



EuroElone[®]
serving science through innovation

BIOFLUX 200

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Junior Product Specialist

Cell biology



CELBIO

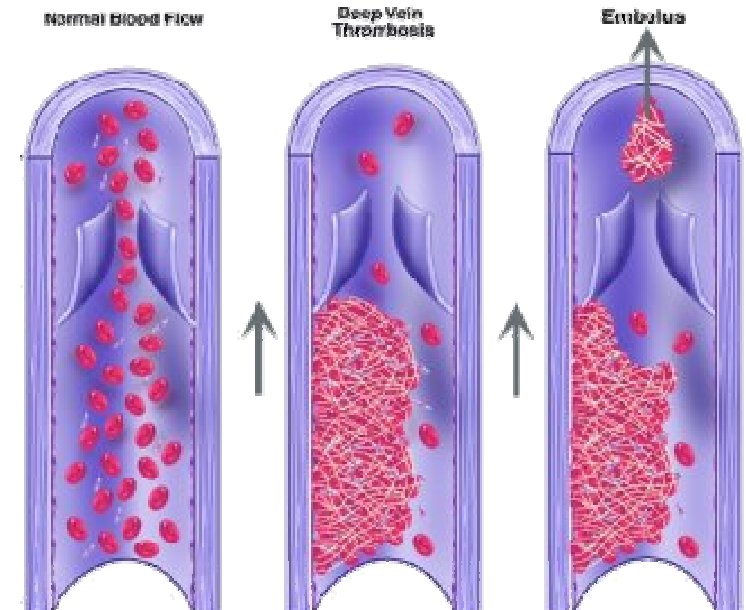


BIOFLUX Automated system for functional cell assays under shear flow



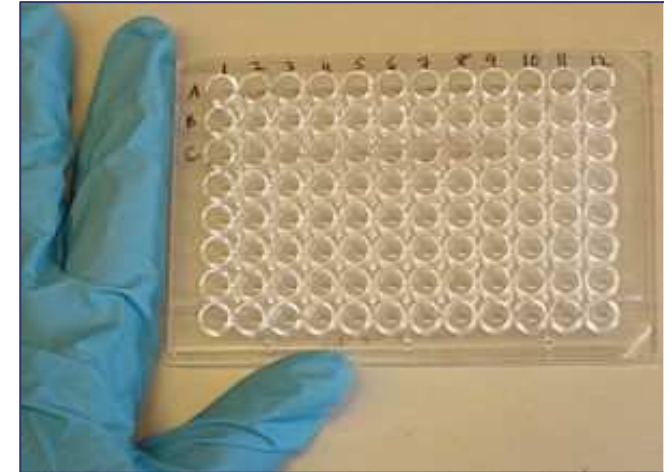
Importance of shear stress assays

- Simulate physiological conditions
- Perform nutrient/media exchange
- Analyze cellular interactions
- Physiological range:
 - 0.1 dyne/cm² small veins
 - 20 dyne/cm² aorta
 - >20 dyne/cm² diseased vessel



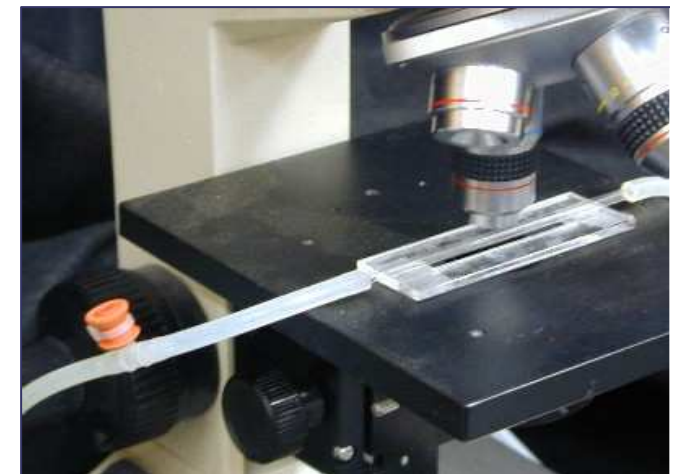
Microtiter plate assays

- Advantages
 - High throughput
 - Simple
- Disadvantages
 - Can't easily perfuse media or remove waste
 - Flow is not controlled
 - May not adequately simulate desired condition



Conventional Flow Cells

- Advantages
 - Adhesion under flow
 - Greater physiological relevance
- Disadvantages
 - Low throughput
 - Complexity
 - High reagent volumes



BioFlux 200 is a simple to use integrated and automated system which combines the ease of use and throughput of a well plate assay with the biological relevance of a controlled shear stress experiment

BioFlux 200 presents an accurate representation of environmental or physiological conditions by precisely controlling shear flow and can bridge the gap between *in vitro* e *in vivo* assays





**Inverted Microscope
CCD Camera**

BioFlux controller

BioFlux Plates

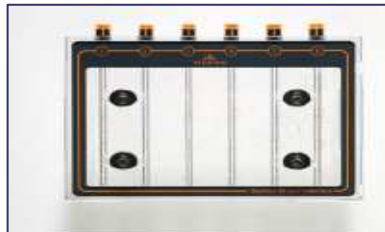
Heated plate holder





BioFlux 200 controller

Provides experimental control over shear flow, temperature and compound addition



BioFlux 200 interface

Transmits pressure to the plates



Heating Stage

Controls temperature of the experimental zones of the plates

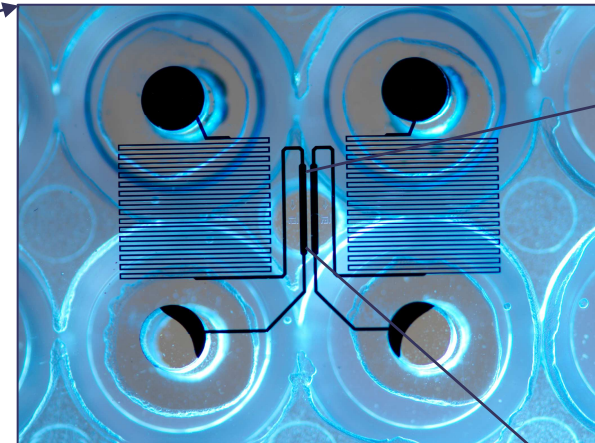
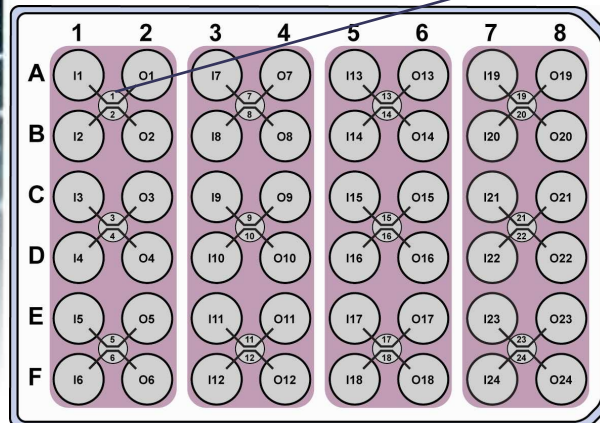


BioFlux Plates

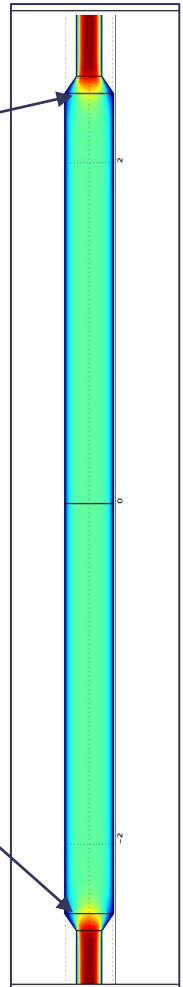
Microfluidic experimental channels integrated into SBS-standard plates.



BioFlux Plates – 48 well 0-20dyne/cm²



70μ x 370μ

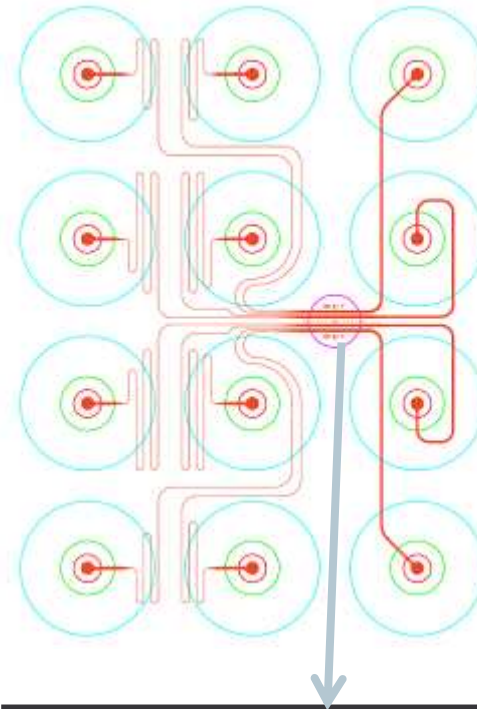
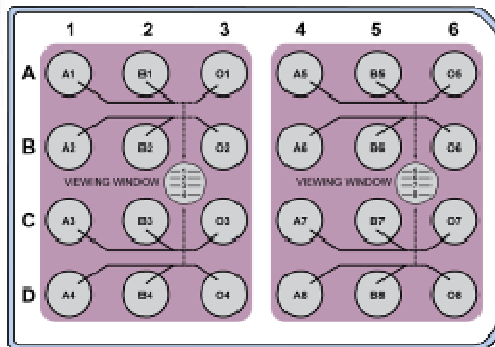


24 channel plate

- Tops and sides of channels are made in poly dimethyl siloxane (PDMS)
- Bottom of the channel is a standard 180μm cover slip glass
- 8 channels plate: 2 input wells 1 output well
- 24 channels plate: 1 input well and 1 output well



BioFlux Plates – 24 well 0-20dyne/cm²



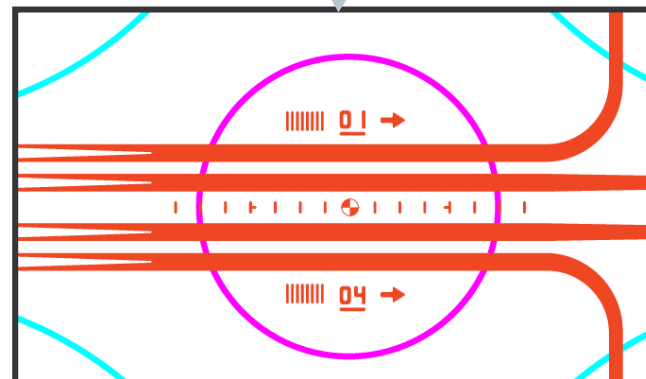
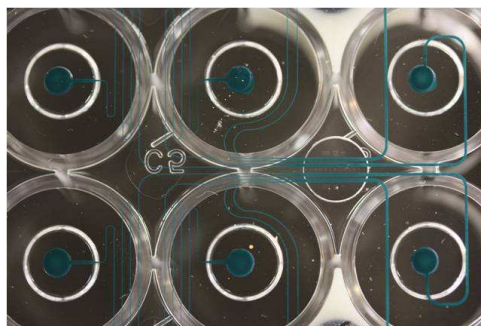
24-well BioFlux Plate

0-20 dyne/cm²

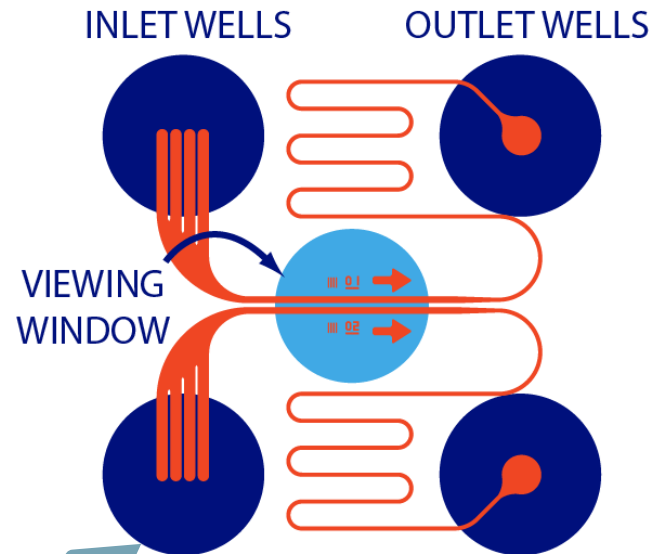
8 experimental channels

2 input wells per channel/3 ml per well

Dynamic switching of media, compounds, wash buffers, etc. between input wells or parallel flow



BioFlux Plates - 48-well High Shear 0-200dyne/cm²



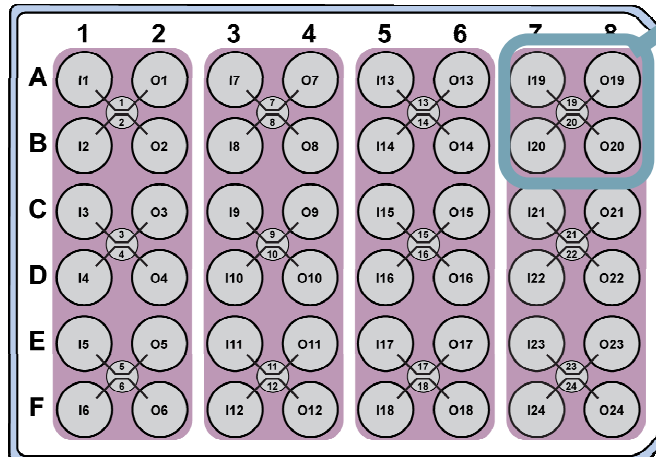
48-well High Shear BioFlux Plate

0-200 dyne/cm²

24 experimental channels

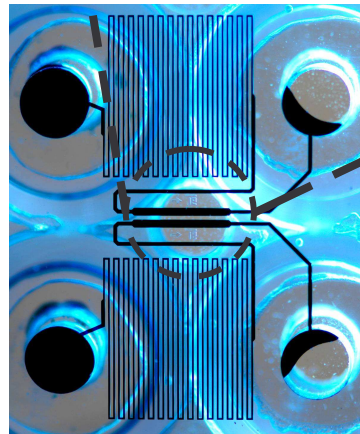
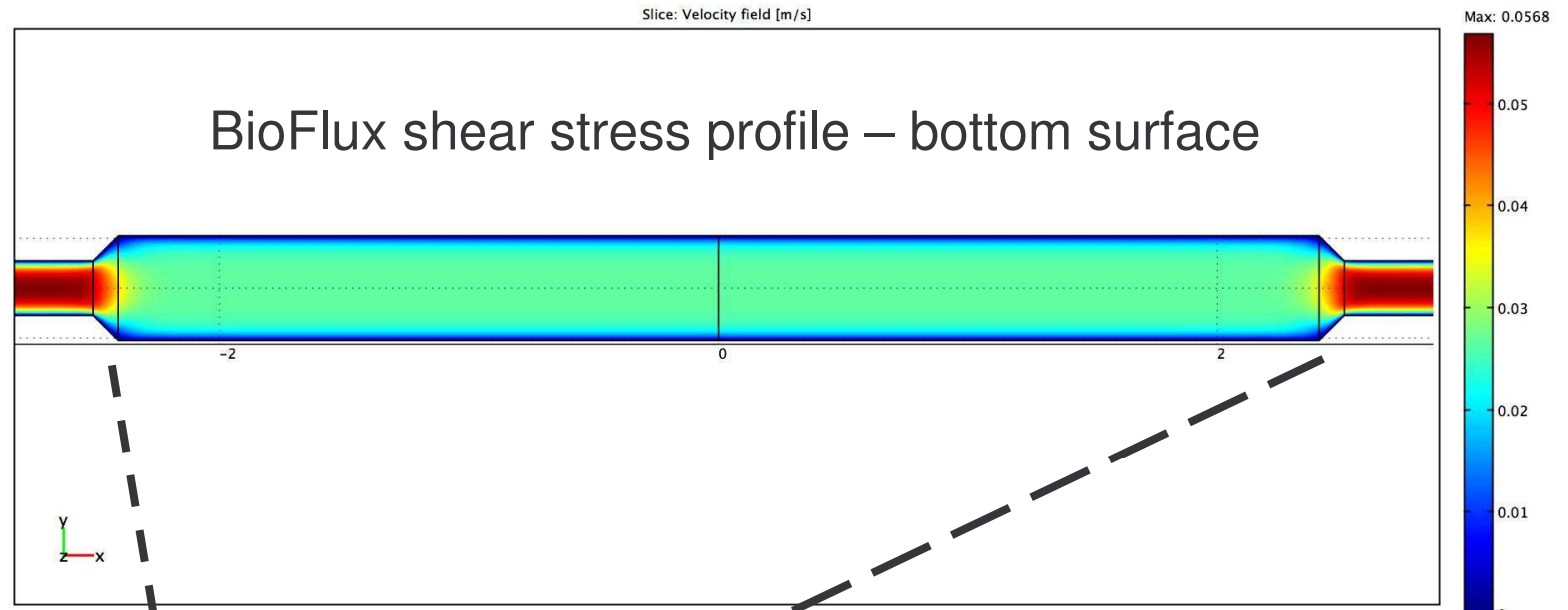
1 input well per channel

High shear range for platelet studies, adhesion, compound screening



Note: Serpentine on back end for this plate format

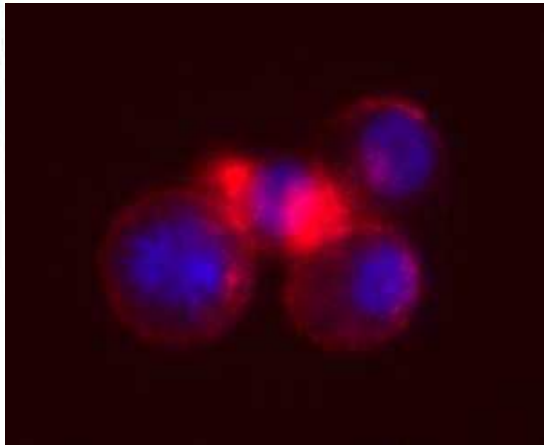




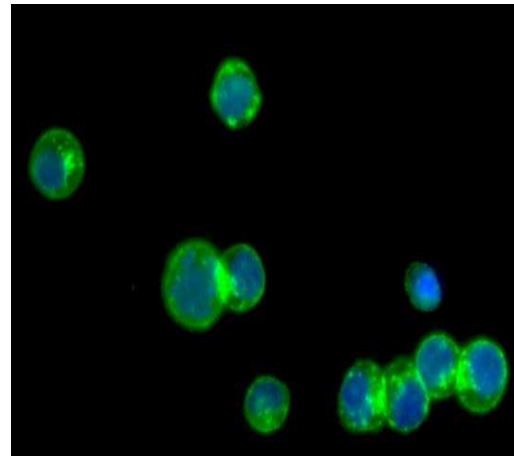
Uniform, laminar flow
within the region of interest



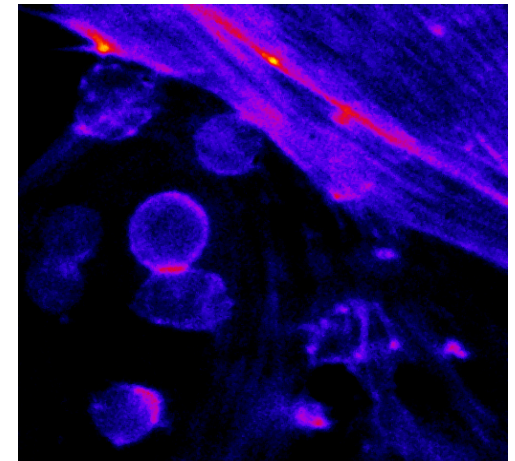
High Content Imaging for Live/Fixed Cells



Fibroblasts
epifluorescence



T-lymphocytes
epifluorescence



T-cells/ HUVEC
swept field confocal
Nikon Center, UCSF

Imaging can be performed on inverted microscopes,
compatible with confocal, brightfield, and epifluorescence

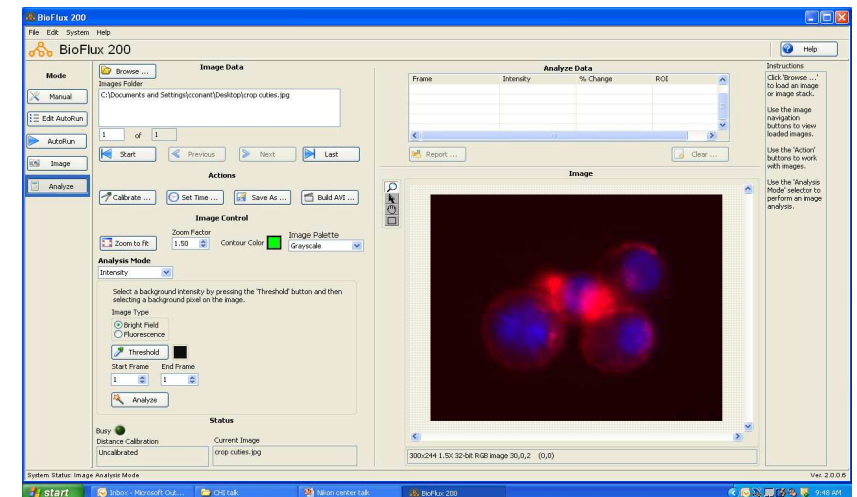
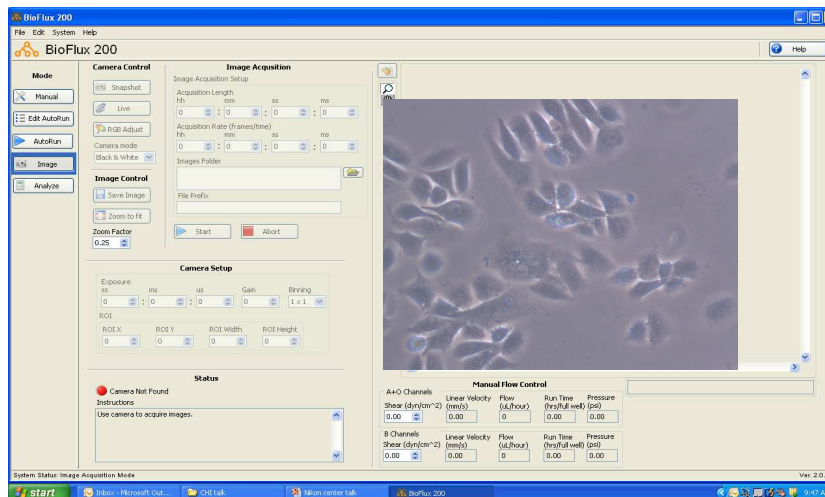


Controls the operations of the experimental channels and allows powerful image acquisition and analysis capabilities

Integrated Image Capture

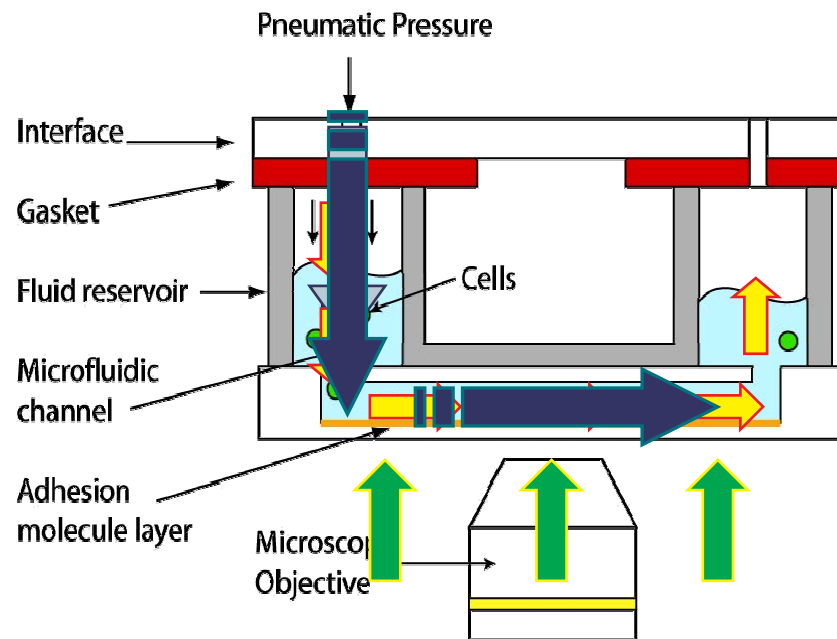
&

Image Analysis



- Fluorescence Intensity
- Rolling Velocity
- Image Stacking
- AVI export





- **Fluidic reagents loaded in BioFlux Plate**
- **Pressure applied via Controller**
- **Reagents flow through network of channels underneath plate**
- **Certain regions dedicated as viewing windows**



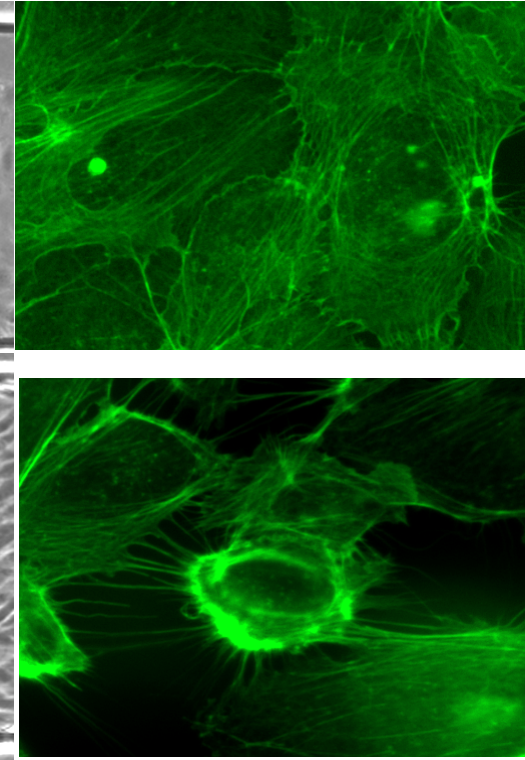
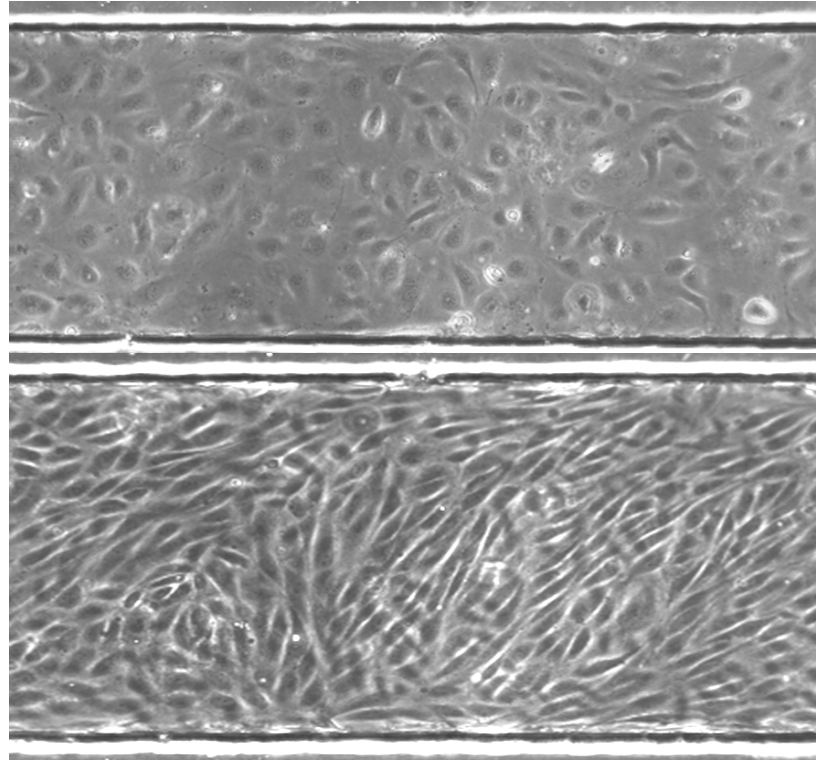


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Applications

Cellular Response to Shear Flow

0hr
16hr @ 2dynes/cm²

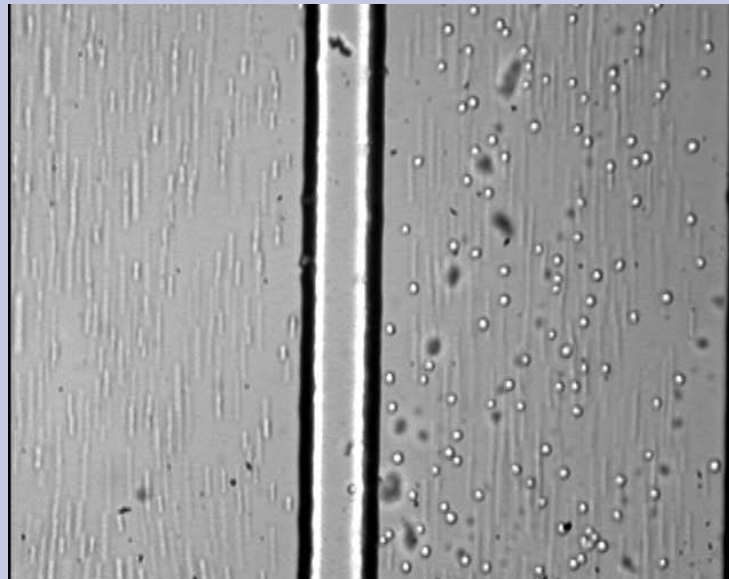


Stress fiber formation under shear flow



Lymphoma Cell Adhesion to rhVCAM-1

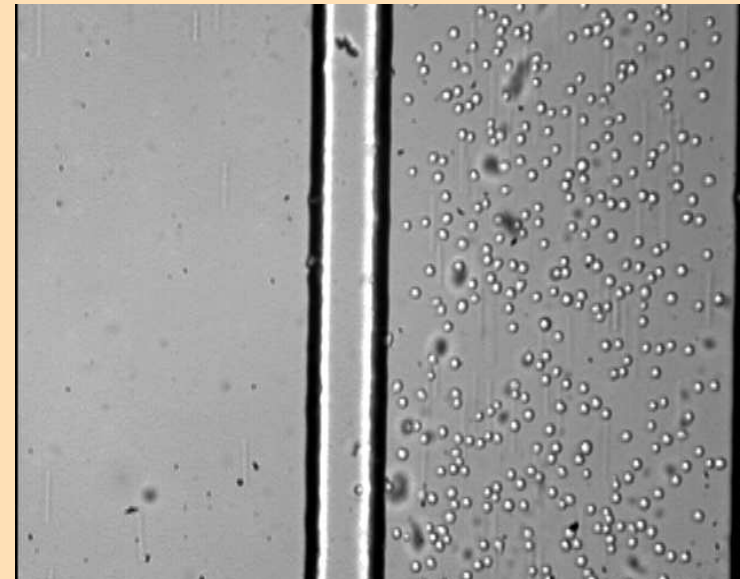
3 min



Channel 1

Channel 2

10 min



Channel 1

Channel 2

Channel 1: 1 µg/ml VCAM (control)

Channel 2: VCAM + 100 nM chemoattractant





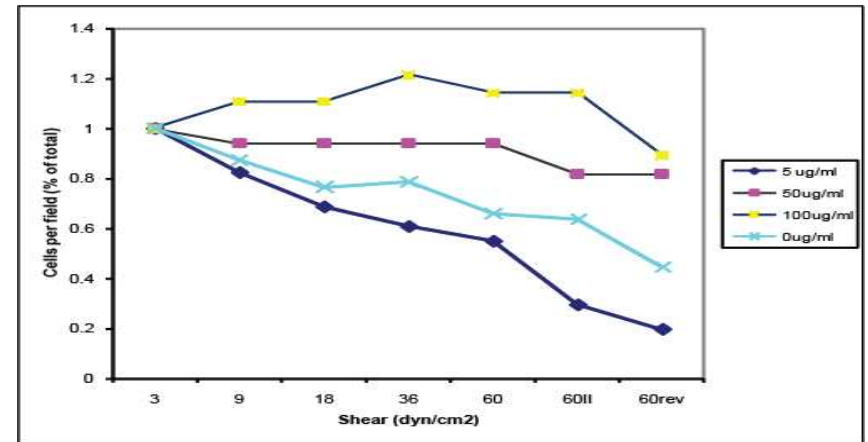
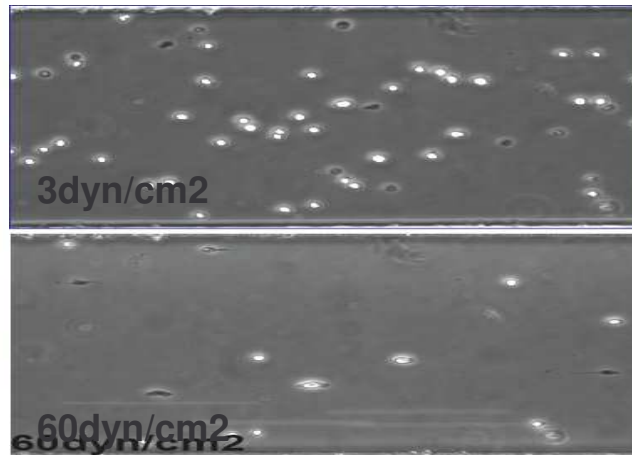
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Applications

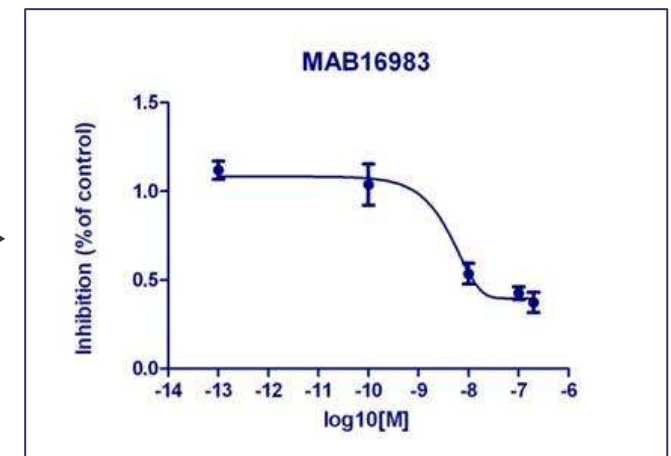


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Adhesion Strength on Purified Ligand

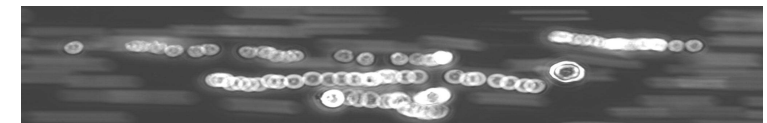
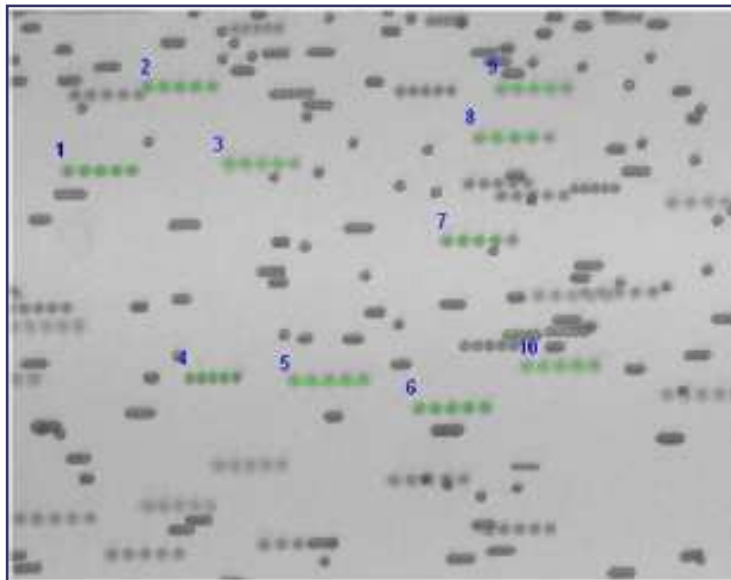


Dose-response curves for compounds or inhibitors



Cellular rolling velocity

- Assays the earliest phase of cell adhesion when cells start to roll across surface
- Can be done on ligand or cell monolayer
- Image stacker module compiles images into stacked frames, automatic calculation of rolling velocity



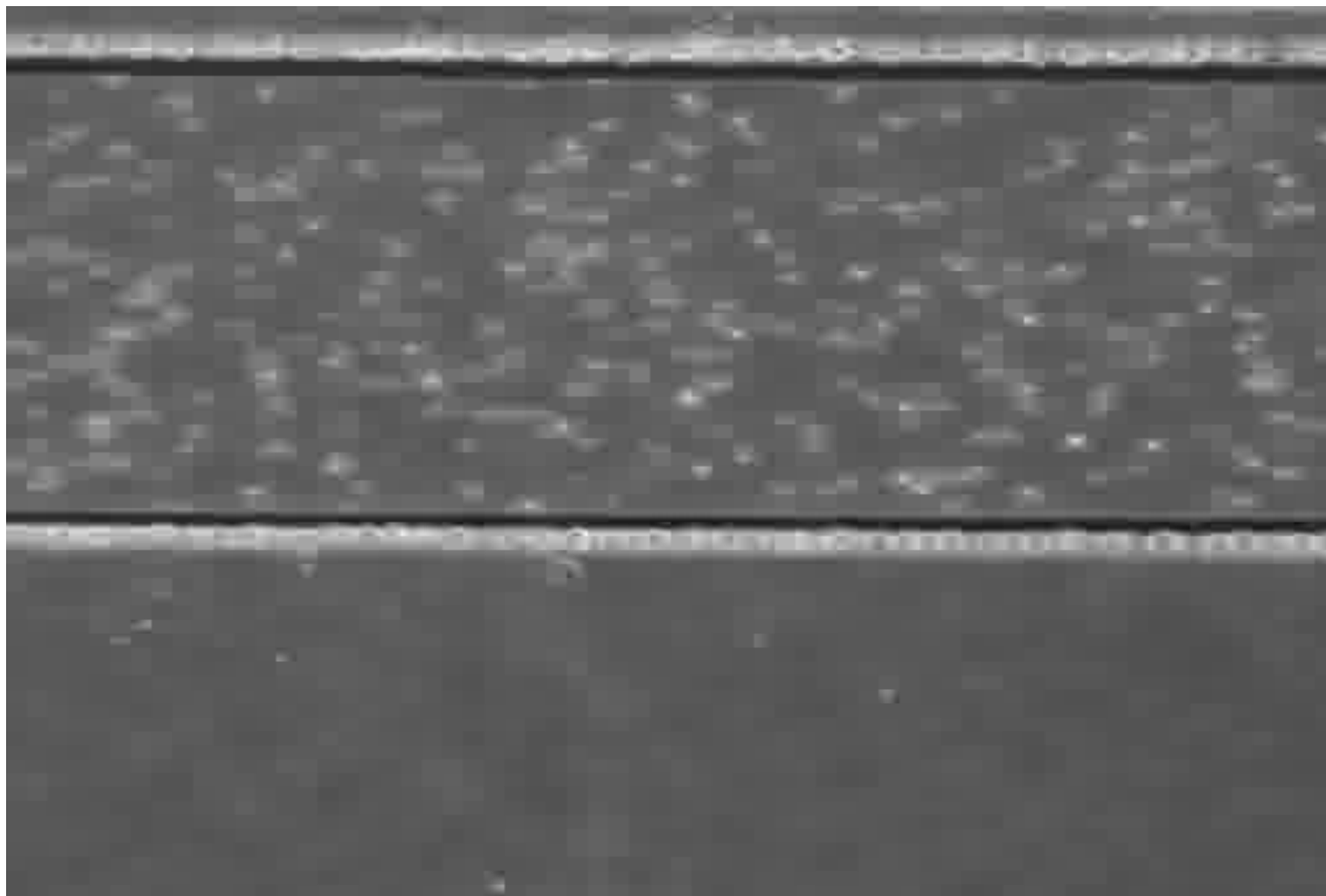
Contour	Distance (um)	Velocity (um/s)
1	27.22	206.23
2	24.44	185.19
3	26.67	202.02
4	18.33	138.89
5	30.00	227.27
6	29.44	223.06
7	23.33	176.77
8	26.11	197.81
9	26.11	197.81
10	26.11	197.81
AVERAGE	25.78	195.29
STD	3.29	24.95





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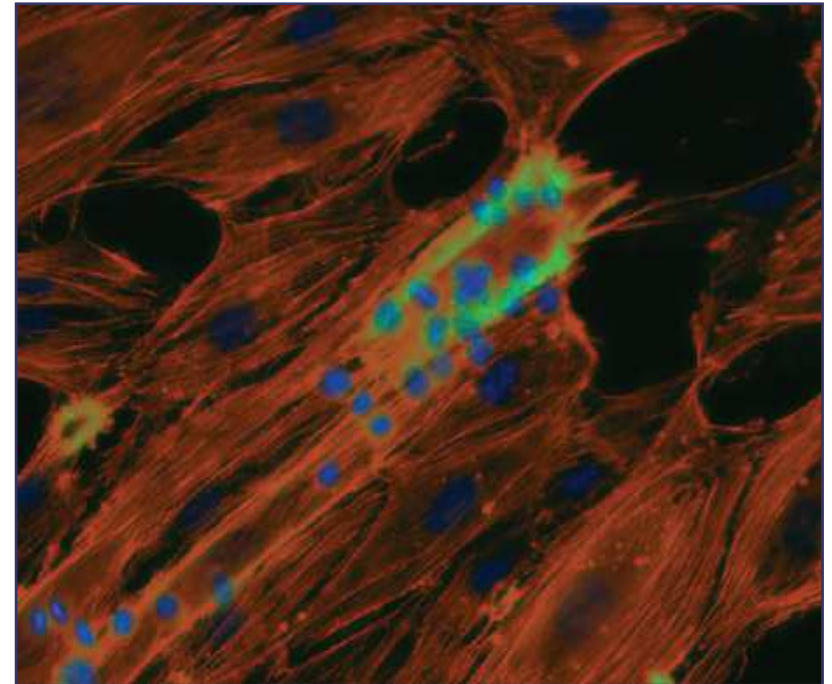


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Cell-cell adhesion

- Very common assay to study cell-cell interaction
- Cell monolayer applied to channels as a coating
- Circulating cells introduced under shear flow
- Types of analyses:
 - Cell-cell interactions

Applications

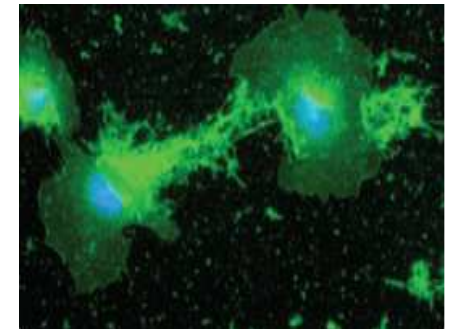


Interaction between Jurkat cells and HUVEC



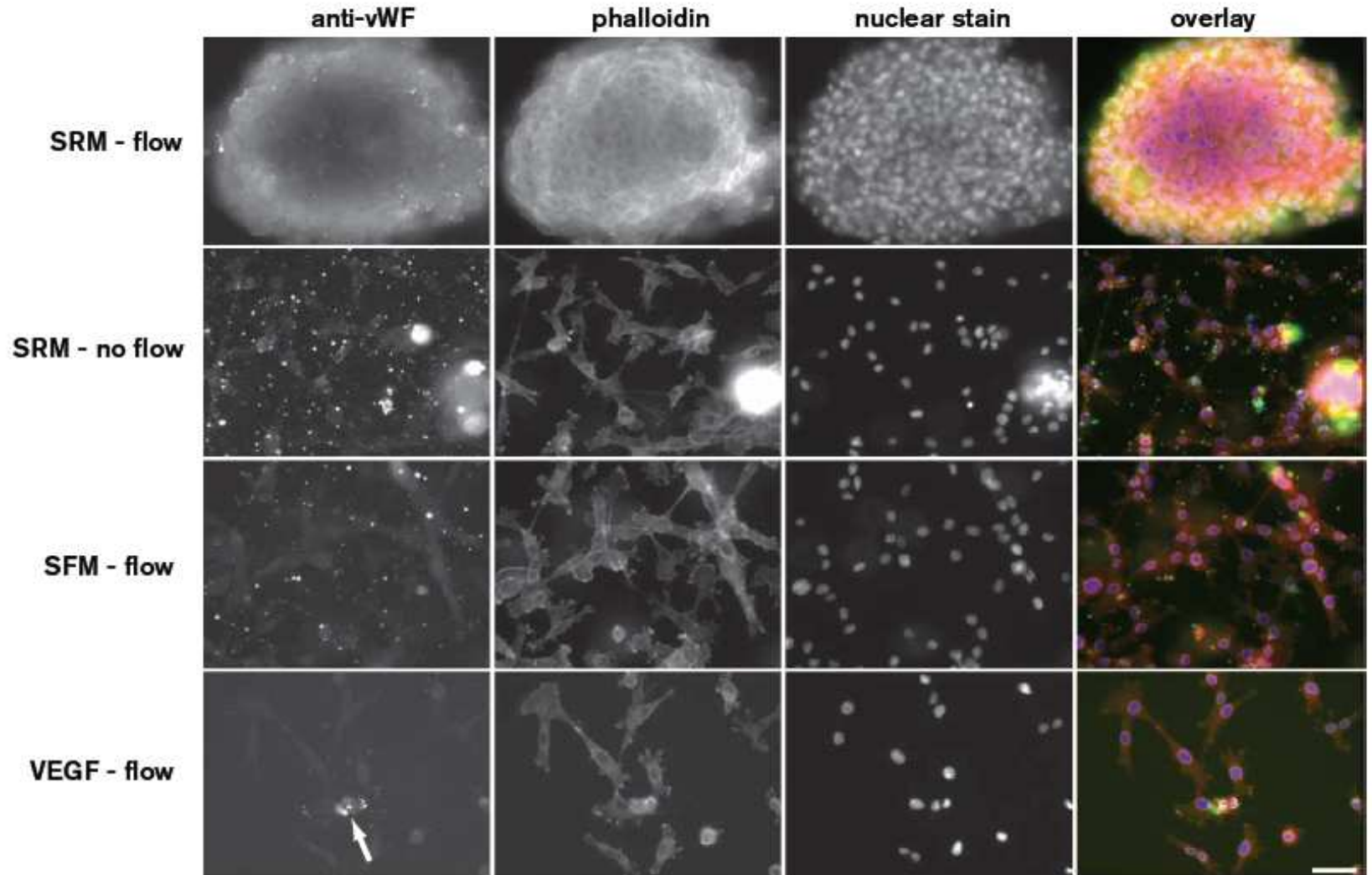
Stem Cells

- Many stem cell applications call for shear flow to differentiate cells
- Other applications call for media exchange to influence stem cell behavior (prevent/enable differentiation)
- Stem cells can be recruited to sites and transmigrate through a cell layer (chemokine modulated)
- Stem cell applications benefit from BioFlux:
 - Fewer cells and reagents needed per assay
 - Tight control over cellular microenvironment
 - Many assays run in parallel for data throughput and consistency





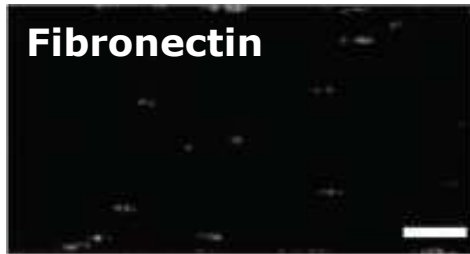
Differentiation of mesenchymal stem cells into endothelial cells



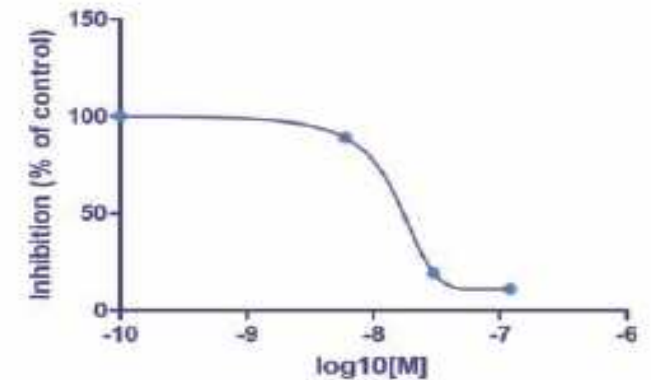
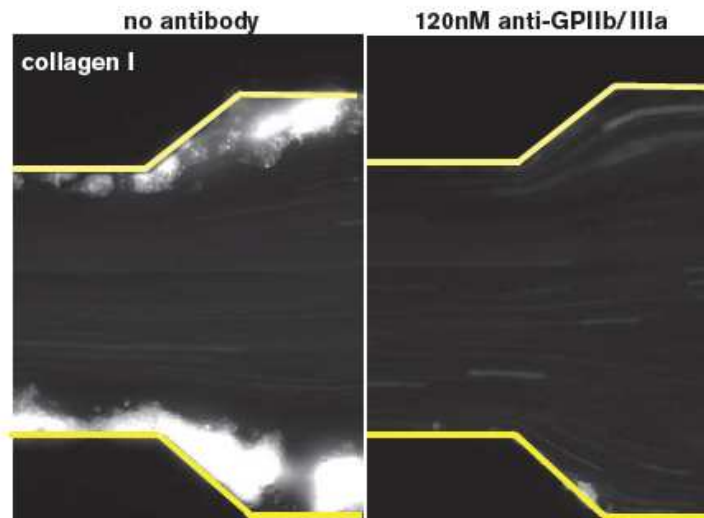
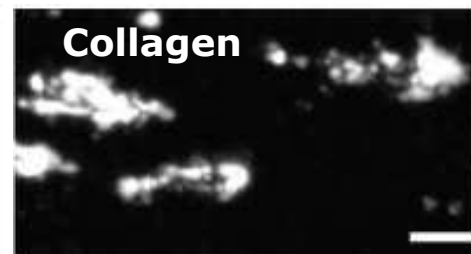
Platelets adhesion: up to 200 dyn/cm² with 48 WPM

Vessel	Diameter (cm)	Wall Shear Rate (s ⁻¹)	Wall Shear Stress (dyn/cm ²)
Ascending aorta	2.3 - 4.5	50 - 300	2 - 10
Femoral artery	0.5	350	10
Large veins	0.5 - 10	200	7
Inferior vena cava	2.0	50	2

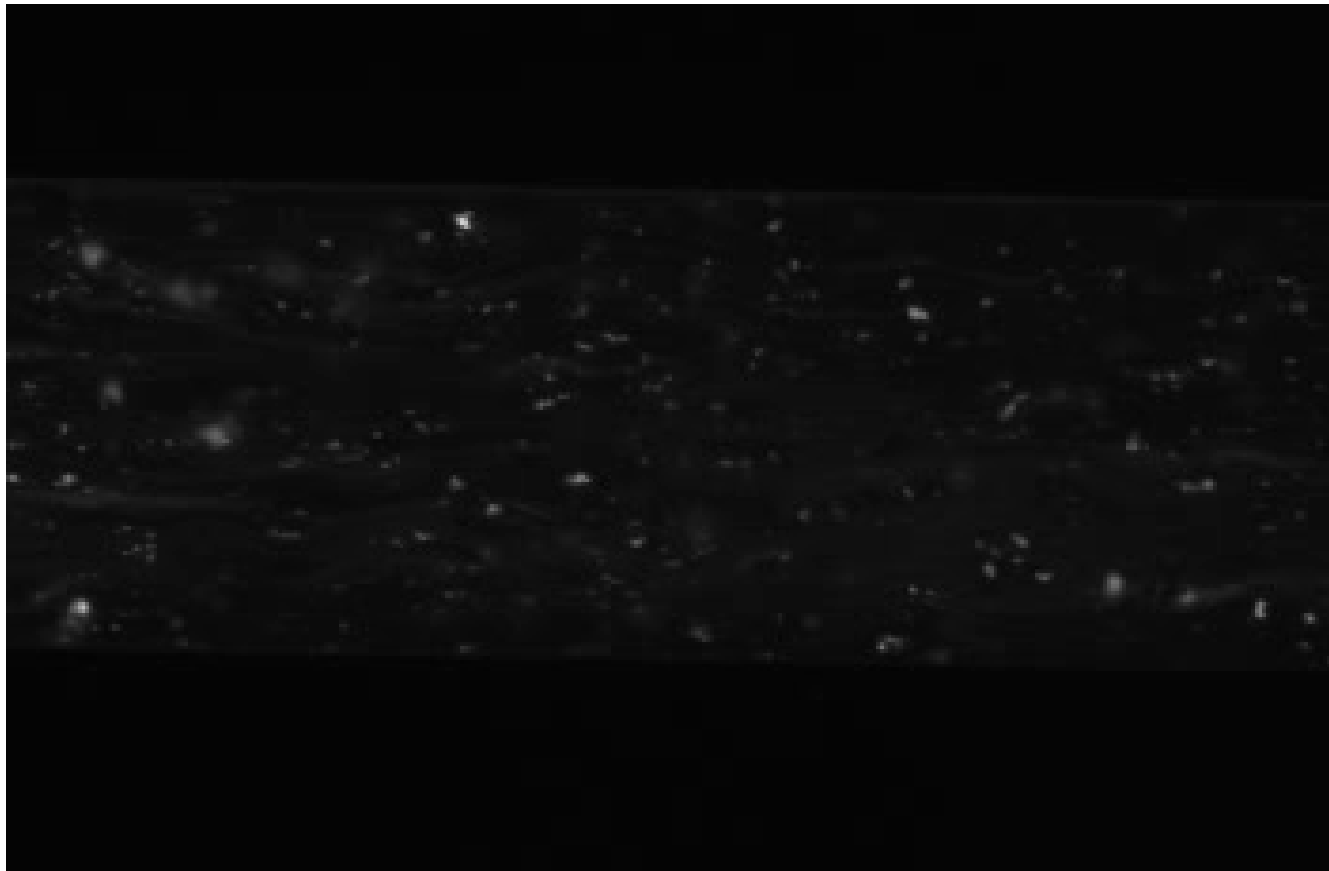
Fibronectin



Collagen

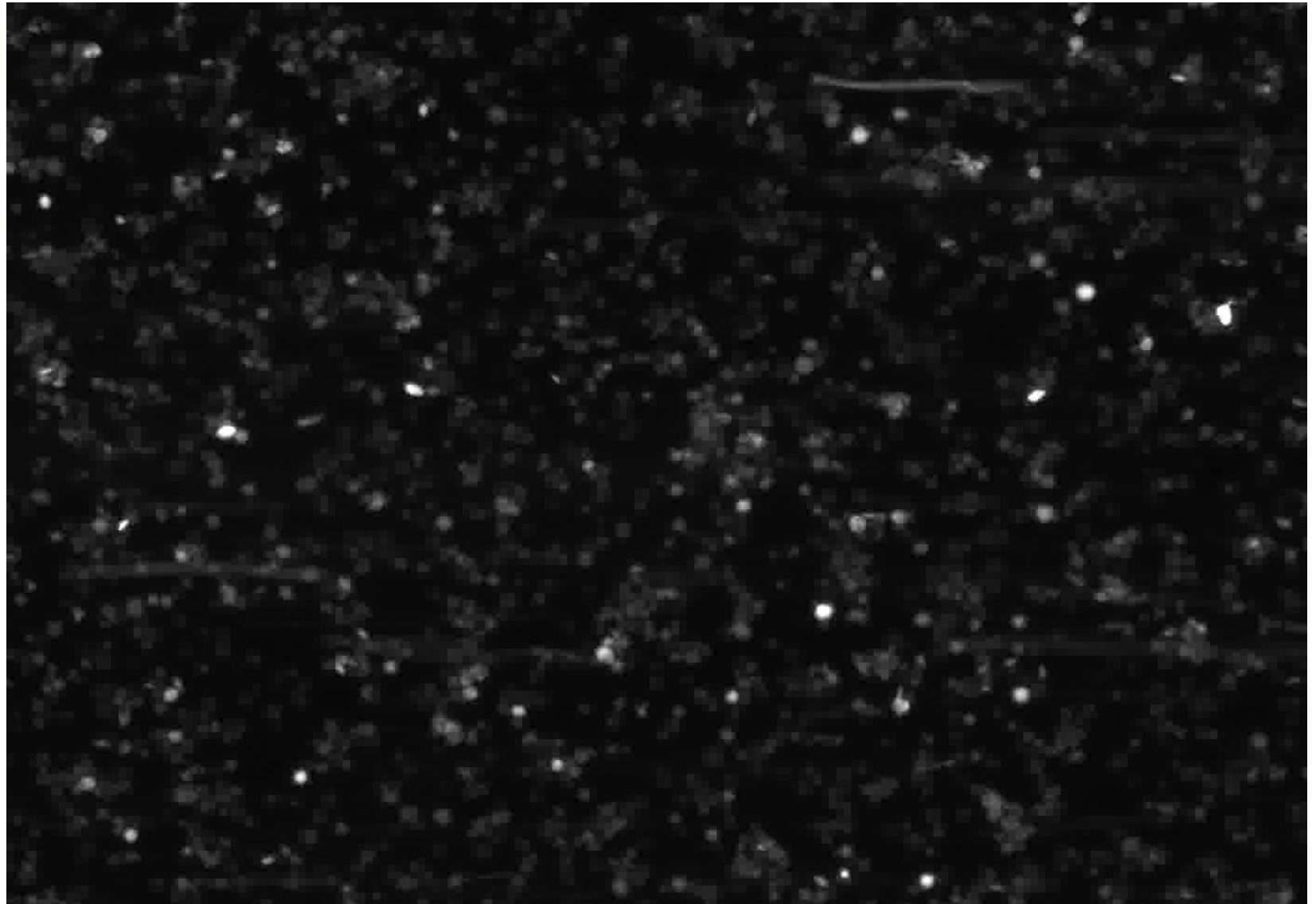


Platelets aggregation assay on Col-1 using Calcein AM stained platelets



Applications

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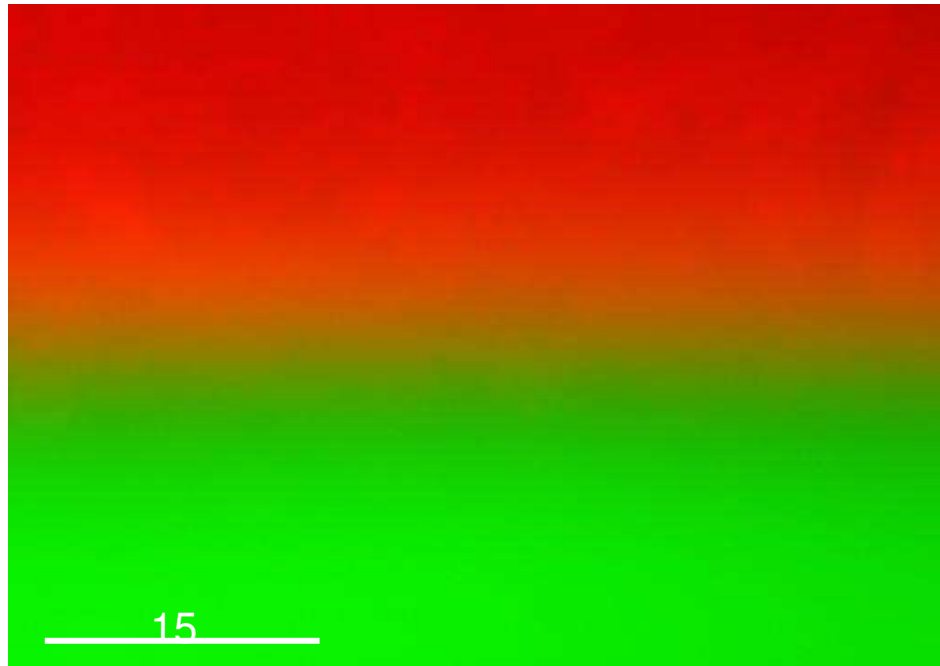


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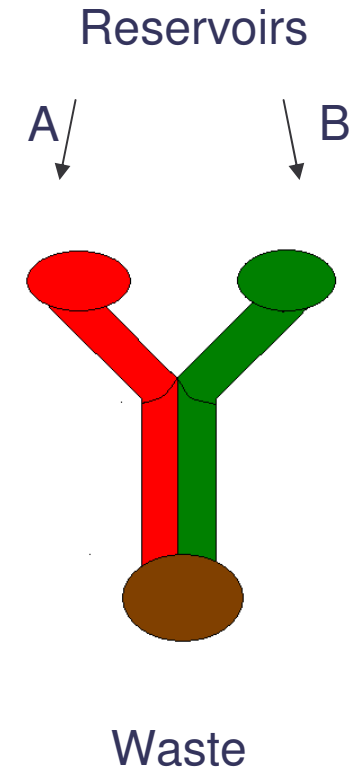


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Wound Healing



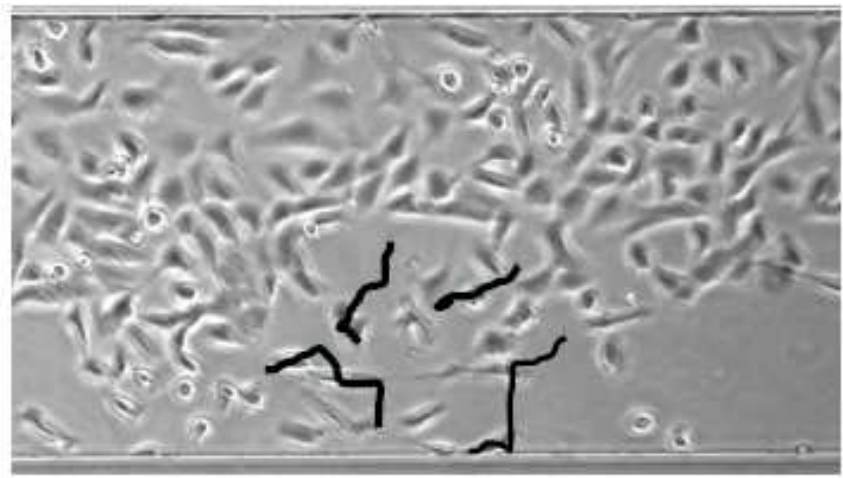
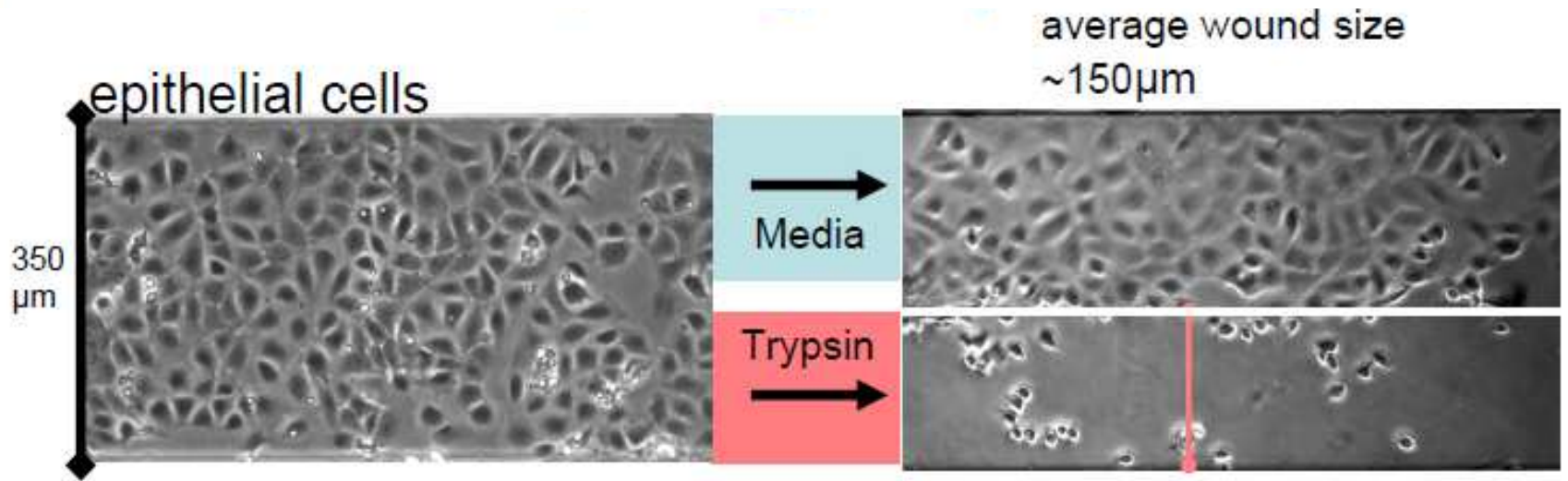
Applications



- Use 24 well plate
- Grow monolayer of cells
- Flow Trypsin from reservoir A and Media in reservoir B at equal shear.
- Cells in A are removed with <10% variability of wound size



Wound Healing



6hr

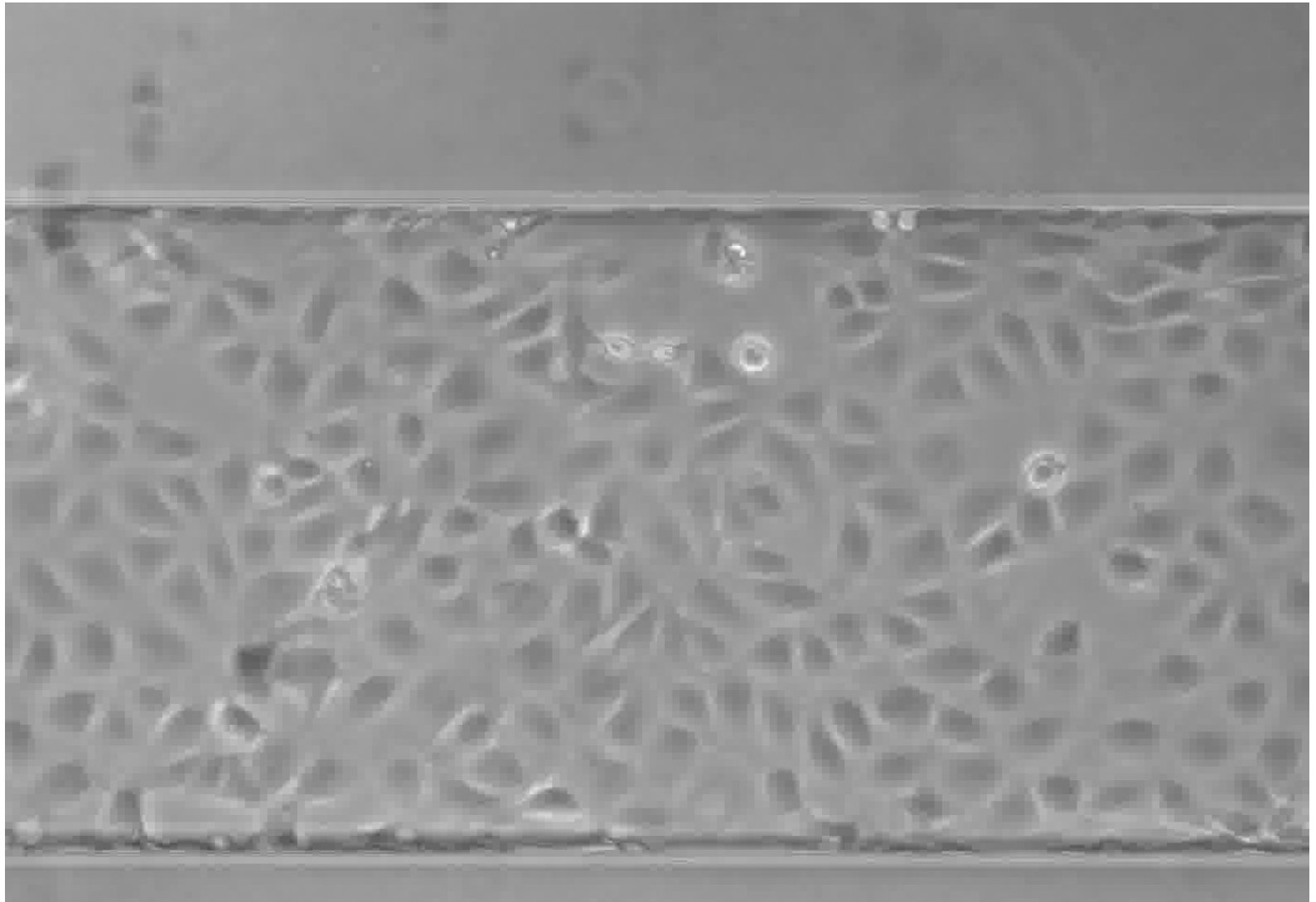
healing





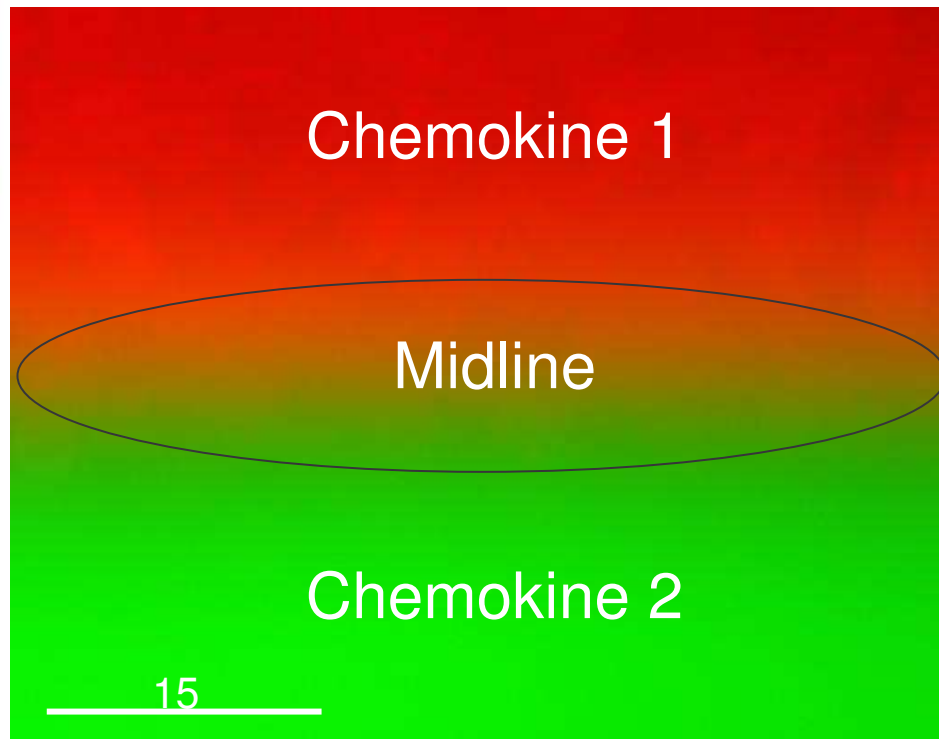
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Applications



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Migration



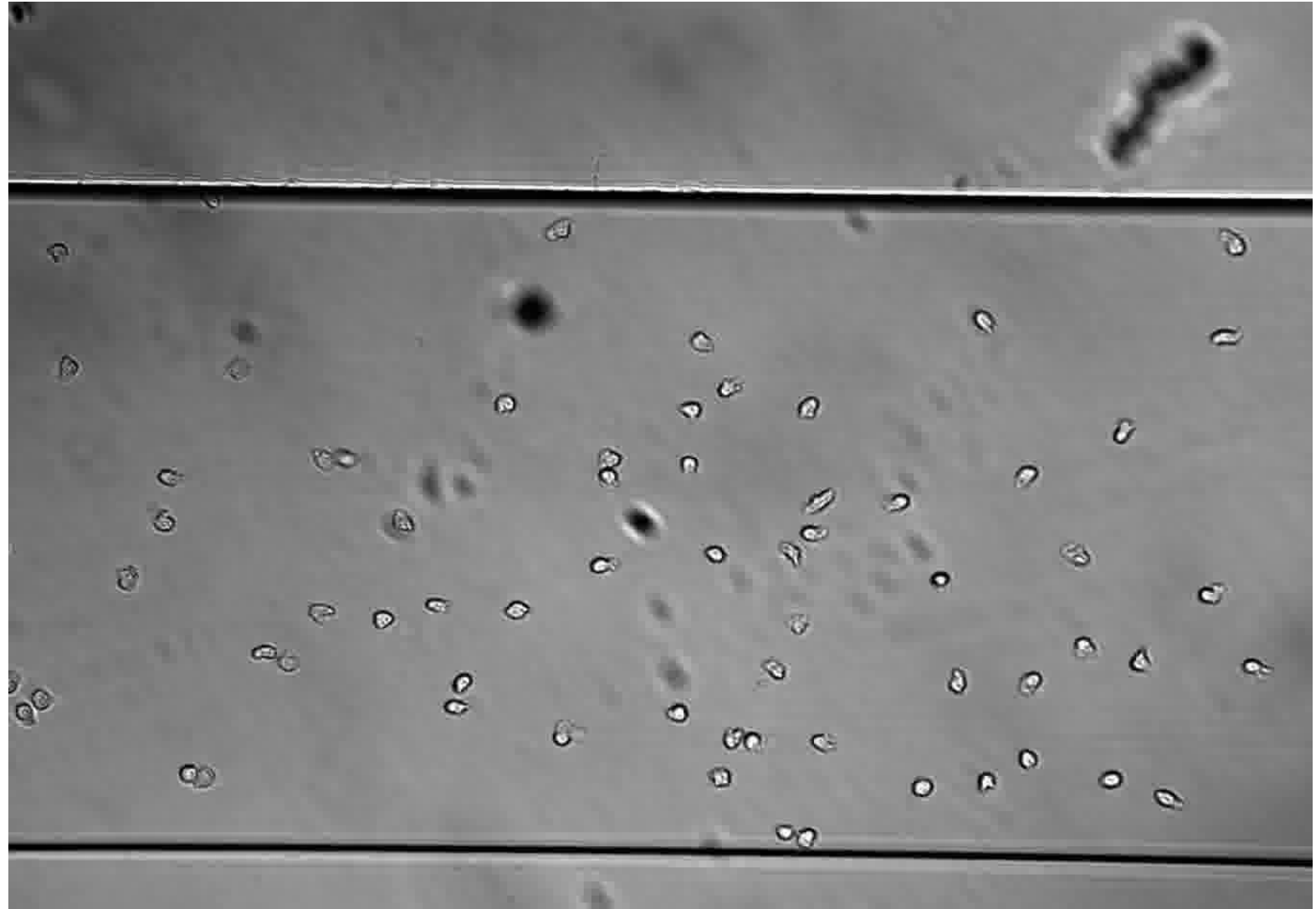
- Use 24 well plate
- Adhere cells
- Run 2 chemokines simultaneously in both inlet reservoirs
- Diffusion gradient in midline where two liquids meet
- Midline can be moved and spread out based on ratio of flow
- Cells migrate towards favored Chemokine
 - Observe cells in midline





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Applications



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Migration

Chemokine

Midline

15

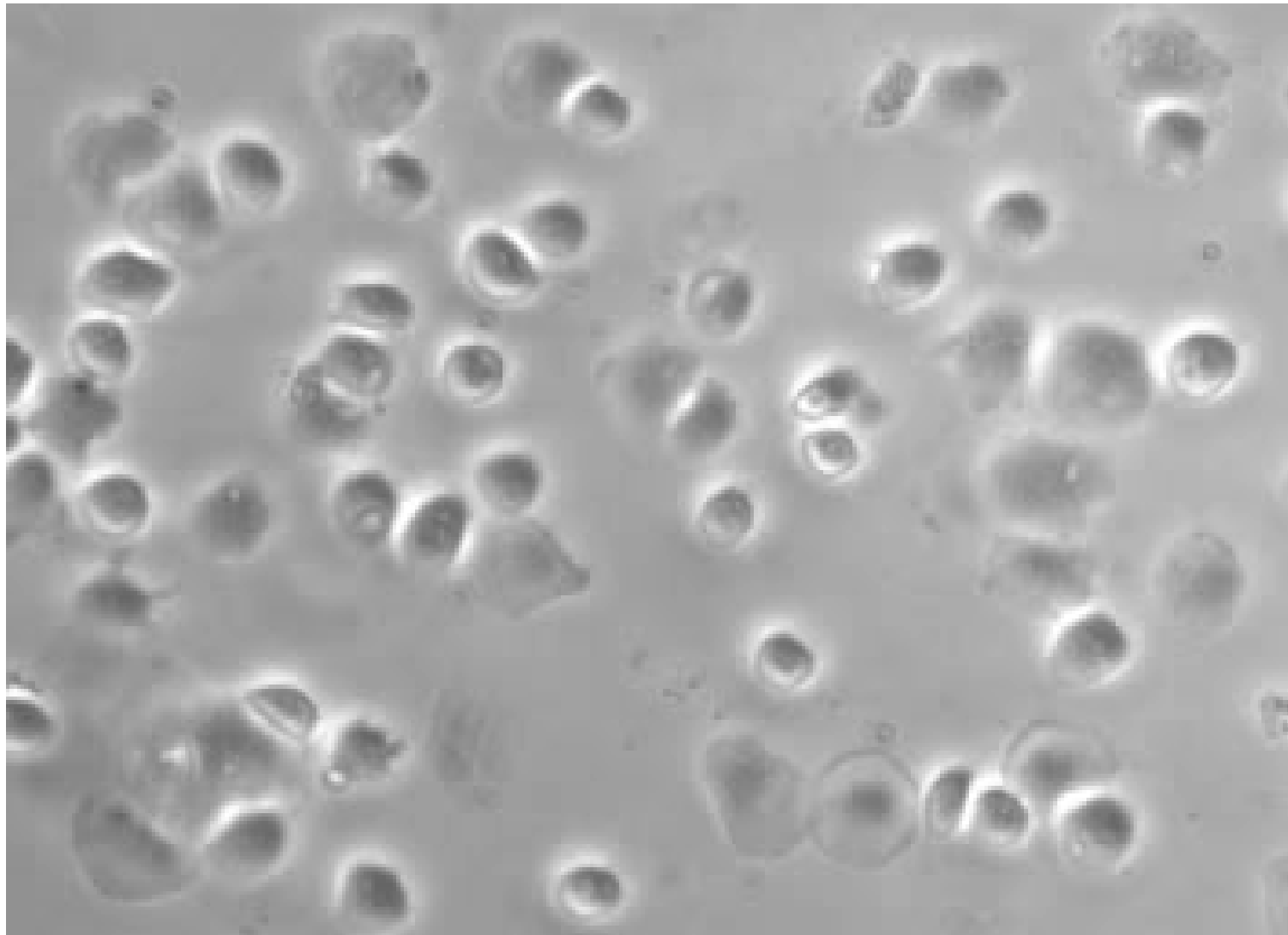
- Neutrophils adherent to E-Selectin
- Chemoattractant- bacterial peptide fMLP run on upper portion of channel.
- 5 dynes/cm² for 60 min.
- Diffusion gradient in midline where two liquids meet
- Migration toward active chemokine can be seen





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Applications

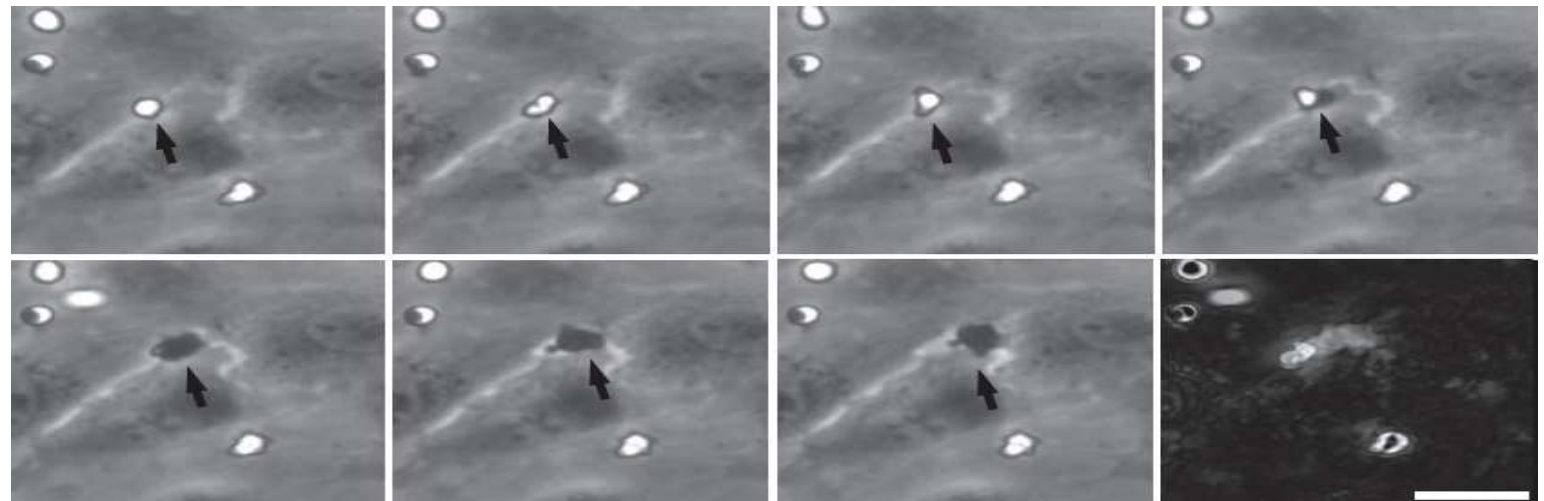


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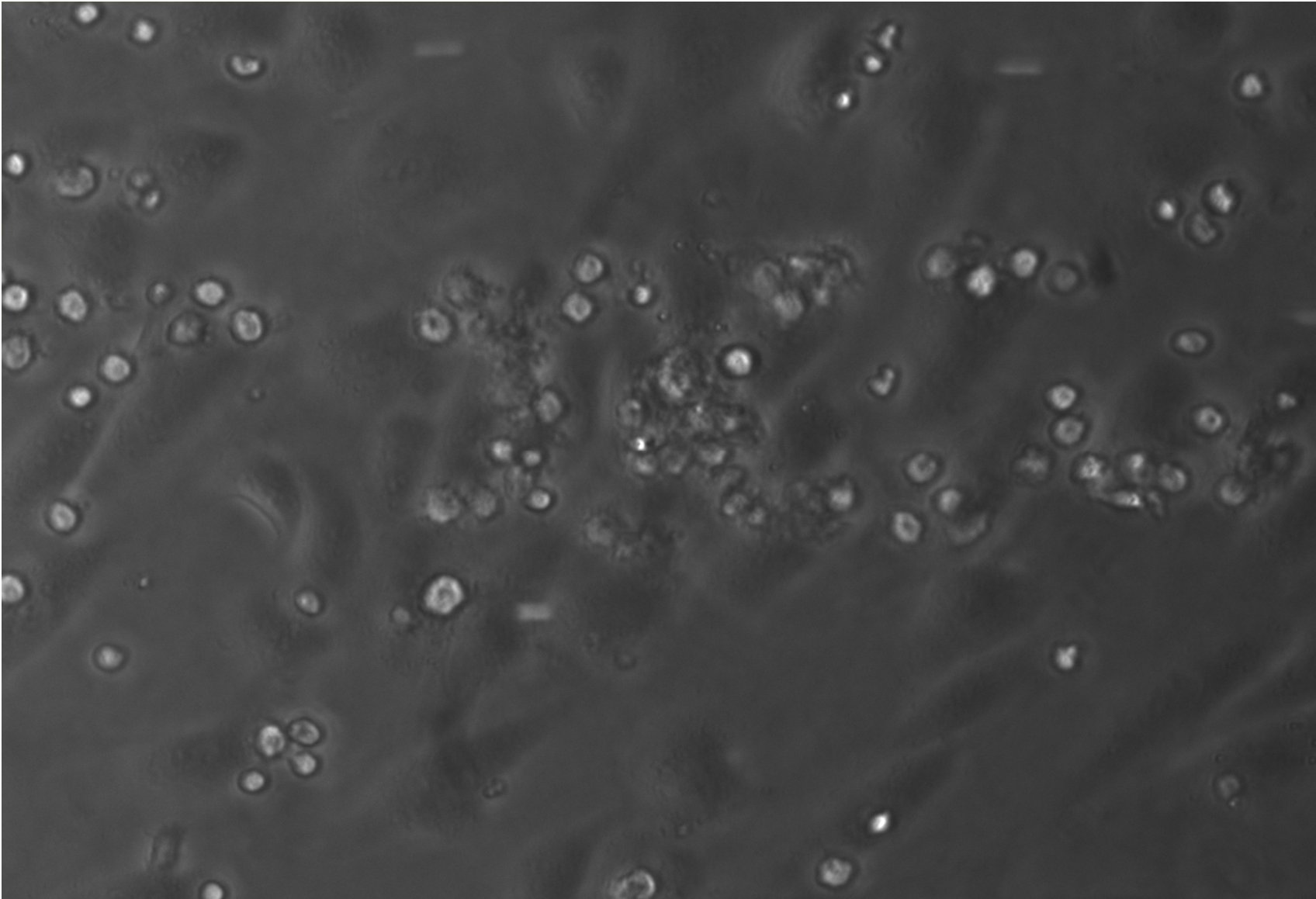


Transmigration

- Looks at the final stage of adhesion as the cells migrate across the endothelial layer
- Usually mediated by chemokines and interactions with the endothelial layer
- Typical analyses:
 - Number or % of cells which migrate across cell layer



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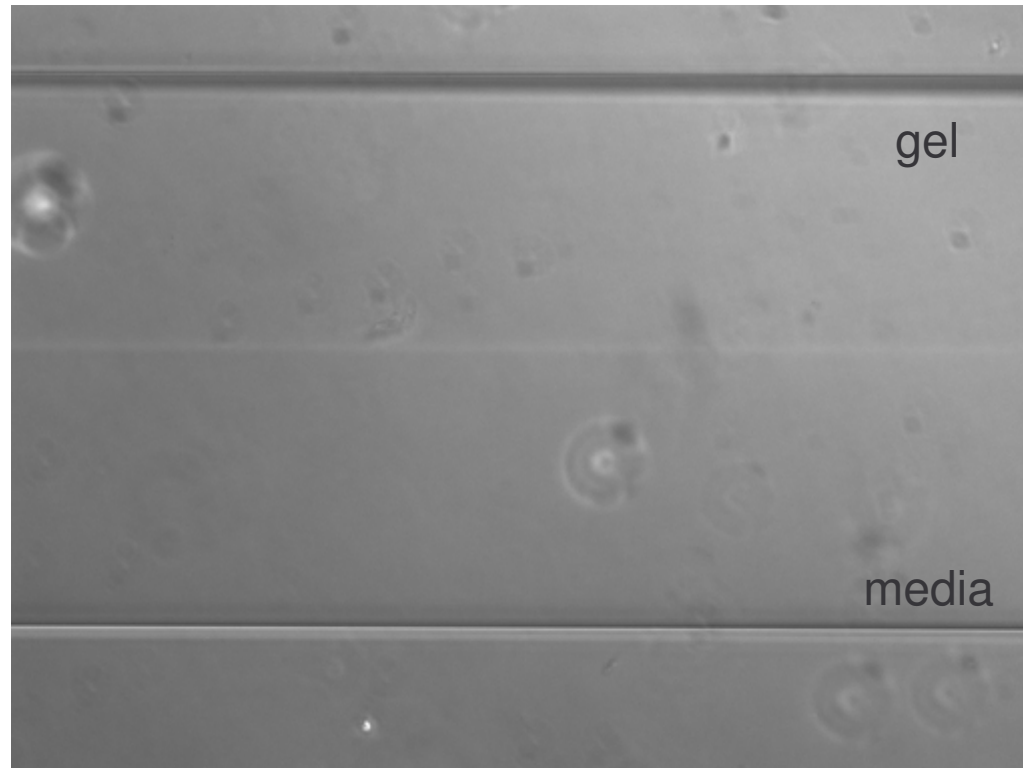




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Applications

Invasion & Angiogenesis assay



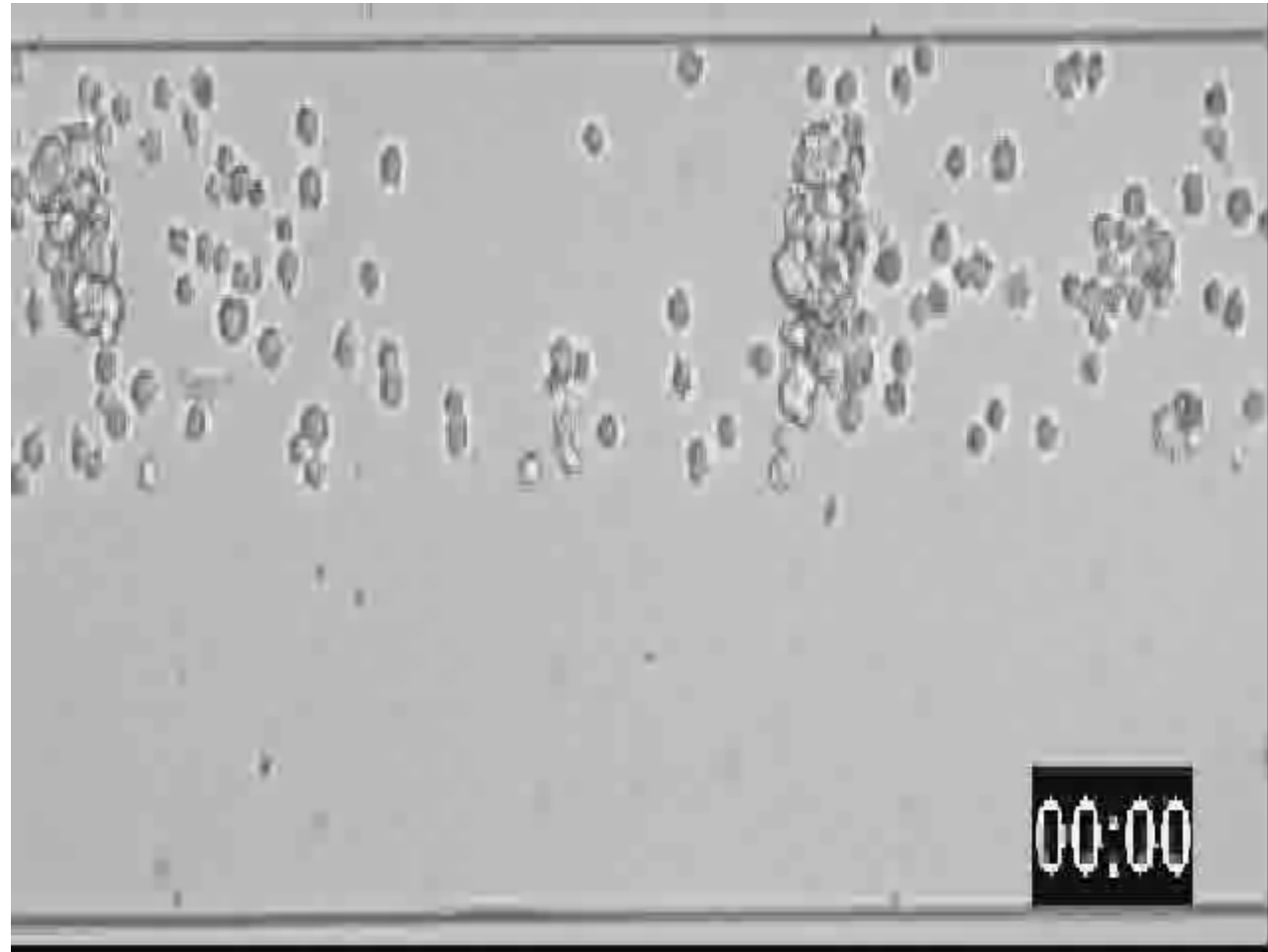
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Applications

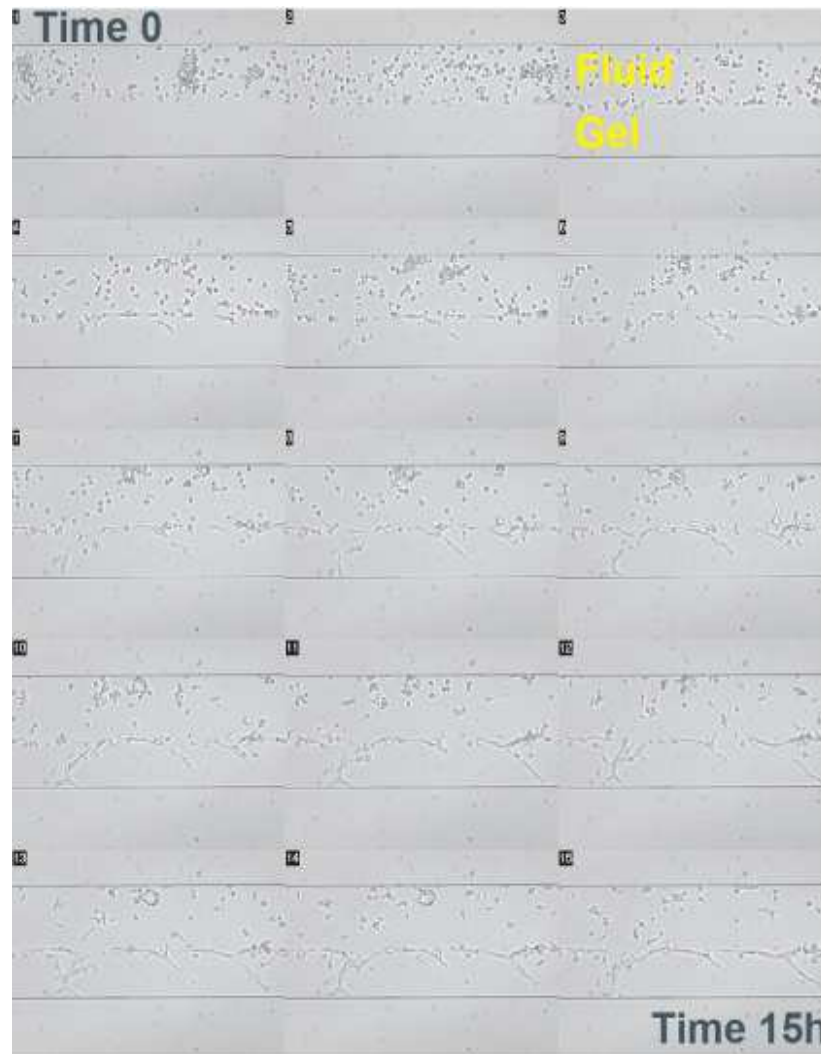
Invasion & Angiogenesis assay



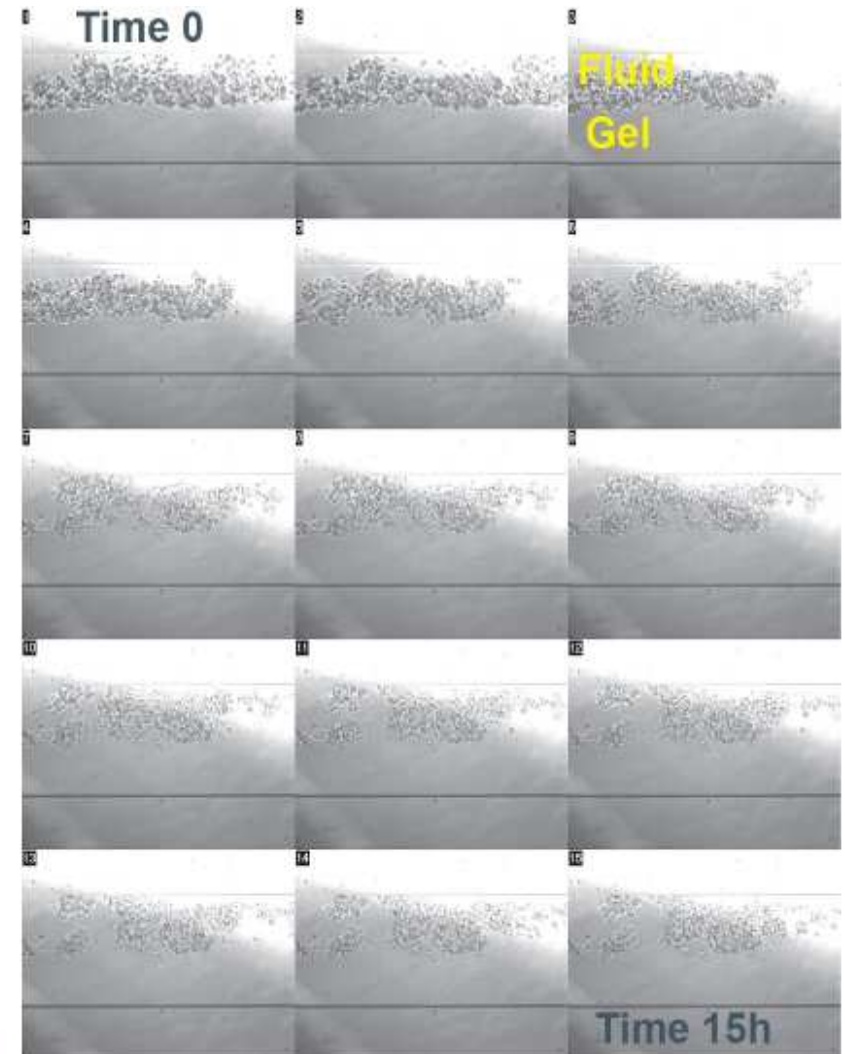
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Invasion & Angiogenesis assay



HT1080_Colon Carcinoma Cells



MCF7_Breast Cancer Cells



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