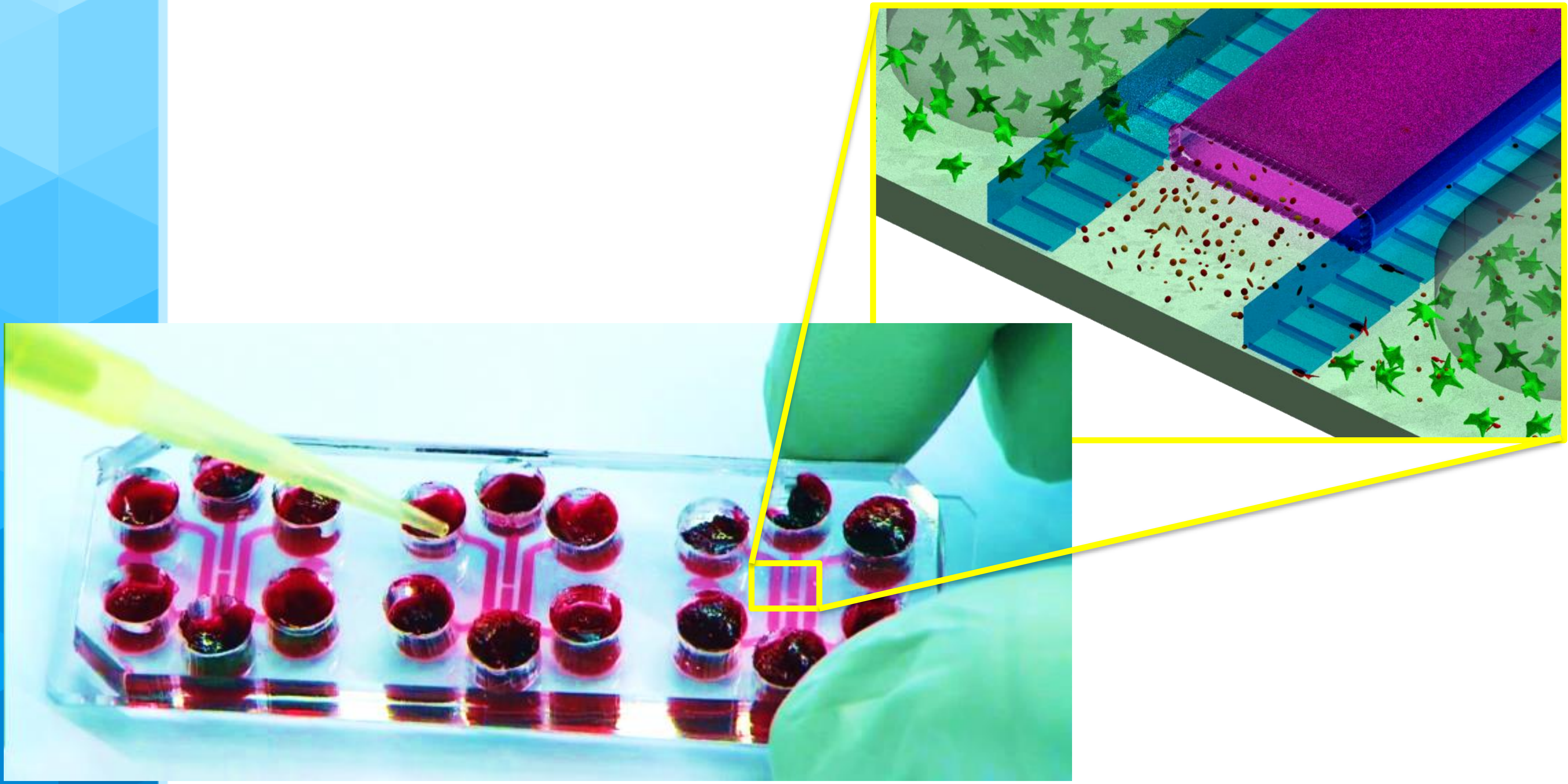
≠



"You have to remember
that a worm,
with very few exceptions,
is not a human being"
Prof. F. Frankenstein

IFN Institute for Photonics and Nanotechnologies
National Research Council

Microfluidcs for Organs-On-Chip & Micro Physiological Systems



IFN Institute for Photonics and Nanotechnologies
National Research Council

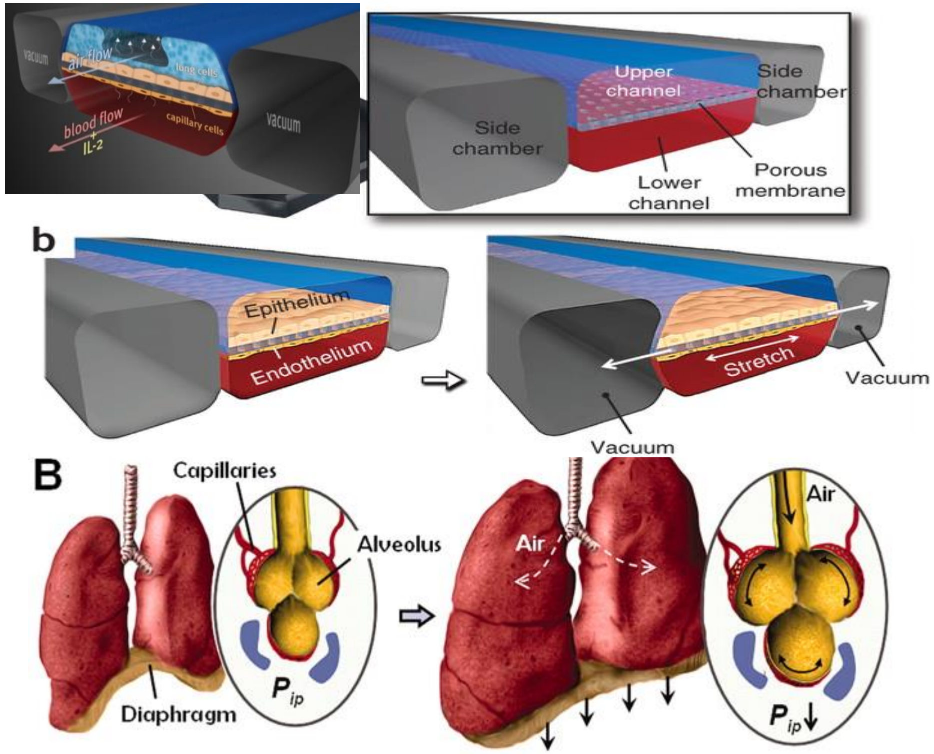
Definitions

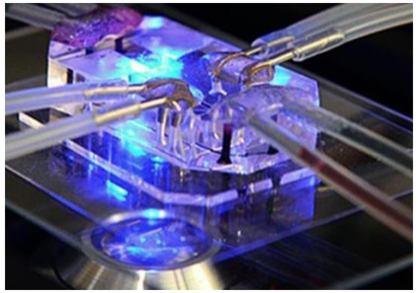
Microfluidic devices lined with living human cells for drug development, disease modeling, and personalized medicine

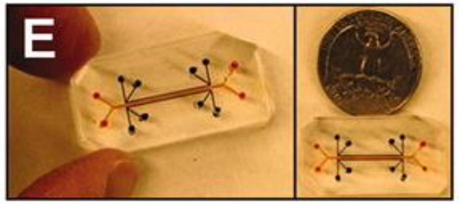
An Organ-on-Chip (OoC) is a fit for purpose fabricated microfluidic-based device, containing living engineered organ substructures in a controlled micro- or nanoenvironment, that recapitulate one or more aspects of the dynamics, functionality and (patho)physiological response of an organ in vivo, in real-time monitoring mode

OoC can be classified into 2 distinct types

- **Single-organ systems**: emulating key functions of single tissues or organs







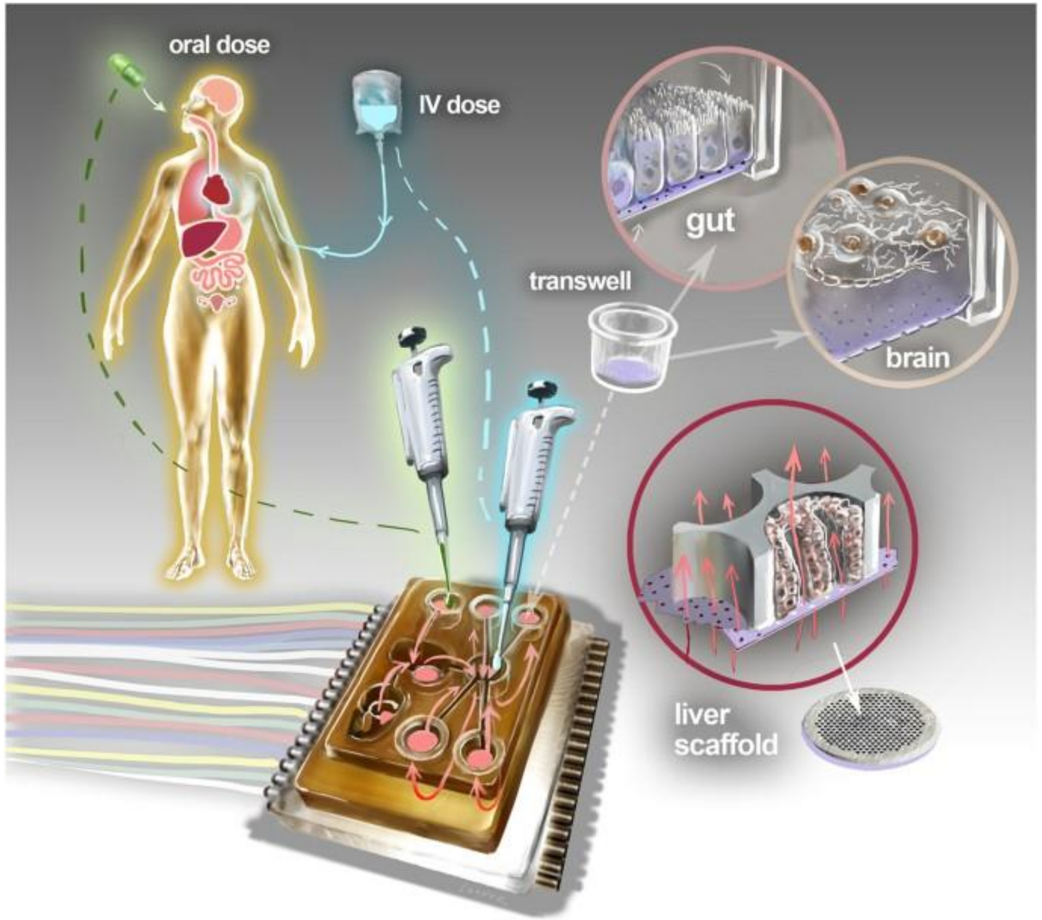
Reconstituting Organ-Level Lung Functions on a Chip

Huh D, Matthews BD, Mammoto A, Montoya-Zavala M, Hsin HY, Ingber DE. *Science 328, 2010.*

OoC classification

OoC can be classified into 2 distinct types

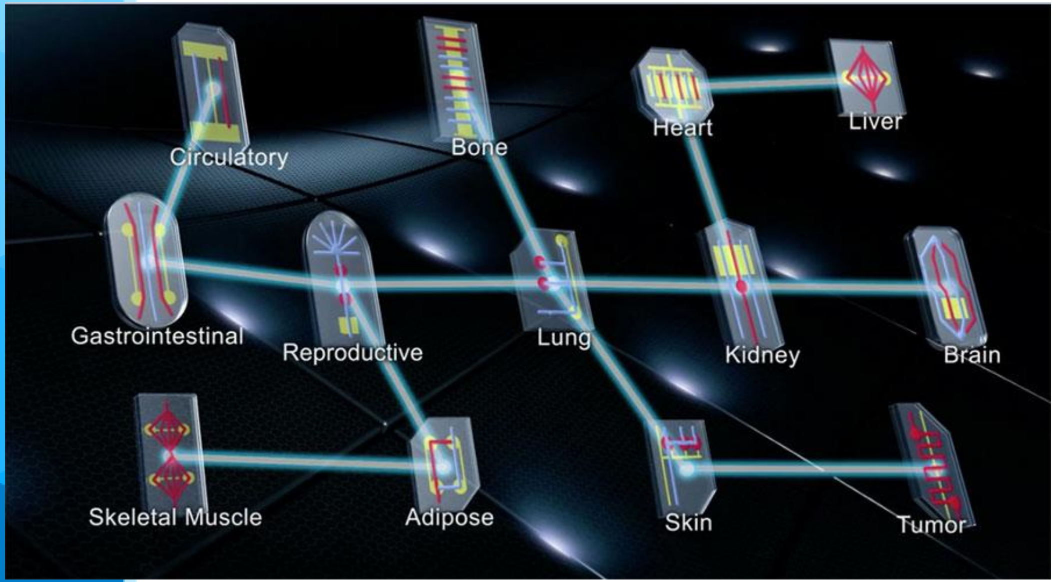
- **Multi-organ systems**: combining multiple organ/OoC to reproduce the systemic interactions that occur *in vivo*.



IFN Institute for Photonics and Nanotechnologies
National Research Council

Science Fiction? Look at Tissue Chip Project

NIH National Center for Advancing Translational Sciences



Start:	2012
Status	active
funding	>100M$
Partners	11
Approach	Annual awards
subprojects	>60

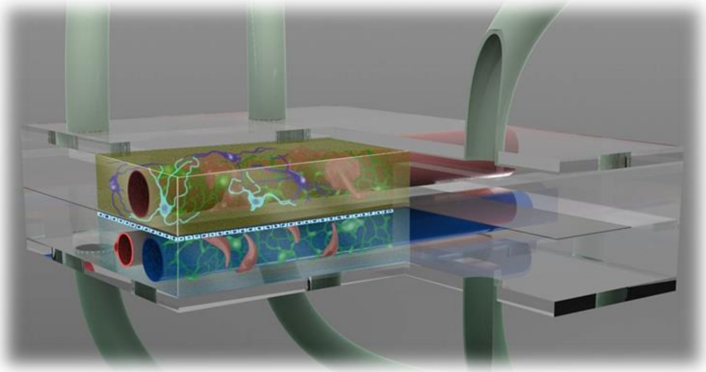
https://www.youtube.com/watch?v=zVlEr8c-OJk&feature=youtu.be

US scenario: Meet the chips

- Meet Chip: Brain
- Meet Chip: Heart
- Meet Chip: Muscle
- Meet Chip: Lungs
- Meet Chip: Liver
- Meet Chip: Kidneys
- Meet Chip: Gastrointestinal System
- Meet Chip: Female Reproductive System
- Meet Chip: Blood Vessels
- Meet Chip: Fat (Adipose)
- Meet Chip: Skin
- Meet Chip: Disease Models

https://ncats.nih.gov/tissuechip/chip

Tiny Organs in Orbit



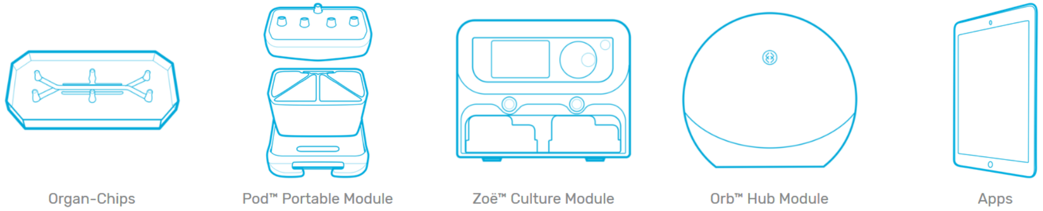
Concept of Planar BBB Neuro Vascular Unit Chip (Vanderbilt)

IFN
Institute for Photonics and Nanotechnologies
National Research Council

US scenario: Companies

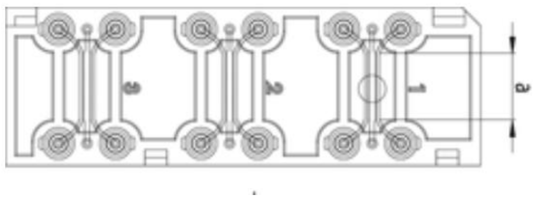
A technological echosystem

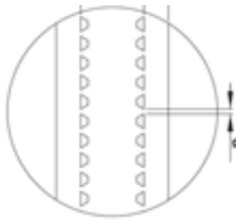
Emulate Bio



https://vimeo.com/398591225

AIM BIOTECH
ADVANCED • INTEGRATED • MICROFLUIDICS



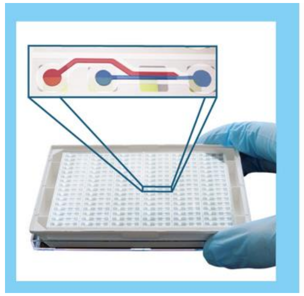


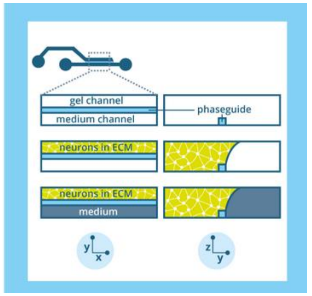
https://www.aimbiotech.com/videos.html

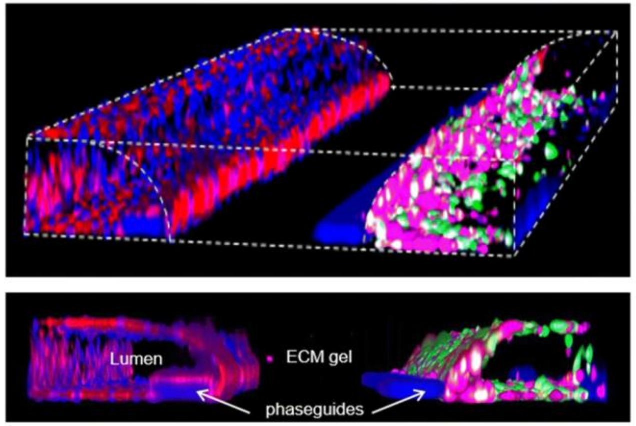
IFN Institute for Photonics and Nanotechnologies
National Research Council

EU Scenario - Companies

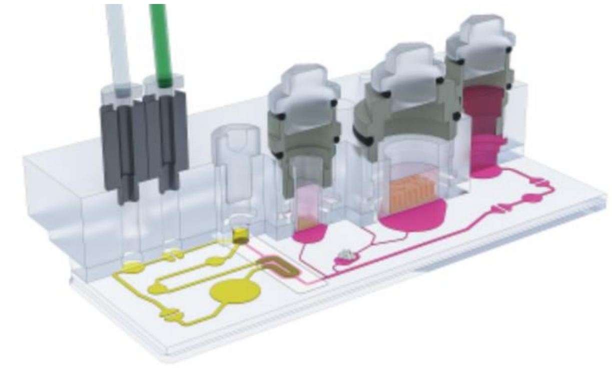
MIMETAS
the organ-on-a-chip company







TISSUSE
Emulating Human Biology



Organs on chip companies

Body on-a-Chip

Company	Scientific founders	Selected products
Hesperos®	Michael Shuler James Hickman	Multi-Organ Chip (2, 4 organs) (5-10 organs)*
TISSUSE Emulating Human Biology	Uwe Marx	2-Organ-Chip (2-OC) 4-Organ-Chip (4-OC) Human-on-a-Chip (HoC)*
CNBio innovations	Linda G Griffith	LiverChip® LiverChip® 36
DRAPER	Joseph Charest	Microphysiological Systems

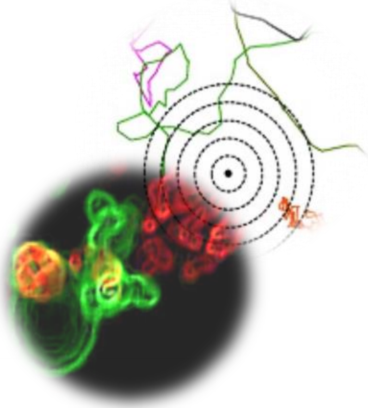
Tissue interface on-a-Chip

Company	Scientific founders	Selected products
emulate	Donald Ingber	Lung on-a-Chip Airway on-a-Chip Gut on-a-Chip Kidney on-a-Chip Bone Marrow on-a-Chip
AlveoliX In-vitro models inspired by nature	Olivier Guenat	Lung-on-a-chip array
NORTIS	Thomas Neumann	Kidney on-a-Chip Vessel on-a-Chip
Quorum	Axel Guenther	Artery on-a-Chip
MIMETAS the organ-on-a-chip company	Jos Joore Paul Vulto Thomas Hankemeier	OrganoPlates®
SynVivo	Kapil Pant B. Prabhakar Pandian	SynTumor SynBBB SynRAM SynTox
4DBio 4Design Biosciences	G. Wesley Hatfield Christopher Hughes Steven George Abraham Lee	Vascularized micro-organ (VMO) platform
AIM Biotech	Roger Kamm	3D cell culture chips

Parenchymal tissue on-a-Chip

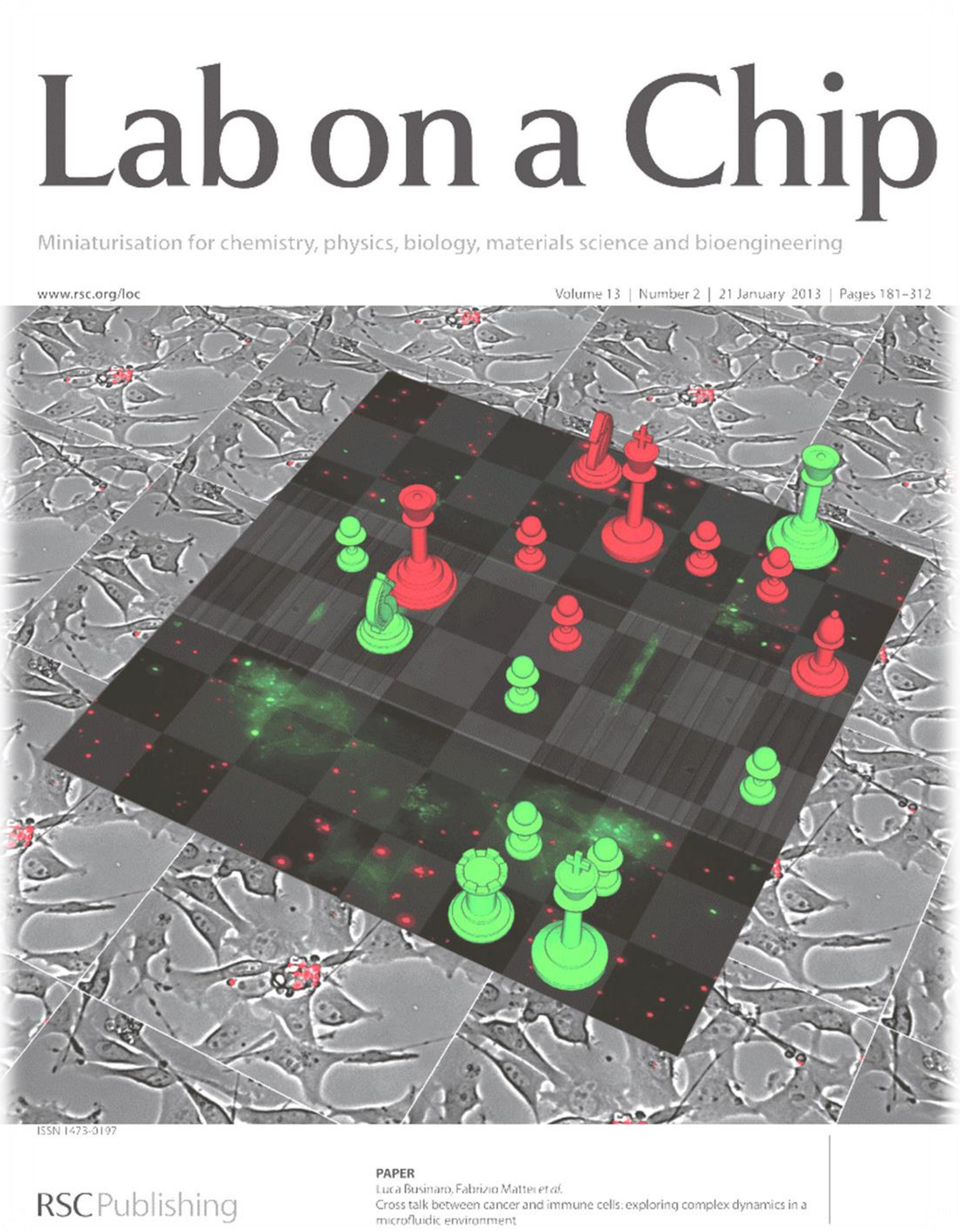
Company	Scientific founders	Selected products
Hepregen	Sangeeta Bhatia	HepatoPac® HepatoMune™
organovo™	Gabor Forgacs Keith Murphy	ExVive3D™ Liver ExVive3D™ Kidney*
Aspect biosystems	Tamer Mohamed Konrad Walus Sam Wadsworth Simon Beyer	Lab-on-a-Printer™ 3DBioRing™ Airway
insphero	Jan Lichtenberg Jens M. Kelm Wolfgang Moritz	3D Insight™ Liver 3D Insight™ Islet 3D Insight™ Tumor
3D Biomatrix™ Three-Dimensional Cell Culture	Nicholas Kotov	PERFECTA3D® HANGING DROP PLATES
HµREL Corporation	Greg Baxter Robert Freedman	HµRELhuman™ HµRELflux™ HµRELTox™ HµRELflow™
KIYATEC®	Matthew R. Gevaert	3DKUBE™
VAXDESIGN	William L. Warren	MIMIC® Technology
TARA	Milica Radisic Gordana Vunjak-Novakovic	Cardiac Biowire™ II AngioChip*
µOrgano	Kevin Healy	µOrgano
EHT Technologies	Thomas Eschenhagen	Engineered Heart Tissue (EHT)
myriamed	Wolfram-Hubertus Zimmermann	3D Cardiac Systems
AxoSim	Michael Moore	Nerve-on-a-Chip™
xona microfluidics	Noo Li Jeon Carl W. Cotman Anne Taylor	Standard / Triple Chamber Neuron Device
MicroBrain BT	Bernadette Bung	Neuronal Diode
ananda™	Margaret Magdesian	Neuro Device

Microfluidics for Immuno-Oncology Recreating and Measuring cells battle in the tumor ecosystem



Istituto di Fotonica e Nanotecnologie - Institute for Photonics and Nanotechnologies

Consiglio Nazionale delle Ricerche
National Research Council


Lab on a Chip

Miniaturisation for chemistry, physics, biology, materials science and bioengineering

www.rsc.org/loc

Volume 13 | Number 2 | 21 January 2013 | Pages 181–312

ISSN 1473-0197

RSC Publishing

PAPER
Luca Businaro, Fabrizio Mattei *et al.*
Cross talk between cancer and immune cells: exploring complex dynamics in a microfluidic environment

IFN Institute for Photonics and Nanotechnologies
National Research Council

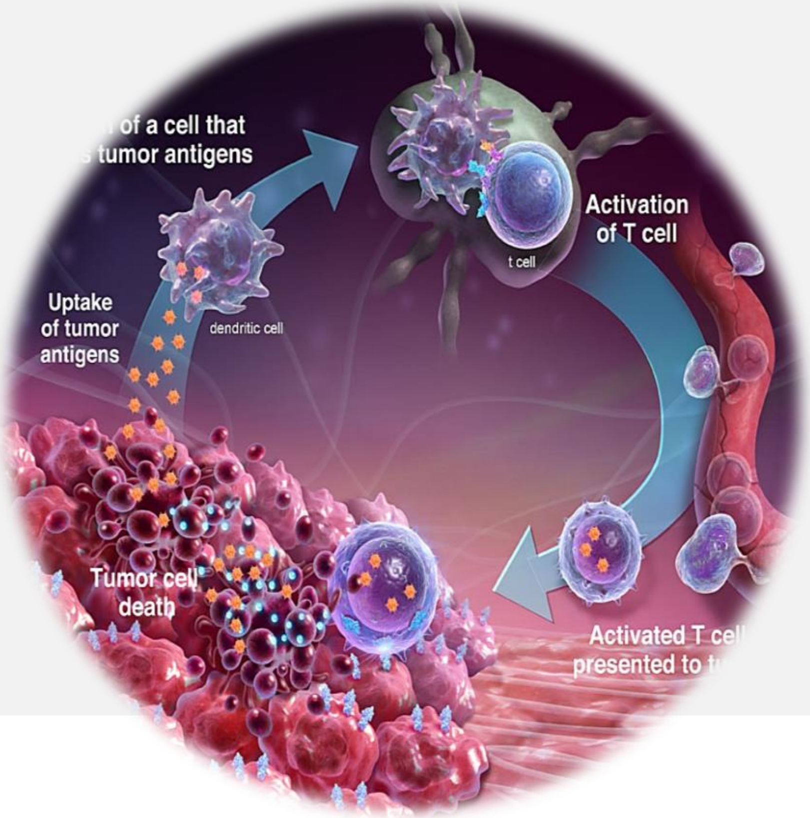
OUTLINE

- **Chemotherapy-induce immune response and genetic mutations**

- Drug-resistance tumor heterogeneous microenvironments

- Immunotherapeutic strategies

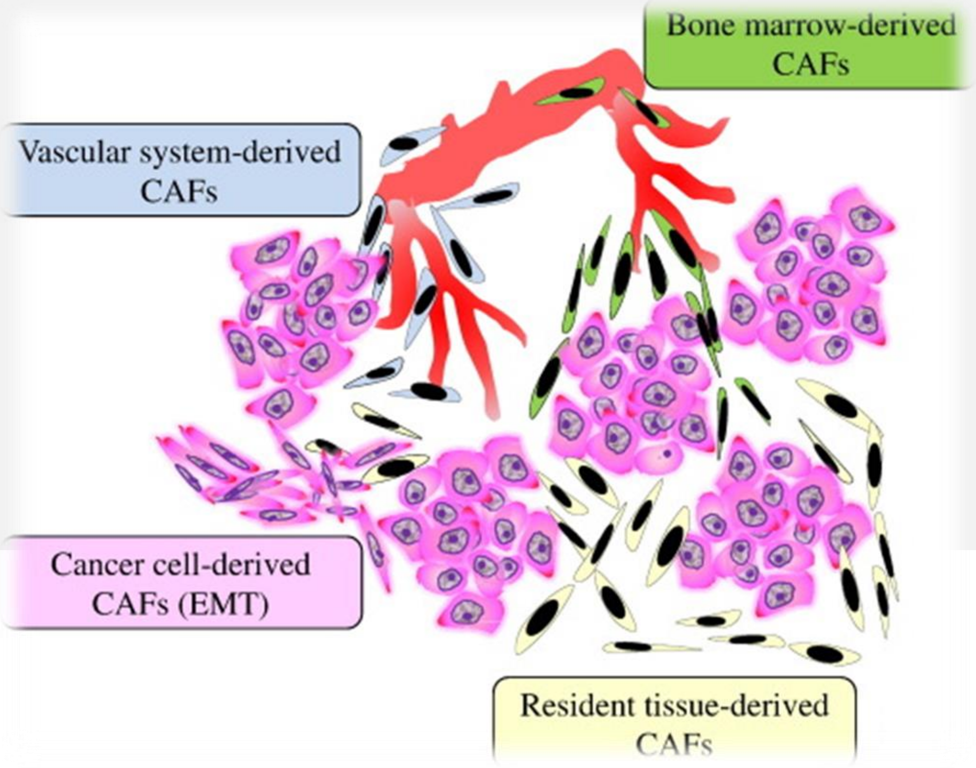
- Mechanism of Tumor immune escape



Vacchelli et al., Science 2015

OUTLINE

- Chemotherapy-induce immune response and genetic mutations
- **Drug-resistance tumor heterogeneous microenvironments**
- Immunotherapeutic strategies
- Mechanism of Tumor immune escape

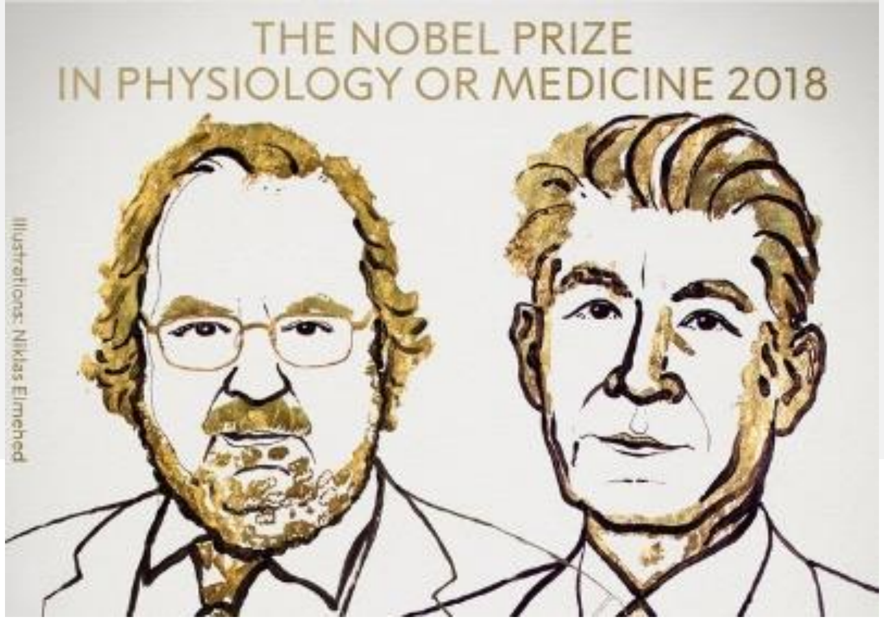


Nguyen, De Ninno A et al., Cell Reports 2018

IFN
Institute for Photonics and Nanotechnologies
National Research Council

OUTLINE

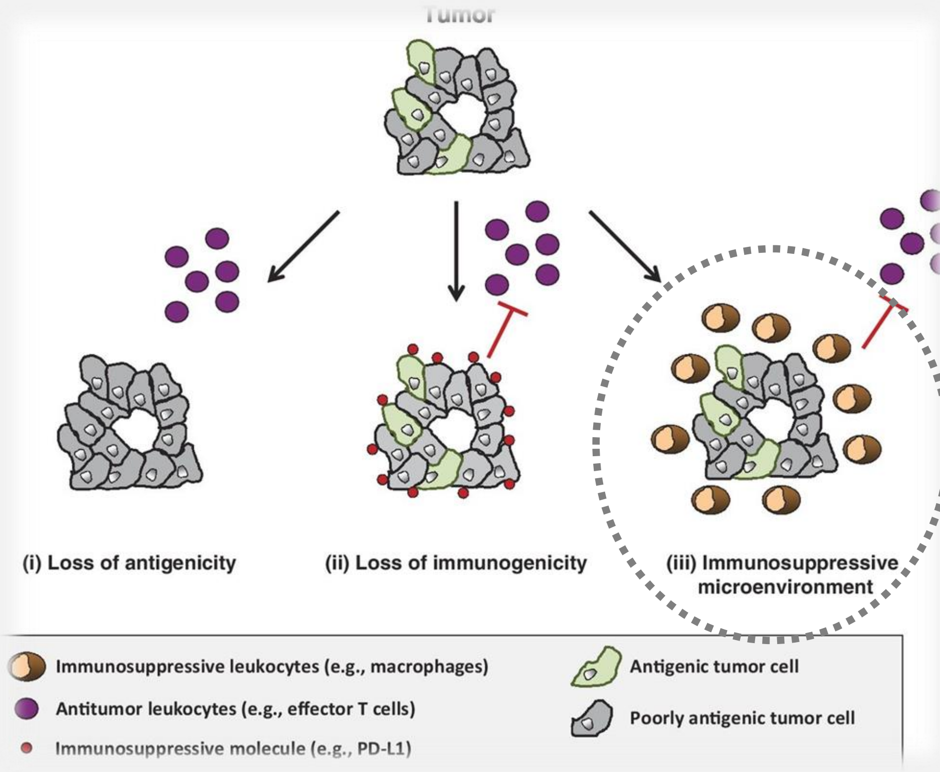
- Chemotherapy-induce immune response and genetic mutations
- Drug-resistance tumor heterogeneous microenvironments
- **Immunotherapeutic strategies**
- Mechanism of Tumor immune escape



Parlato et al., Sc Reports 2017

OUTLINE

- Chemotherapy-induce immune response and genetic mutations
- Drug-resistance tumor heterogeneous microenvironments
- Immunotherapeutic strategies
- **Mechanism of Tumor immune escape**

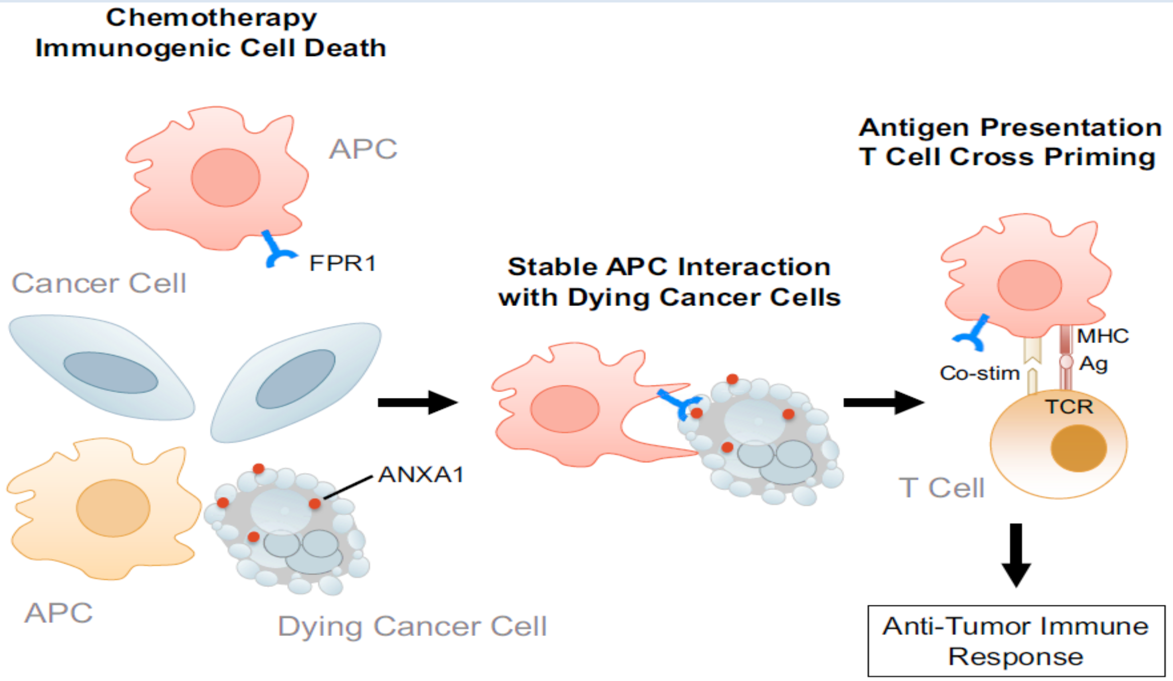


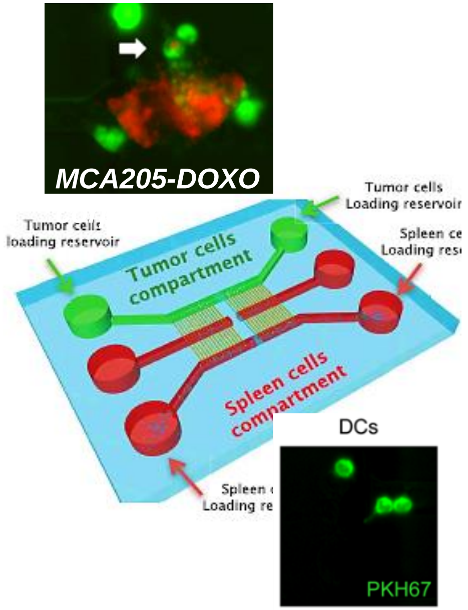
Racioppi et al. Nat Comm 2019

How dying tumor cells get noticed

Chemotherapy-induced anticancer immune response

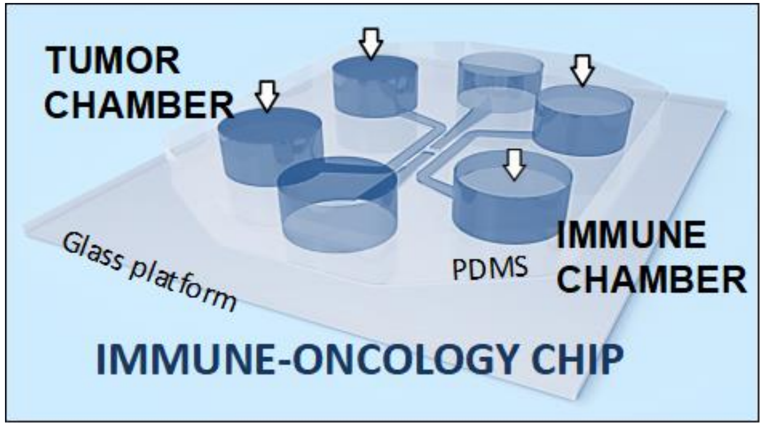
Chemotherapy-induced antitumor immunity requires formyl peptide receptor 1. Erika Vacchelli †, Yuting Ma †, Elisa E. Baracco, Antonella Sistigu, David P. Enot, Federico Pietrocola, Heng Yang, Sandy Adjemian, Kariman Chaba, Michaela Semeraro, Michele Signore, Adele De Ninno, Valeria Lucarini, Francesca Peschiaroli, Luca Businaro, Annamaria Gerardino, Gwenola Manic, Thomas Ulas[13], Patrick Günther, Joachim Schultze, Aicha Goubar, Gautier Stoll, Céline Lefebvre, Sylvie Rusakiewicz, Sylvain Ladoire, Monica Lucattelli, Fabrice André, Lorenzo Galluzzi, Ilio Vitale, Giovanna Schiavoni, Fabrizio Mattei *, **Laurence Zitvogel*, & Guido Kroemer***. ***Science*** *2015*.

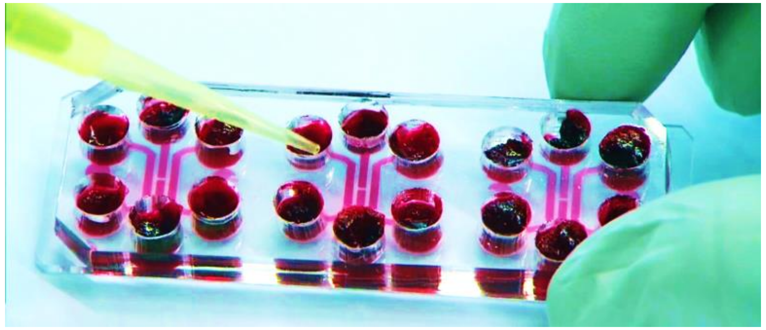


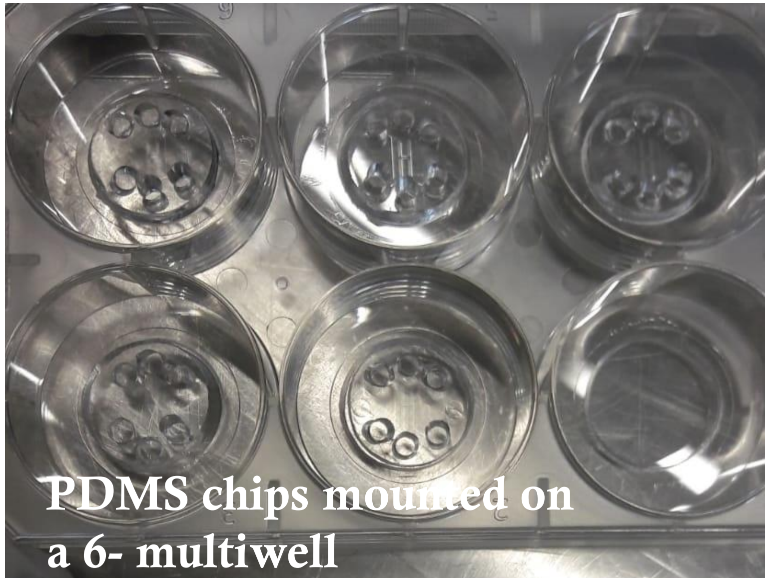


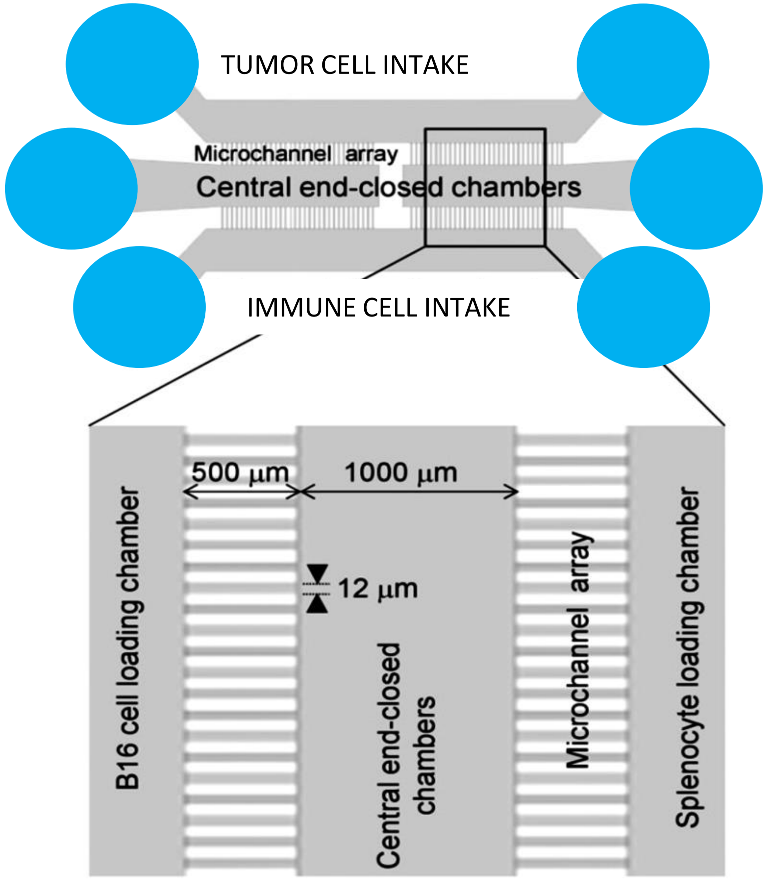
- **Major finding:** Anthracycline-induced antitumor immunity requires immune cells to express the protein formyl peptide receptor 1 (FPR1)
- **Mechanism:** FPR1 and its ligand ANXA1 mediate stable contacts between dendritic cells and dying cancer cells. In mice cancer cells growing in Fpr1–/– hosts were resistant to anthracyclines . Failure of DCs lacking FPR1 to approach.
- **Impact:** Breast or colon cancer patients expressing a variant of FPR1 and treated withanthracyclines showed poor metastasis-free and overall survival

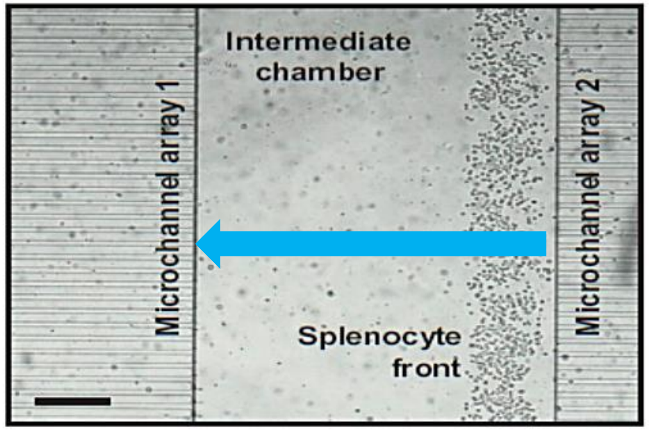
2D COCULTURE CHIP: ADHERENT AND FLOATING CELLS

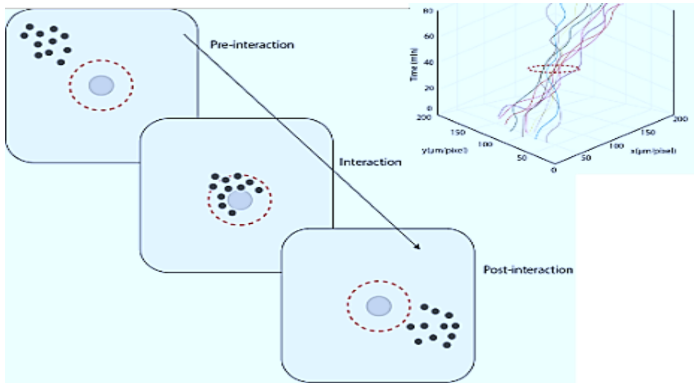






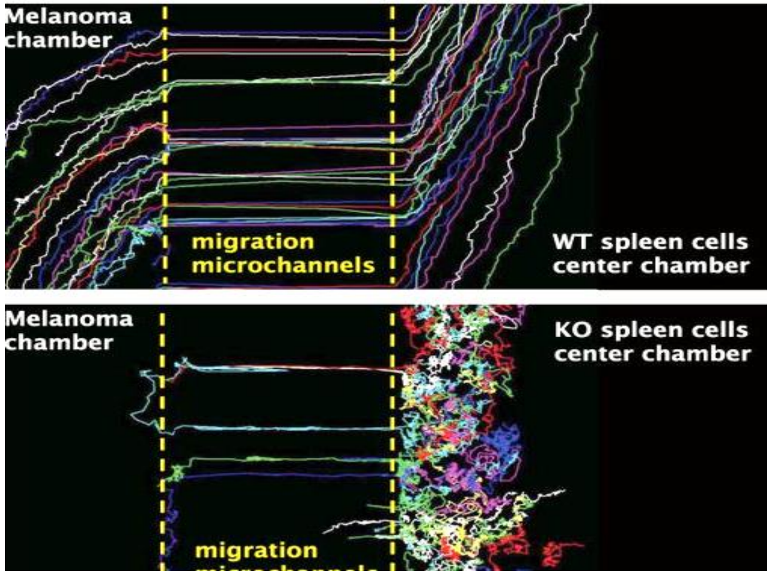






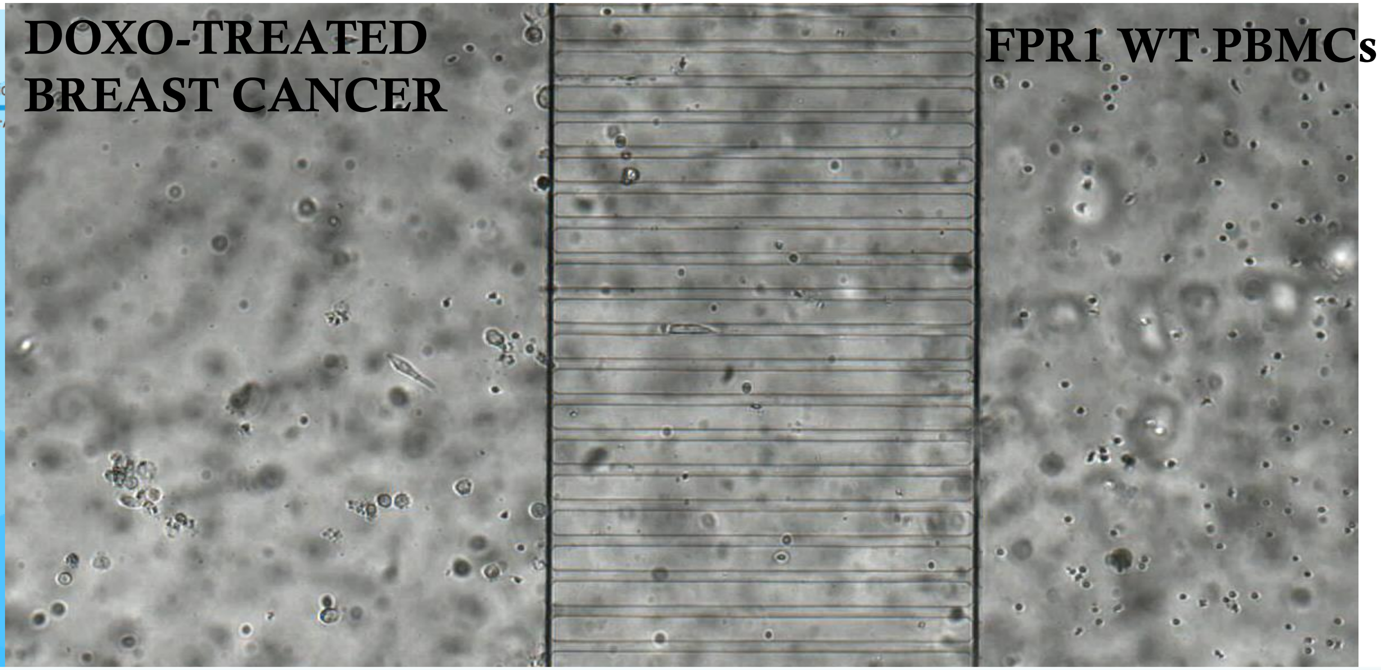
TRACKED TRAJECTORIES:

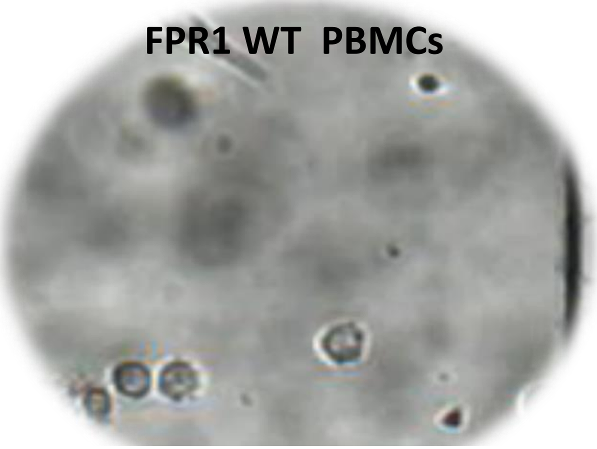
MOTILITY AND INTERACTION PARAMETERS



Vacchelli et al., Science, 2015
Businaro,De Ninno et al., Lab chip, 2014
De Ninno et al, Meth in Enzimatology 2019

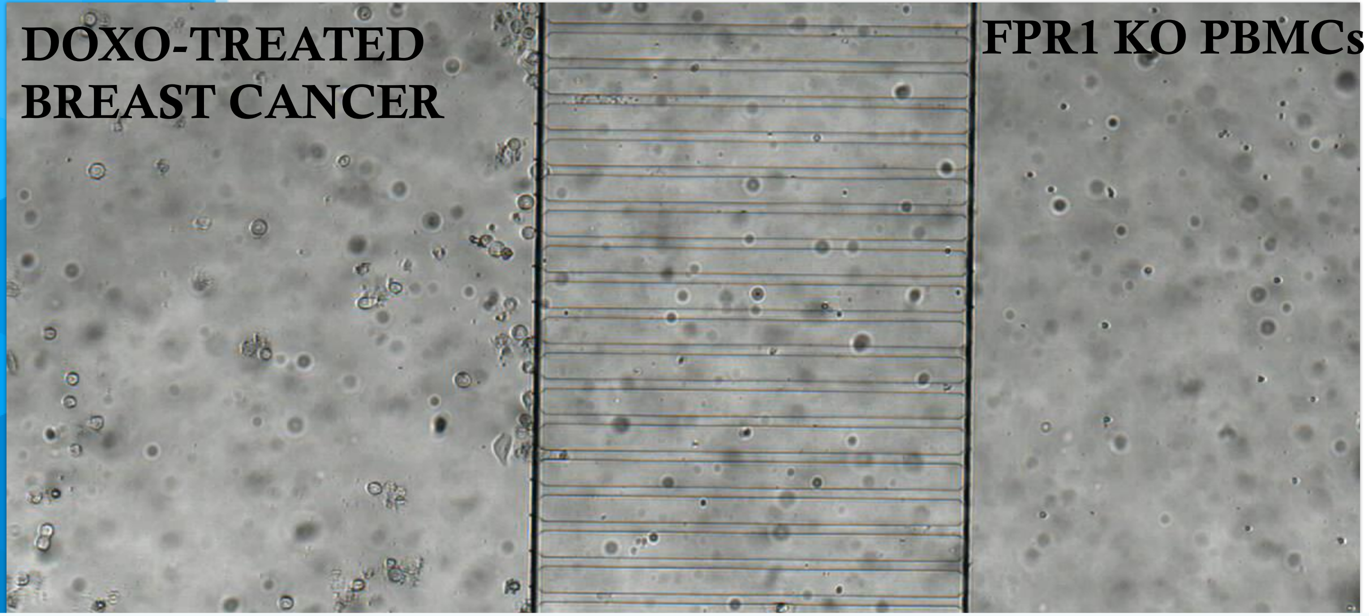
Vacchelli et al., Science, 2015

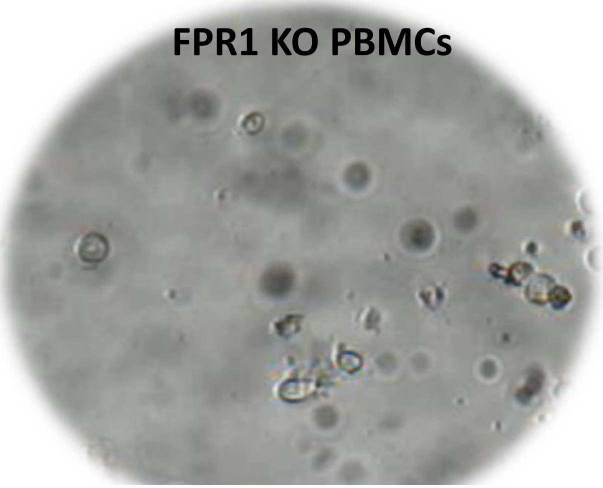




MASSIVE PBMCs ATTACK

WT immune cells in close proximity of cancer ones

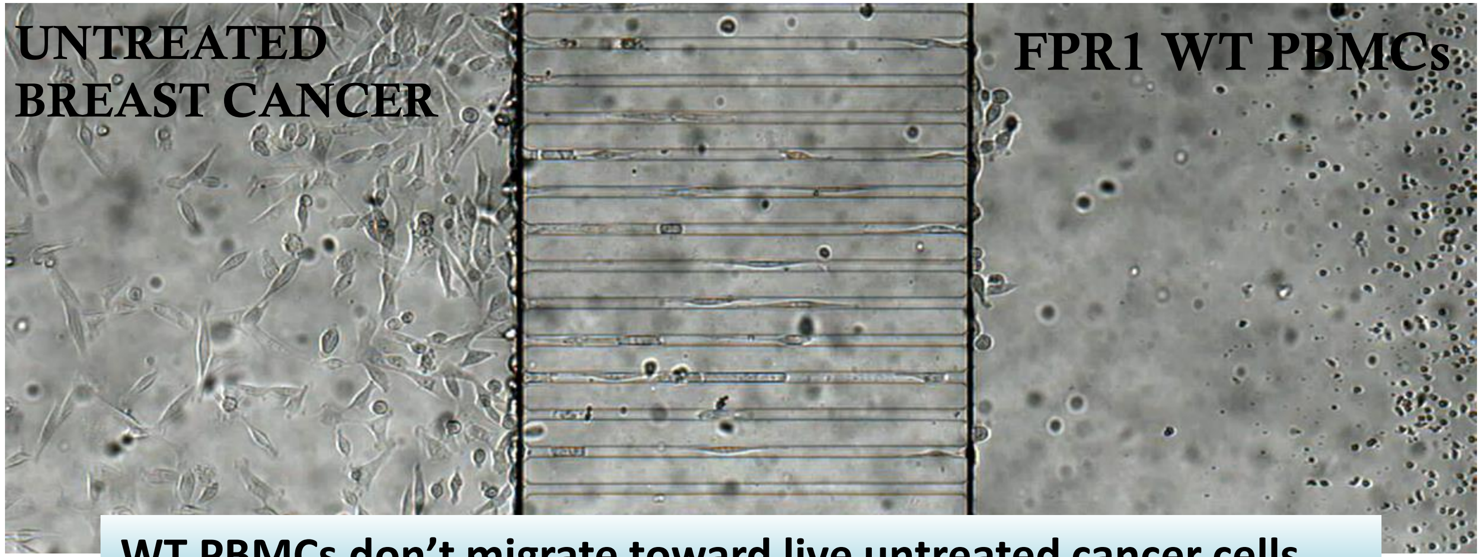




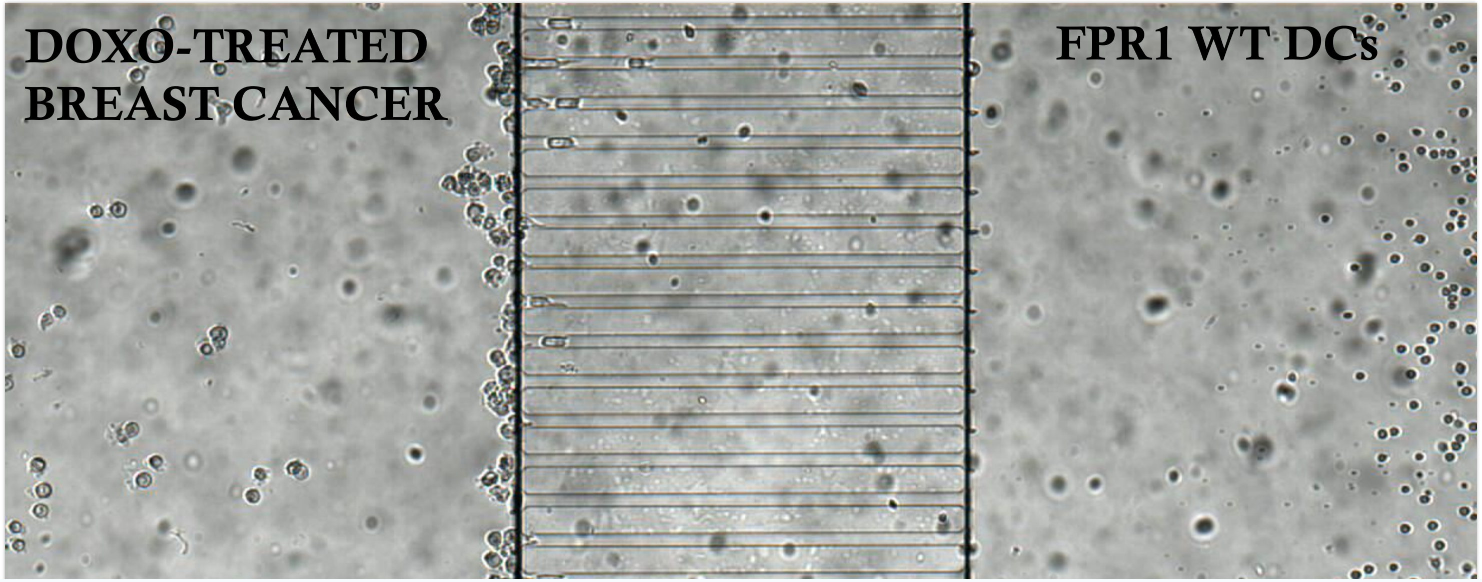
INEFFICIENT INTERACTION

Immune cells deficient in FPR1 interacted less with dying cancer cells treated with doxorubicin than functional PBMCs

IFN Institute for Photonics and Nanotechnologies
National Research Council

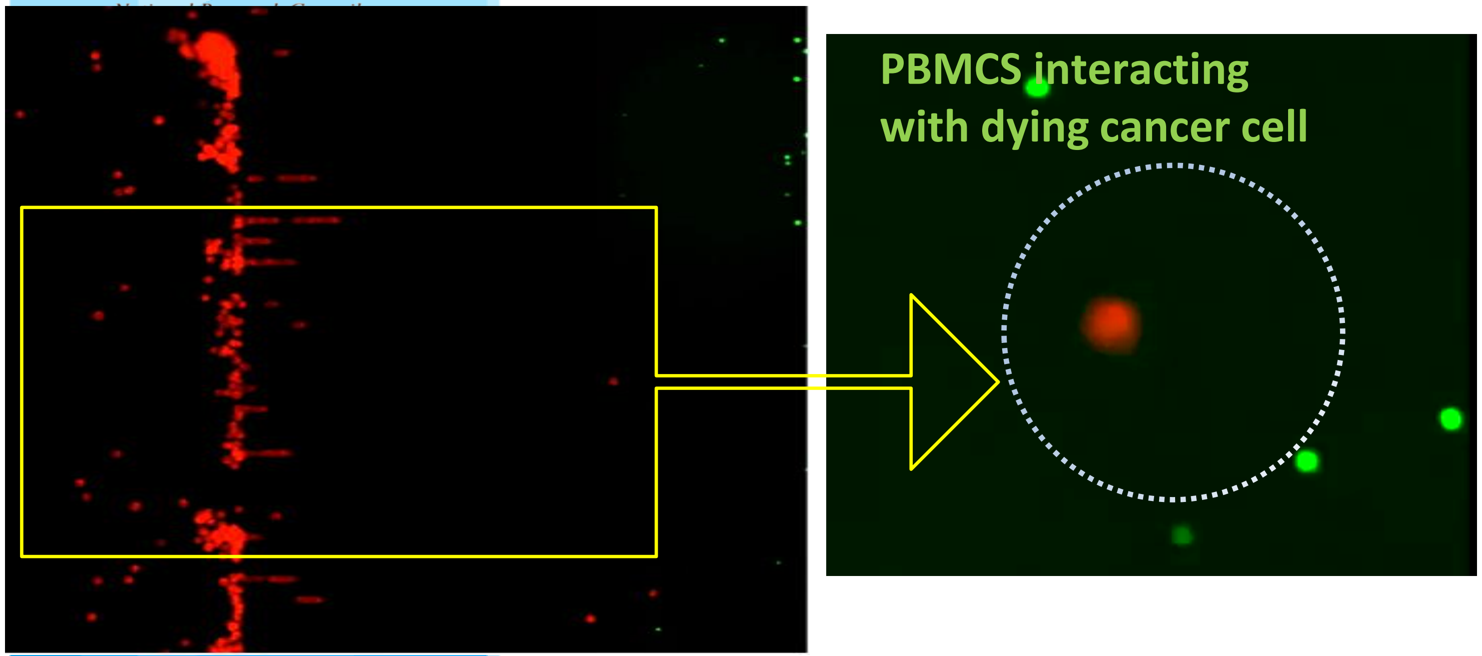


WT PBMCs don't migrate toward live untreated cancer cells

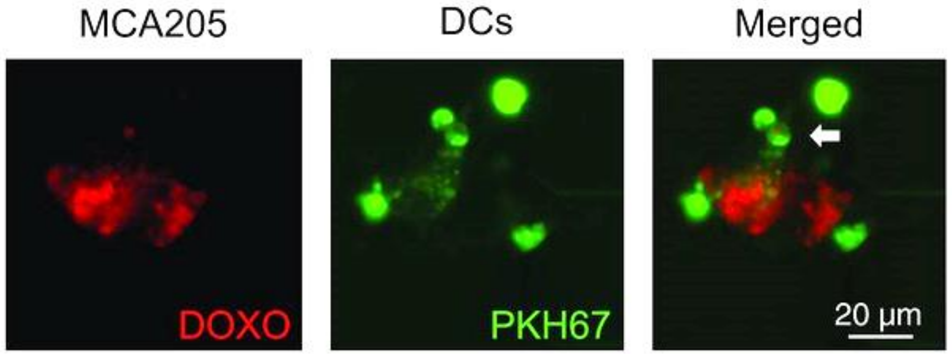


Only DCs isolated from the pool of PBMCs don't migrate toward dying cancer cells

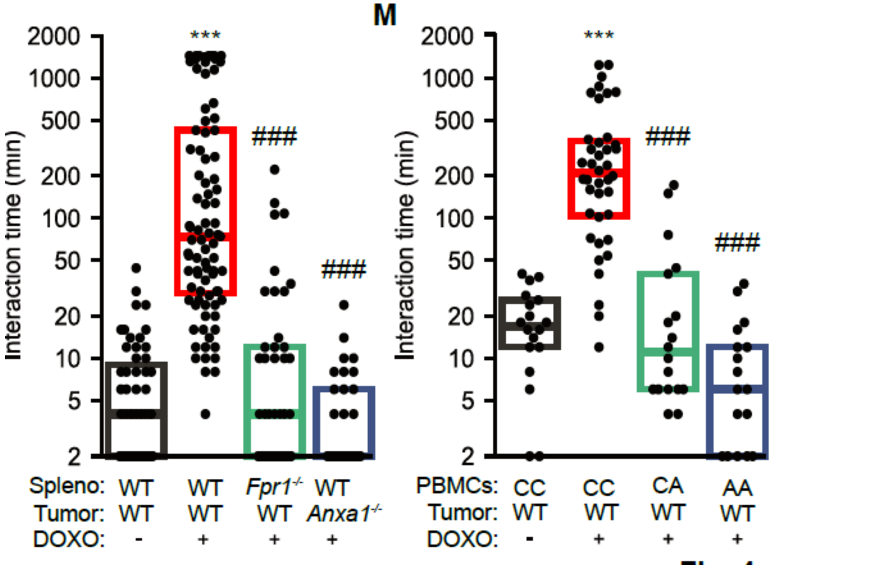
Vacchelli et al., Science 2015



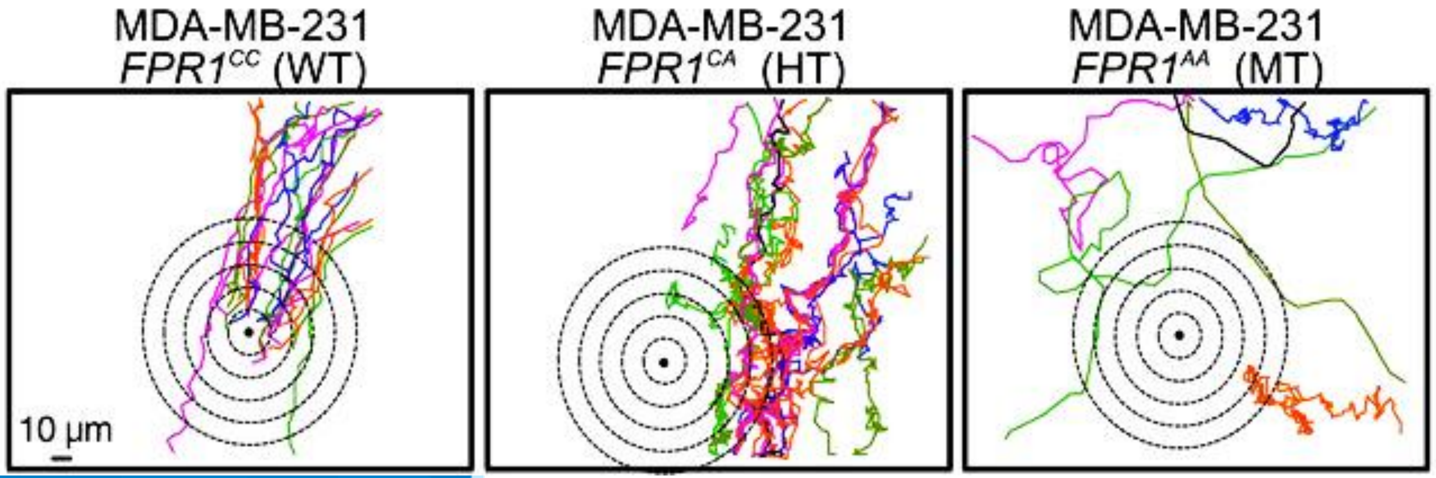
Stable conjugates between dying tumor cells and human DCs prolonged (> 60 min) juxtaposition



Interaction times between dying cancer cells and immune cells

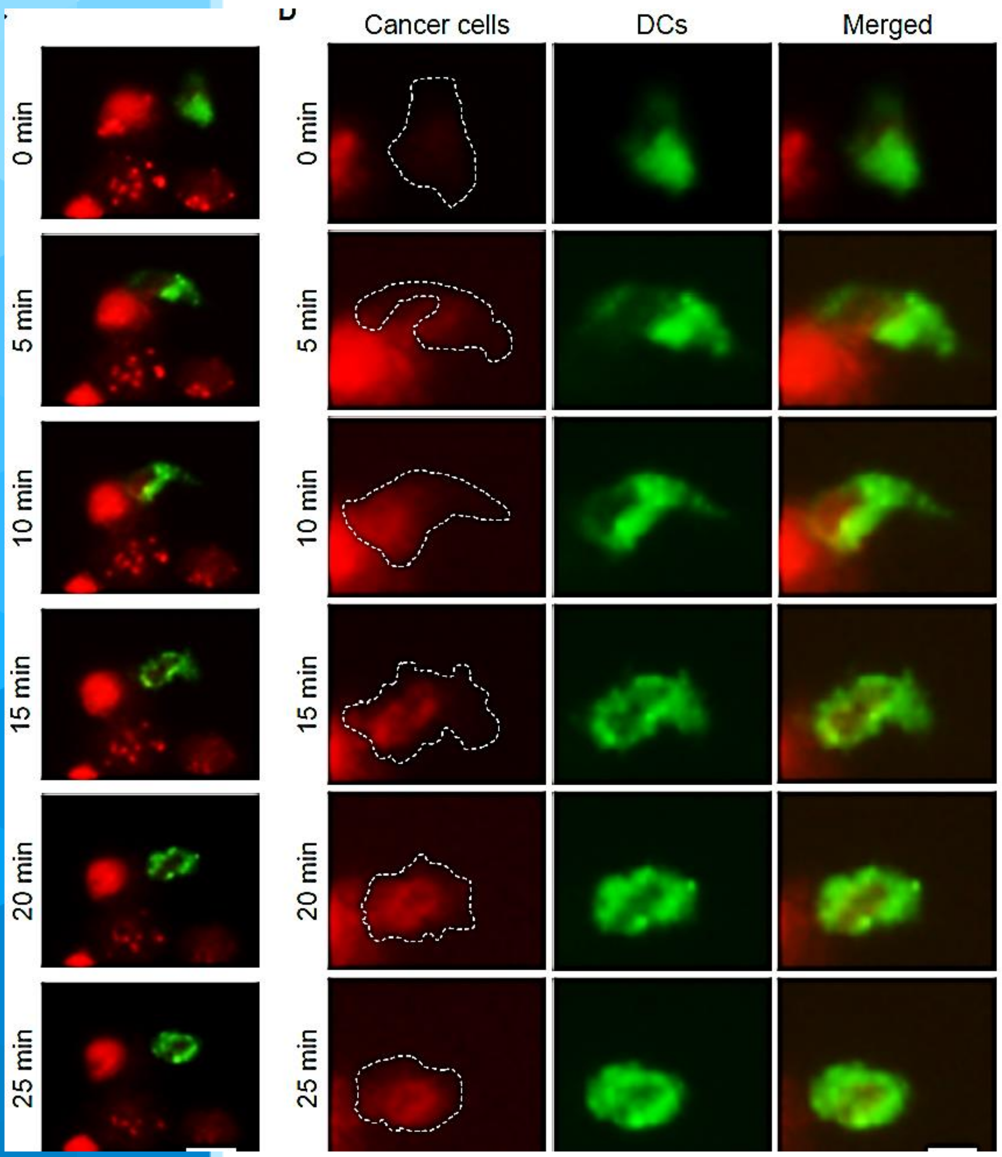


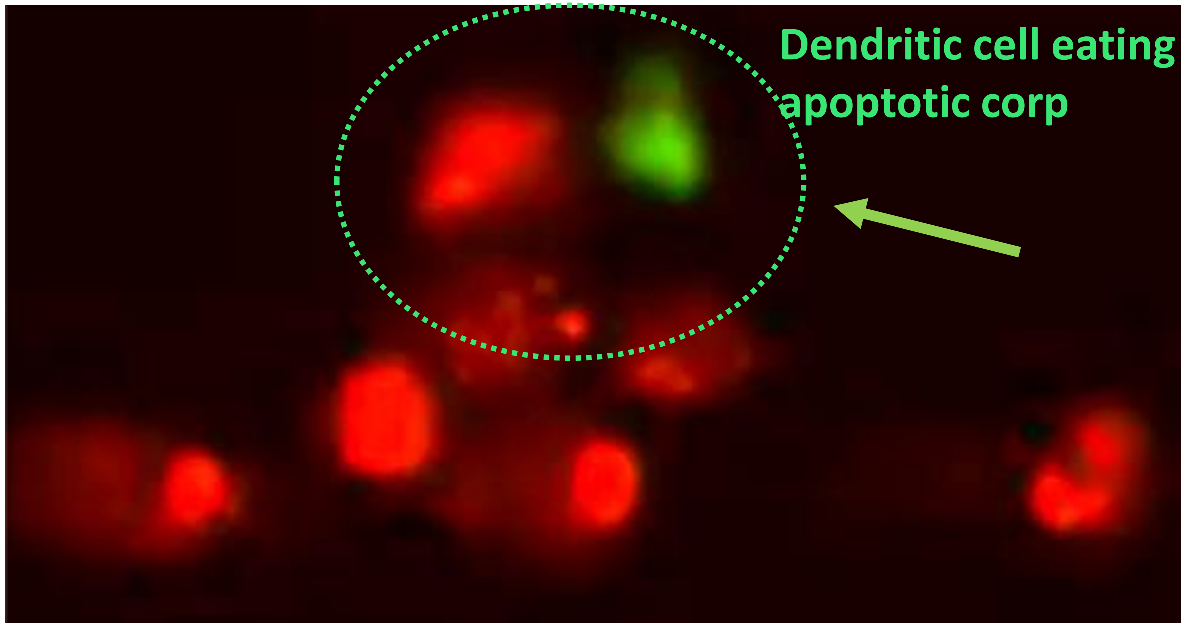
Tracking patterns of human immune cells toward apototic target tumor

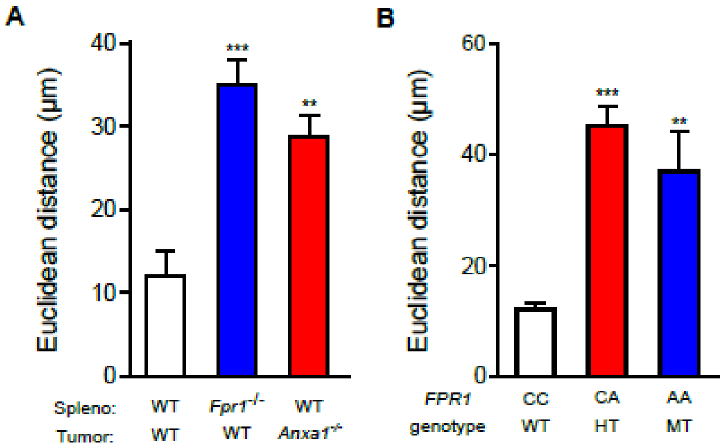


Vacchelli et al., Science 2015

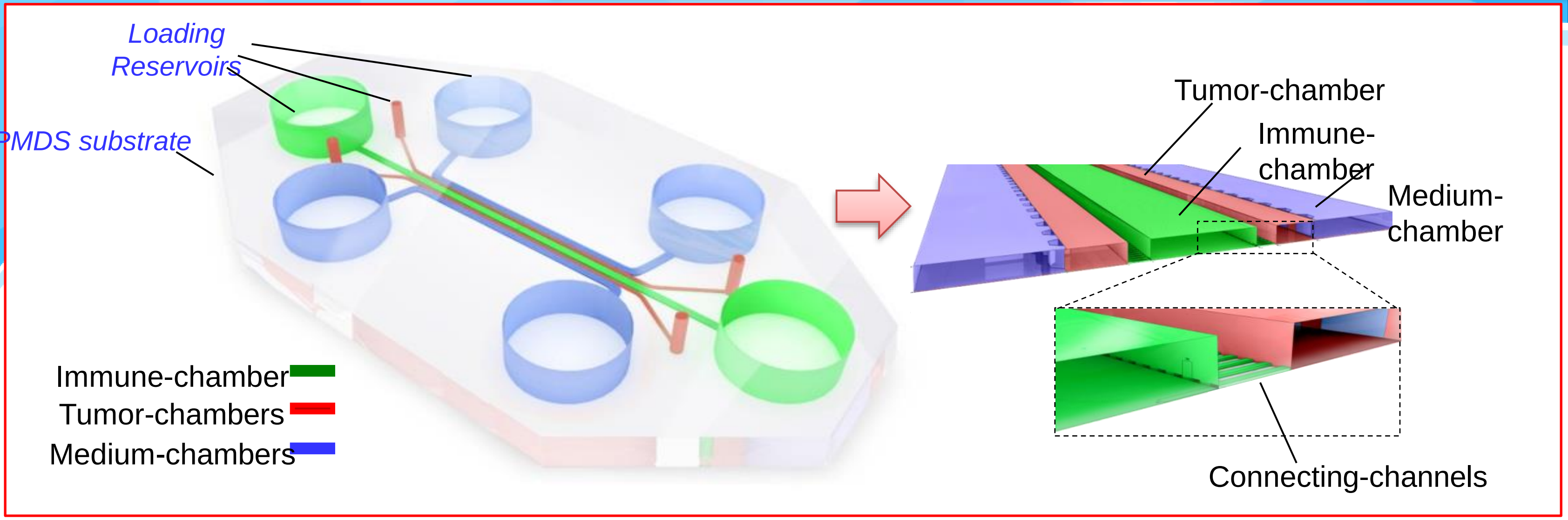
IFN
Institute for Photonics and Nanotechnologies
National Research Council



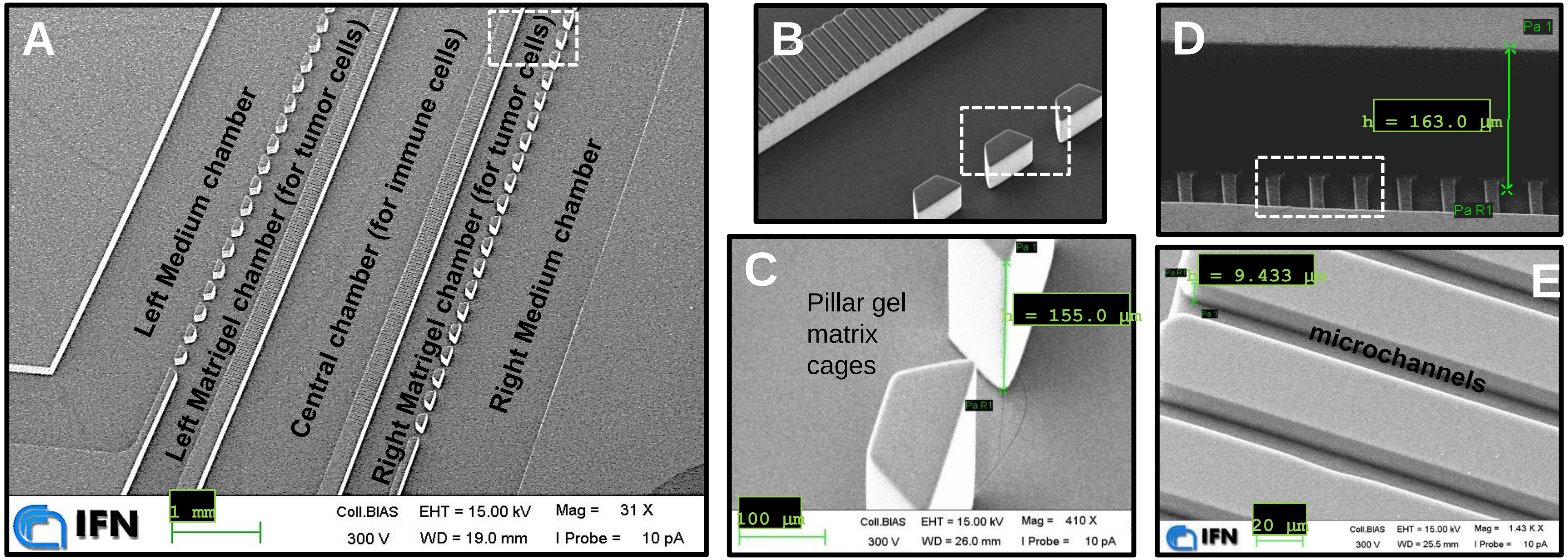


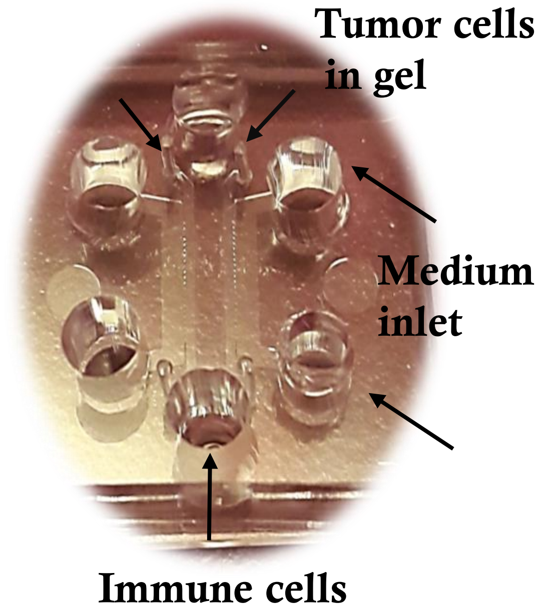


3D CANCER-IMMUNE MODEL ON CHIP FOR IMMUNOTHERAPIC APPROACHES

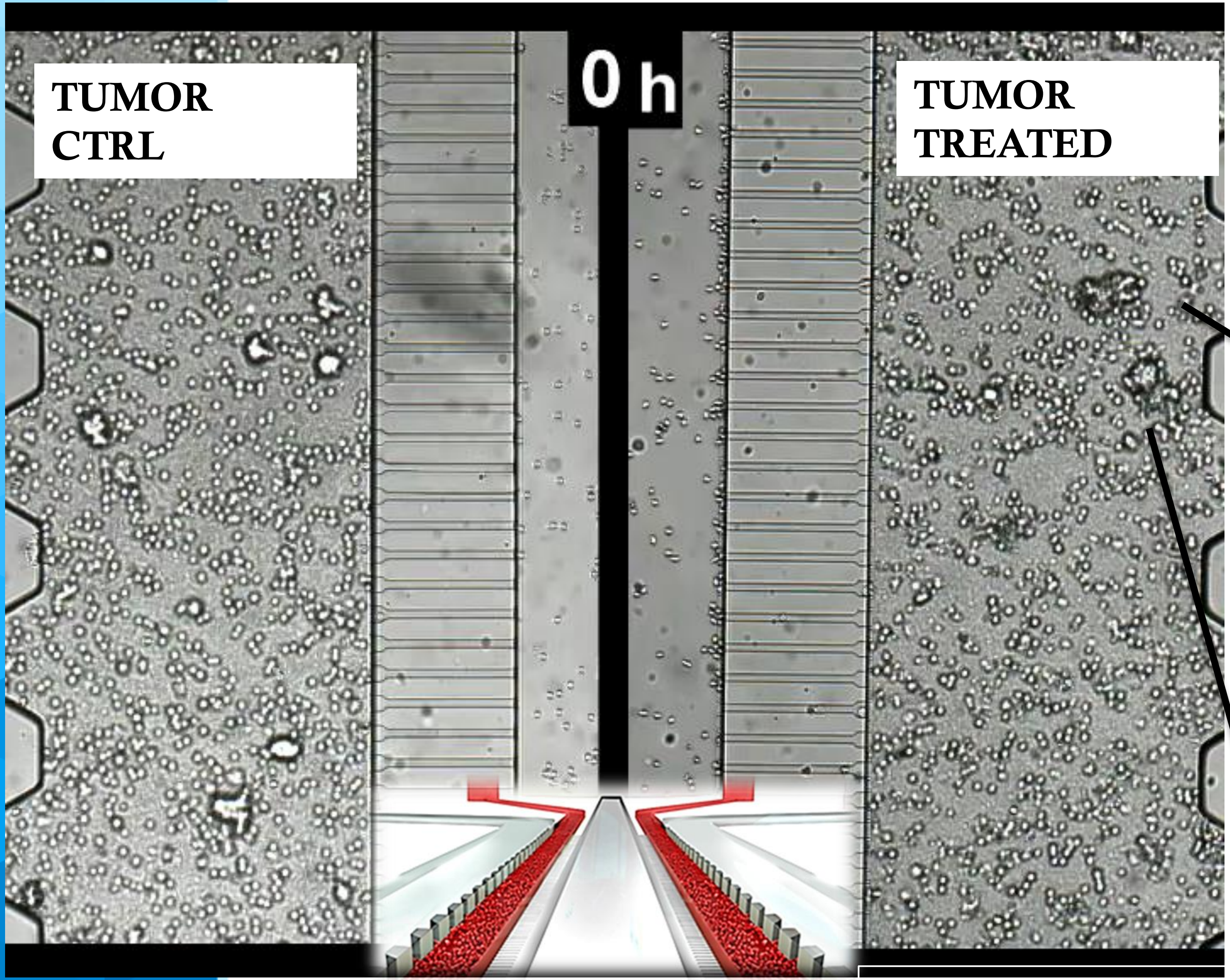


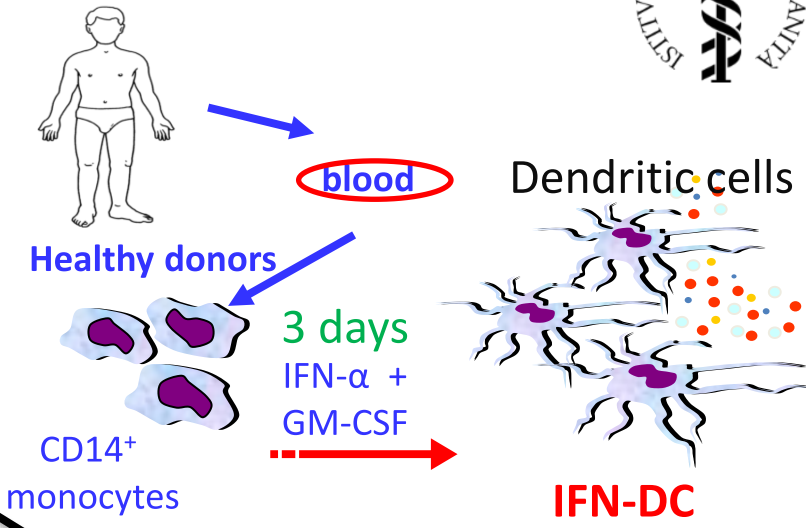
Racioppi et al.,Nat Comm 2019
De Ninno et al., Met in Enzimology 2019
Parlato et al., Sc Reports 2017
Lucarini et al., JID 2017

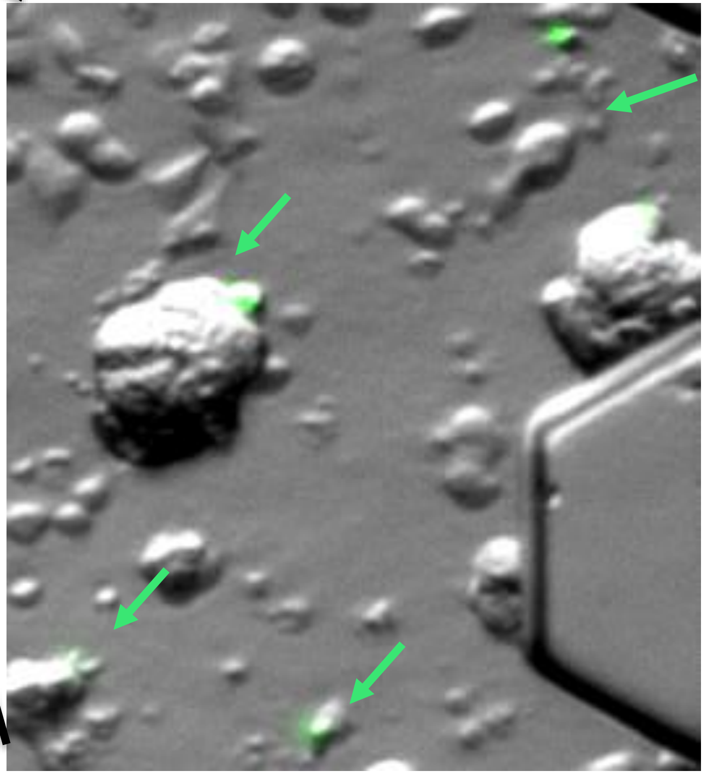




IFN-DC sense environmental signals and adjust their motion towards cancer cells undergoing RI-induced apoptosis







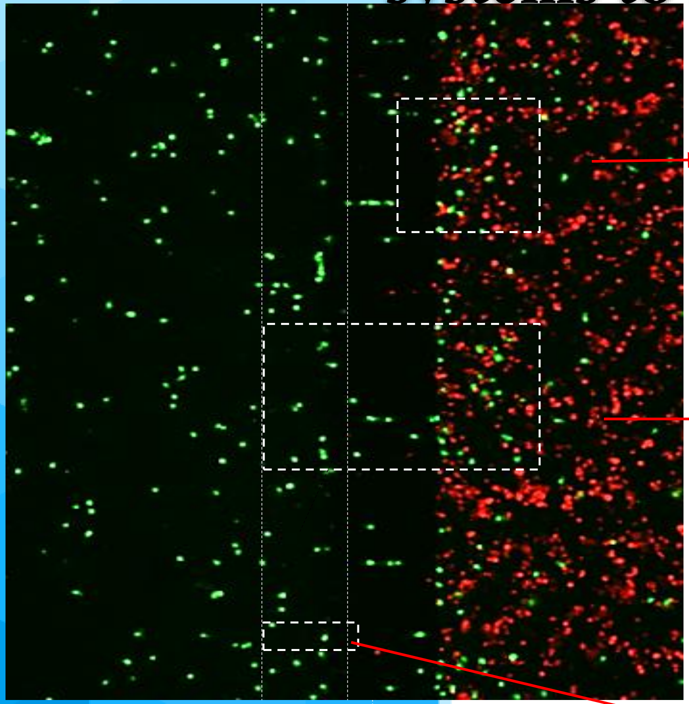
Parlato et al., Sc Reports 2017

COMPETITION

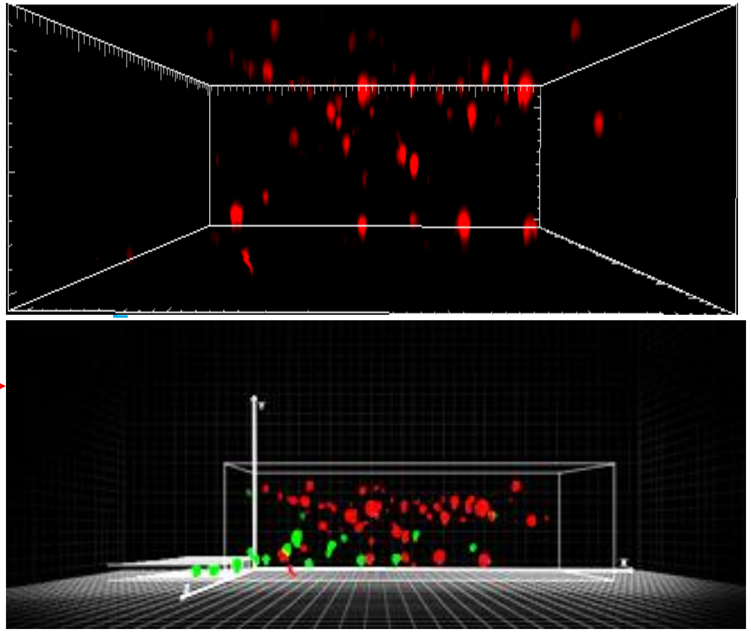
IFN Institute for Photonics and Nanotechnologies
National Research Council

3D microfluidic platform recapitulates interconnected immune-tumor systems to track DC-cancer cell dialogue

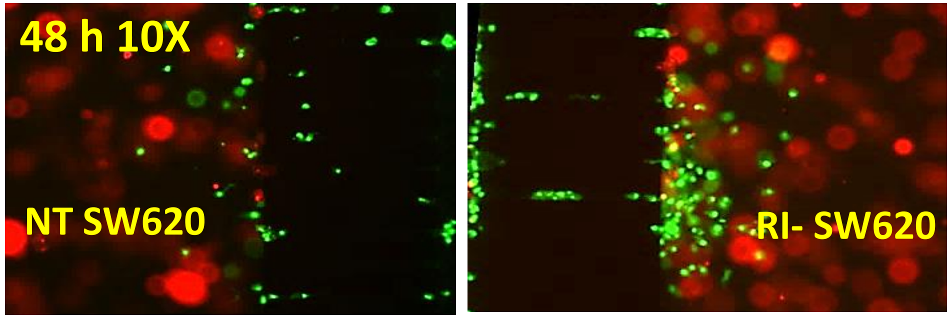
IFN-DC/SW620

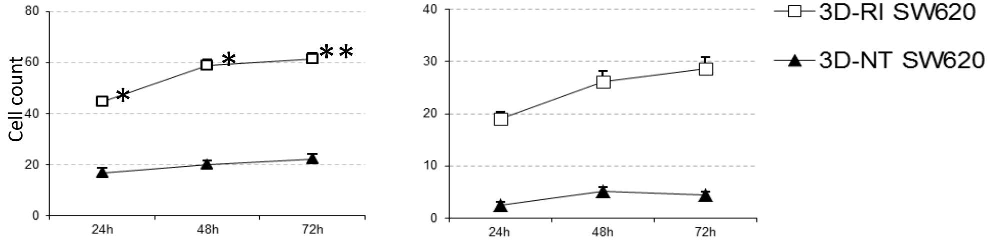


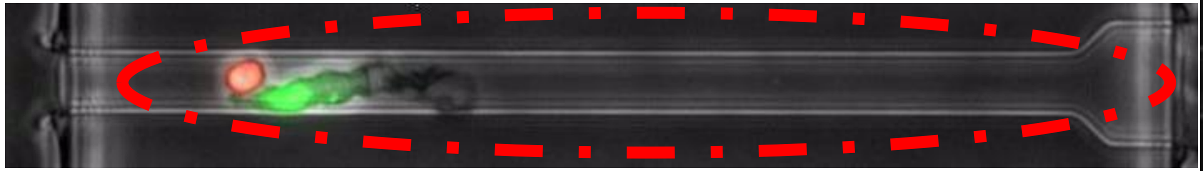
Immune-chamber
Tumor-chamber
Connecting-channels

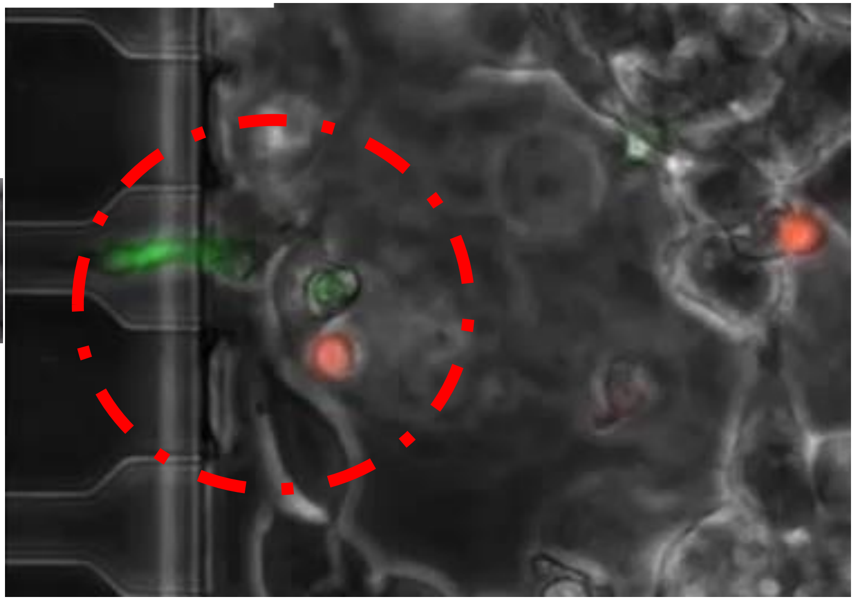


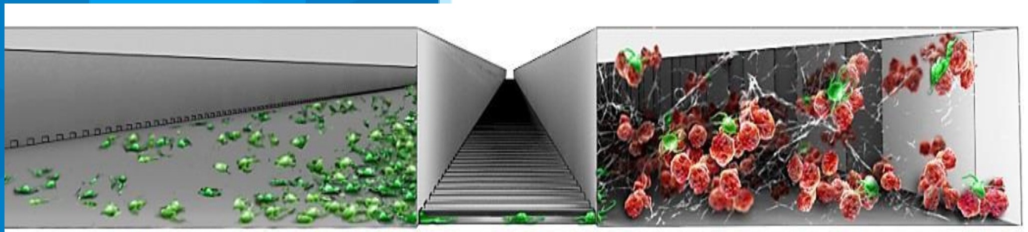
DC confined stretched









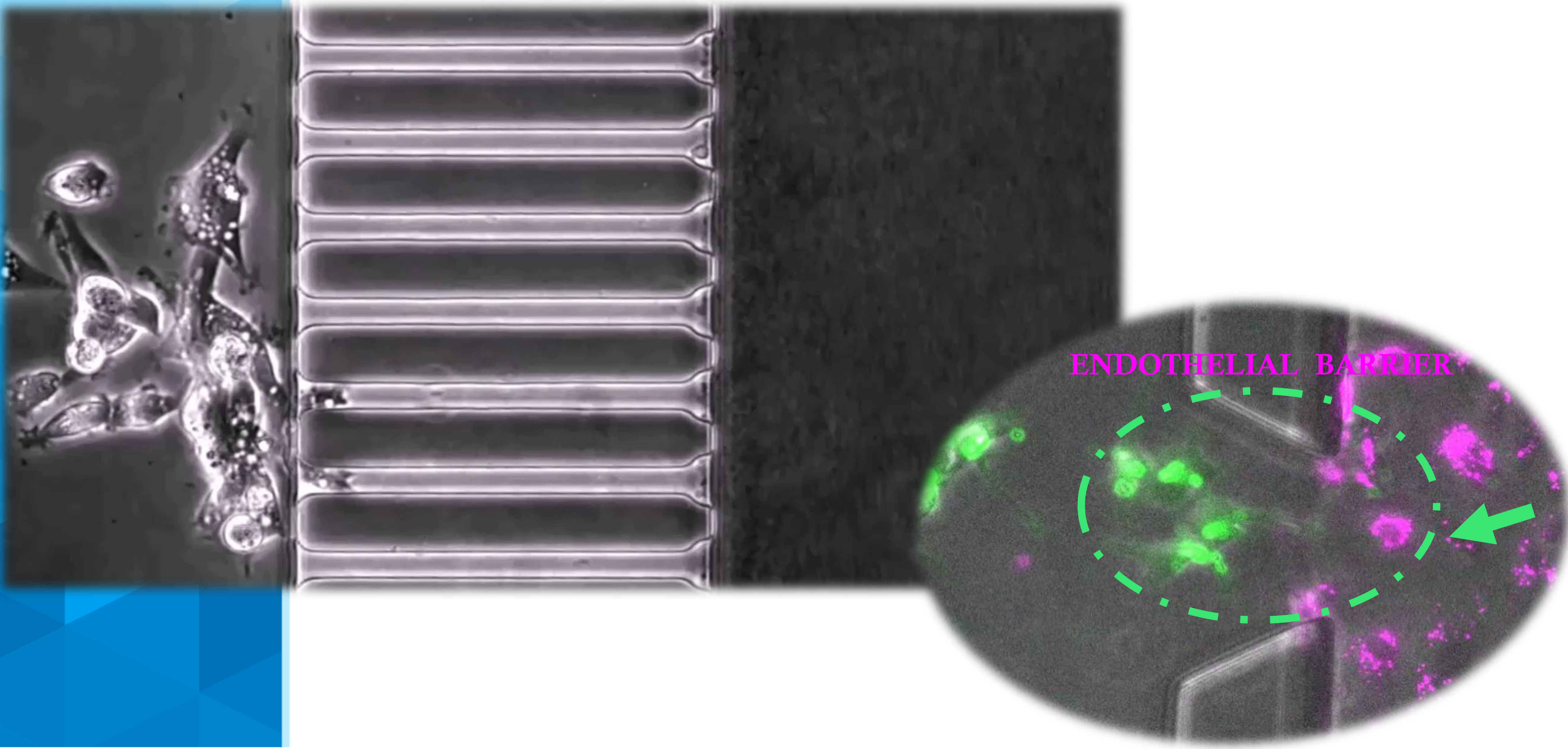


Immune-chamber (floating cells)
Confinement in u-channels
Invasion in Tumor site 3D - Collagen

Parlato, De Ninno et al., Sc Reports 2017

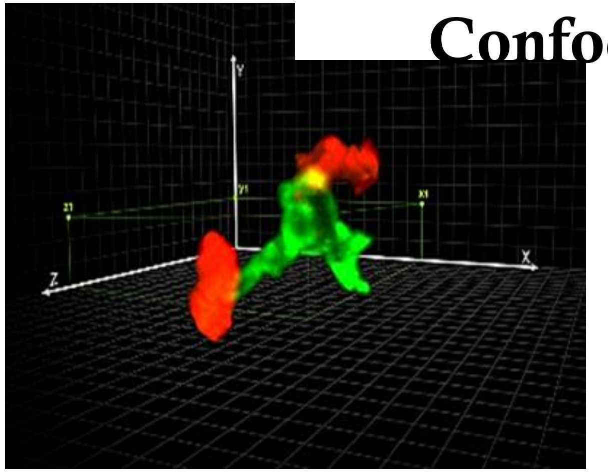
IFN Institute for Photonics and Nanotechnologies
National Research Council

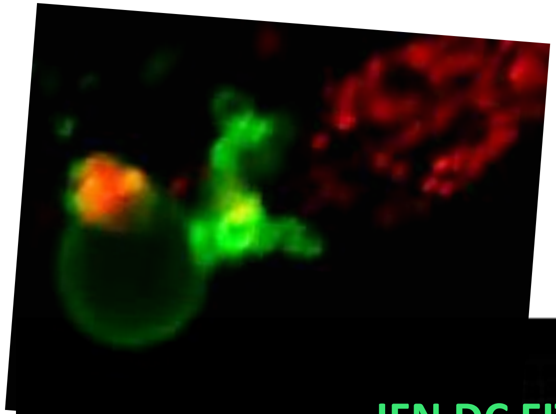
Motility in 2D and 3D

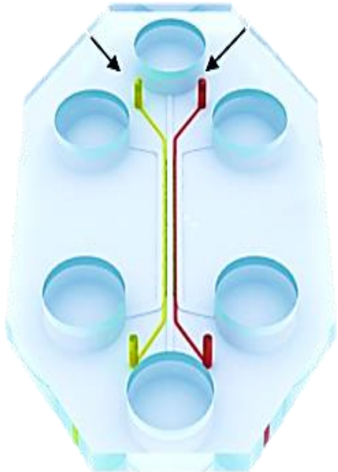


Clearing the Dead

Confocal optical sections of IFN DCs in RI tumor site



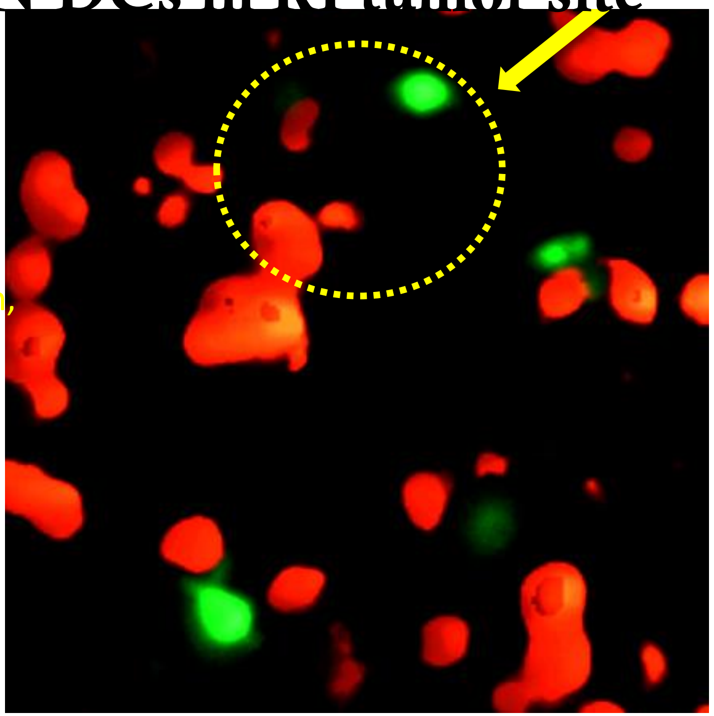


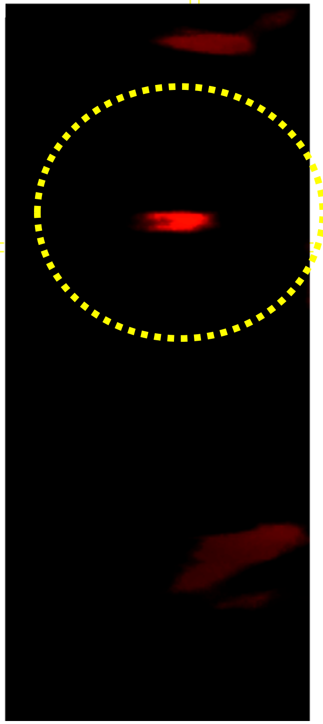


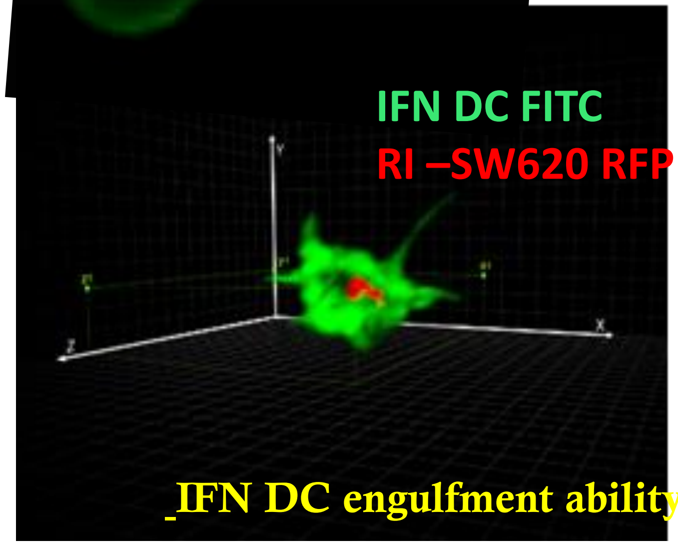
interval time 30min,
15h duration

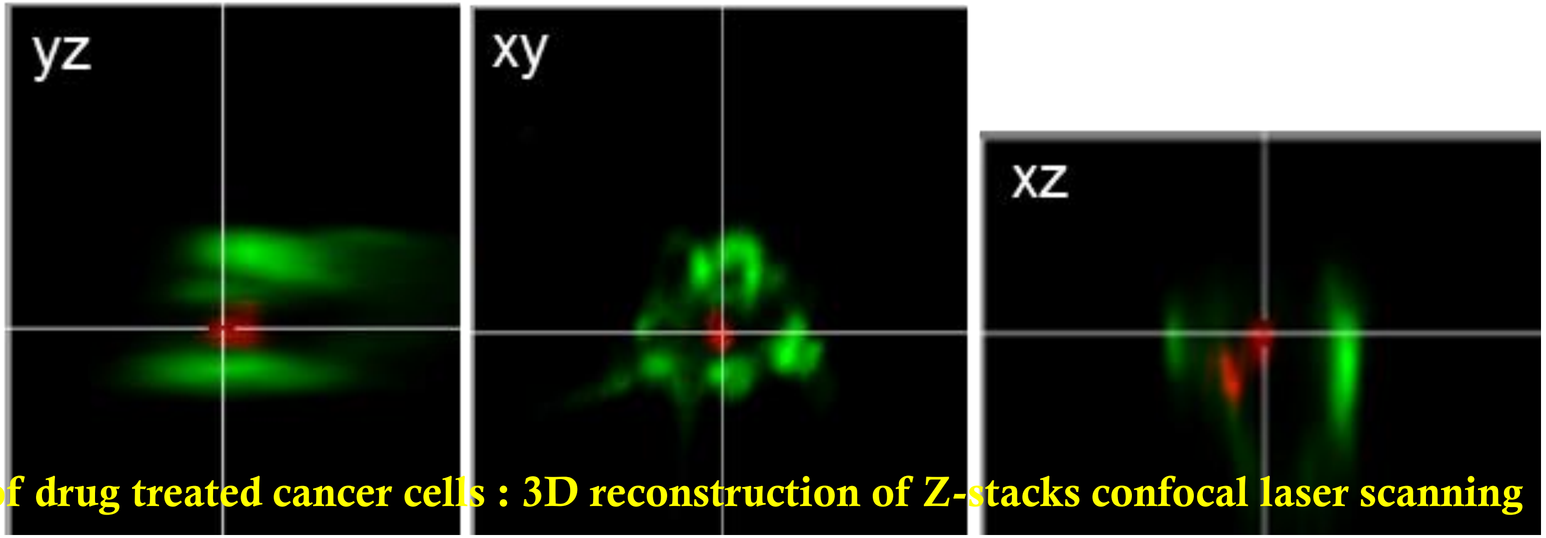
IFN DC FITC

RI –SW620 RFP







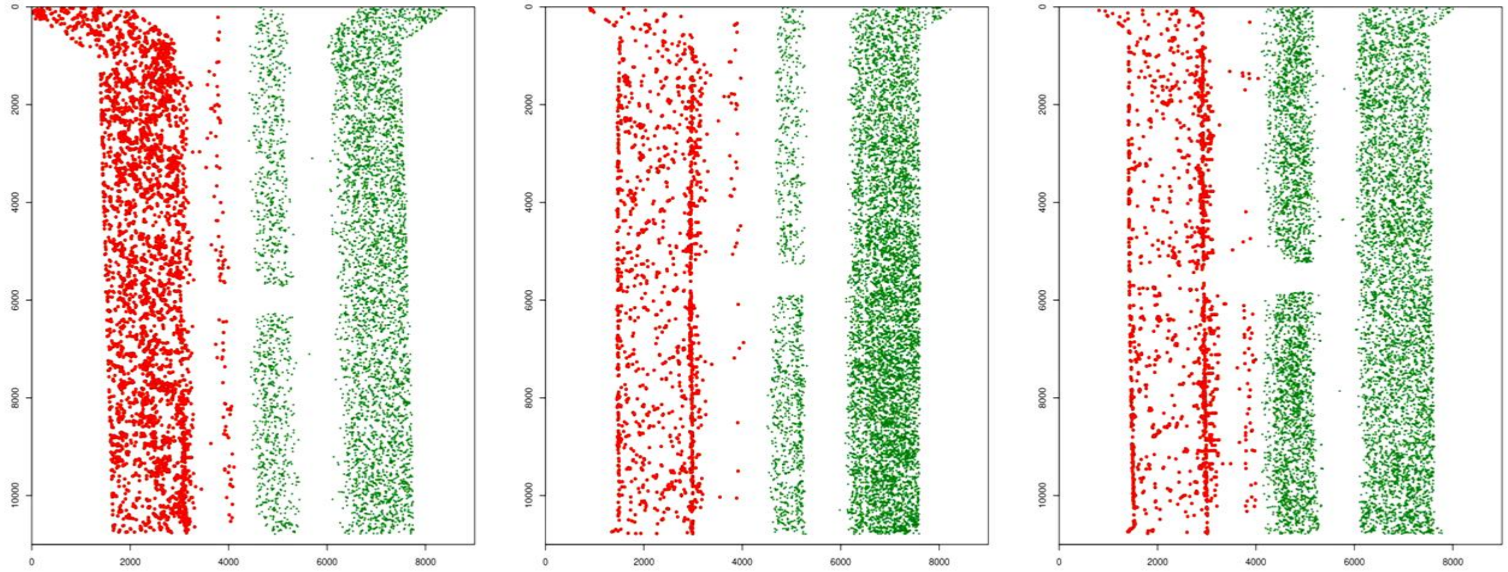


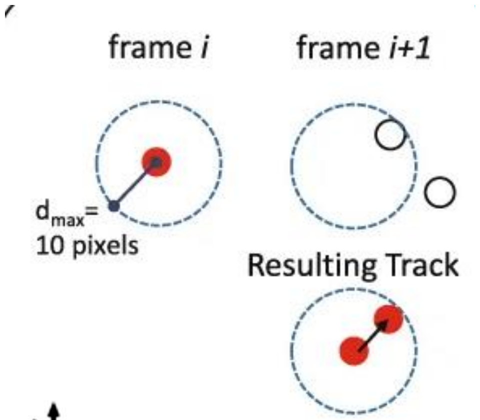
IFN DC engulfment ability of drug treated cancer cells : 3D reconstruction of Z-stacks confocal laser scanning

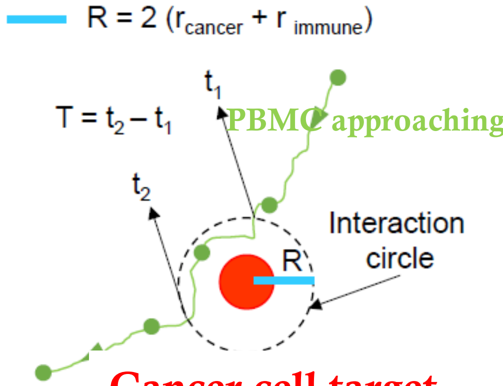
Automated tracking Cell Hunter software :
Measuring un-labeled cancer-immune interactions in multi-cell type context

Cancer and immune cell centre discrimination

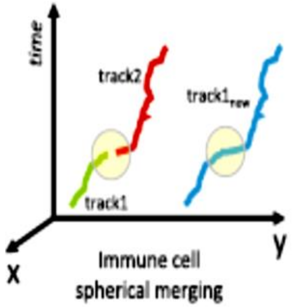
MC Comes et al. Scientific reports 2019
MC Comes et al. IEEE TNNLS *submitted*
Agliari et al. Scientific reports 2018

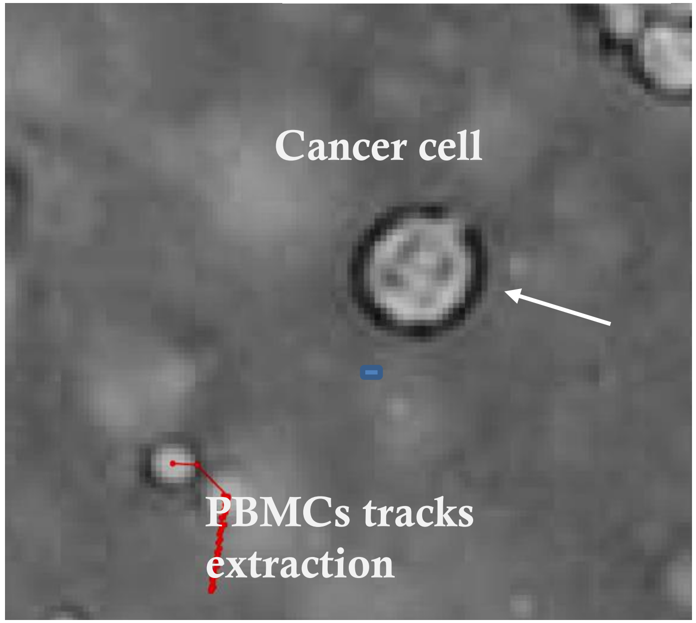


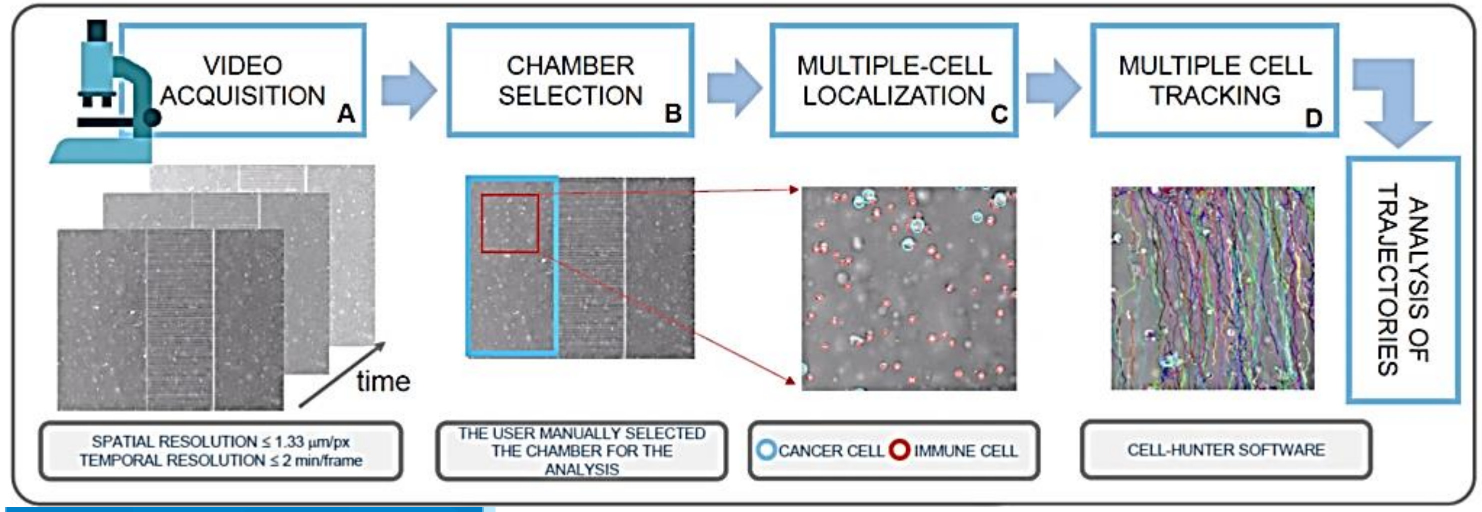




Cancer cell target

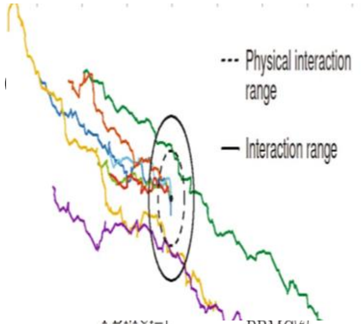




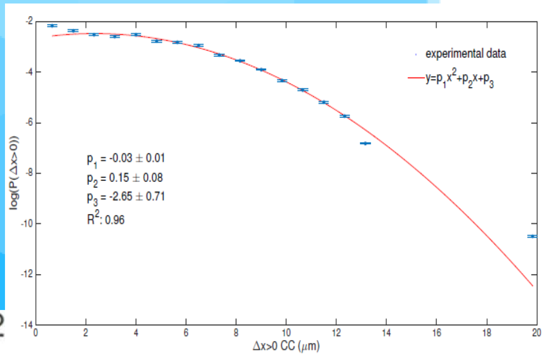


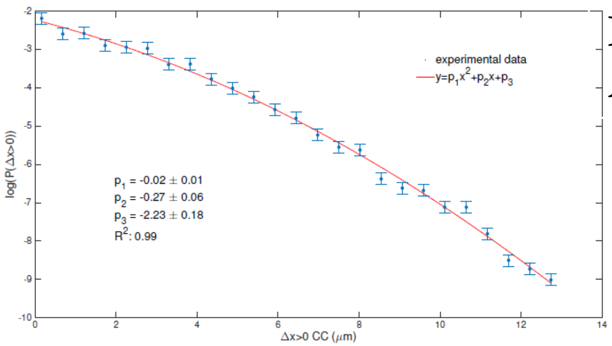
Statistical Mechanics & Biological Complexity

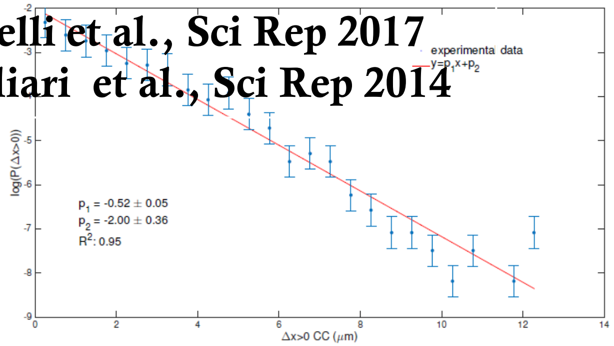
Migratory ability and the mutual interaction of human immun dynamical systems theory

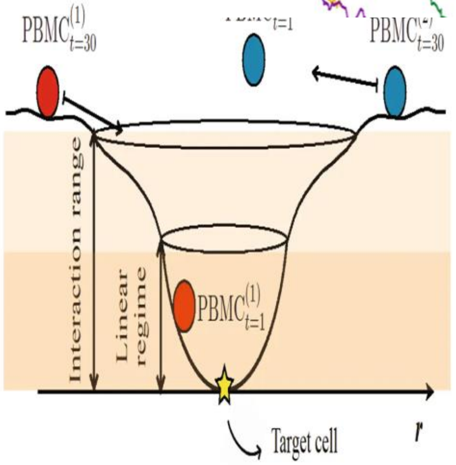


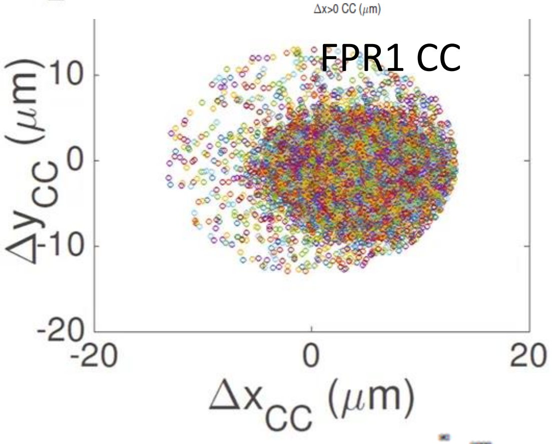
Biselli et al., Sci Rep 2017
Agliari et al., Sci Rep 2014

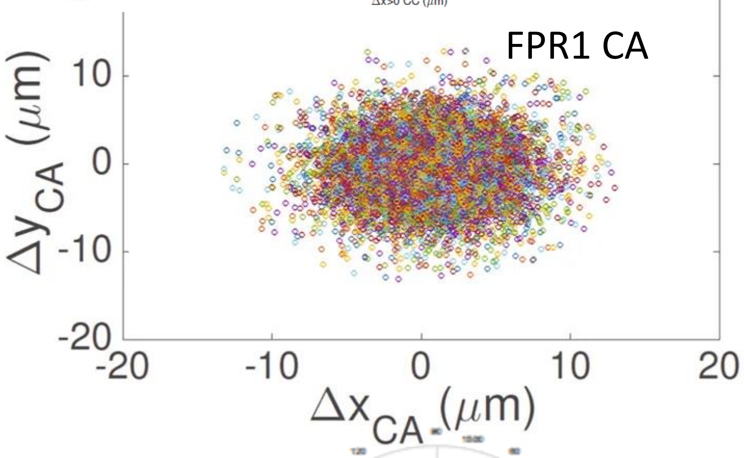


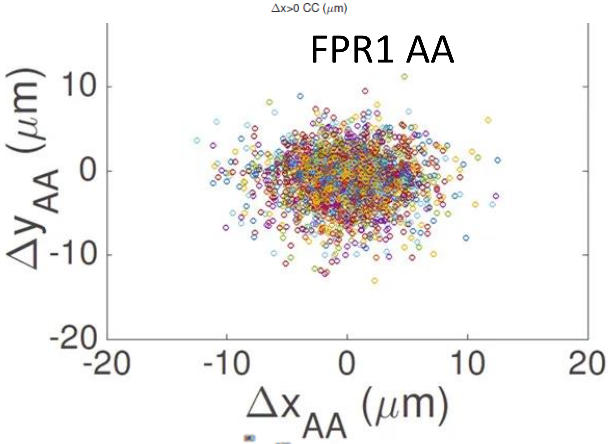


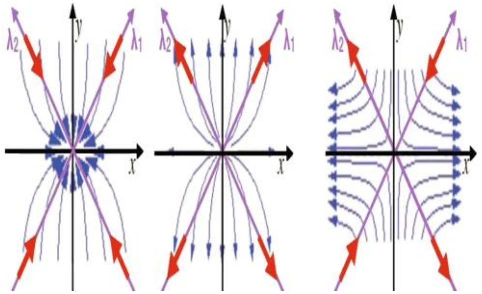


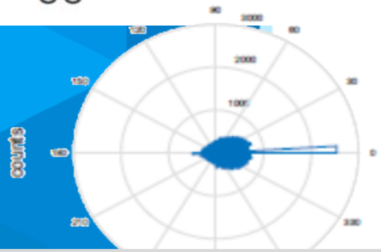




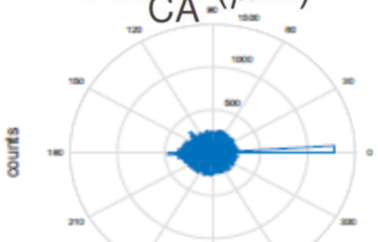




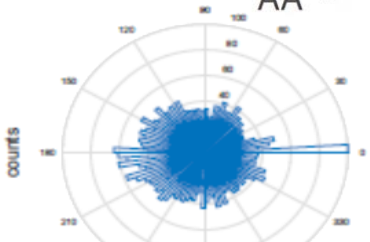




Biased Random walks

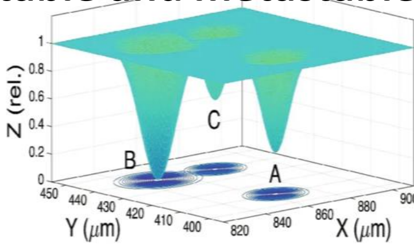


Weaker Bias random walks



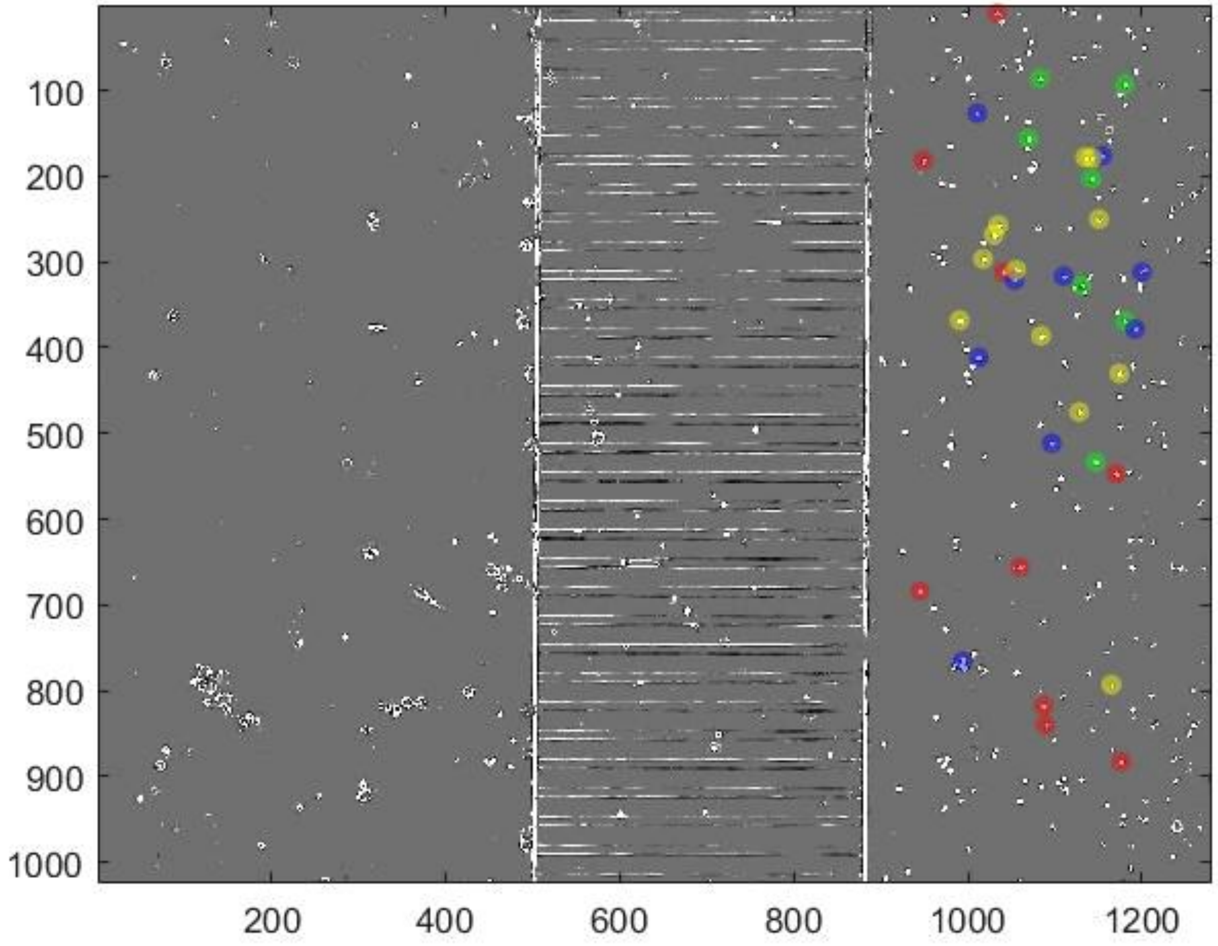
Uncorrelated Random walks

stable, unstable and metastable dynamics



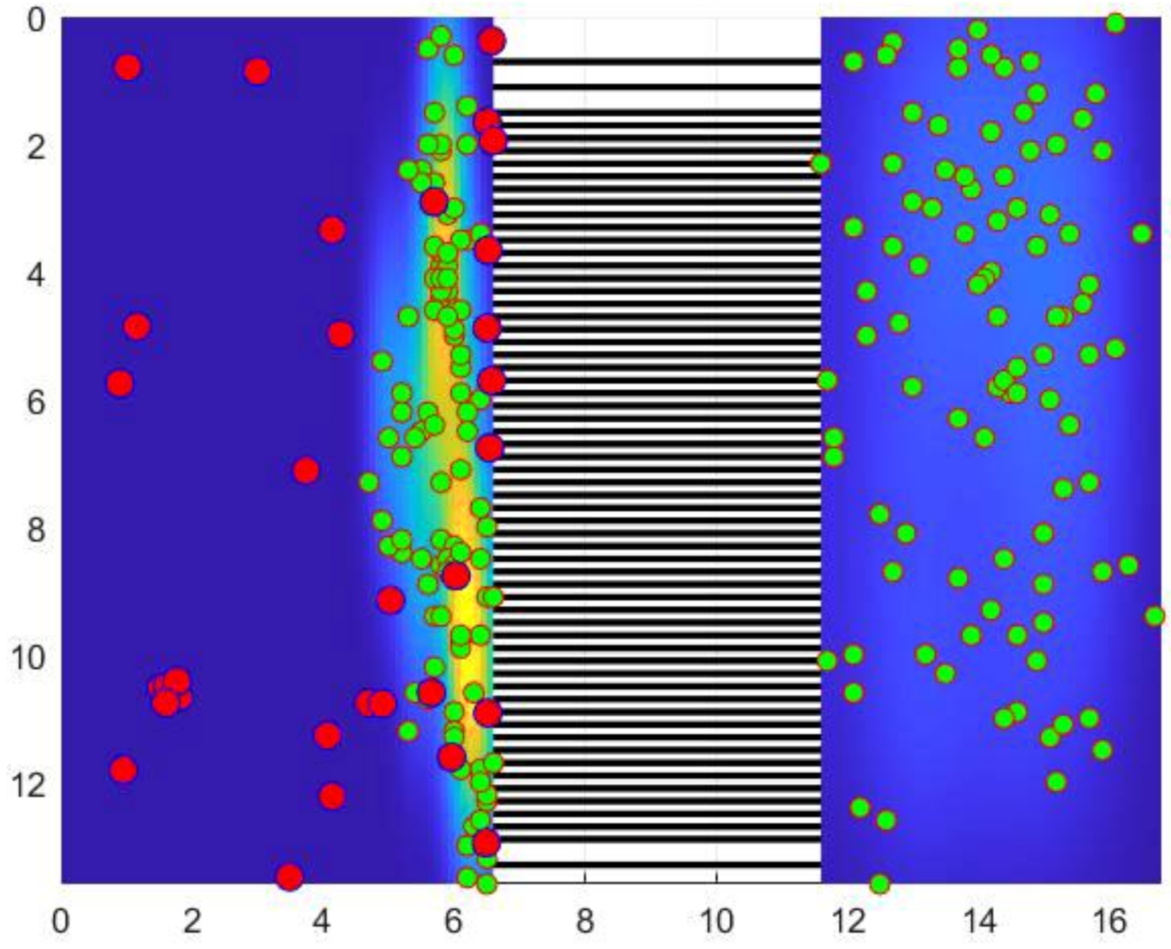
Towards in-silico staining

Very Preliminary data!!



Unpublished: curtesy of CNR-IAC (Roberto Natalini, Gabriella Bretti, Elishan Brown, Paola Stolfi, Davide Vergni

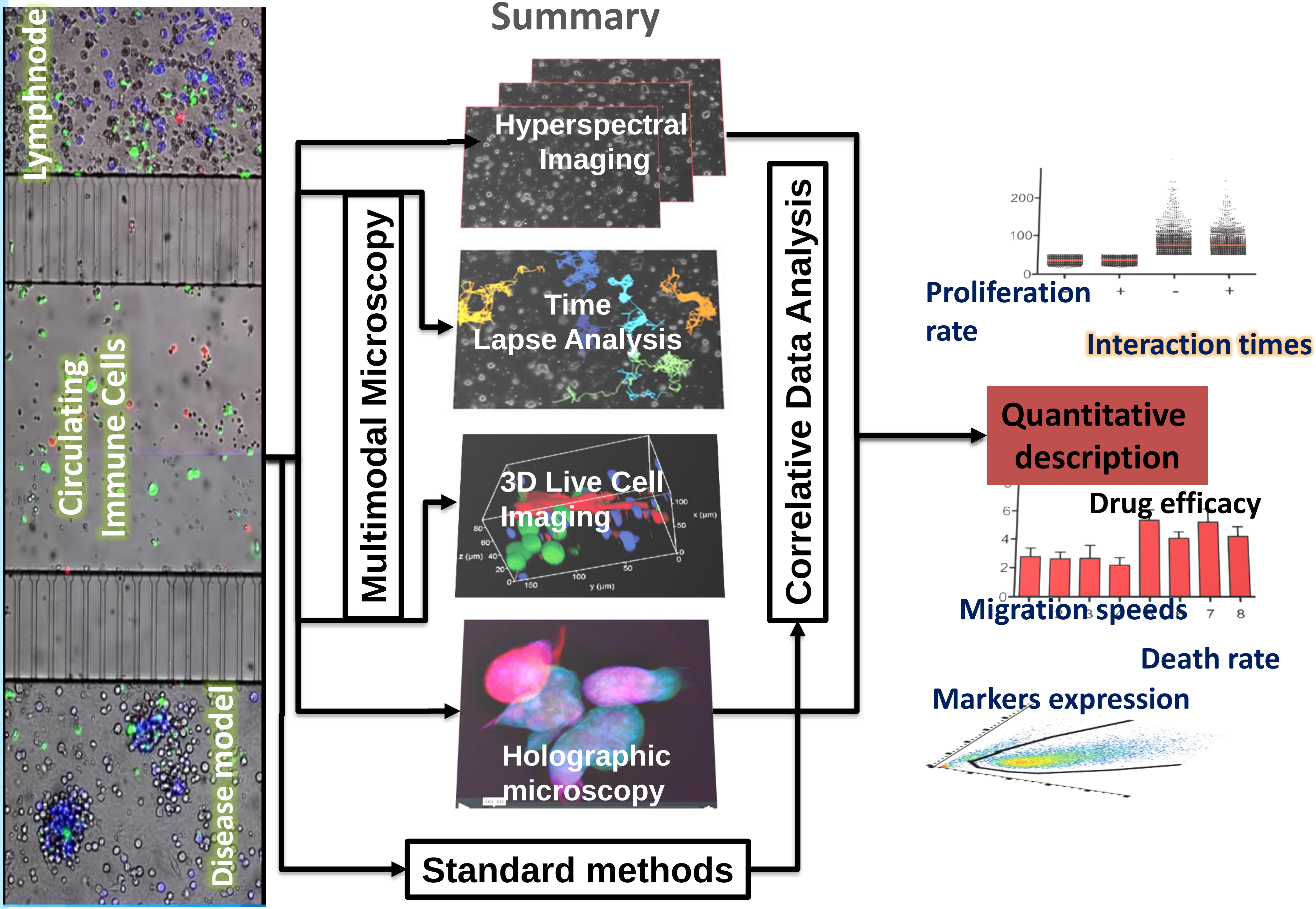
Towards in-silico /on-chip experiment merging



Unpublished: curtesy of CNR-IAC (Roberto Natalini, Gabriella Bretti, Elishan Brown, Paola Stolfi, Davide Vergni

IFN
Institute for Photonics and Nanotechnologies
National Research Council

Summary



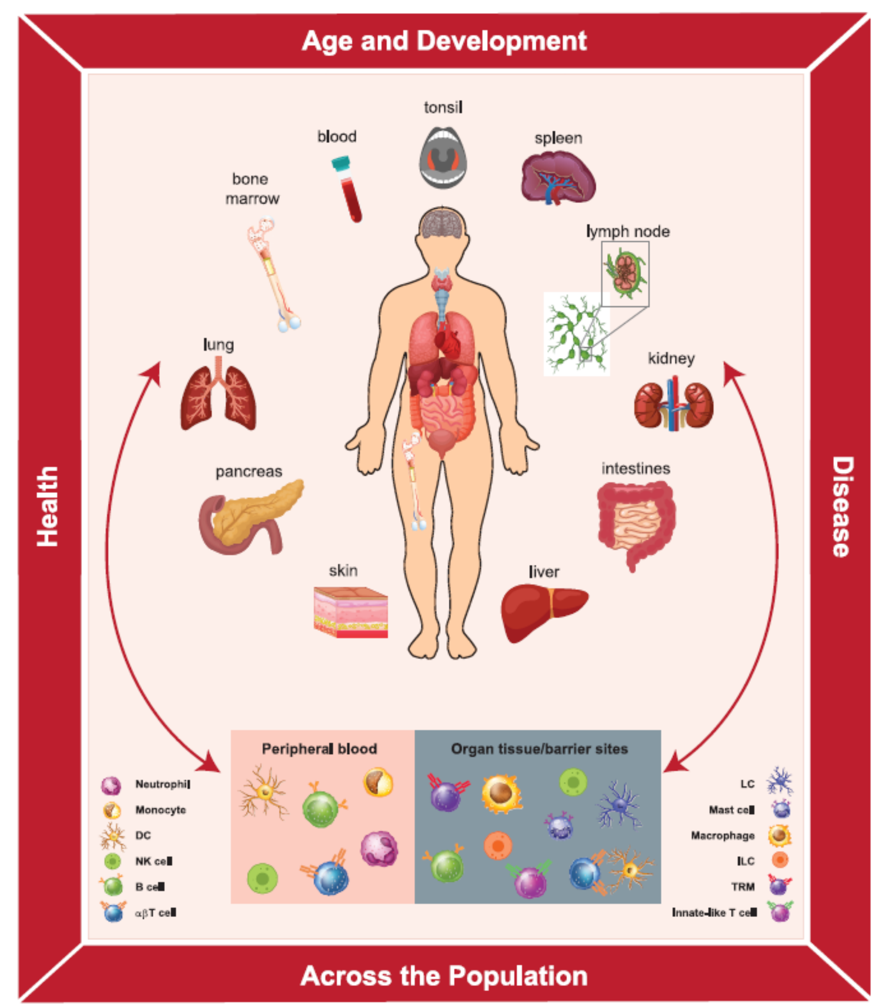
IFN Institute for Photonics and Nanotechnologies
National Research Council

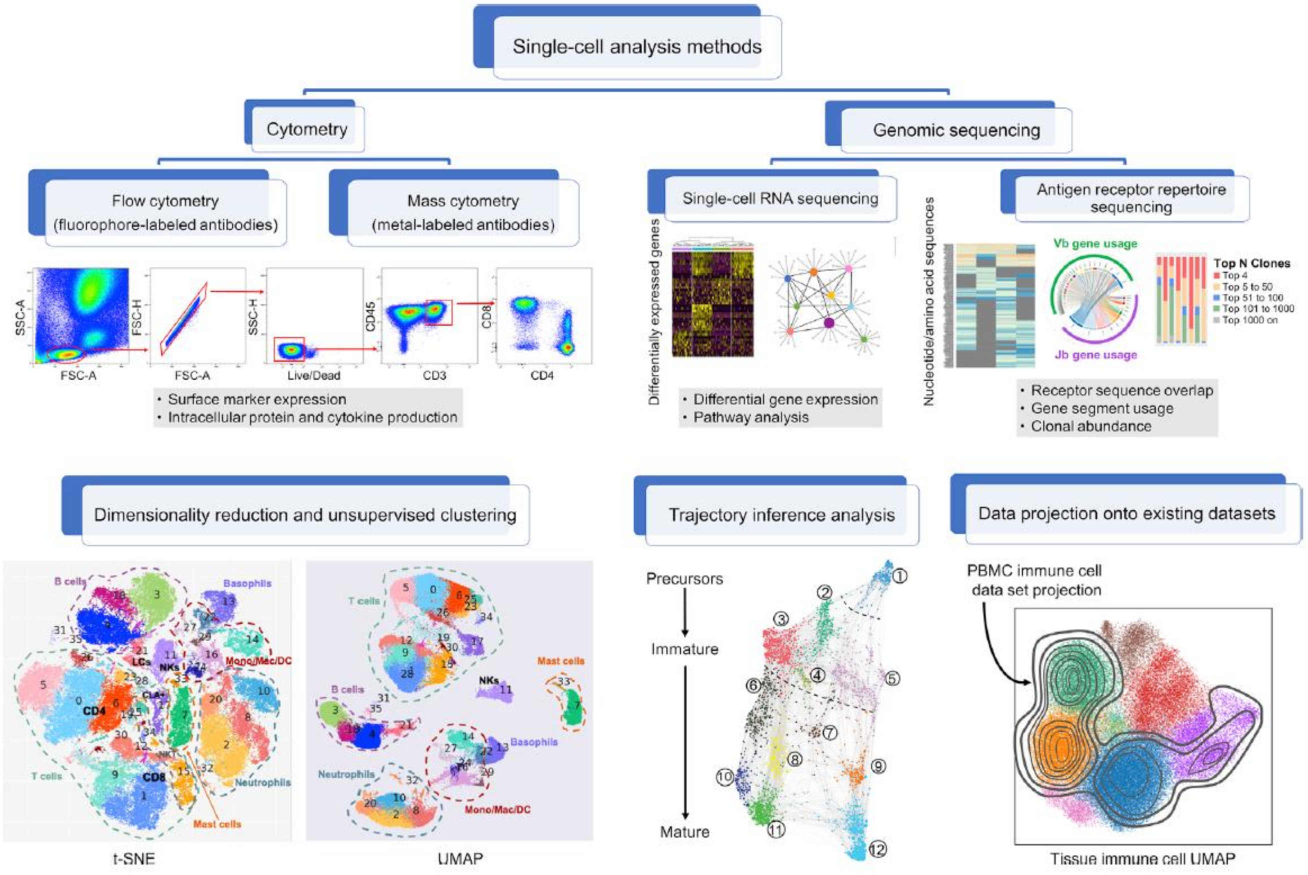
Synergy with Systems Biology

iScience 23, 101509, September 25, 2020

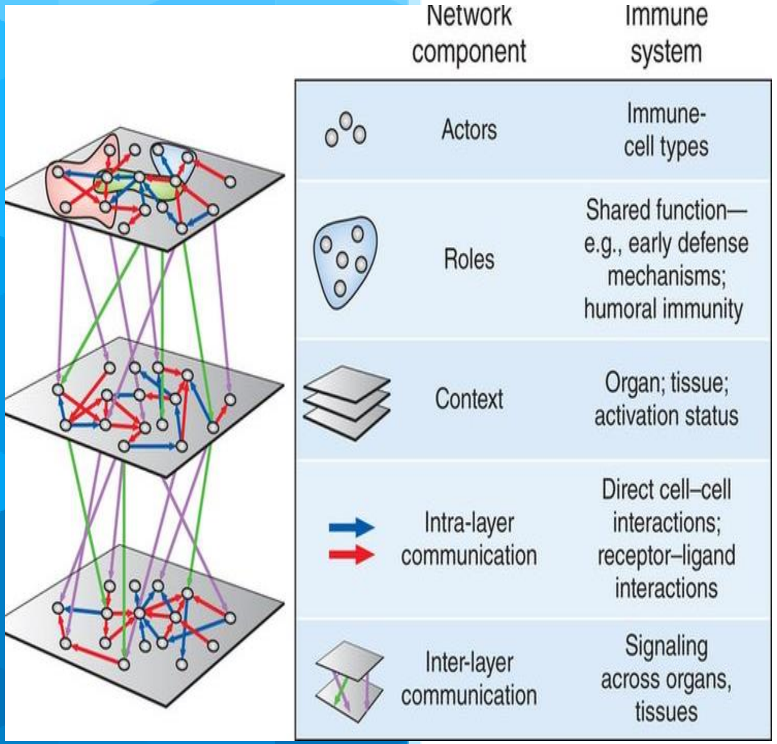
The Whole Body as the System in Systems Immunology

Maya M.L. Poon[1,2] and Donna L. Farber[1,2,3,*]

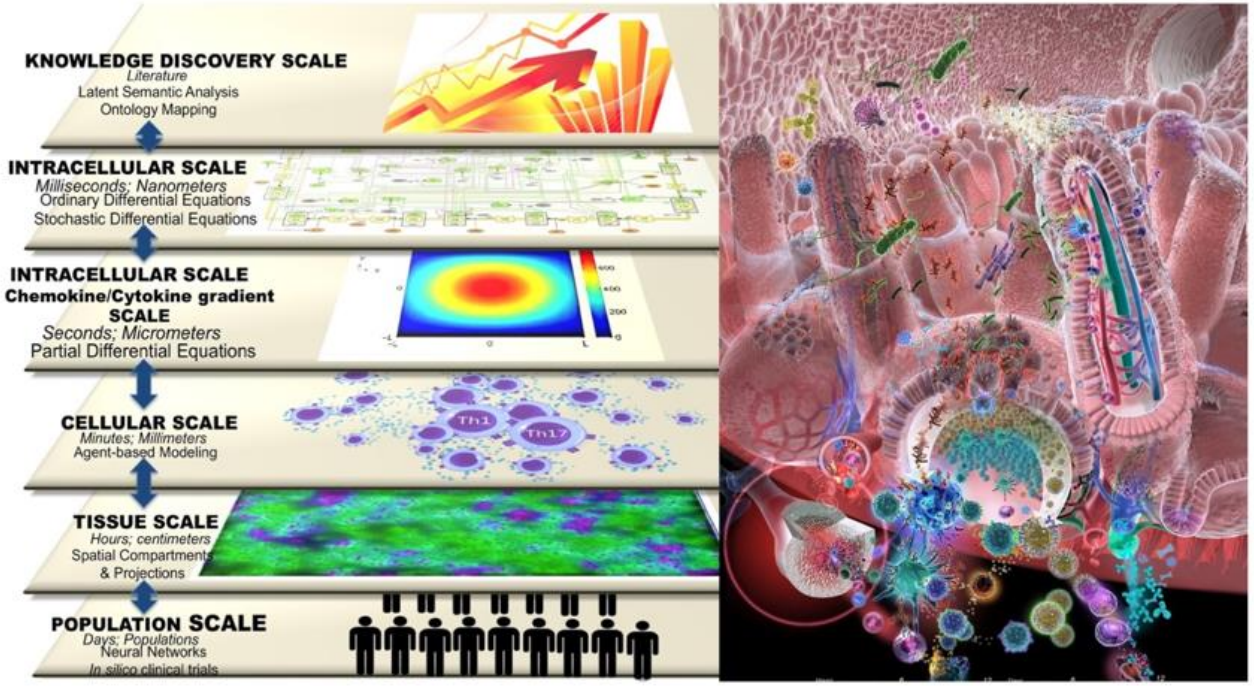




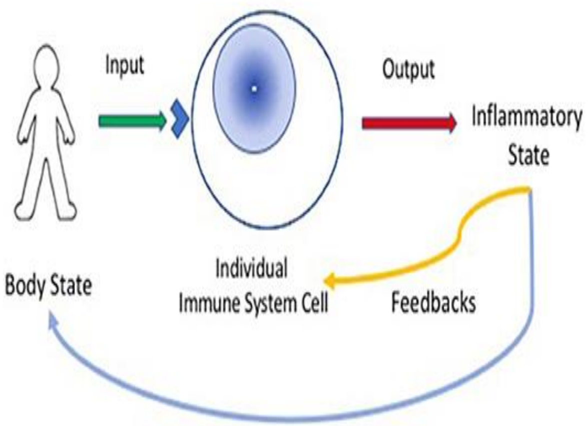
IFN
Institute for Photonics and Nanotechnologies
National Research Council



Andreas Bergthaler Nat Immunology 2017

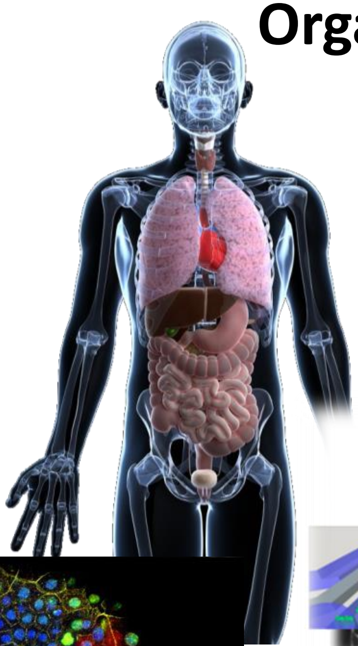


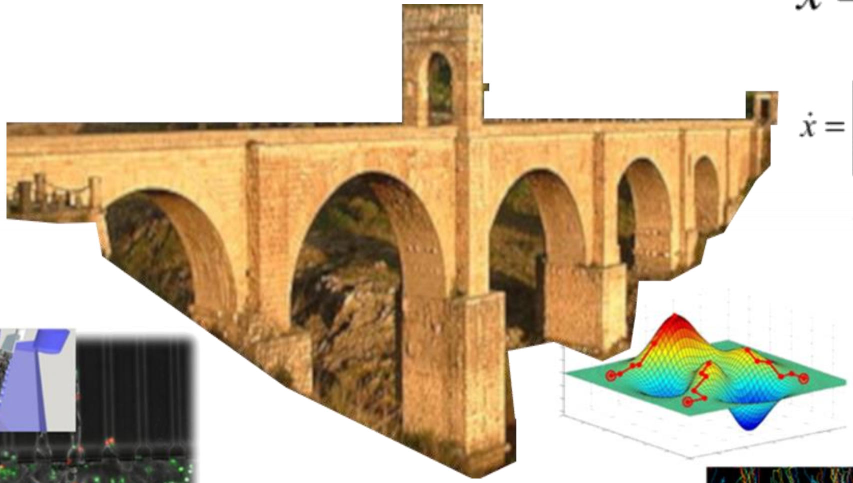
Immune Cell Computation



Cohen Front. Immun 2019

Organs on chip, imaging and computation for biological discovery



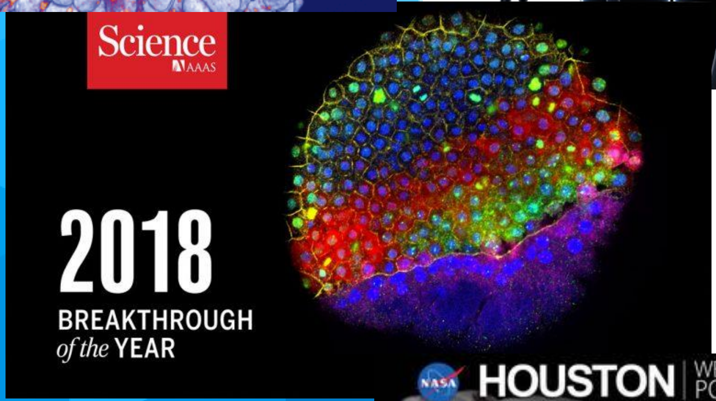


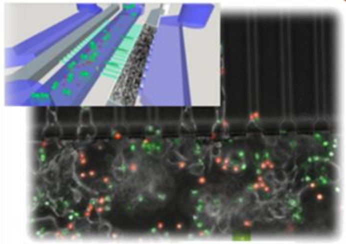
$$\dot{x} = f(x(t),u(t),t) \quad f(x_e,0,t) = 0,$$

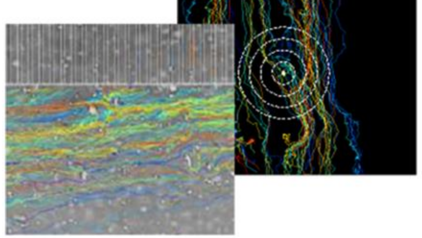
$$\dot{x} = \begin{bmatrix} 0 & 1 \\ -2 & -3 \end{bmatrix} x + \begin{bmatrix} 1 \\ 1 \end{bmatrix} u(t)$$

$$(t) = 0$$

$$\begin{bmatrix} & 1 \\ & -3 \end{bmatrix} \begin{bmatrix} x_{1e} \\ x_{2e} \end{bmatrix} = 0 \Rightarrow \begin{bmatrix} x_{1e} \\ x_{2e} \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \end{bmatrix}$$





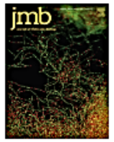


Take a look !!

jmb

Journal of Molecular Biology

Volume 431, Issue 14, 28 June 2019, Pages 2485-2486



Editorial Overview

Seeing Your Way to New Insights in Biology ☆

Charles Reilly [1], Donald E. Ingber [1, 2, 3]

⊞ Show more

https://doi.org/10.1016/j.jmb.2019.04.033

Get rights and content

Thanks to:

UCBM CAMPUS BIO-MEDICO UNIVERSITY OF ROME
Gut on chip
Liver On Chip

institut Curie
Ensemble, prenons le cancer de vitesse.
Tumor microenvironment reconstitution

150 1869 2019 Bambino Gesù OSPEDALE PEDIATRICO
Organoids,
Immunotherapy

Department of Medicine
Duke University School of Medicine
Immunosurvelliance

SAPIENZA UNIVERSITÀ DI ROMA
Statistical Mechanics
& Biological
Complexity

Institut de cancérologie GUSTAVE ROUSSY
VILLEJUIF - www.igr.fr
Onco-immunology

Università di Roma Tor Vergata Sensors Group
Image analysis
Machine Learning

IAC CONSIGLIO NAZIONALE DELLE RICERCHE
In-silico models

Contacts: luca.businaro@cnr.it
adeledeninno@gmail.com