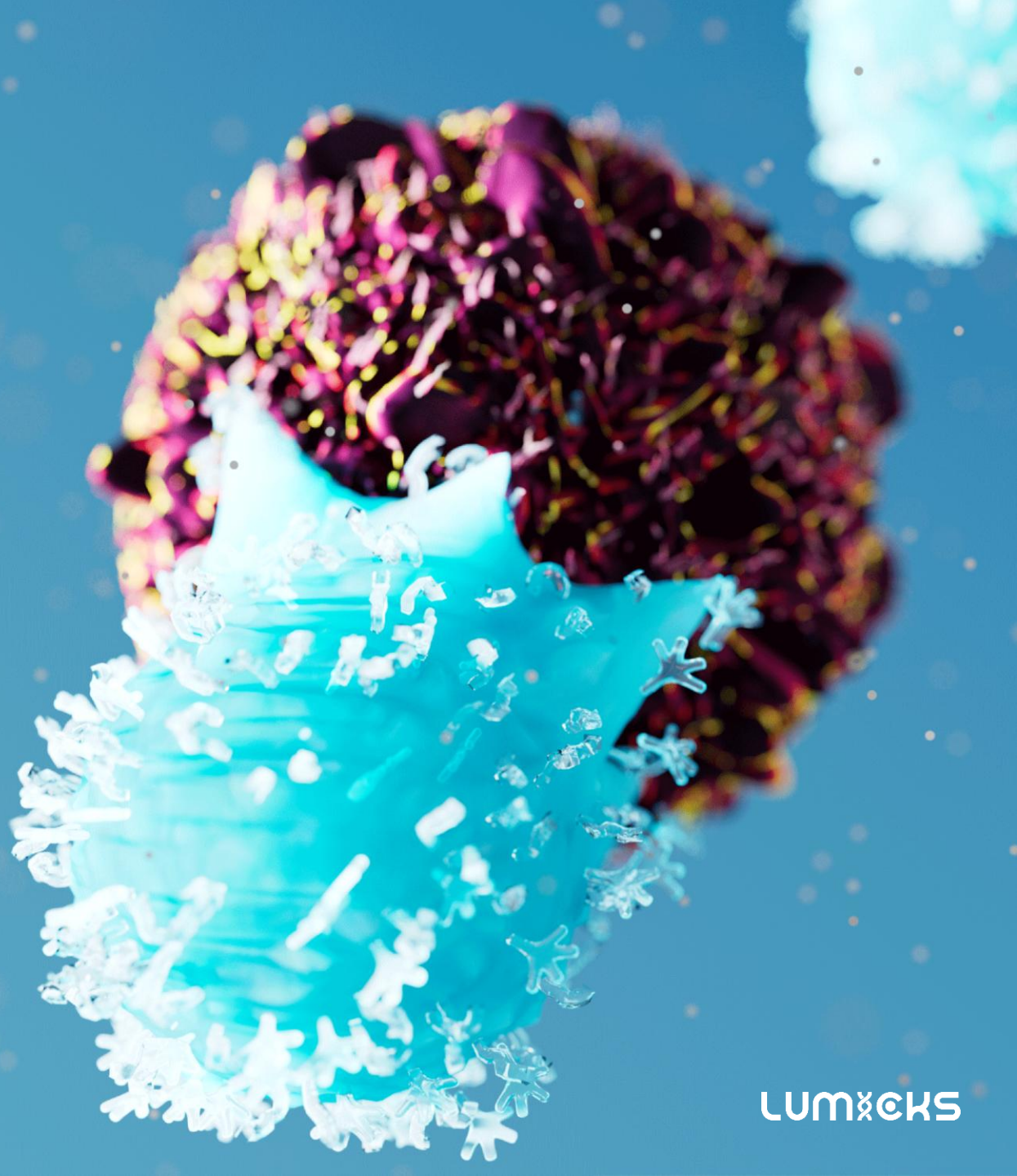
Welcome

z-Movi training presentation

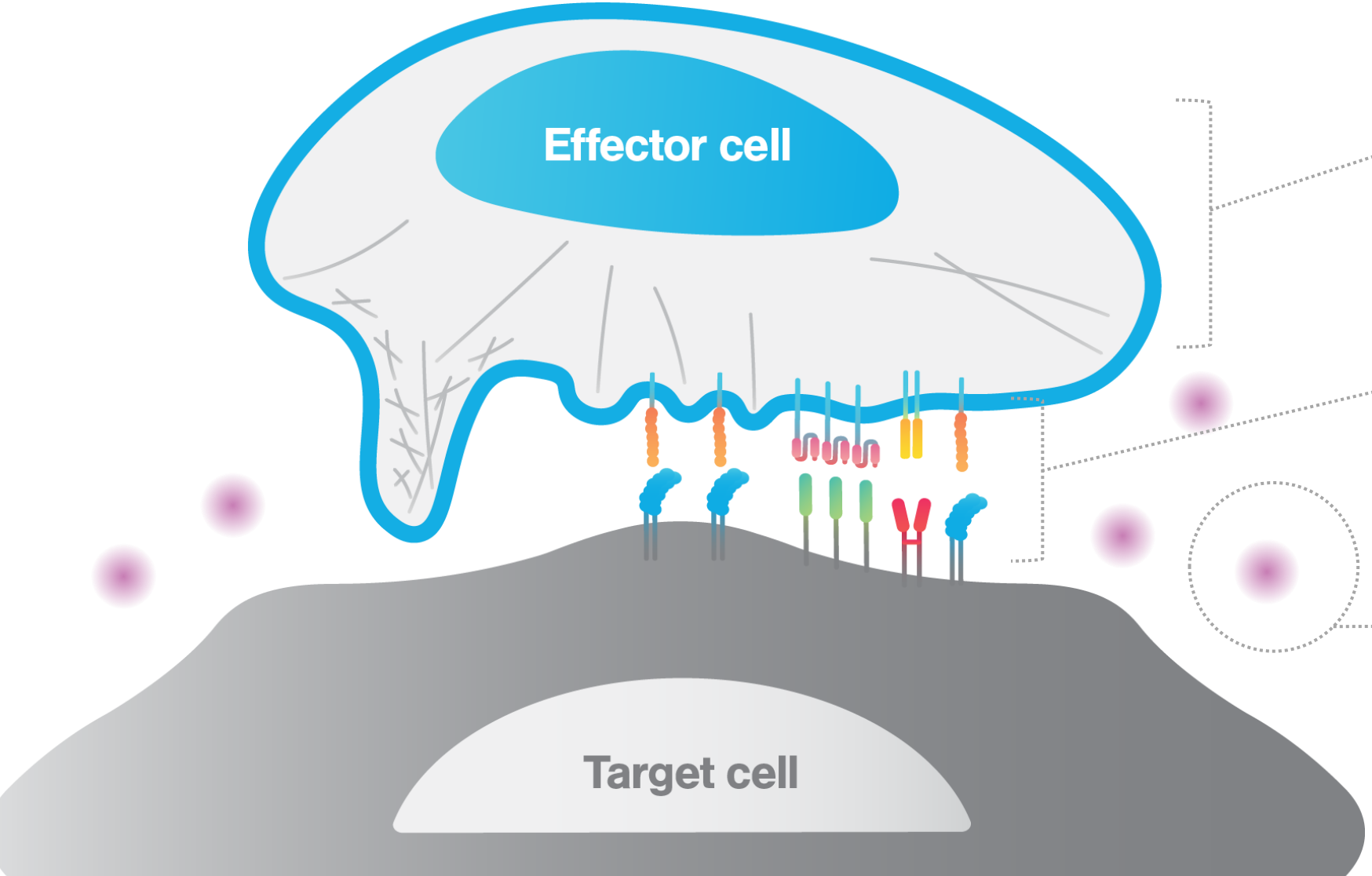
Ray Wang | FAS CS

LUMICKS



Technology

Cell-cell binding is governed by a complex and dynamic interplay between the multitude of receptor-ligand binding and cell activity in the context of the physiological environment



Cell activity
- Cytoskeletal activity
- Activation state
- Viability

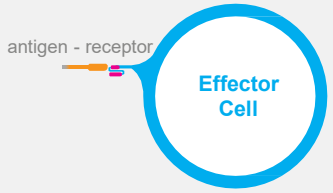
Receptor-Ligand
- Population
- Abundance
- Accessibility
- Valency
- Affinity

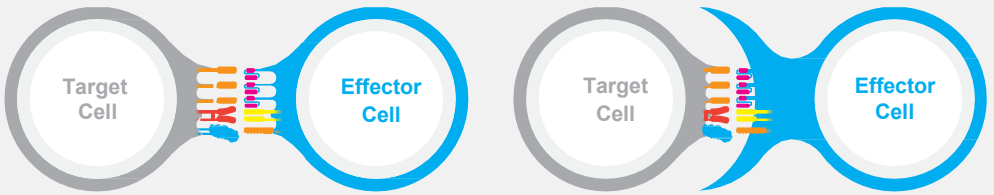
Physiological environment:
- TME acidification
- ECM composition
- Glycocalyx modification

Cell Avidity measures cell-cell binding, a critical step in the Mechanism of Action

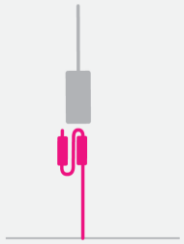
Key Steps of the Mechanism of Action

Molecular binding | Cell-Cell Binding | Biological outcome





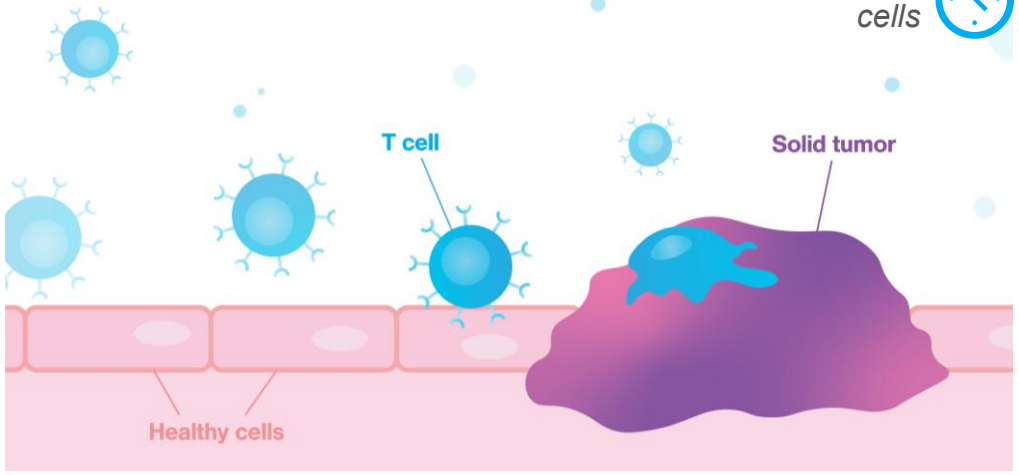
Alternative tools:



Surface plasmon resonance

Tetramer binding assays - flow cytometer

*Cell Avidity measures the **time-sensitive handshake** between cells*



Alternative tools:

Single-cell functional analysis

Single-cell functional proteomics

Microscopy Functional analysis

In vivo imaging mouse model

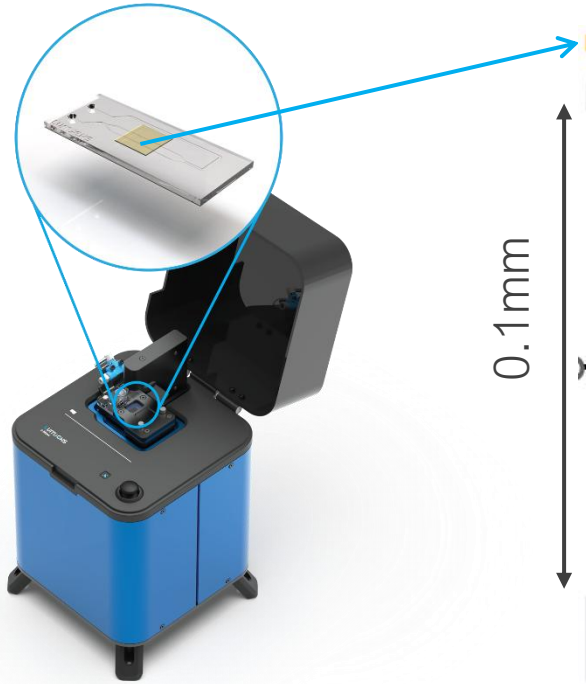
LUMICKS

“Need for technology that allows direct, fast and high throughput measurements of T cell binding avidity.”

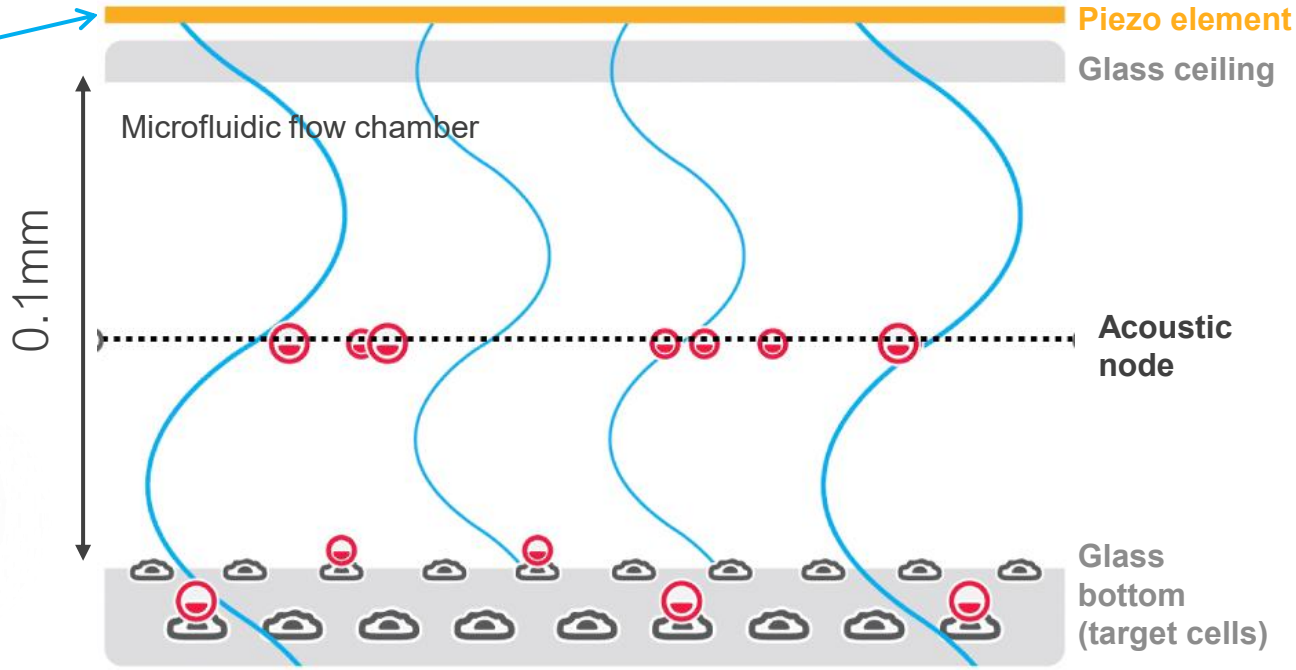
z-Movi® Cell Avidity Analyzer

z-Movi a complete benchtop solution using acoustic force technology in a chip

Let's zoom in!

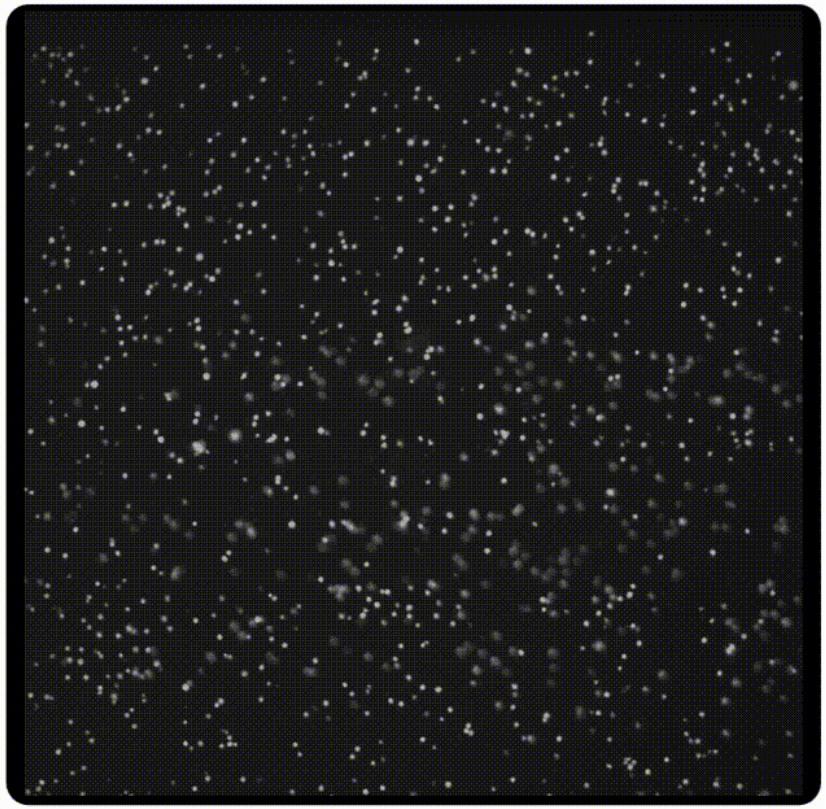


Chip technology:

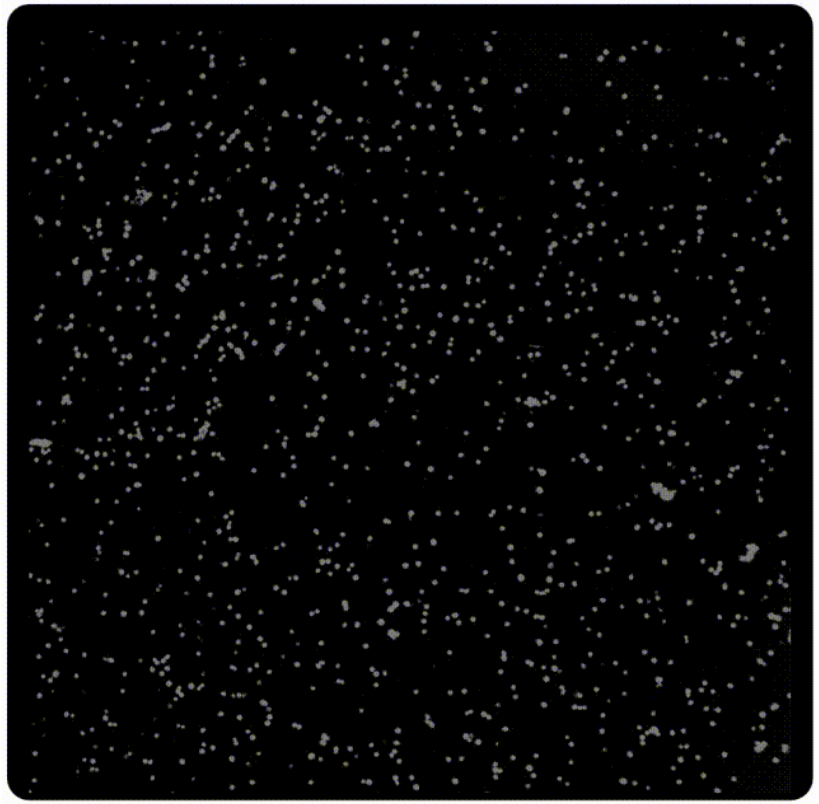


References
Sitters et al., Nature Methods 2015 – Acoustic technology principle | Kamsma et al., Cell Reports 2018 – CD4+ T cell adhesion to fibronectin

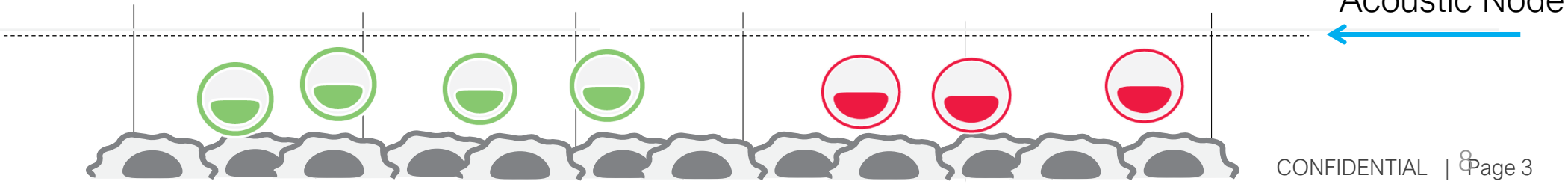
Fluorescence imaging is used to determine if T cells are bound or not to the tumor monolayer



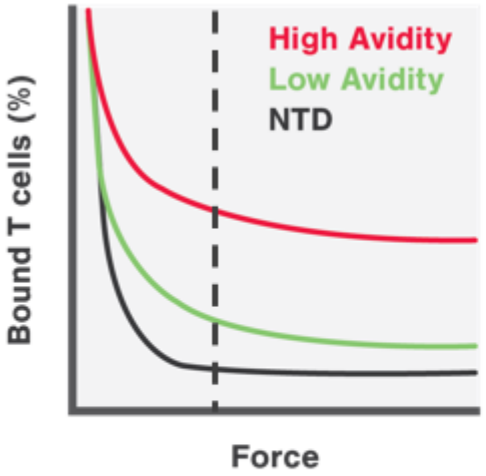
Non tumor targeting T cells

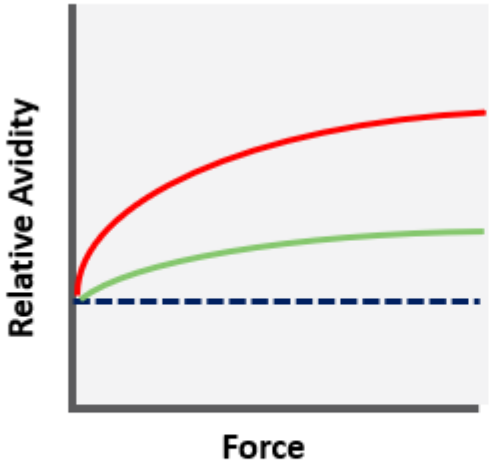


Tumor targeting T cells



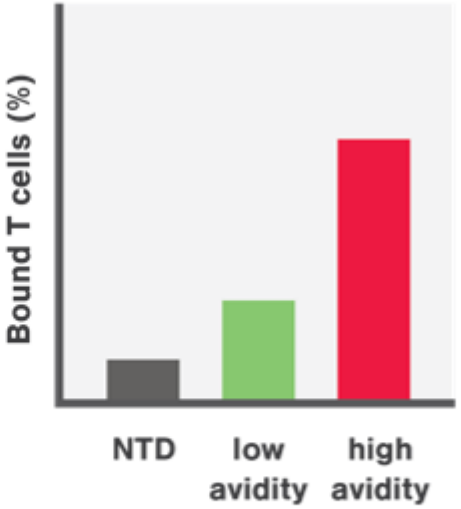
Easy to interpret avidity curves to rank order desired functional interactions





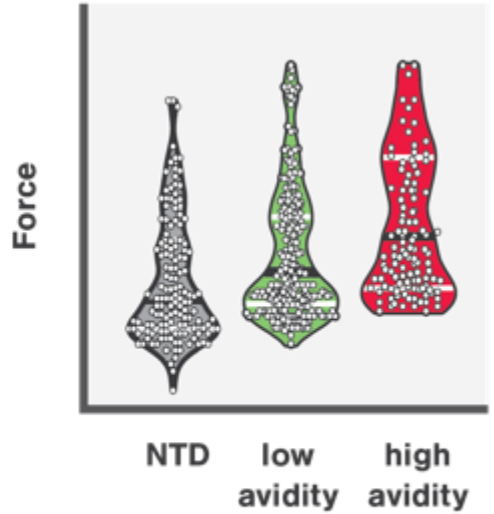
Avidity curves

Percentage of bound T cells as a function of force (negative control, NTD)



Bar graphs

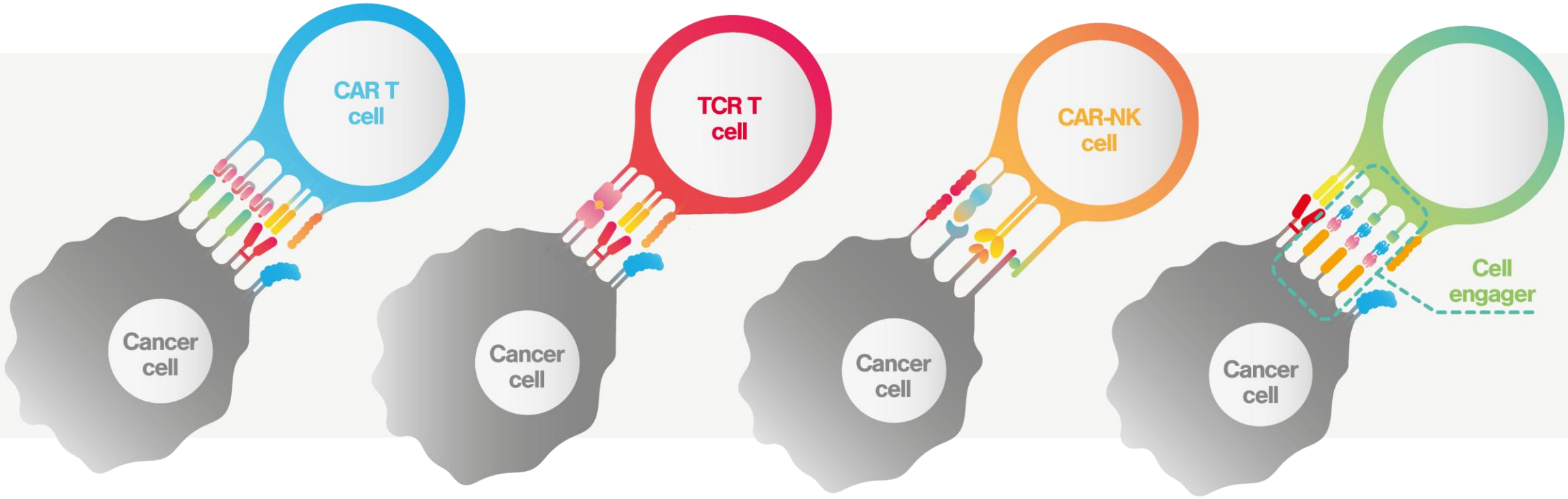
Percentage effector cells that still bind to the monolayer at the end of the force ramp (after application of 1000pN)



Violin dot plots

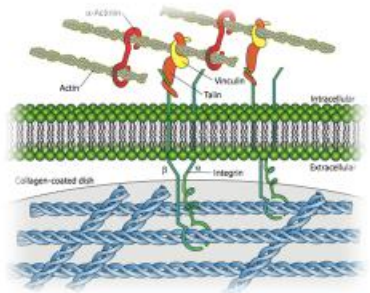
Track the force-resistance of individual events, allowing insights into population-level distribution data

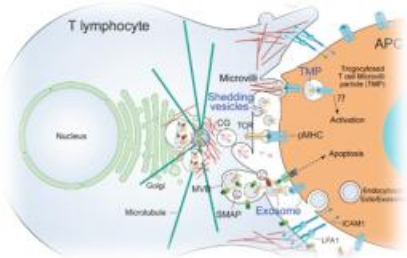
Different applications for
cell avidity measurements

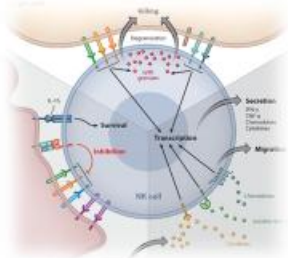


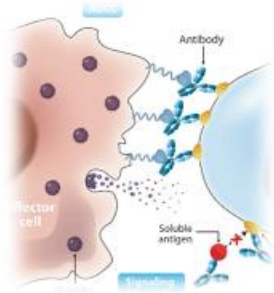
Avidity Governs Diverse Classes of Cell Interfaces

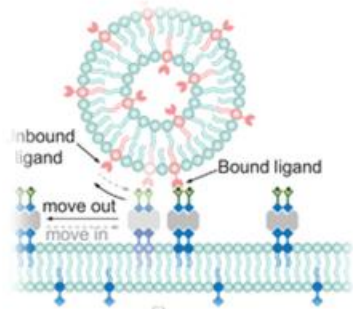
Cell interactions fall into distinct mechanistic classes











	1. Native adhesion	2. Immune Synapses	3. Multivalent driven Engagement	4. Fc-Mediated Immune Engagement	5. Nanoparticles & Viral delivery
examples	*Integrin-ICAM (or ECM)*	*TCR-pMHC*	*Receptor co-clustering (non-synaptic)*	*ADCC*	*LNP delivery*
	aGPCRs	*NK KIR-HLA*	*Biparatopic antibody induced clustering*	*ADCP*	*Viral infections*
	CD44-HA	*TCE/CAR synapse*	*Death receptor clustering*	*Complement engagement*	*Liposome – Cell*

Avidity changes reflect how biological or engineered perturbations alter these interactions:

Biological state & regulation

- Genetic perturbations (KO, knock-in, expression level, mutations)
- Cell Metabolism & state (eg activated/exhausted/glycosylation)
- Signalling-driven receptor activation or redistribution

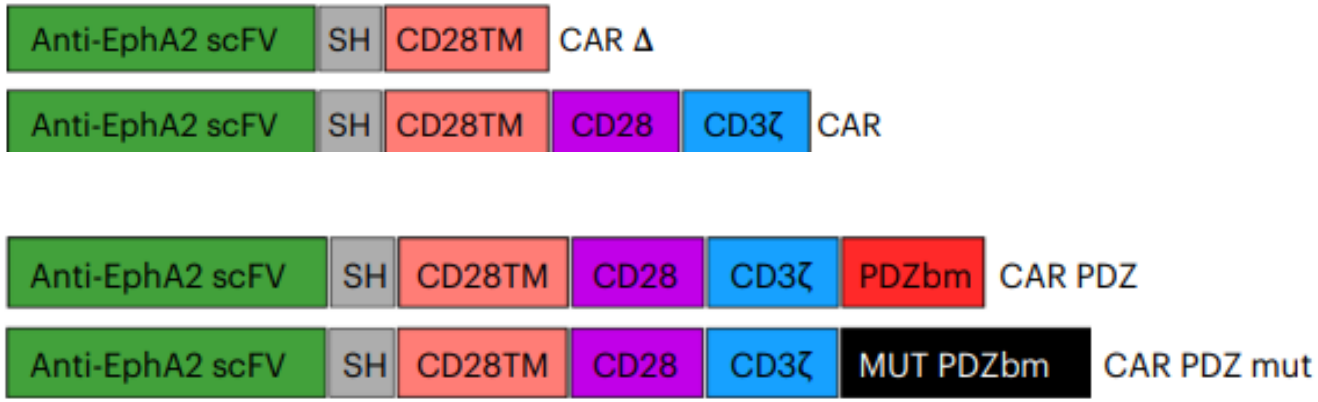
Therapeutic & engineered inputs

- Small molecules and biologics
- Engineered receptors or ligands (e.g. CARs, TCEs)
- Particles and delivery systems (e.g. antibodies, LNPs)

Immune-Oncology | CAR-T/NK | Mechanism of Action

Functional Enhancement

Limited options to measure CAR design changes on NK cells to enhance synaptic-binding



Problem

CAR-NK cell efficacy is limited by weak immune synapses and a *lack of rapid reproducible assays to assess design changes* that affect this important cell-cell interaction.

Hypothesis

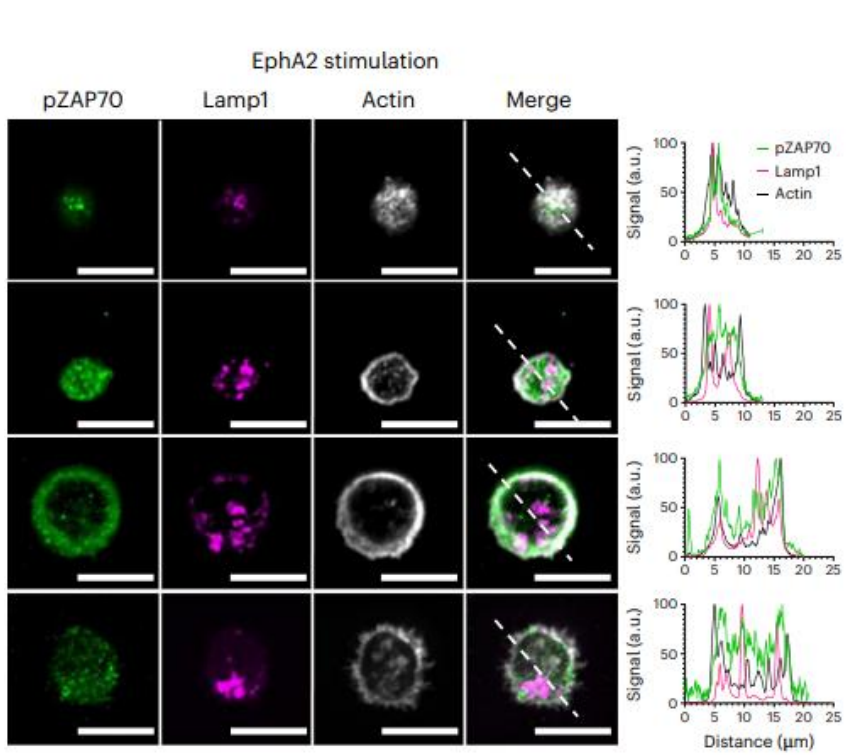
Adding an intracellular PDZ domain to the CAR will *enhance NK synapse formation*, improving organization, strength, and tumor binding, *only measurable by cell avidity*.

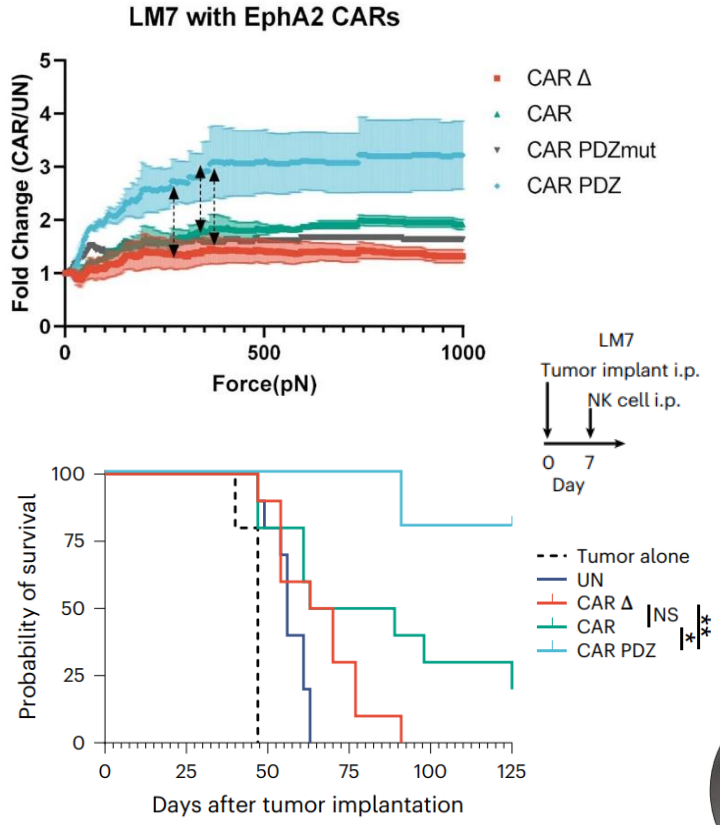
Dr. Stephan Gottschalk
Endowed Chair in Bone Marrow Transplantation & Cellular Therapy
St. Jude Children's Research Hospital

LUMICKS

Chockley et al., Nat. Biotech., 2023

Synapse-tuned CARs demonstrated enhanced cell avidity and anti-tumor activity in CAR-NK cells





Outcome

PDZ-modified CAR-NKs showed *enhanced immune synapse signaling*, increased cell avidity to solid tumors, and improved in vivo efficacy in mouse models.

Dr. Stephan Gottschalk
Endowed Chair in Bone Marrow Transplantation & Cellular Therapy
St. Jude Children's Research Hospital

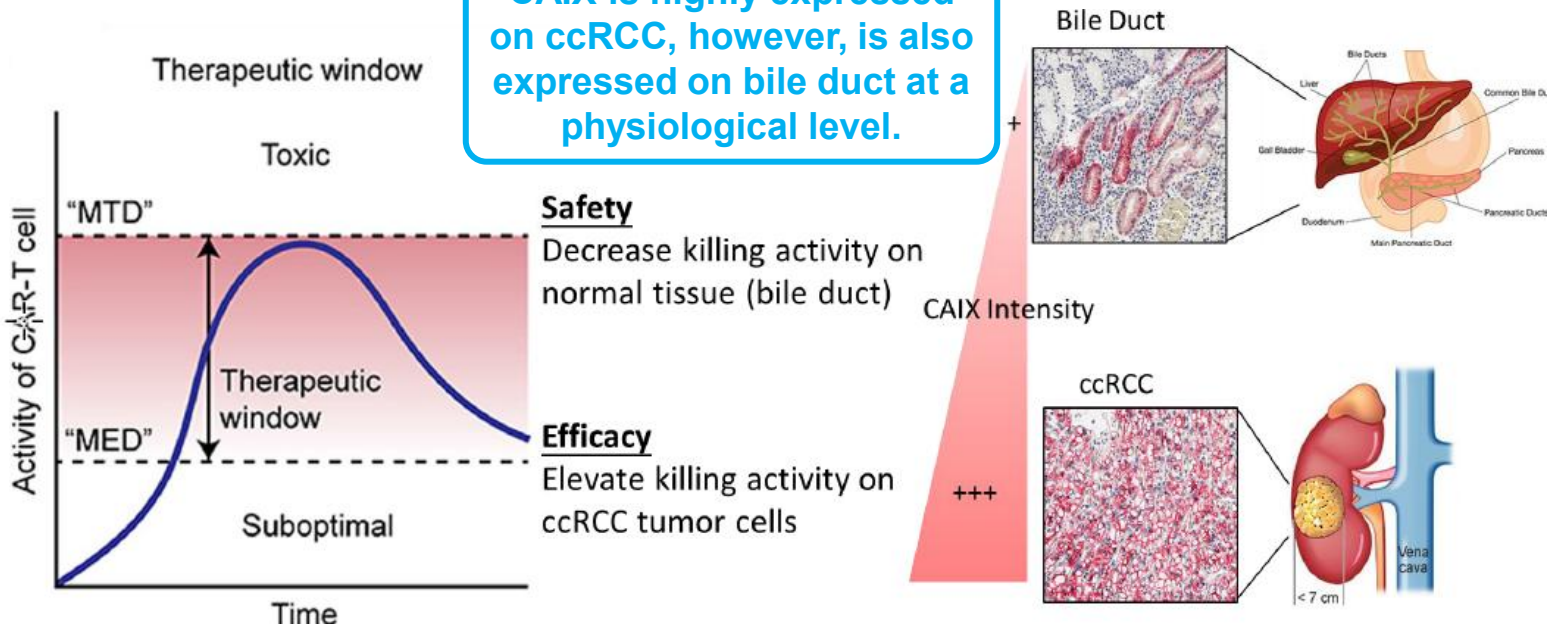
LUMICKS

Chockley et al., Nat. Biotech., 2023

Avidity-tuning for improved safety of CAR T therapy in solid tumors

Dr. Yufei Wang
Sr. Postdoctoral researcher
Dana Farber Cancer Institute

CAIX is highly expressed on ccRCC, however, is also expressed on bile duct at a physiological level.



Problem

Affinity measurements or in vitro cytotoxicity cannot predict on-target-off-tumor binding in a cellular context, which could lead to wrongful dismissal of ideal CAR T candidates, or it requires elaborate and extensive in vivo testing.

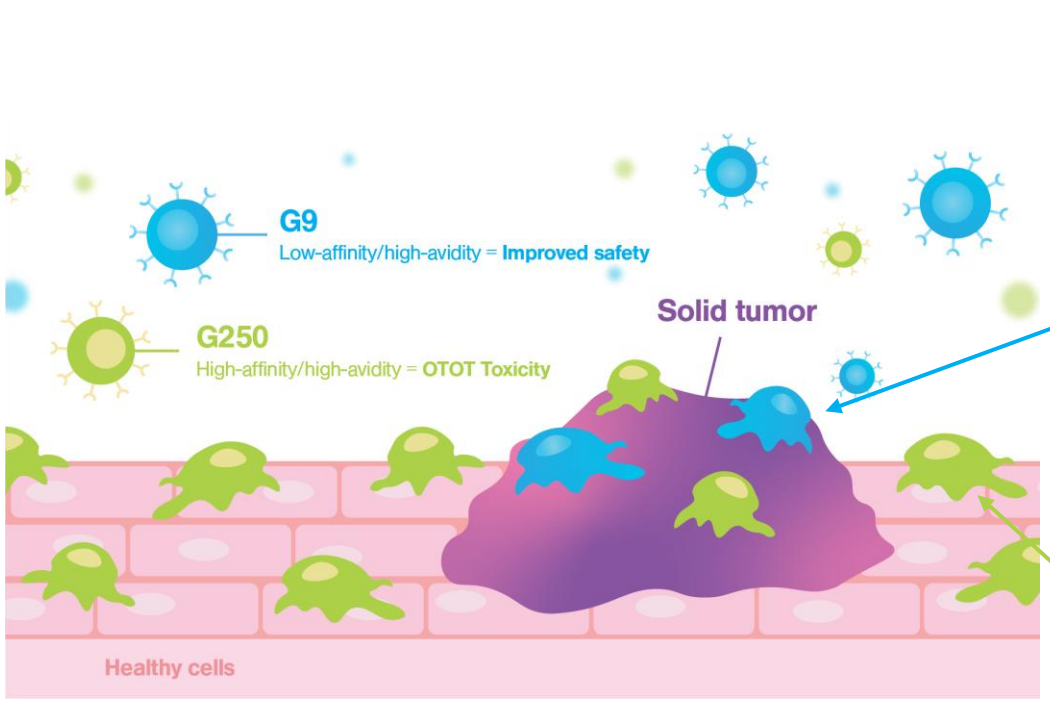
Hypothesis

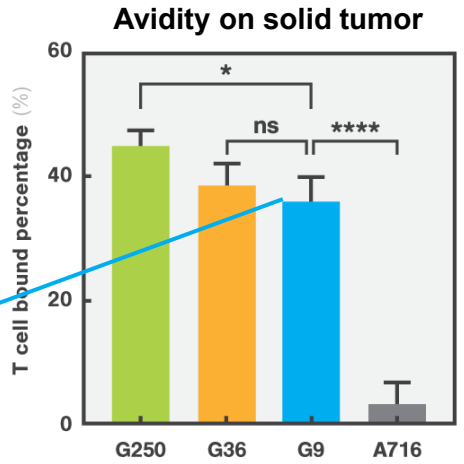
Incorporating a lower-affinity scFv domain will allow sufficiently potent binding of tumor cells, while mitigating on-target off-tumor bindings.

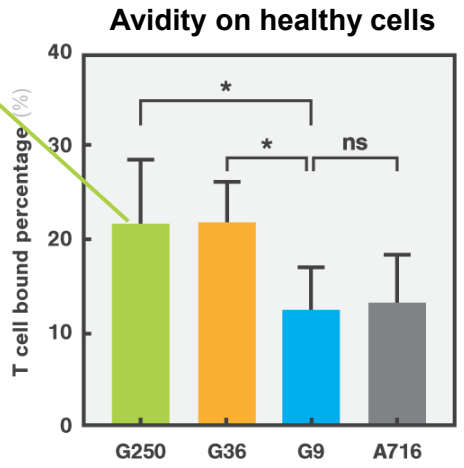
LUMICKS

Wang et al., Mol. Cancer, 2024

Avidity analysis demonstrates safe CAR-T binding to healthy cells







Outcome

G9 CAR-T product **shows strong and potent binding** to **solid tumor cells, comparable to G250**. In addition, as opposed to the clinically toxic G250, G9 anti-CAIX CAR-T demonstrates **low and safe** binding to **healthy cells**

LUMICKS

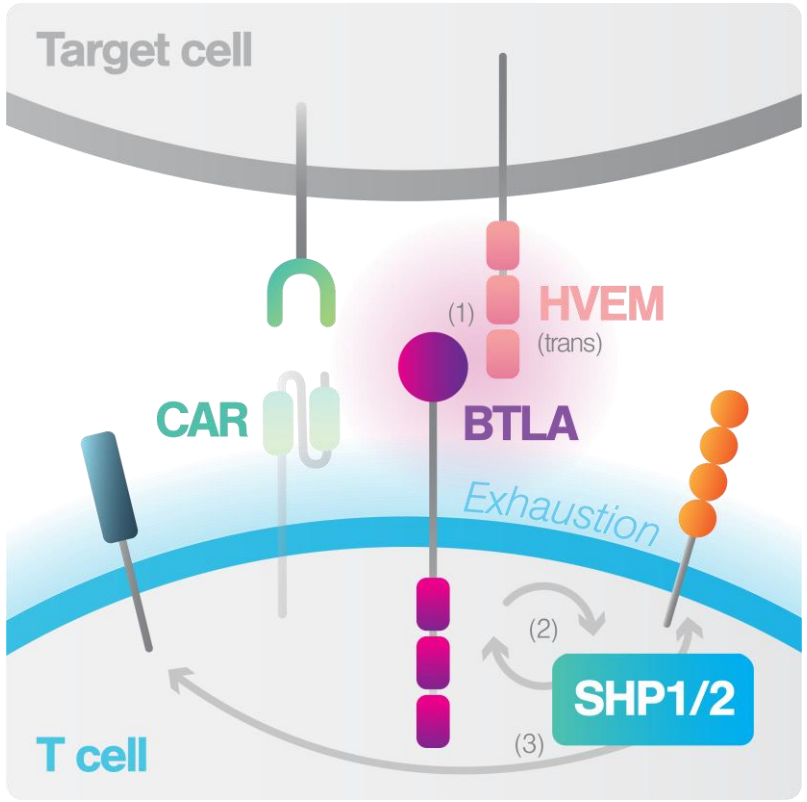
Wang et al., Mol. Cancer, 2024

Immune-Oncology | CAR-T/NK | Design Choice | Avidity Ranking

Functional Enhancement

Cell Avidity uncovers a critical binding mechanism impairing CAR-T potency in the TME

Dr. Marco Ruella
Associate Professor of Medicine
Hospital of the University of Pennsylvania



Problem

A key mechanism deployed by the TME is the **upregulation of immune checkpoint molecules, which affect cell binding**. The BTLA-HVEM interaction between CAR-T cells and tumor cells has emerged as a critical factor in **T-cell inhibition** and **exhaustion**. Proofing the mechanism of action is hard applying current technologies.

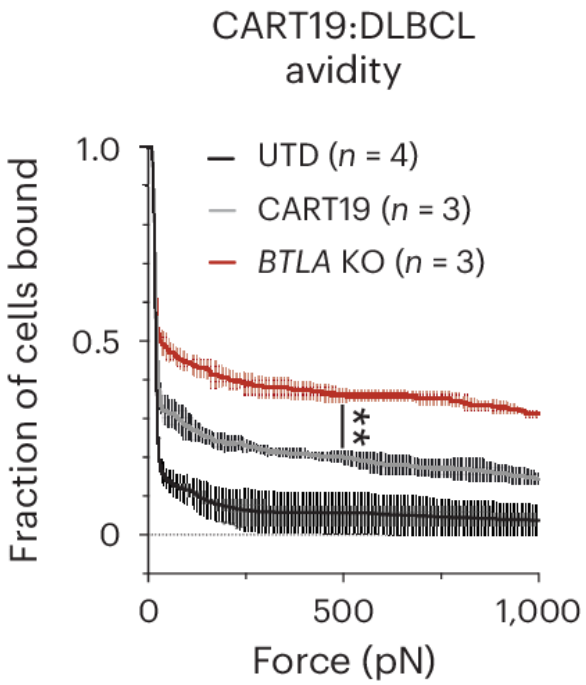
Hypothesis

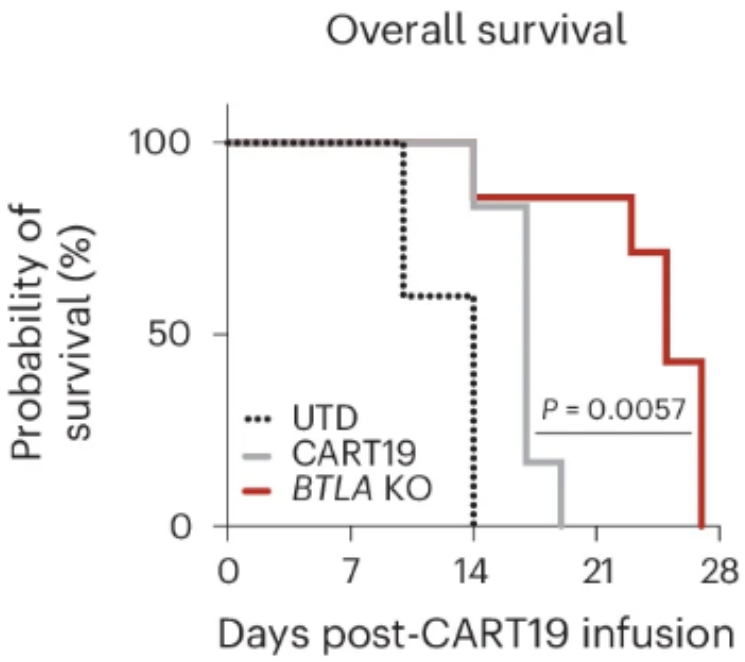
Avidity enhancement can be achieved by deleting BTLA expression on CAR-T cells to improve synapse formation, expansion, cytokine secretion and potency *in vivo*.

LUMICKS

Guruprasad et al., Nature Immunology, 2024

Avidity enhancement by deleting BTLA improves CAR-T potency in the TME



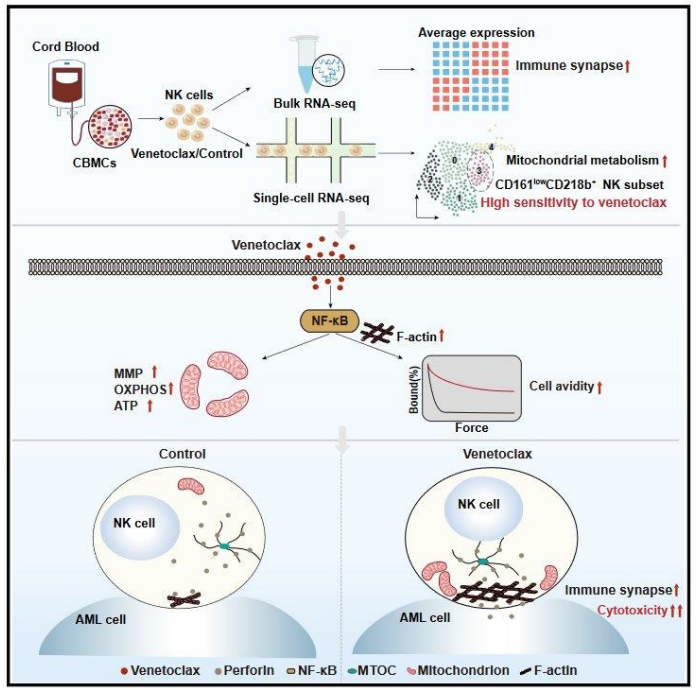


Outcome

BTLA-deficient CAR-T cells exhibited increased cell avidity to tumor targets when using CD19-targeting CAR-T cells leading to **improved functionality** ***in vivo***

LUMICKS

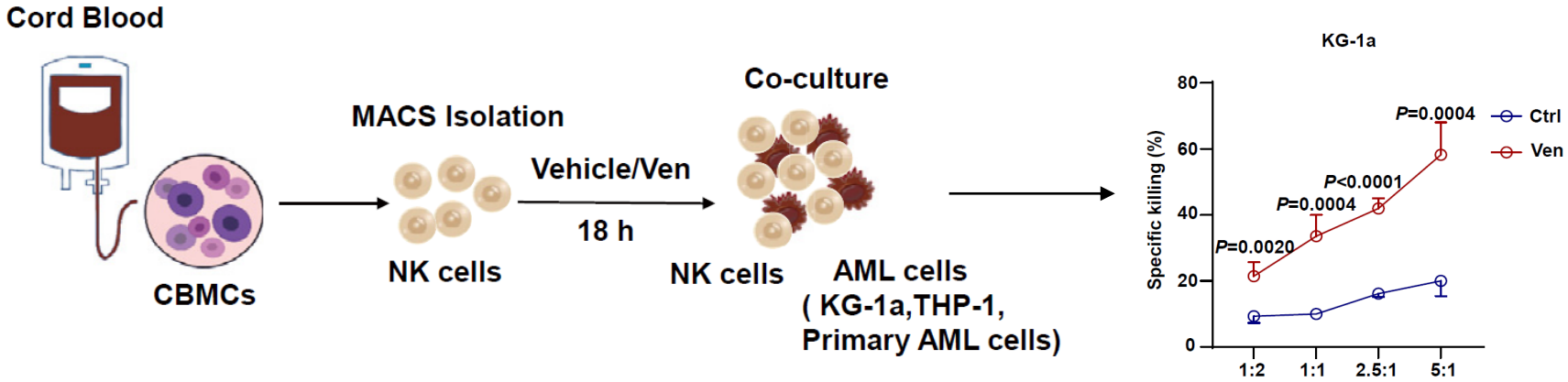
Guruprasad et al., Nature Immunology, 2024



Cell Reports
Medicine

Article

Venetoclax acts as an immunometabolic modulator to potentiate adoptive NK cell immunotherapy against leukemia

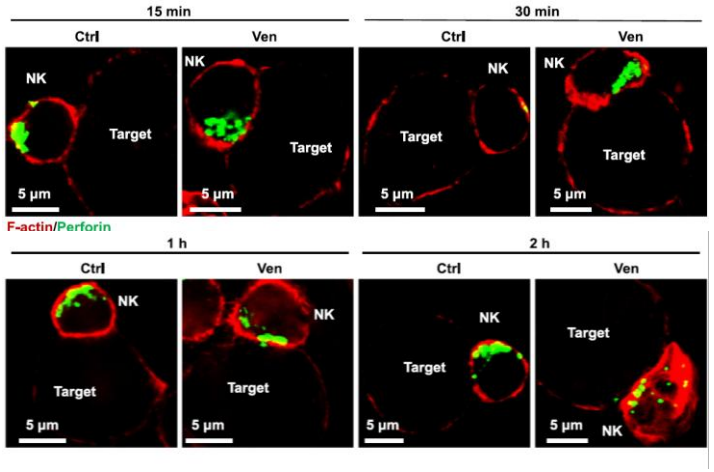


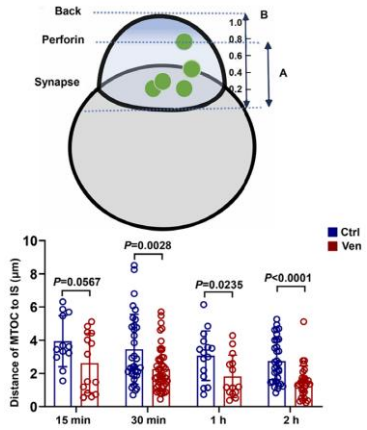
Venetoclax (BCL-2 inhibitor) enhances NK cell cytotoxicity in AML patients

References
Cell reports. Medicine, 101580. 21 May. 2024

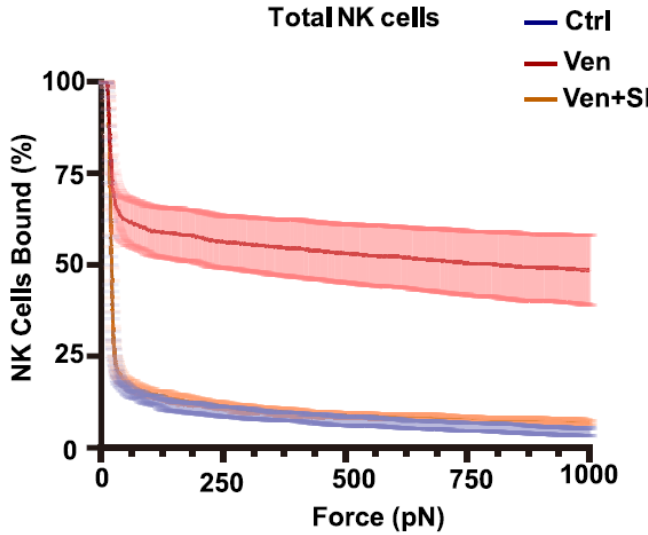
Venetoclax increases NK cell avidity for AML cells and improves IS formation via NF-κB pathway

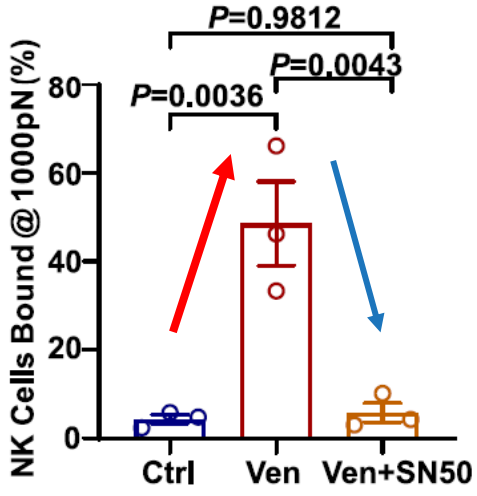
lytic granule polarization and IS formation was increased





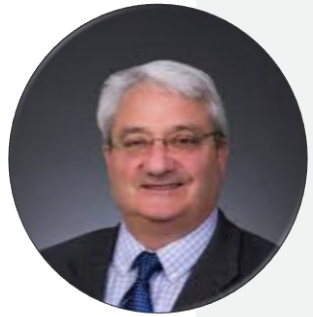
Enhanced avidity of NK cells to AML cells by Venetoclax was suppressed by NK-kB inhibitor SN50





References
Cell reports. Medicine, 101580. 21 May. 2024

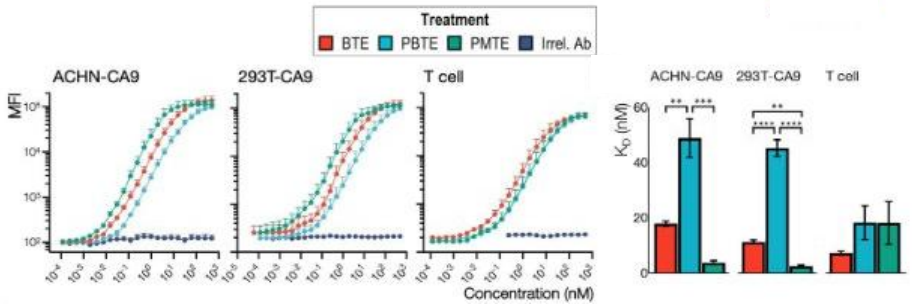
Introducing multivalency upon Fc-domain inclusion restores cell avidity for CA9 on ccRCC



Dr. David Weiner
Executive Vice President & Director of Vaccine and Immunotherapy Center, Wistar Institute

half-life

3.4 hours | 4.5 days | 3.9 days



Problem

Current technologies lack the ability to fully characterize functional trade-offs between first- and second-generation bispecific T-cell engagers or do not allow the assessment of the impact of structural modifications, like the Fc domain in PBTE formats, on mechanism of action.

Hypothesis

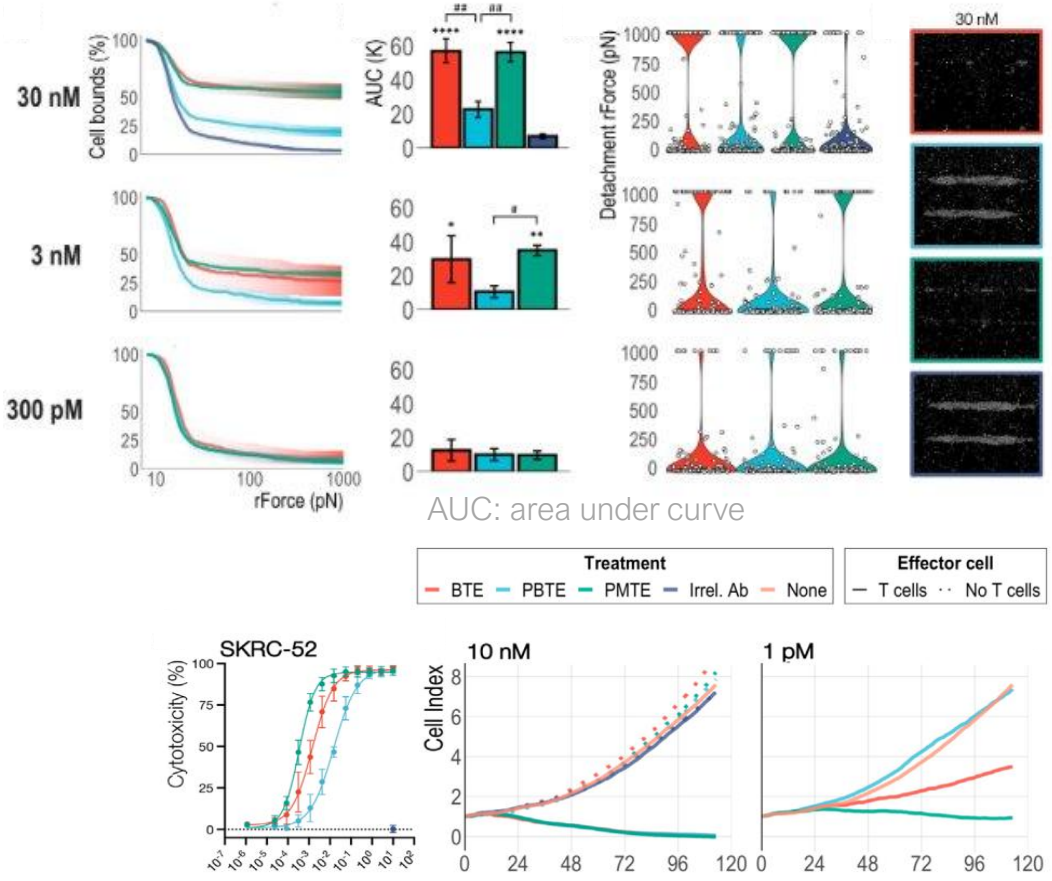
Adding an extra tumor-targeting (PMTE format) domain, thereby increasing cell avidity, restores the engager's function, creating a potent format with Fc-enhanced half-life.

BTE: bispecific T cell engager; PBTE: persistent BTE; PMTE: persistent multivalent TE

LUMICKS

O'Connell et al., JITC, 2024

Format-tuning of CE by including an Fc-domain for increased half-life is only beneficial upon introducing multivalency for tumor-targeting

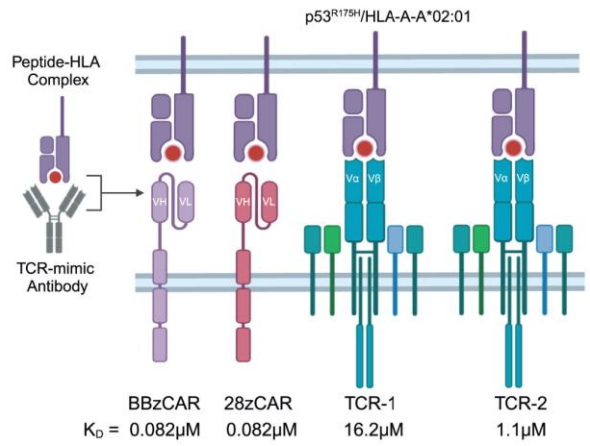


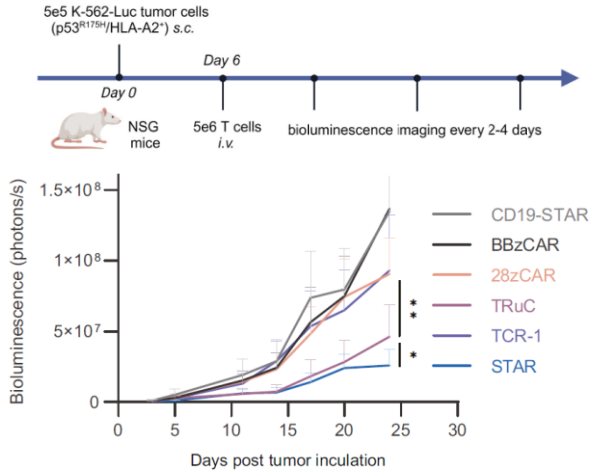
Outcome

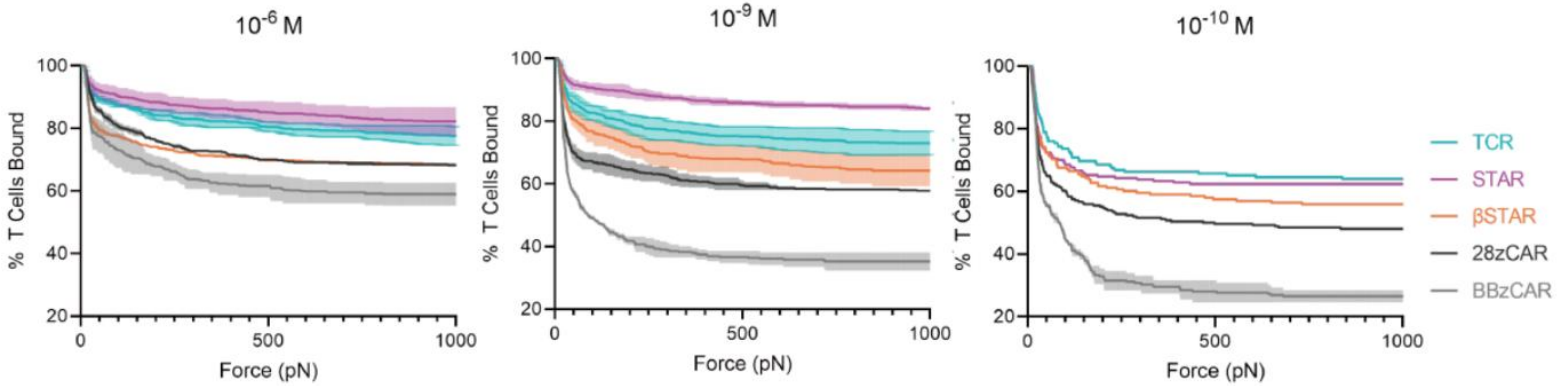
PMTE's additional Ag binder **restores** PBTE's Fc-domain related reduced CA9 (target on ccRCC) **avidity**. The repercussions of the PBTE's parallel reductions in flow binding appeared to take shape with weaker synapses while the PMTE's higher avidity for CA9 lent itself to **stronger synapses**.

O'Connell et al., JITC, 2024

STARs and TCRs tend to form tighter immune synapses with target cells than CARs







Problem

CARs exhibit 10–100 times lower sensitivity than T cell receptors TCRs when targeting human leukocyte antigen (HLA) class I-presented p53R175H neoantigen

Hypothesis

Introduced T cell receptor fusion constructs (TRuCs) and synthetic TCRs and antigen receptors (STARs) can enhance CAR functionality

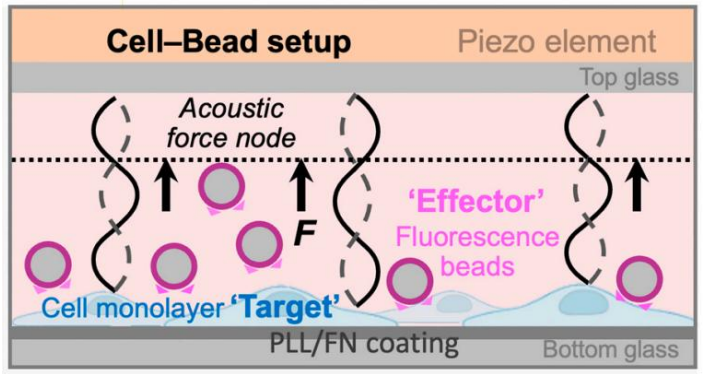
Dr. Xin Lin
School of Basic Medical Sciences
Tsinghua University

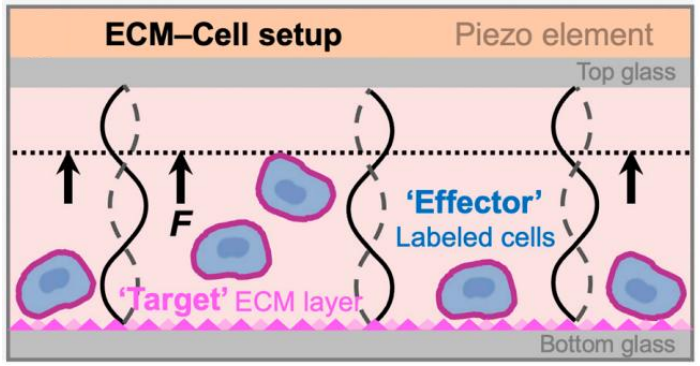
LUMICKS

Huang et al., Cell Reports, 2024

Protein-on-glass Avidity as a novel tool for mechanistic insights into ECM-cell interactions for metastasis

Dr. Lining (Arnold) Ju.
USYD, School of Biomedical Engineering





Problem

Current approaches to measure cancer cell-ECM interactions are either **low throughput**, **not quantitative**, or with **poor sensitivity** and **reproducibility**.

This makes it difficult to obtain robust data and uncover the MoA behind the metastatic potential of cancer cells.

Hypothesis

Cancer cell – ECM interactions directly correlates to the metastatic potential of cancer cells.

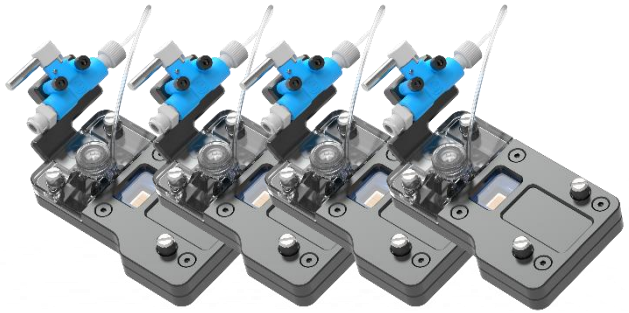
LUMICKS

Wang et al., 2023 (Biosensors)

z-Movi Cell Avidity Analyzer

- z-Movi® Cell Avidity Analyzer
  - z-Movi Microscope
  - z-Movi Controller
  - 5x z-Movi Chips
  - Workstation (desktop, screen, mouse, keyboard, external SSD)

- Oceon™ Avidity Software



z-Movi Instrument – Microscope

Specification overview

Number of cells analyzed	~400 cells*
Maximum force applicable on 10 µm polystyrene beads	Up to 1 nano-Newtons
Brightfield/fluorescence illumination	LED (670 nm)/(635 nm)
Objective/Magnification	10x/6.25x
Field of view	1 mm x 2 mm
Fluorescence detection bandwidth	660 – 750 nm
Brightfield/fluorescent frame rate	5 Hz
Dimensions - w x h x d	200 mm x 320 mm x 200 mm
Weight	7.0 kg

*typically 200-300 individual cells are analyzed

z-Movi Instrument – Controller box

- The controller box drives the microscope, and enables the communication between the z-Movi Microscope and the computer.
- Separation of controller and microscope electronics ensures the most stable functioning of the microscope.
- Power cables are equipped with the European plug

Specification overview

Dimensions - w x h x d	250 mm x 80 mm x 225 mm
Weight	2.6 kg

Chip glossary

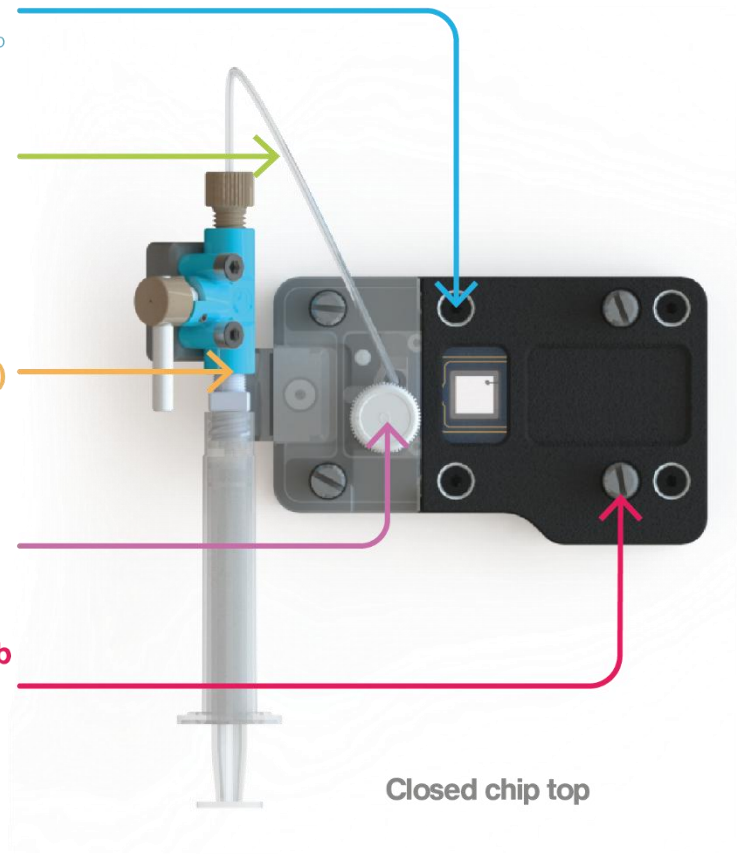
Chip screws – 4x
- Four chip screws hold the chip together and closed

Tubing (outlet)
- Media/cells flow out

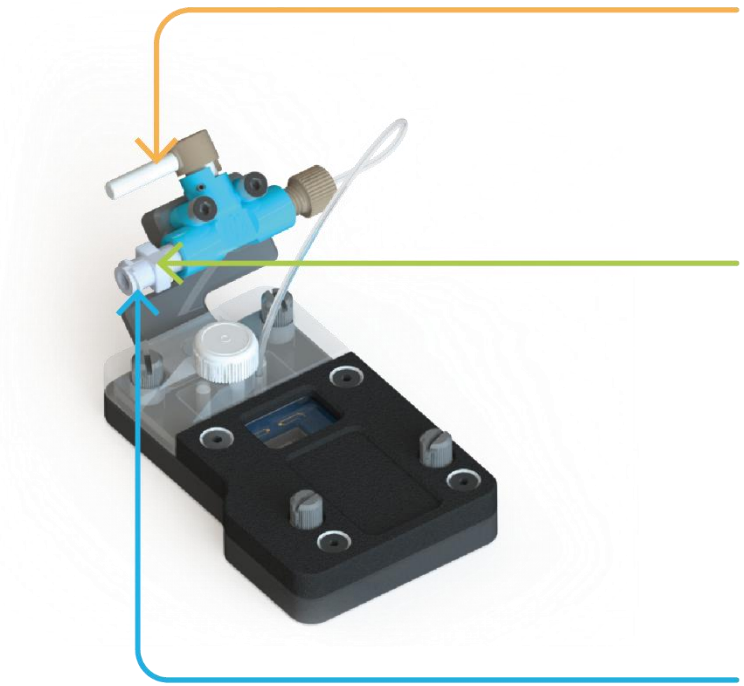
Manual fluidics control (valve adaper)
- Flow control (open/closed)
- Zero flow

Inlet through trans-parent reservoir

Captive screw (thumb screws – 4x)
- Holds the chip in place when placed on the z-Movi



Closed chip top



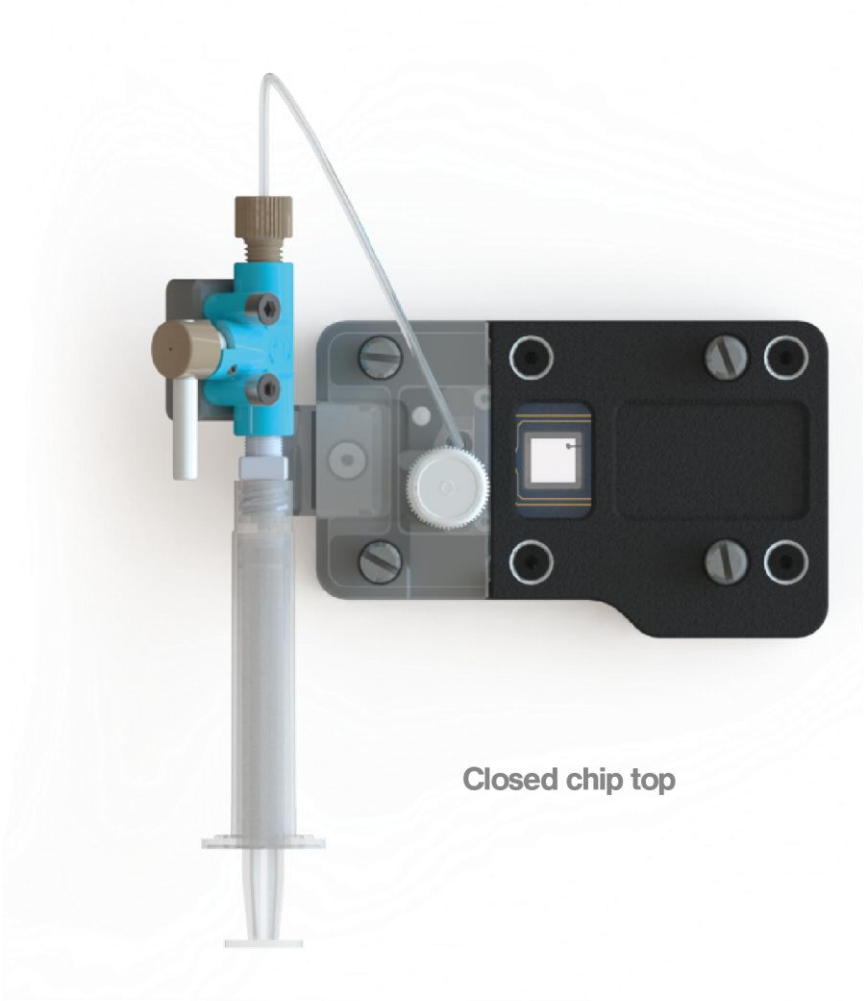
Closed chip isometric view

Valve
- Control the flow of the liquid from the reservoir to the flow cell

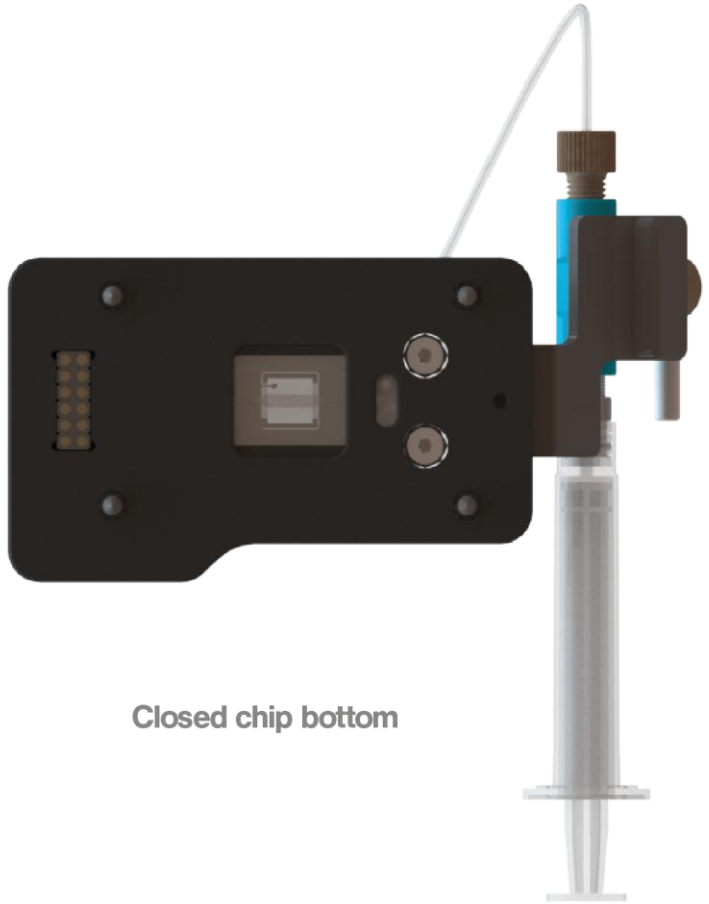
Valve adaptor
- Connects the white adapter for placing the syringe in the Luer lock adapter

Luer lock adapter in the valve
- Makes it possible to turn the syringe and having an airtight connection between the valve and the syringe

Chip glossary

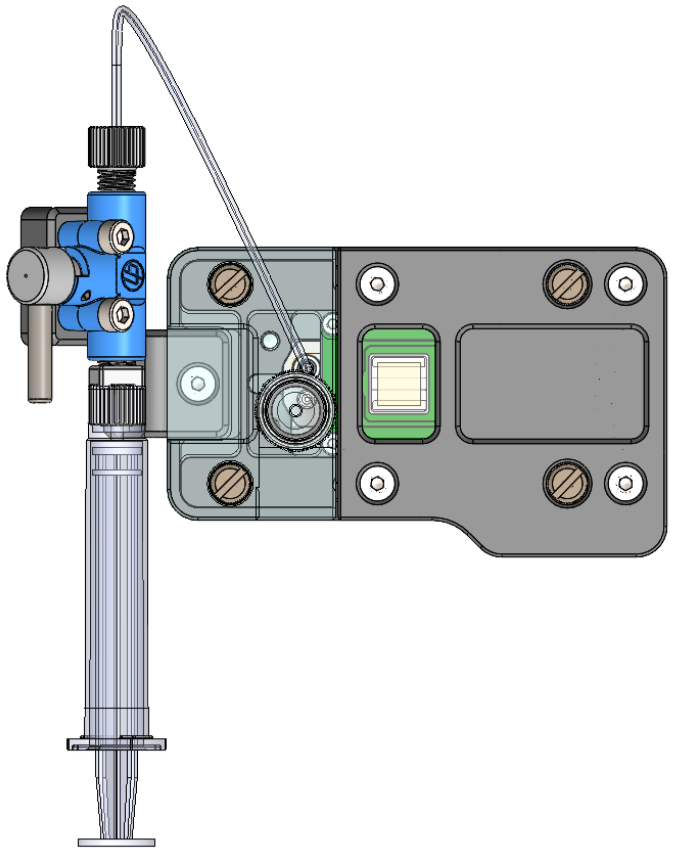


Closed chip top



Closed chip bottom

z-Movi Chips



z-Movi Chip Warranty

- What is a run? An experimental force ramp that lasts over 10s.
- What is not a run? Monolayer testing: will use fixed forced.

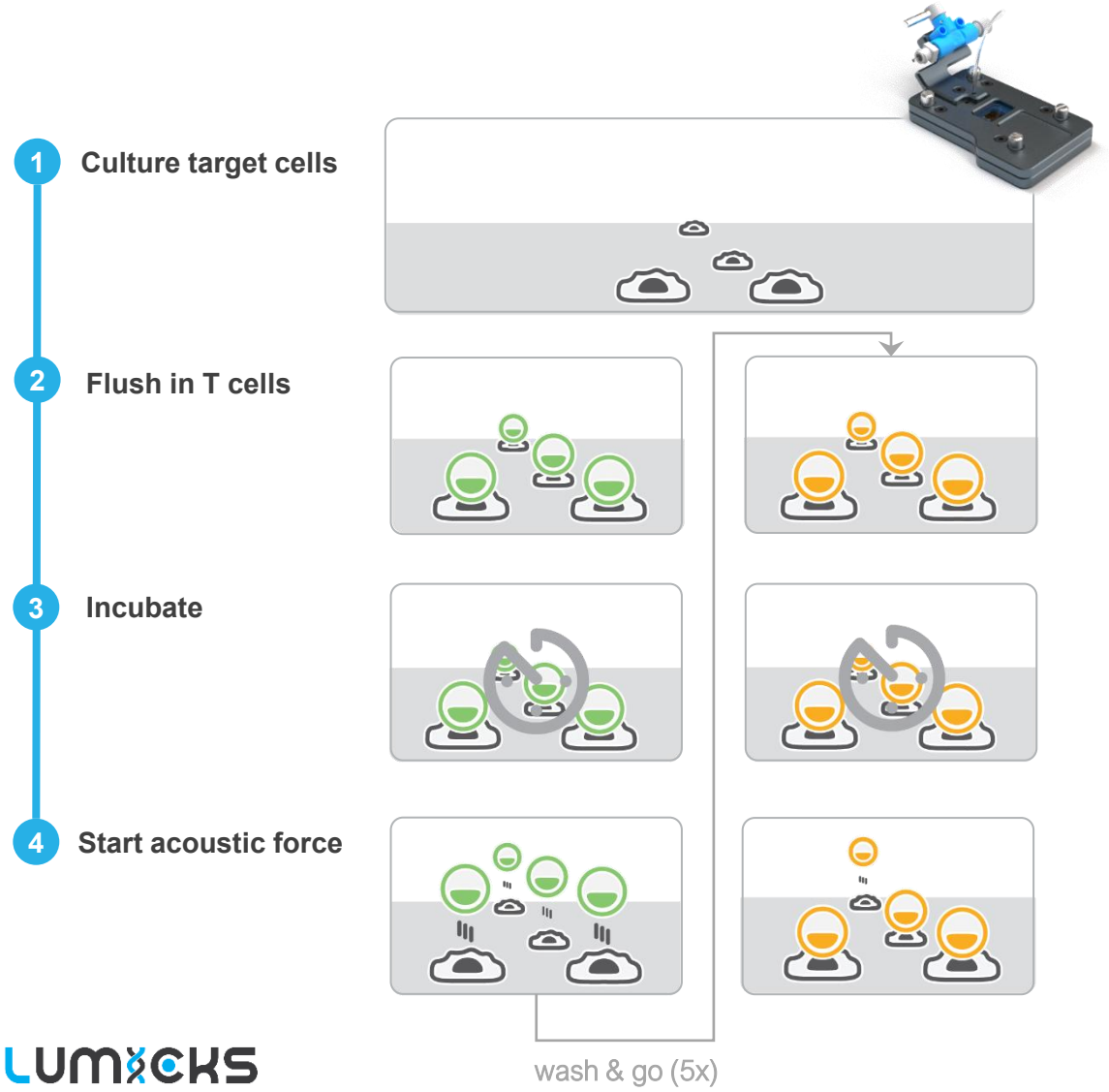
Workflow

Avidity Experiment Workflow

1. **Chip preparation** (1hrs)
   - Chip cleaned/1M NaOH Functionalization
   - Surface Coating
2. **Chip seeding** (2-3 hrs)
   - Monolayers
3. **Tracked cell staining** (1hrs)
4. **Data Acquisition** (1-3hrs)
5. **Data Analysis** (10min-∞)

Simple workflow to measure binding avidity of multiple effectors on a single target monolayer

Method



1. Culture target monolayer

- Select target cells (could be a panel)
- Typically 100x10^6 cells/mL in max. 10-20uL are needed per chip.
- Seed chips to generate target cell monolayer (~3 hrs)

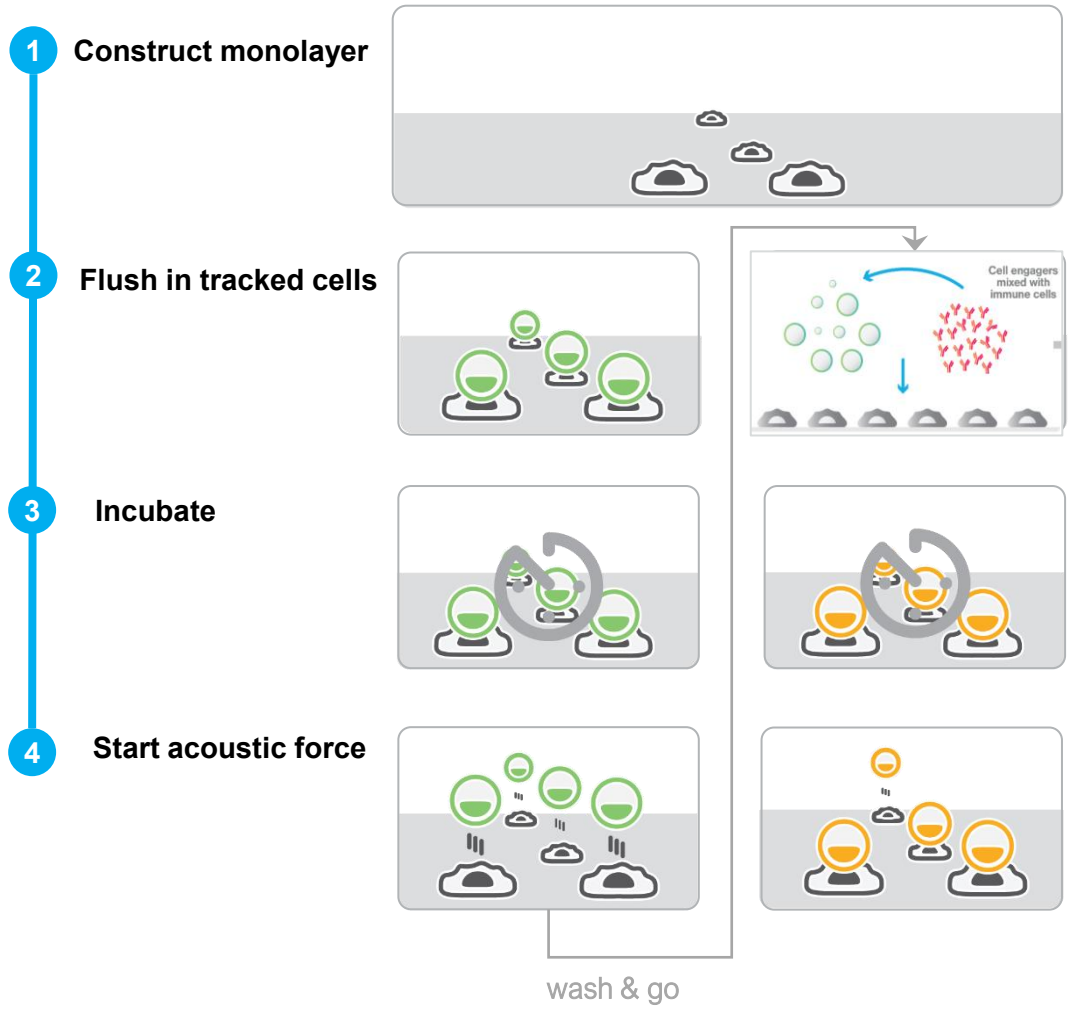
2. Prepare and flush in T cell populations

- Generate engineered T cell populations of interest
- Use fluorescent dye for T cell tracking
- 1-2 x10^5 cells in ~10-20uL are needed per run
- Preparation time for avidity assay: ~1 hour

3-4. Co-culture target cells with T cells

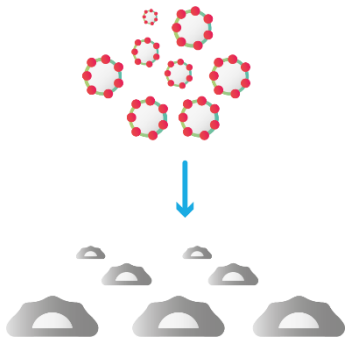
- Incubate engineered T cells with target cells
- Start acoustic force ramp (2.5 min – 0-1000 pN)
- Wash away T cells and incubate next T cell population (n=5 runs)
- Perform avidity measurements: 5 runs ~ 1 hour

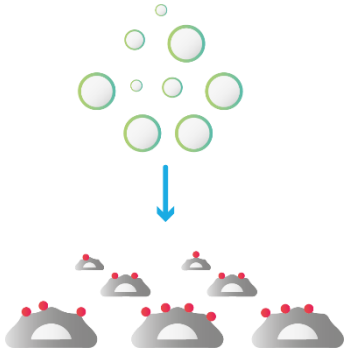
General workflow

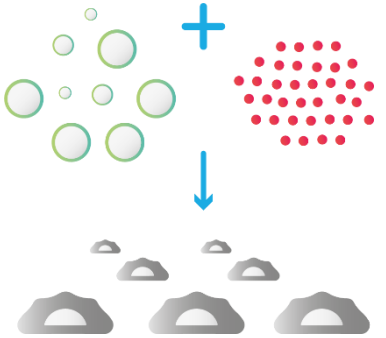


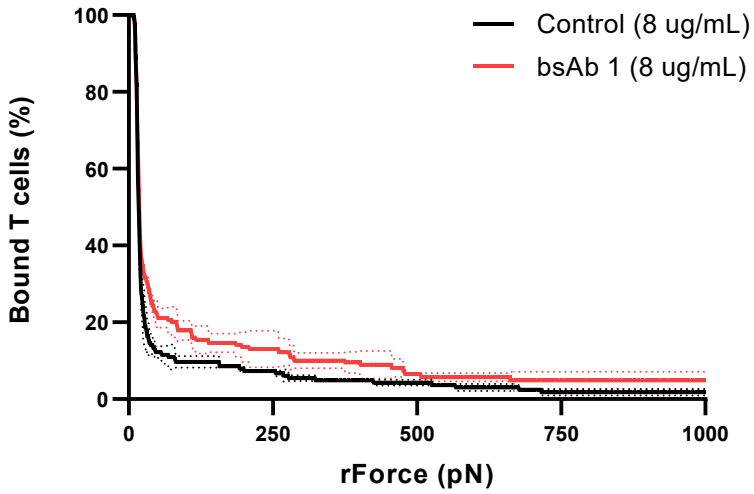
Perform the runs in the following order:
a.No antibody negative control (medium only)
b.Irrelevant antibody negative control (optional)
c.Experimental antibody
*Note: only 1 positive binding antibody can be run per chip

z-Movi cell avidity measurements as a quantitative systems pharmacology (QSP) model for cell engagers

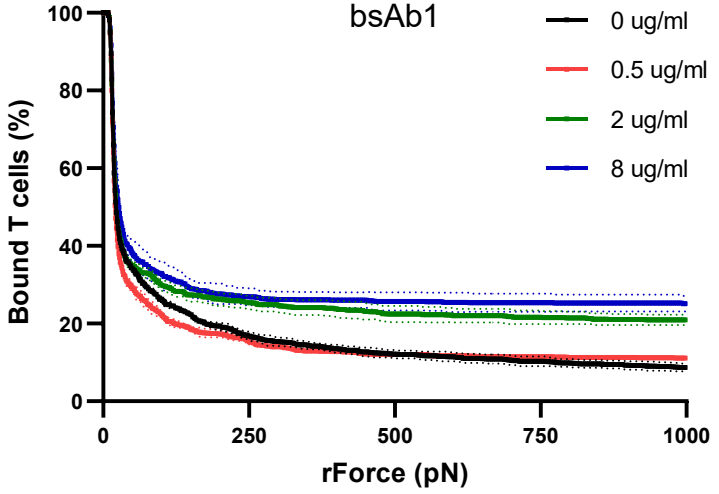




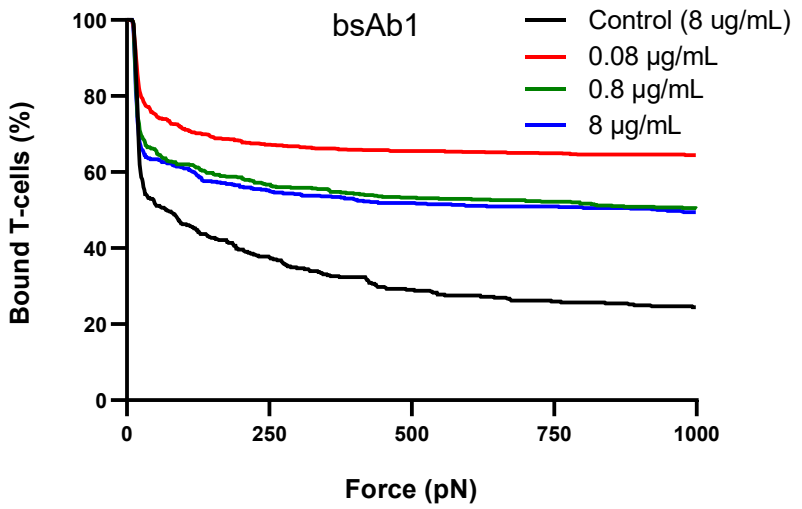




5 min co-culture, n=2



5 min co-culture, n=3



10 min co-culture, n=1

Step 1

Adhere target cells

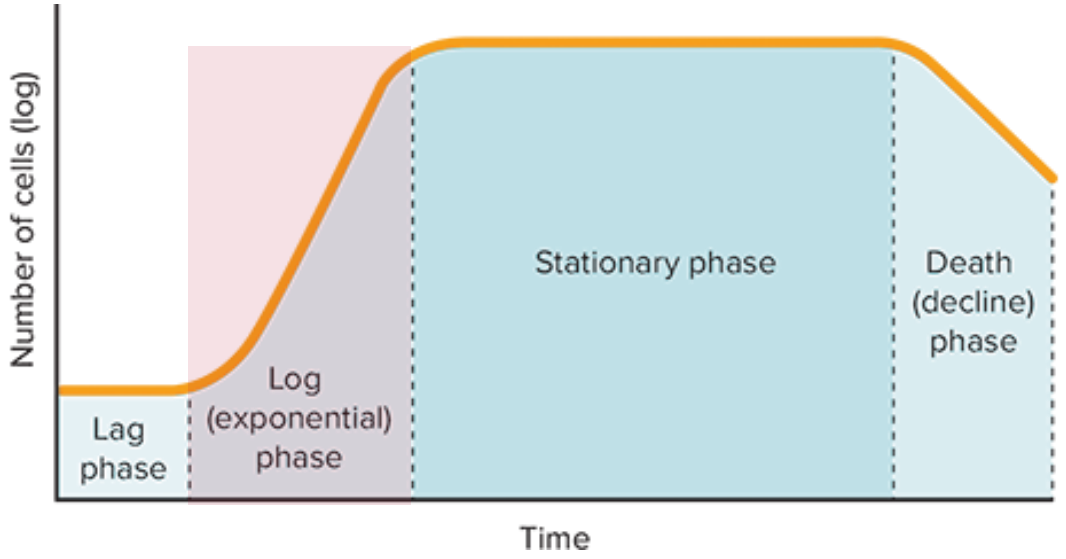
In z-Movi chip. Incubate for at least 2 hours

Material

chip cleaning	dH_2O
cell preparation	PBS
chip coating	Poly-L-lysine (for chip coating) [SigmaAldrich, P4707-50ML] ※
chip coating	Concanavalin A (Sigma-Aldrich, cat. #C2010-25MG) ※
cell staining	CellTraceTM Far Red dye (for staining effectors) [ThermoFischer, C34564] ※
cell preparation	TrypLE (gentle dissociation reagent) [ThermoFisher, 12604013]
cell preparation	HEPES (1M) [ThermoFischer, 15630106] , make 25mM in the medium
chip coating	1M NaOH (0.22 µm filtered)
cell preparation	Culture medium with/without FBS (no other added contents)
chip cleaning	Tween 20 (Sigma-Aldrich cat. # P9416-100ML)
chip cleaning	Bleach (5% w/v or containing 50g/L active chlorine)
chip cleaning	1-Ethyl-3-methylimidazolium dicyanamide (Thermo Fisher, catalog number: H26901.18; Sigma-Aldrich, catalog number: 713384)
※ Please order the same #Catlog reagents for PLL, ConA, Far Red dye and 1-Ethyl-3-methylimidazolium dicyanamide	

Day -2: Cell culture preparation

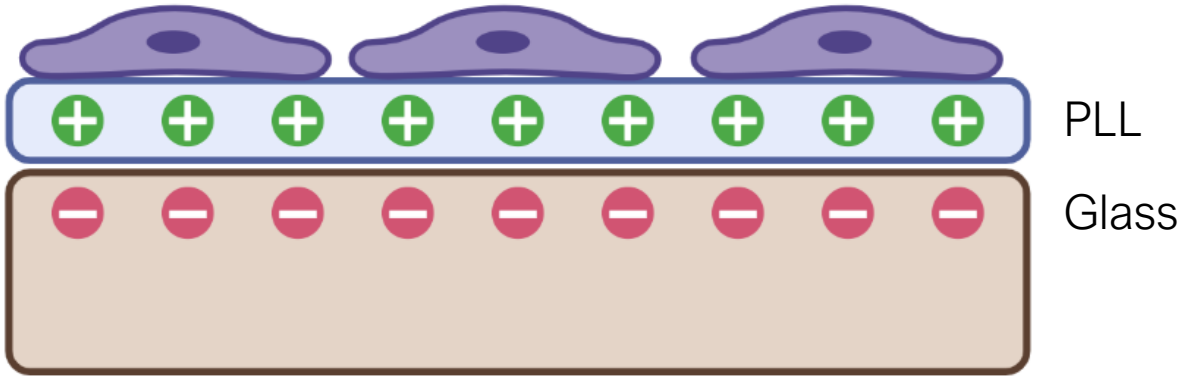
- 70 - 80% cell confluency in the flask at the day of in-chip cell seeding.
- Ensure for best cell quality (viability >95%)



Day -1 or Experiment Day: Chip preparation

Activate glass with NaOH 1M

- 10-minute incubation at RT
- Coat chips within 20 minutes after activation with NaOH!
- Never leave chips incubation longer than 1h maximum!



Coating reagent

- Coat glass with Poly-L-Lysine
  - Flush in PLL and incubate for 10 minutes (or Concanavalin A (1mg/mL or 5mg/mL))
  - Wash twice with PBS
  - Continue seeding OR: Dry chips and store until use (up to 3 days)
    - Rehydrate with PBS/media before in-chip seeding
- Con A is a plant lectin that binds to glycoproteins and glycolipids on the cell membrane.

Con A-coated chips can be prepared 0-1 day before monolayer seeding. If prepared in advance, Con A-coated chips should be stored **wet**, filled with 1X PBS at RT in the dark

Concanavalin A (Con A) (Sigma-Aldrich, catalog number: C2010)

Stock: Con A dissolved in 1X PBS, at 5 mg/mL, frozen in 100 µL aliquots. Do not freeze/thaw more than once.

Working stock: Dilute 100 µL Con A stock solution to 1 mg/mL with 400 µL 1X PBS.

Experiment Day: NALM6

Tissue Culture Conditions for optimal monolayer preparation

Note. *Cells are generally split 1 or two days prior to the experiment in the following ratios to obtain the recommended confluency or concentration.*

Recommended confluency or cell concentration in 1 T75 culture flask on the day of the experiment:
1*10^6/ml

Recommended splitting rate for 1 day prior to experiment: 0.4*10^6/ml

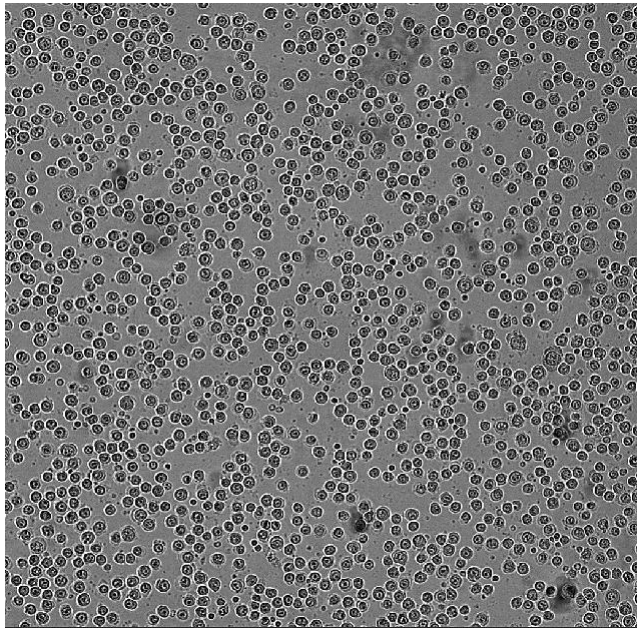
Recommended splitting rate for 2 days prior to experiment: 0.3*10^6/ml

Recommended splitting rate for 3 days (weekend) for culture maintenance: 0.2*10^6/ml

Cells	NALM6
Medium	RPMI + 10% FCS + 25mM HEPES
Coating	PLL
Seeding concentration	180x10^6/ml
Time and conditions after seeding	30 min in serum free medium
Time and conditions after medium exchange	1.5 hours in 10% medium

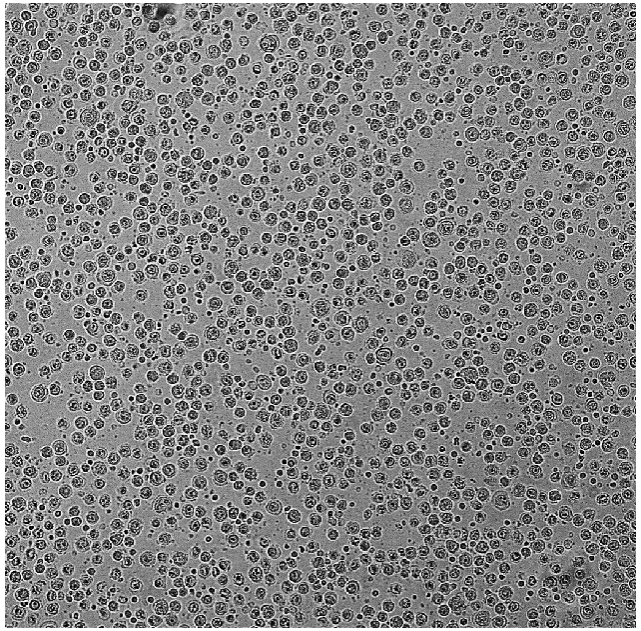
Experiment Day: Bad vs. Good Monolayer

Low confluency



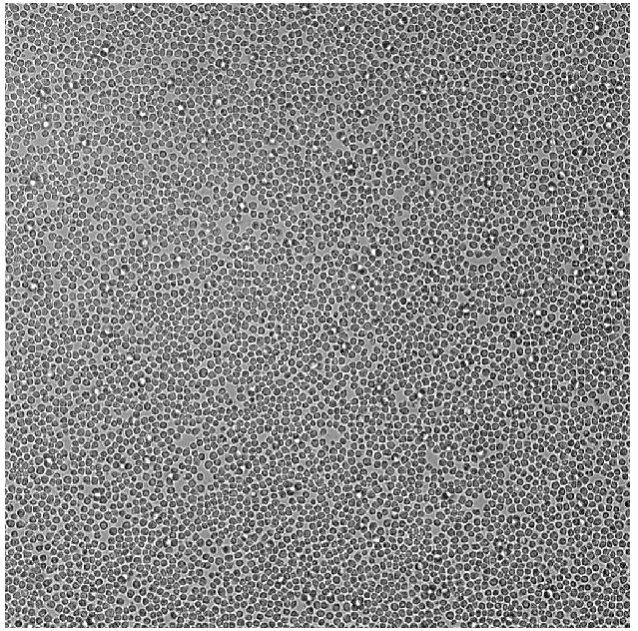
< 50% confluency

Minimum threshold



80% confluency

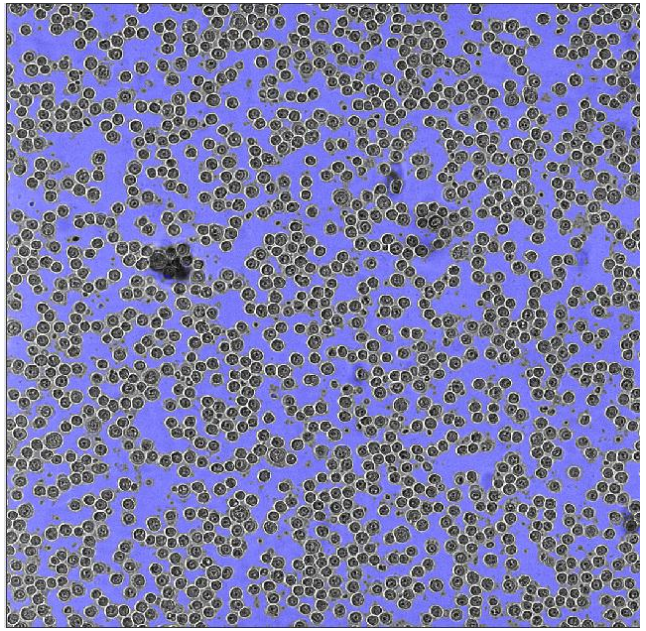
Optimal



>90% confluency

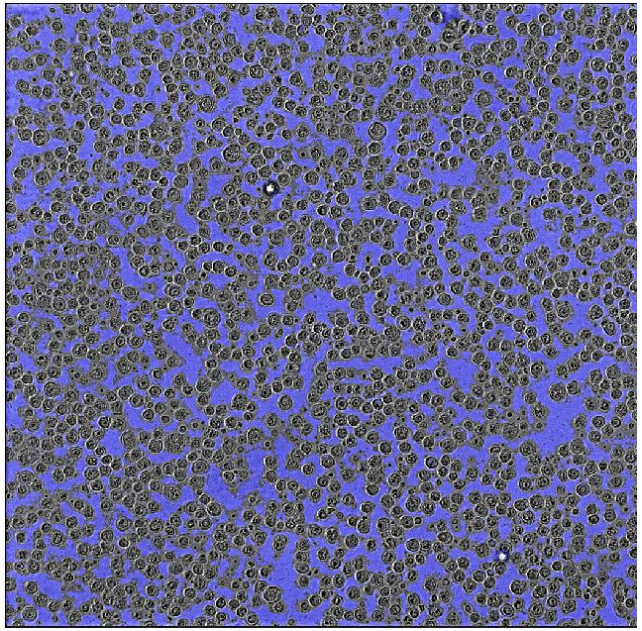
Experiment Day: Bad vs. Good Monolayer

Bad



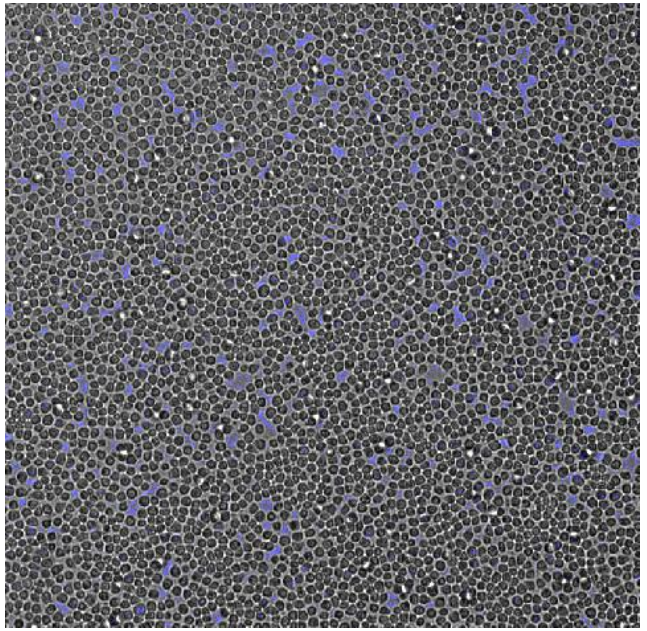
< 50% confluency

Minimum Threshold



80% confluency

Optimal



>90% confluency

Target monolayer: considerations for successful monolayer

A. Pre-seeding cell culture- cell viability, cell confluence in the flask

B. Chip cleaning- chips must be cleaned on the same day and incubation with chemicals

C. Glass coating- even coating using PLL (bubble-free), alternative coating methods if necessary

D. Cell density- optimized monolayer concentration based on cell size and clumpiness

E. Cell adherence time- 1hr is ultimate incubation time goal, however, it is cell line-dependent

F. Monolayer state in the chip- overall monolayer "happiness" in the chip varies depending on cell line. Usually, monolayer is usable 2-4 hours post seeding to assure reproducible data chip-to-chip

Step 2

Flush your tracked cells

Experiment Day: Tracked cell staining

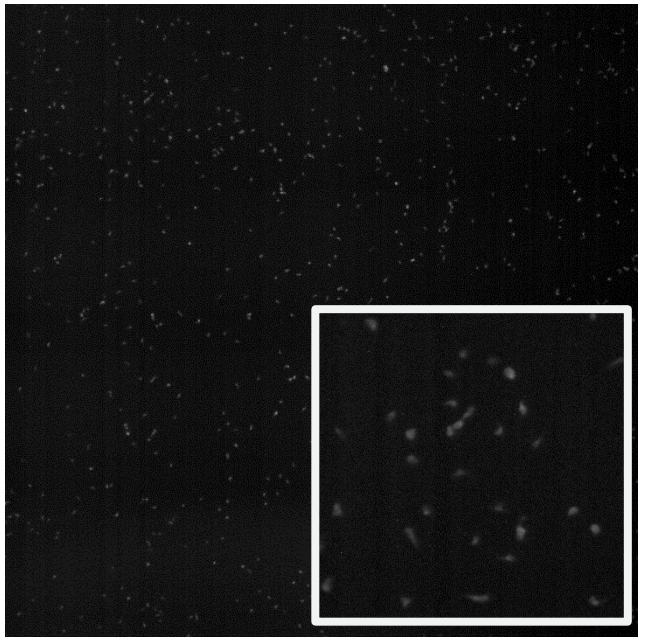
1. Prepare 200,000 tracked cells in 20uL per chip ($10x10^6$ cells/mL suspension).
2. Wash with PBS.
3. Add 1μL of stock CellTrace™ Far Red Dye to cell pellet (1uL of stock dye to 1mL 1X PBS).
4. Incubate for 15 mins in the dark at 37°C.
5. Add 5X volume of complete media to cells and mix well by pipetting.
6. Incubate at 37°C for 5 mins
7. Wash 1X with complete medium.
8. Resuspend stained cells in complete medium at $10x10^6$ cells/mL.

Experiment Day: Determine good staining and concentration of effector cells

Good

Sparse

Dim cells



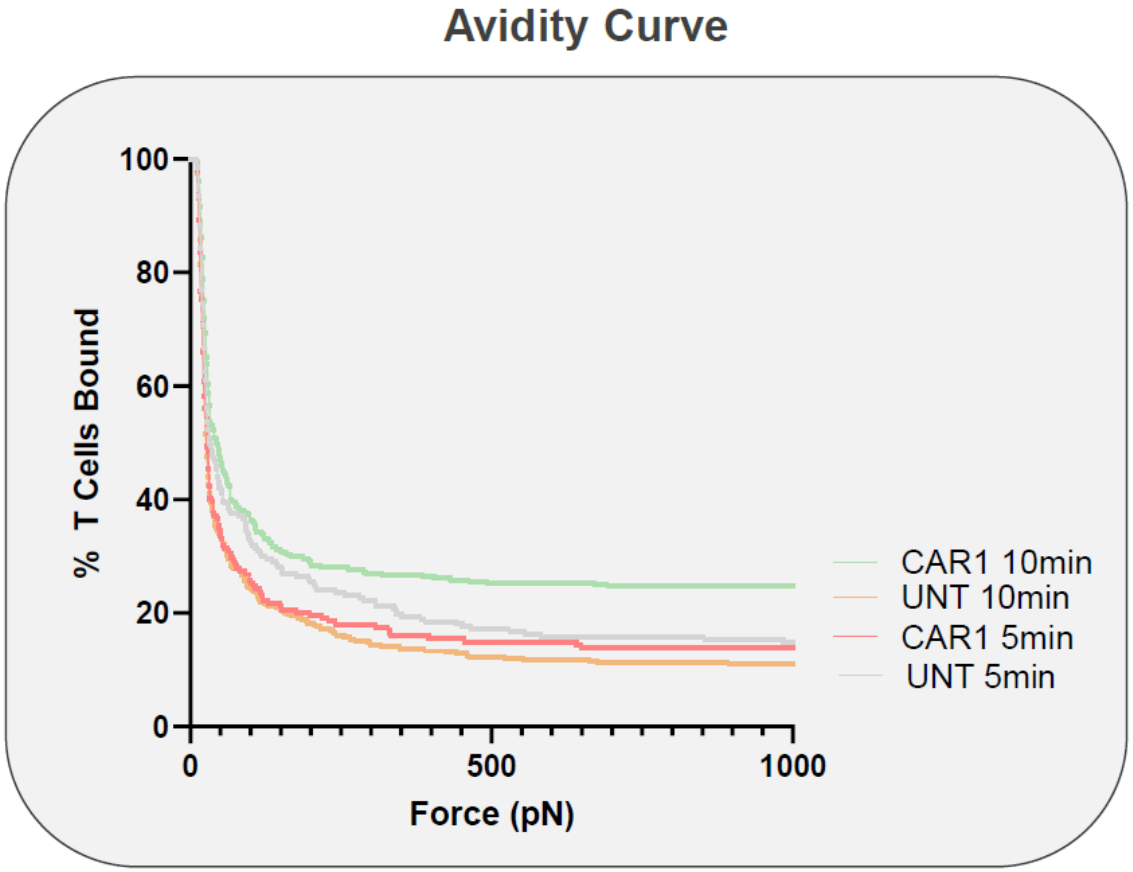
Optimal concentration is about **250-500** cells in FOV

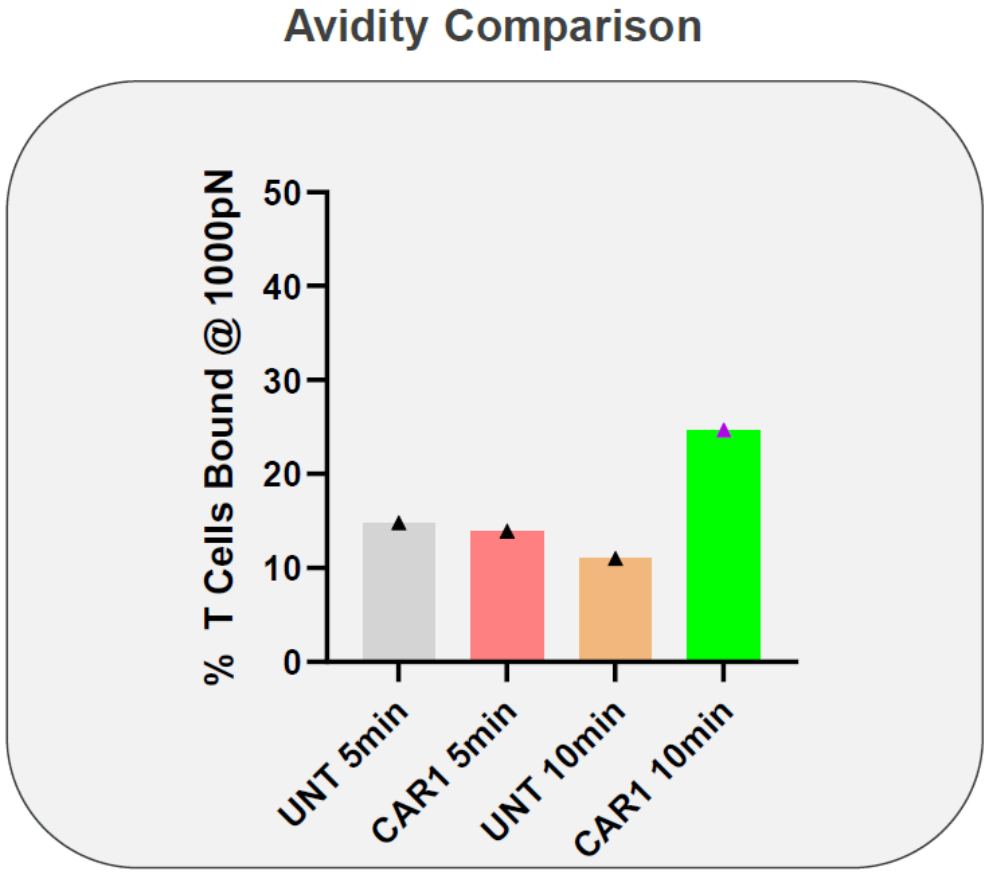
Step 3

Incubation

5 minutes

Find best incubation time: best window

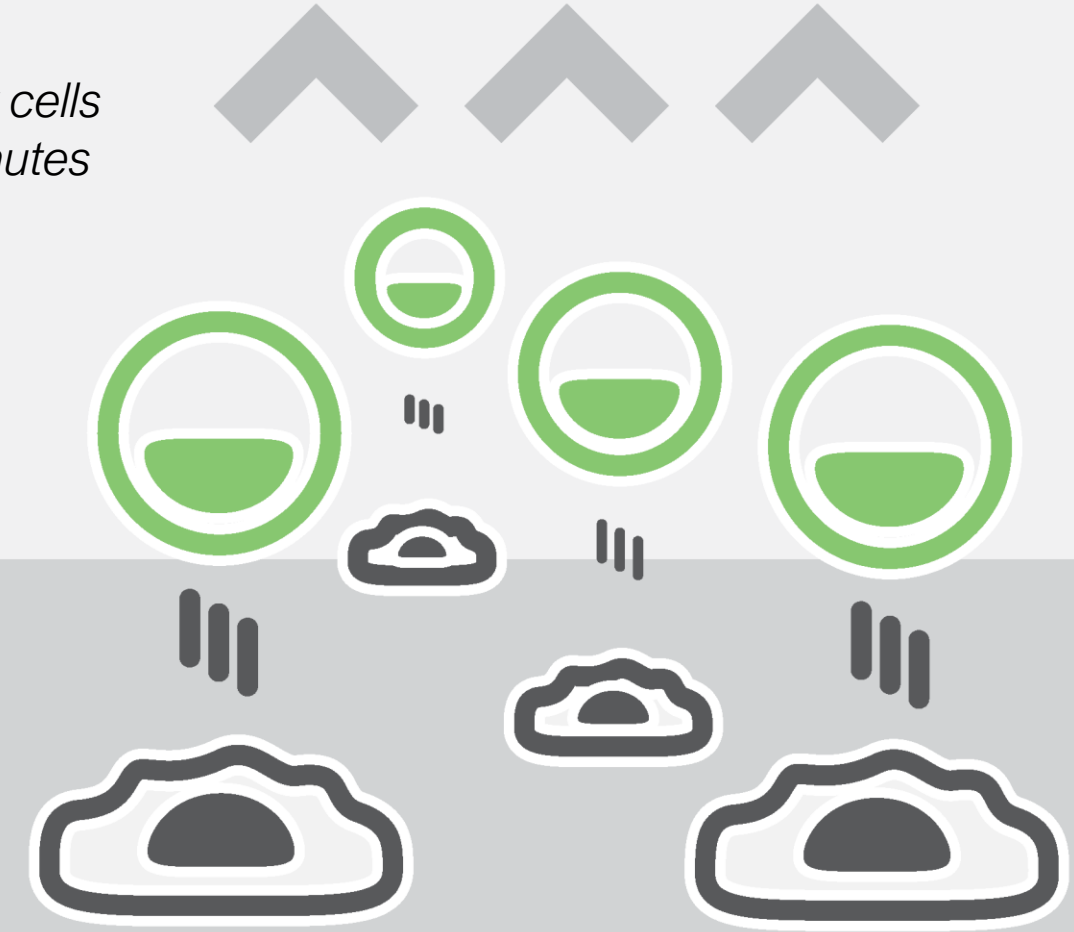




Step 4

Acoustic force

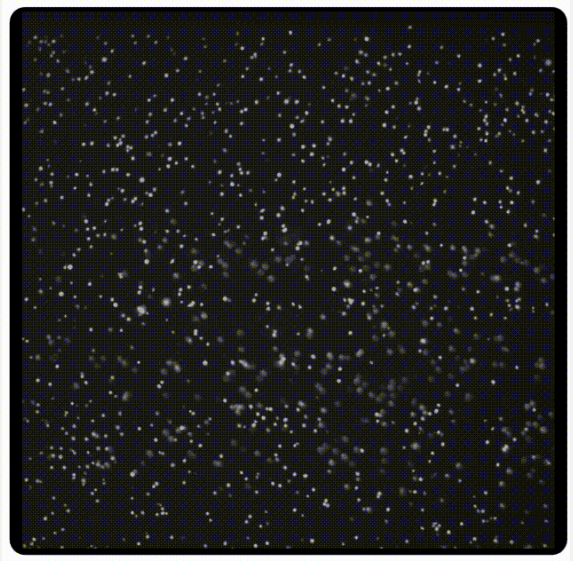
To lift off the tracked effector cells from the target cells. 2.5 minutes



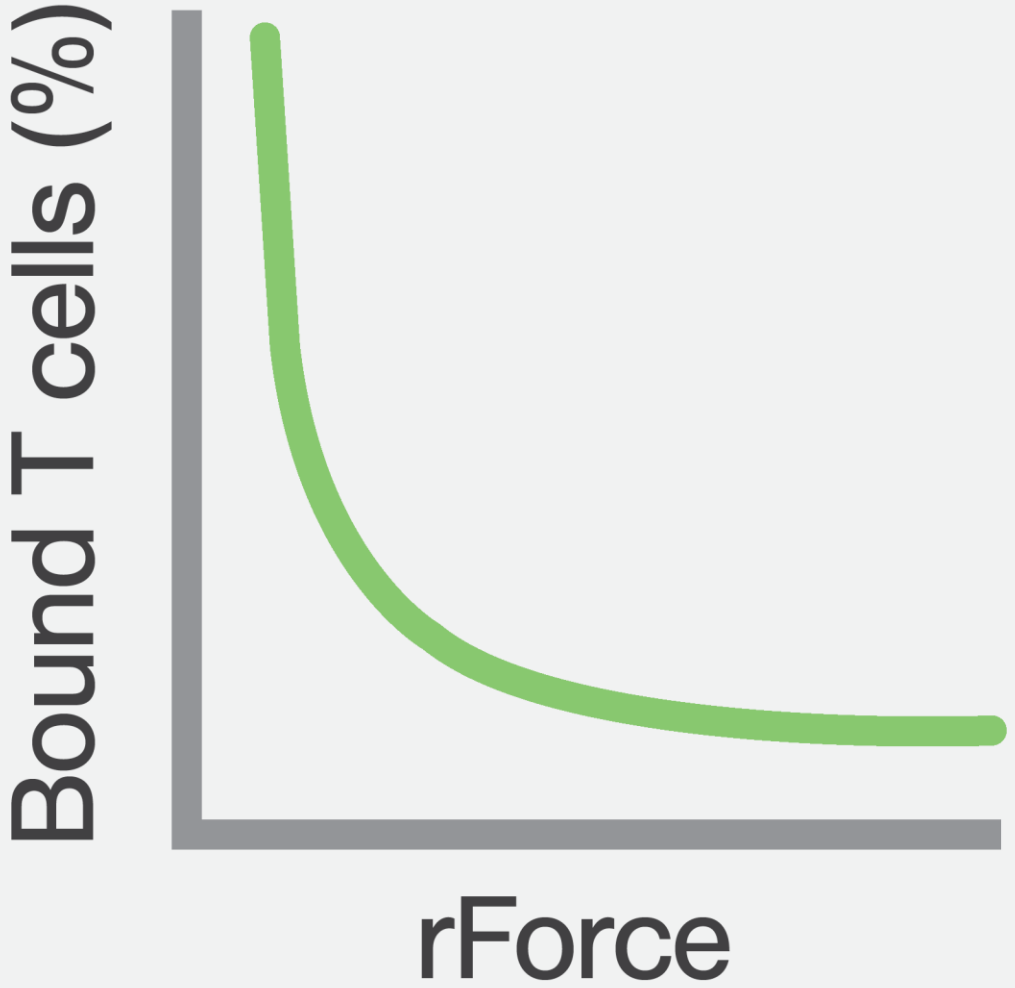
Step 5

Sit back and watch

your avidity measurement results in real-time



Chip top view



Repeat Steps 2-5

Flush in tracked effector cells+ CEs

Repeat with new tracked effector cell population

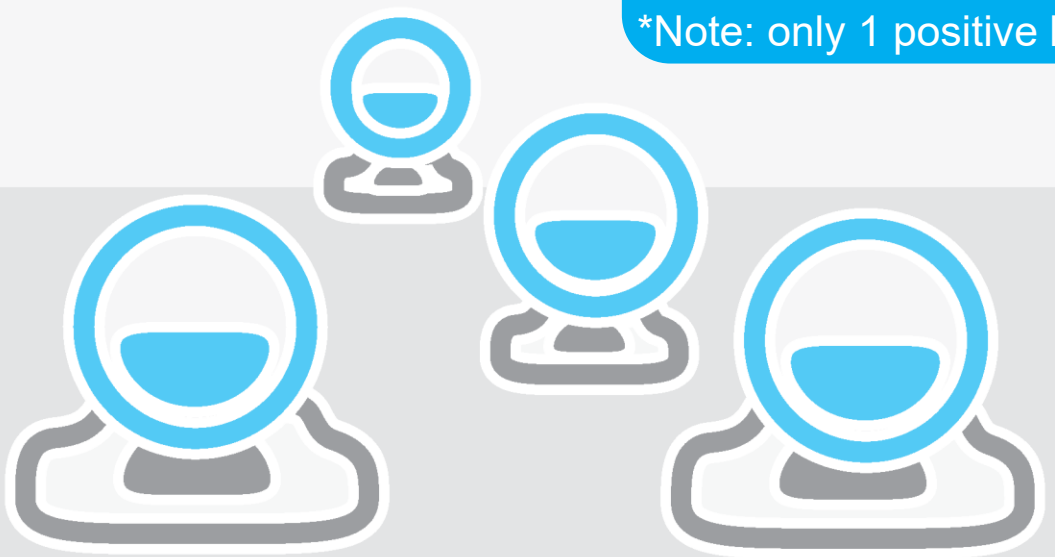
Perform the runs in the following order:

a.No antibody negative control (medium only)

b.Irrelevant antibody negative control

c.Experimental antibody

*Note: only 1 positive binding antibody can be run per chip



Day 0: Cleaning chips

1-Ethyl-3-methylimidazolium dicyanamide

Make sure the flow chamber is bubble free during every incubation step

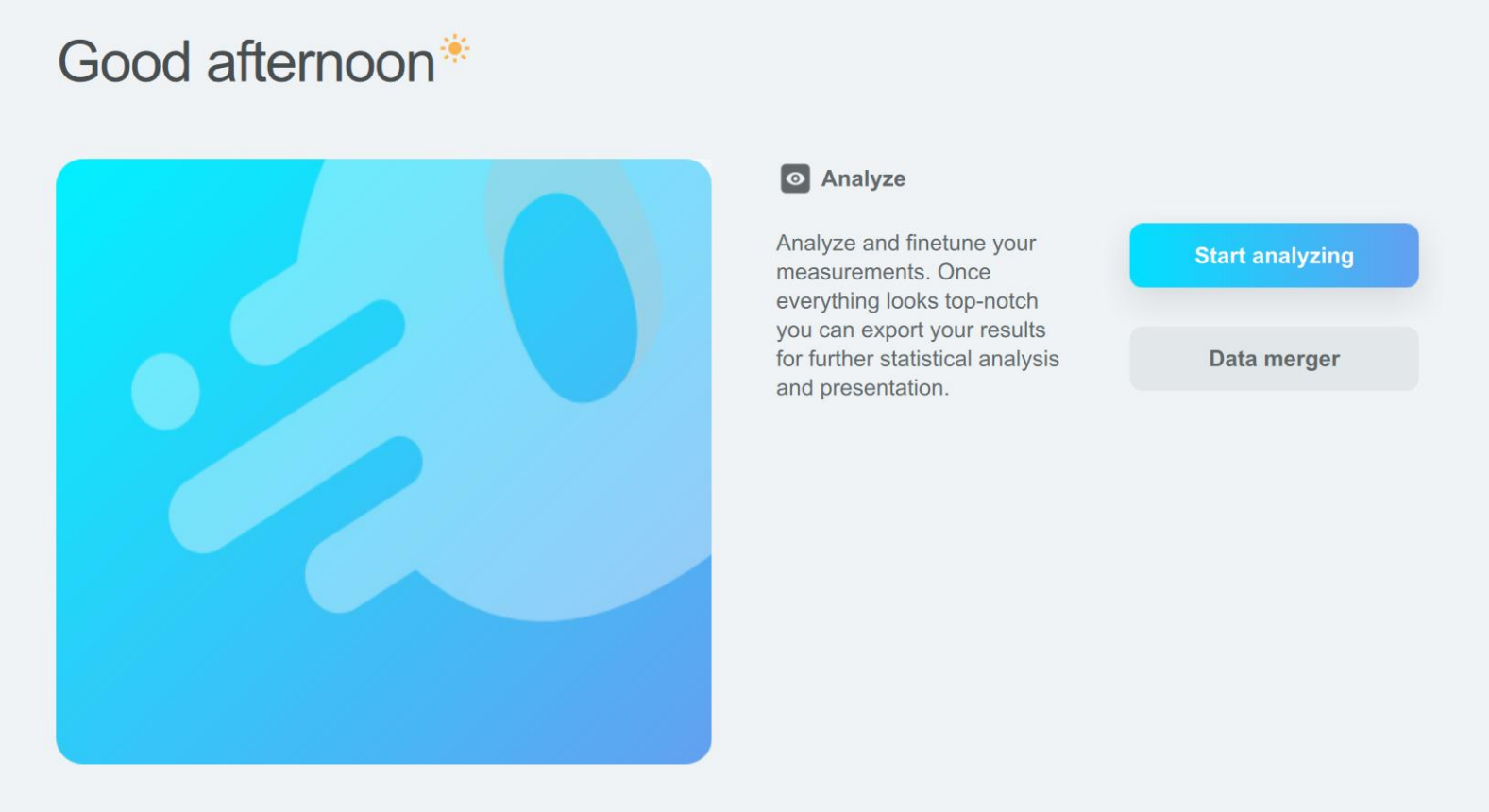
VERY important step. Dirty chips will affect monolayer adherence and introduce more bubbles. Do not rush this!!!

1 cleaning protocol

Step	Chemical	Why
1	H2O	To rinse of any cell media
2	Bleach 5% (10min-30min)	to kill the cells, it always must be done the same day after the experiment
3	H2O	To rinse Bleach
4	**Ionic Liquid (30min~3days)**	**To remove coating/debris from flow channel**
5	**2% Tween 20**	**To get rid of the IL (doesn't dissolve in H2O) residues**
6	H2O	To rinse off all soap residues
7	Coating or Drying	Coating immediately after, or dry for longer storage
7	1M NaOH (1h)	Etching of glass, getting rid of a top layer making the O- groups on glass functional
8	H2O	Rinse NaOH residues and keep glass functional
9	Coating	Coating PLL/ConA

Data analysis

Oceon: Analyze your avidity measurements



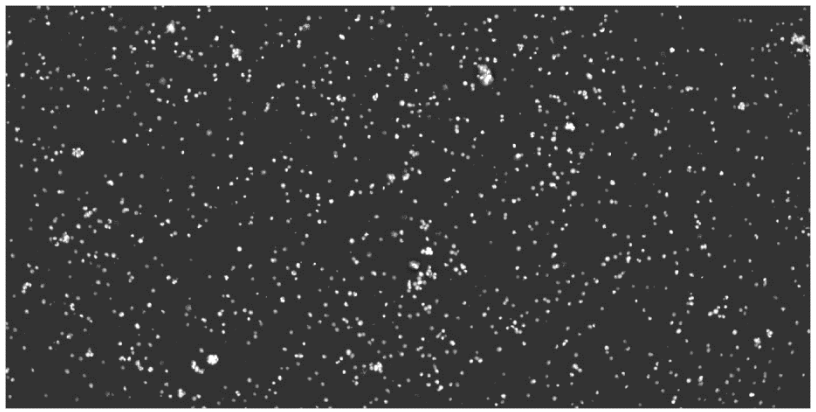
*No need of license for data analysis software

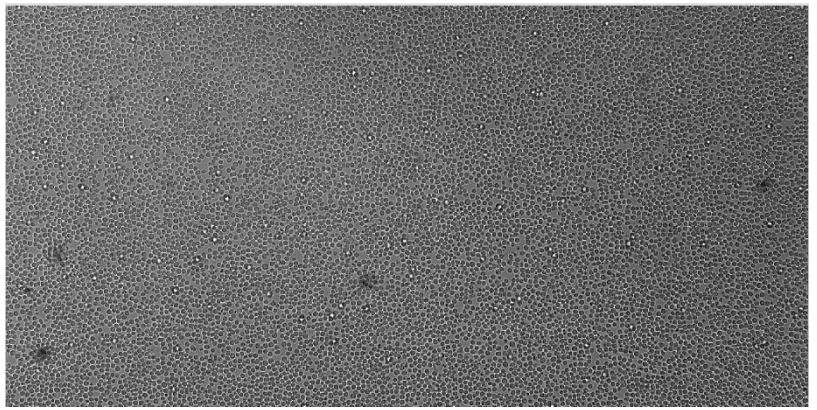
Output videos and analysis

Effector Cells

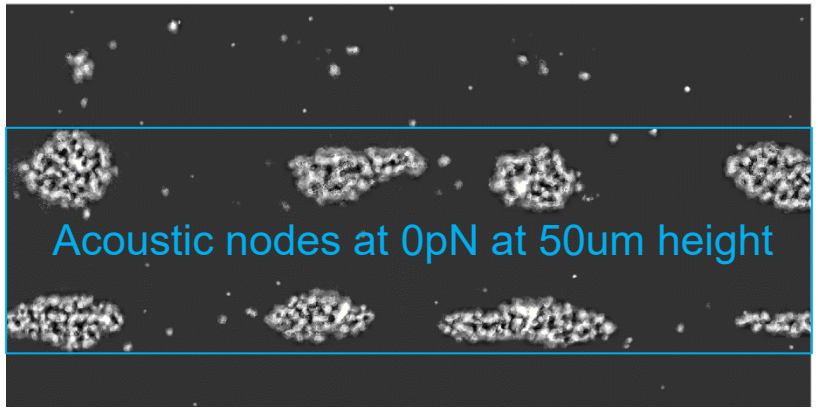
Target Cells

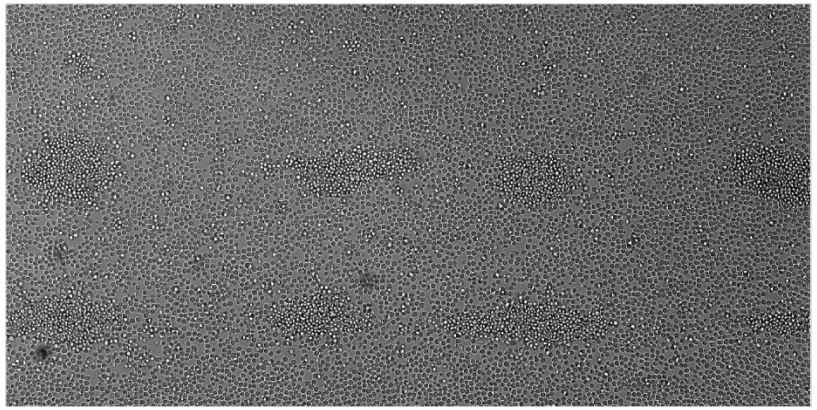
Before Ramp





After Ramp





Data files: Single-cell analysis and avidity curve

Single-cell

9	Total number of selected cells	152	
10	Cells included in avidity curve	114	
11	Monolayer confluency (%)	76	
12	Regular cell count	100	
13	Glass cell count	28	
14	Hinge cell count	14	
15	Covered cell count	10	
16	Regular class	included	
17	Leftover class	excluded	
18	Glass class	excluded	
19	Hinge class (included / treat as bound)	included	treat as bound
20	Covered class	excluded	
21	Custom 1 class (included / treat as bound)	included	
22	Custom 2 class (included / treat as bound)	included	
23	Custom 3 class (included / treat as bound)	included	
24	Custom 4 class (included / treat as bound)	included	
25	Avidity plateau (%)	14.03509	
26	---		
27			
28	Index	Time (s)	Force (pN)
29	0	6	40
30	1	2.4	16
31	2	3.2	21.33333
32	3	6	40
33	4	8.8	58.66667
34	5	bound	bound
35	6	bound	bound
36	8	2.2	14.66667
37	9	37.4	249.3333
38	11	4.4	29.33333
39	12	2.8	18.66667

Avidity Curve

9	Total number of selected cells	152	
10	Cells included in avidity curve	114	
11	Monolayer confluency (%)	76	
12	Regular cell count	100	
13	Glass cell count	28	
14	Hinge cell count	14	
15	Covered cell count	10	
16	Regular class	included	
17	Leftover class	excluded	
18	Glass class	excluded	
19	Hinge class (included / treat as bound)	included	treat as bound
20	Covered class	excluded	
21	Custom 1 class (included / treat as bound)	included	
22	Custom 2 class (included / treat as bound)	included	
23	Custom 3 class (included / treat as bound)	included	
24	Custom 4 class (included / treat as bound)	included	
25	Avidity plateau (%)	14.03508772	
26	---		
27			
28	Force (pN)	Bound cells (%)	
29	0	100	
30	1.333333333	100	
31	2.666666667	100	
32	4	100	
33	5.333333333	100	
34	6.666666667	100	
35	8	100	
36	9.333333333	100	

https://help-zmovi.lumicks.com/

Password: iloveavidity

Troubleshooting

Monolayer