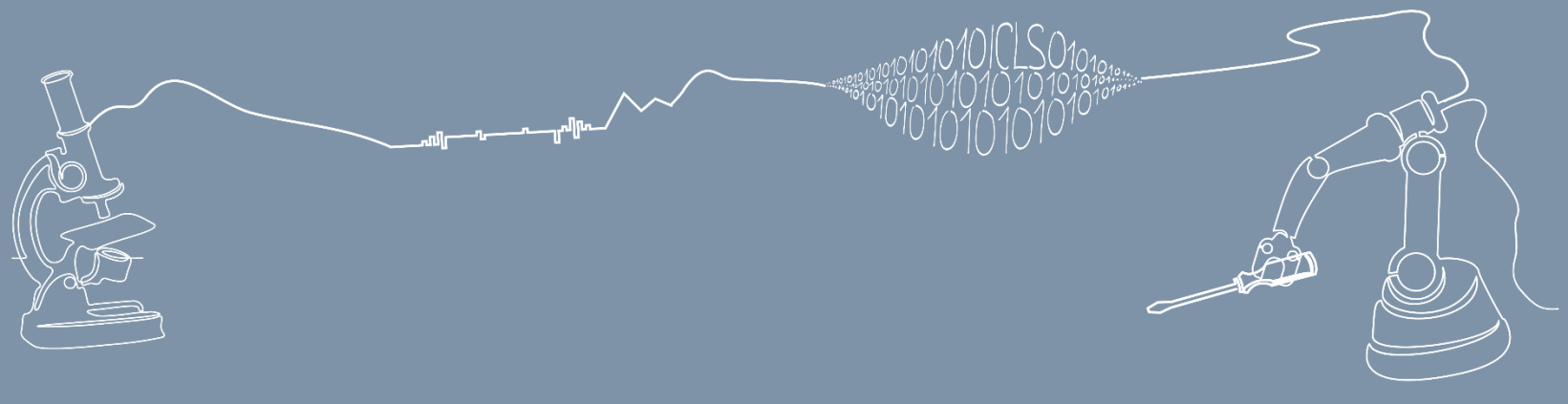
Life Sciences and
Facility Management

Institute of
Computational Life Sciences

Case Studies

Wahlpflichtmodule: Molecular Imaging

Besmira Sabani
sabn@zhaw.ch



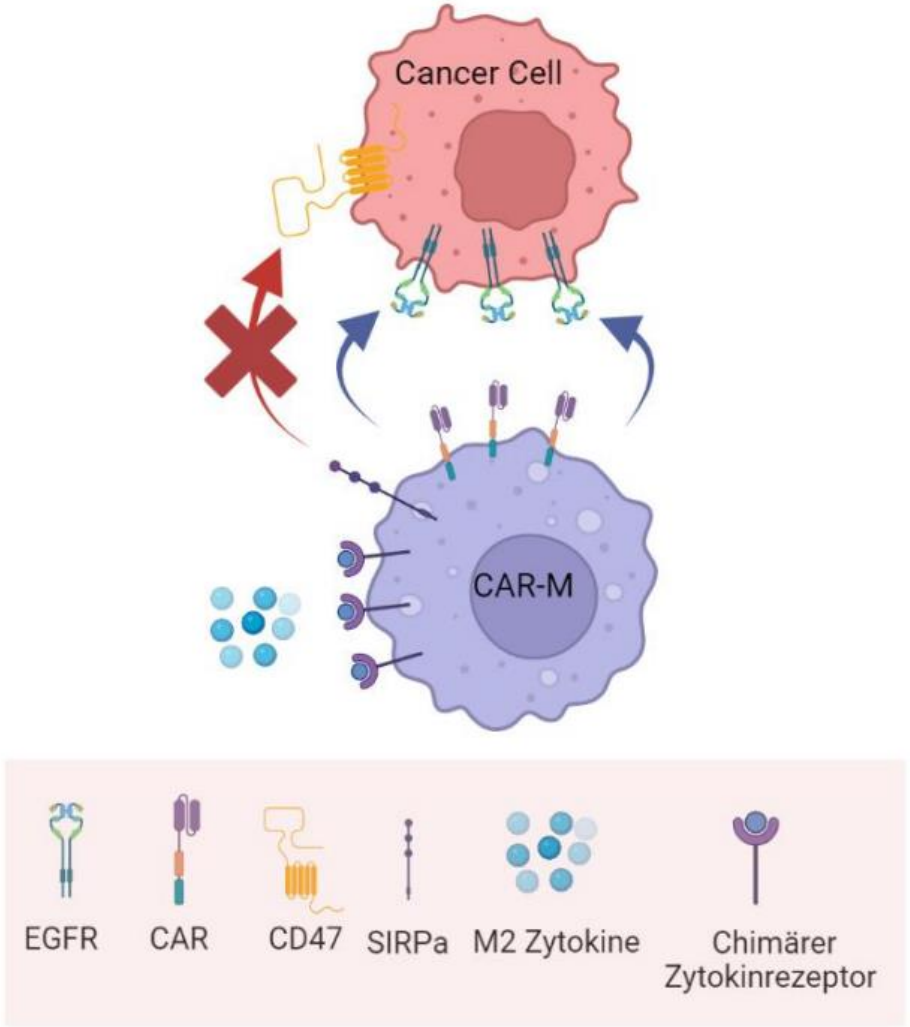
Case Studies

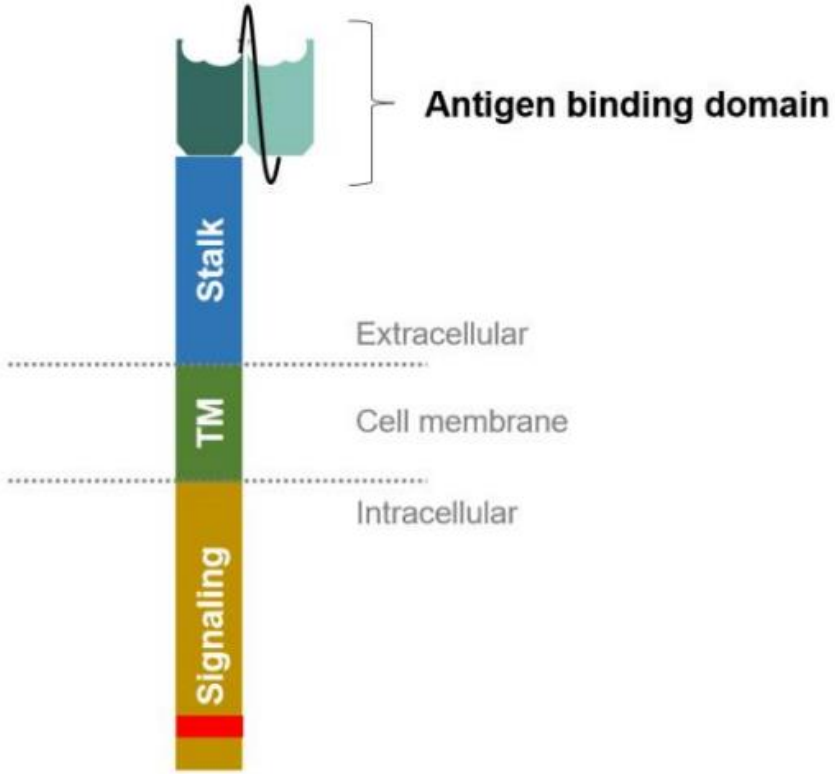
1. Contact-induced cell death of cancer cells by macrophages
2. Quantification of the αSMA expression in patient derived cells cultured in a 3D environment
3. Differentiation of fibroblast to myofibroblast via cell morphology changes
4. Effect of the inflammatory cytokine TGF-β1 on the migratory properties of patient derived cells
5. Zebrafish embryo model for the screening of antimicrobial drugs

Contact-induced cell death of cancer cells by macrophages

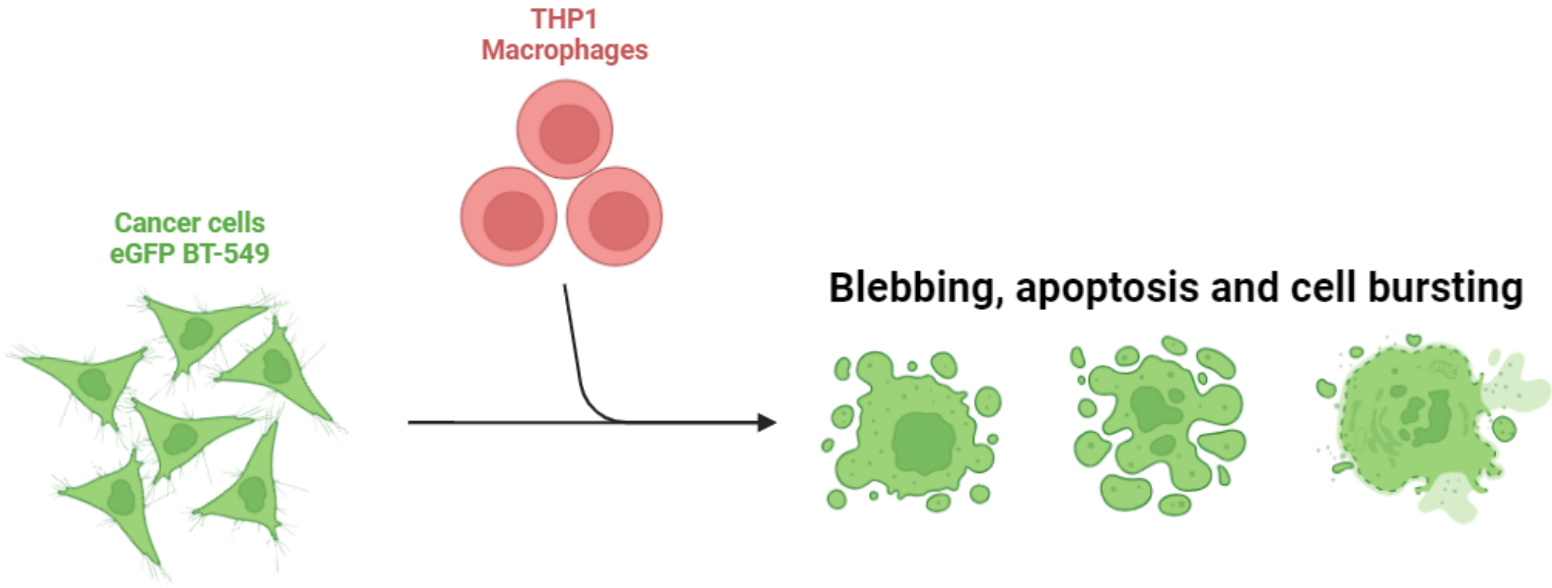
- Cancer is one of the world's largest health problems due to its morbidity and mortality
- Traditional treatments like chemotherapy and radiation directly target cancer
- Innovate approach to treat cancer is with **immunotherapy**
- Immunotherapy works by boosting or modifying immune responses, helping the body recognize and eliminate cancer more effectively
- Examples: CAR cell therapy:
  - Immune cells (T cells or macrophages) are genetically engineered to produce chimeric antigen receptors (CARs) on their surface
  - CARs enable immune cells to better recognize and target cancer cells
  - **immune-cell mediated tumor cell killing**

Contact-induced cell death of cancer cells by macrophages





Contact-induced cell death of cancer cells by macrophages

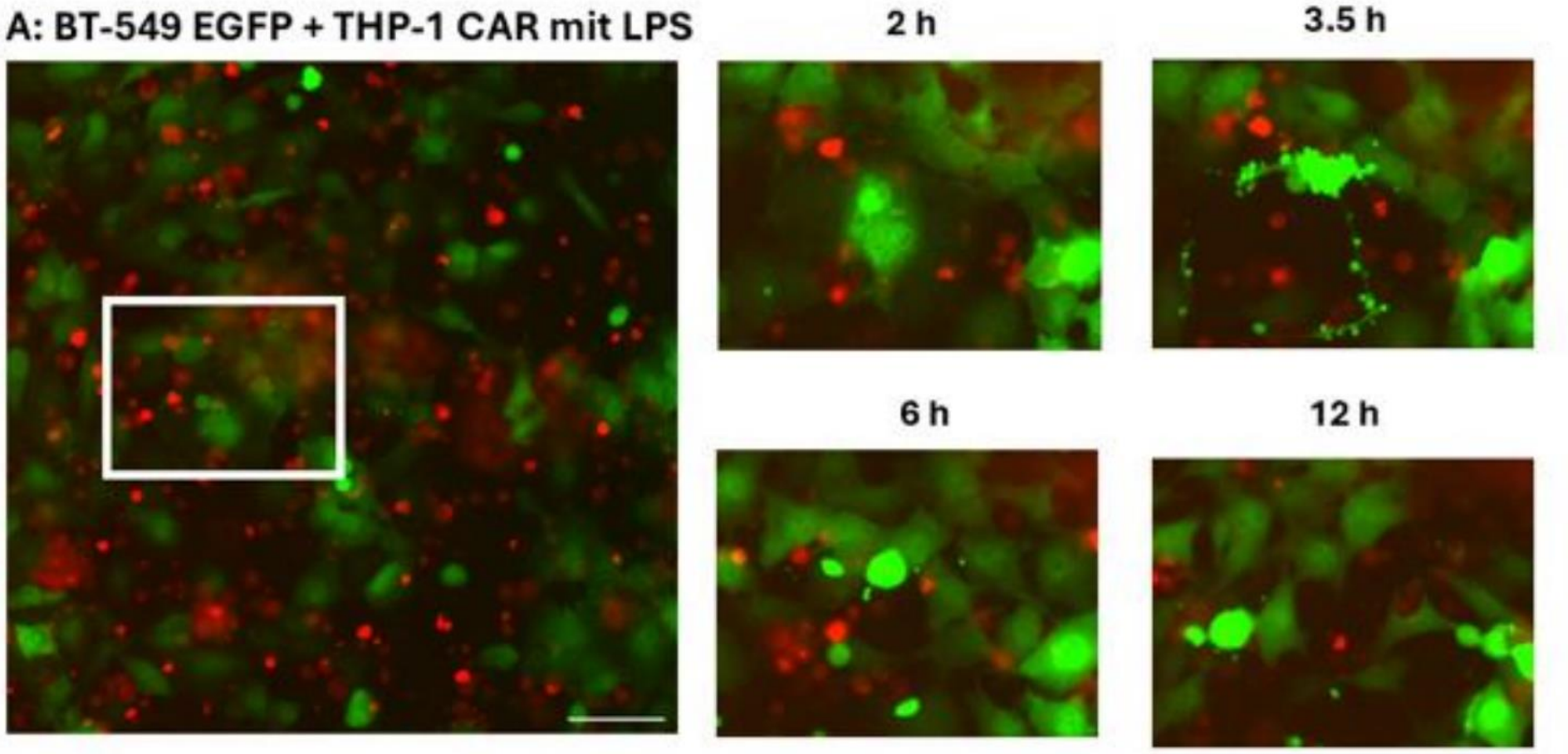


The aim of this experiment was to use live imaging to observe the cell death of cancer cells (BT-549 GFP) after the interaction of with CAR macrophages (CAR-THP-1) in a 2D co-culture system.

Cell death was detected through vesicle formation or cell fragmentation.

Contact-induced cell death of cancer cells by macrophages

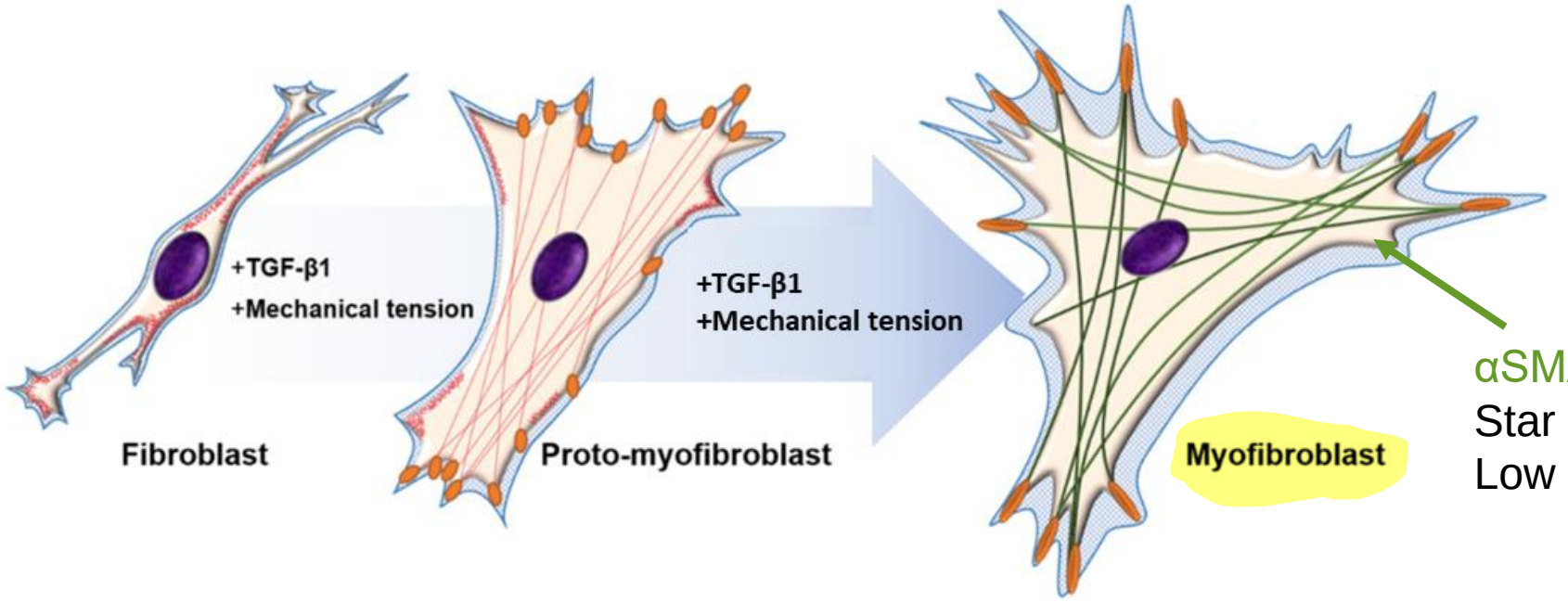
- Cell death was detected through vesicle formation or cell fragmentation
- Your task is to quantitatively analyze the occurrence of cell death by analyzing the cell morphology/cell blebbing/apoptosis/fragmentation and bursting of the cancer green cells over time



Two construct evaluated
L200 and L206

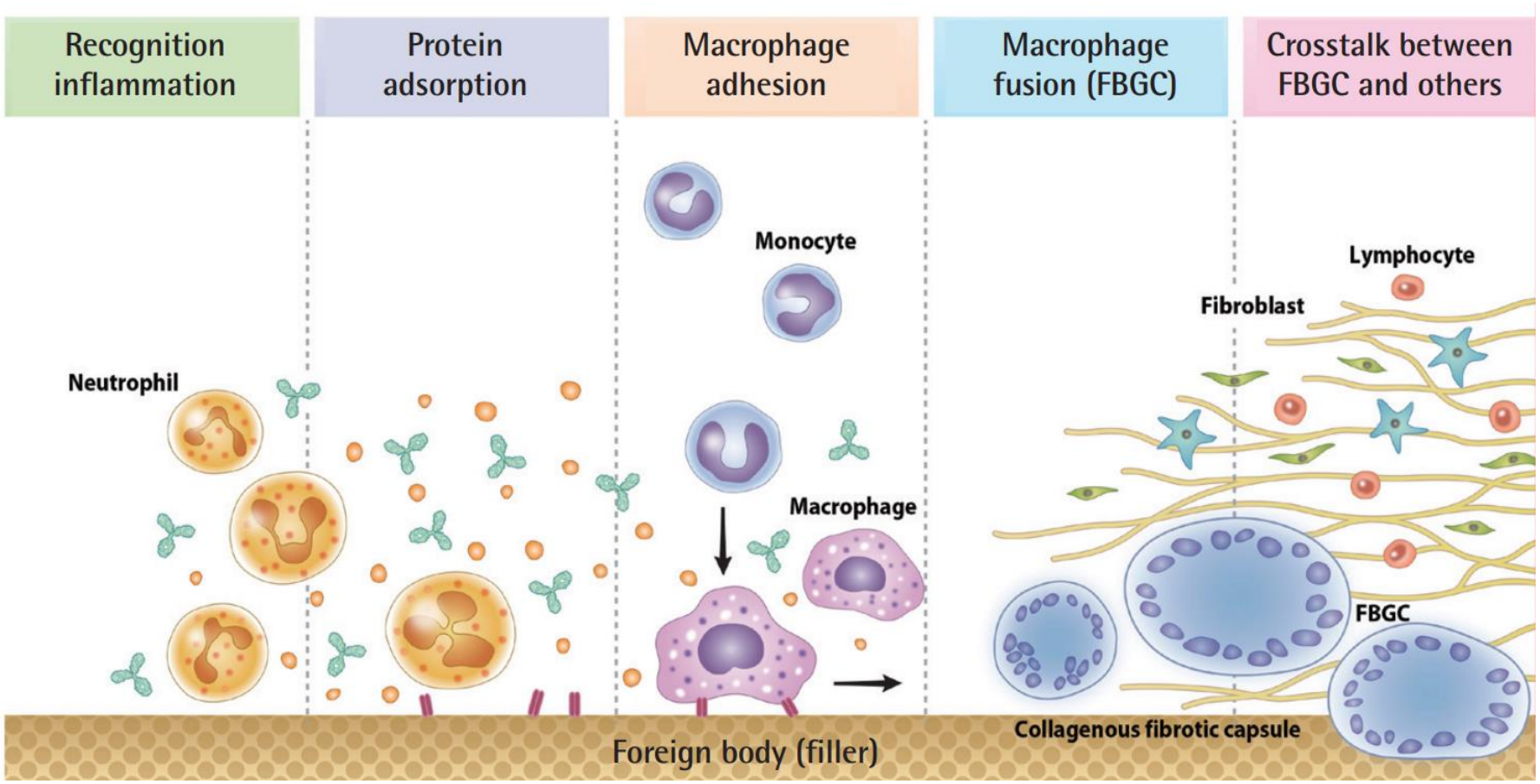
Introduction to Fibrosis (case study 2, 3 & 4)

- In fibrotic disease there is an excess formation of connective tissue in an organ or tissue, often as a reparative response to injury or chronic inflammation
- Over time, excessive fibrosis can lead to organ dysfunction and, in severe cases, organ failure
- Fibrosis is involved in almost 50% of deaths in the western world
- **Efficacious anti-fibrotic drugs are not present**



αSMA expression (case 2)
Star shape (case 3)
Low migratory (case 4)

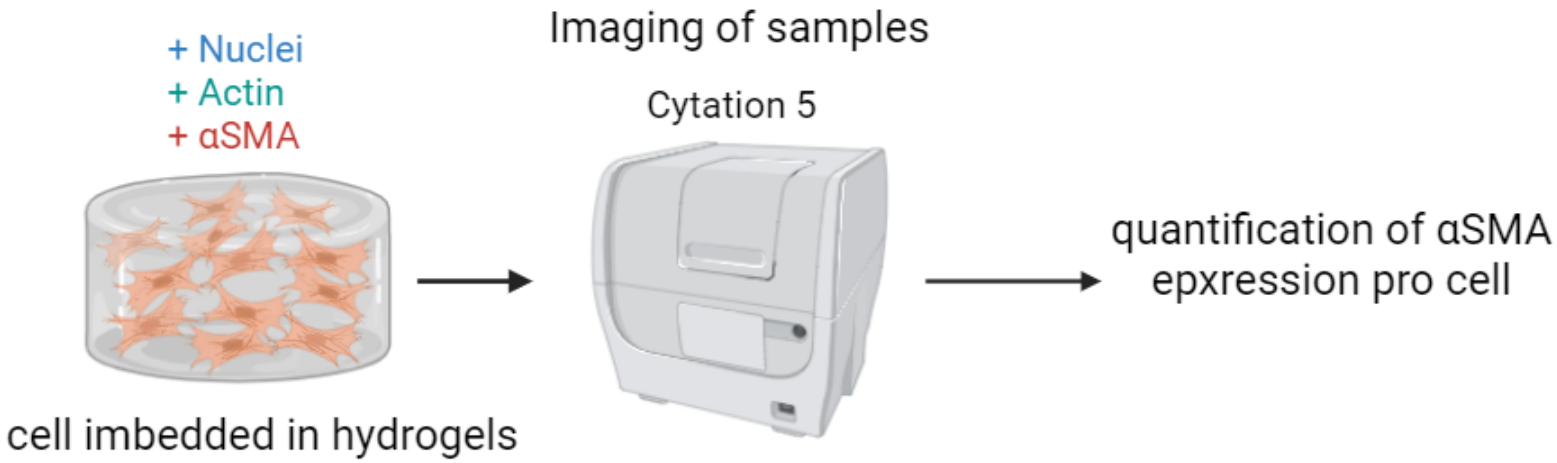
Introduction to Fibrosis (case study 2, 3 & 4)



Isolation of patient derived fibroblast from different fibrosis stages

Quantification of the αSMA expression in patient derived cells cultured in a 3D environment

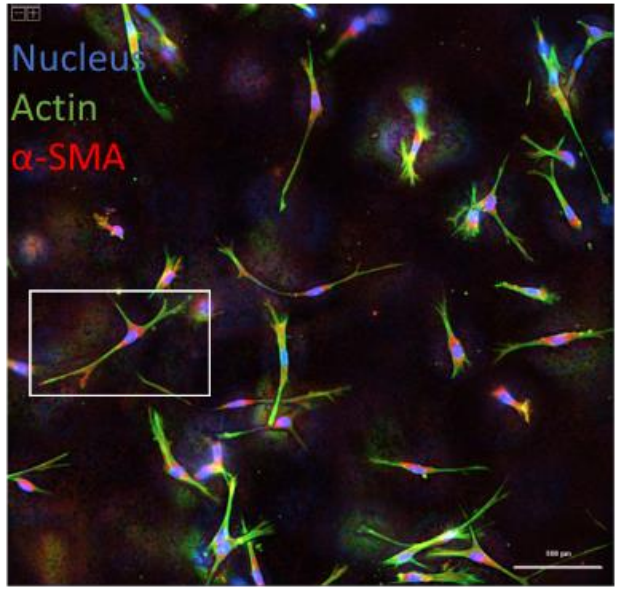
- Primary fibroblasts, isolated from biopsies of patients suffering from implant associated fibrosis, are cultured in a 3D hydrogel pattern, and characterized
- Cells were cultured in 3D hydrogels for 24 hours with or without TGF-β1 and with hydrogels stiffness (2.5mg/ml or 4mg/ml collagen)
- Antibody staining of αSMA was performed, along with additional staining of cell nuclei and actin filaments

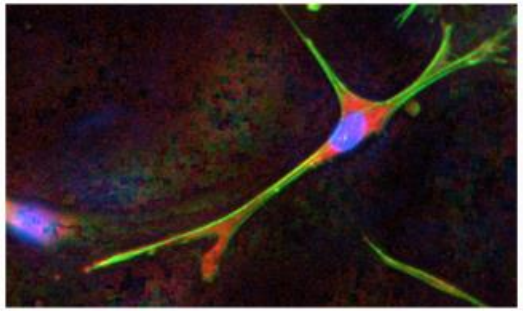


Quantification of the αSMA expression in patient derived cells cultured in a 3D environment

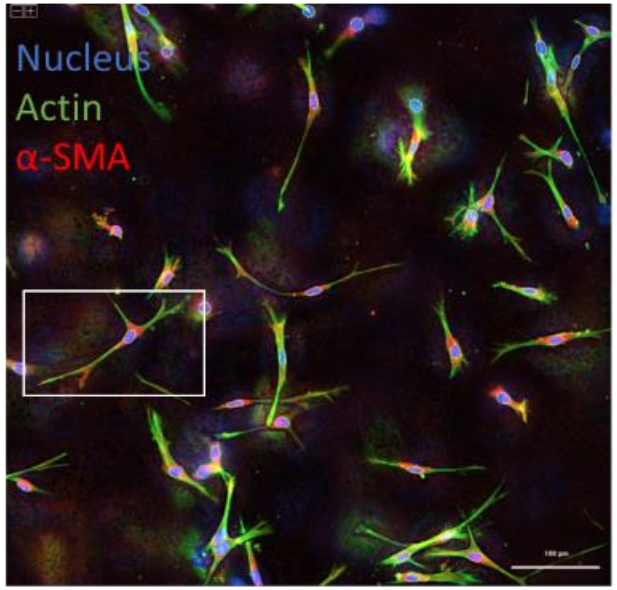
- Your task is to perform cell segmentation and quantify the levels of αSMA/cell in the different settings

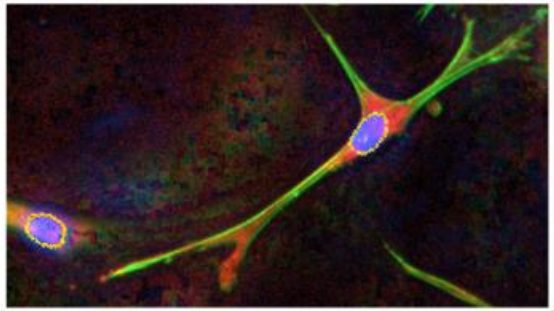
Unmasked



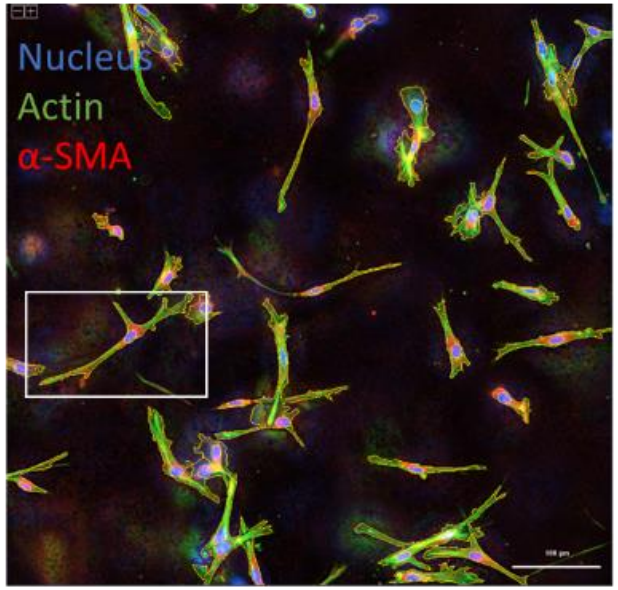


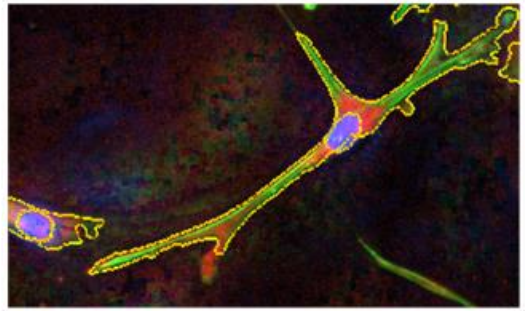
Primary mask





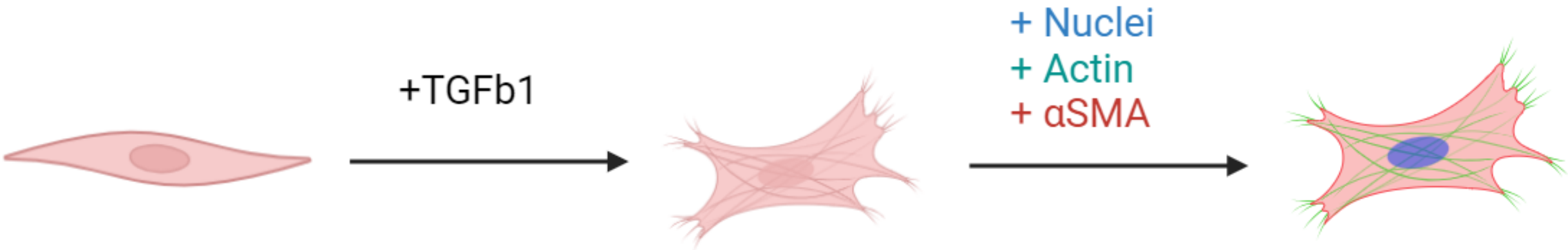
Secondary mask





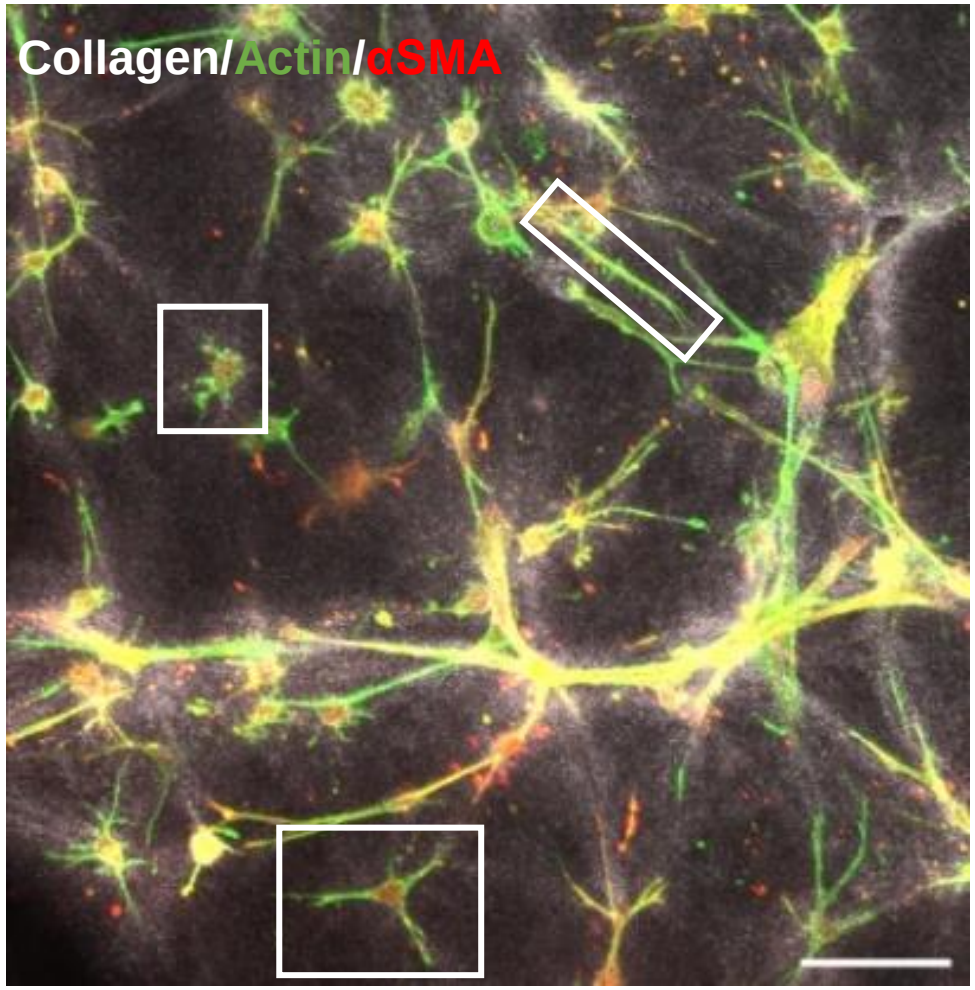
Differentiation of fibroblast to myofibroblast via cell morphology changes

- In addition to the expression of αSMA, fibroblast differentiation into myofibroblasts is associated with changes in cellular morphology/shape (which can include round, elongated, spindle-shaped, or irregular forms)
- While normal fibroblasts show an elongated, spindle-shaped morphology, myofibroblasts develop protrusions and have a star-shaped morphology
- Cells were treated/incubated and stained same as case 2
- The aim of the experiment is to evaluate changes in the morphology of different patient-derived cells at different conditions.



Differentiation of fibroblast to myofibroblast via cell morphology changes

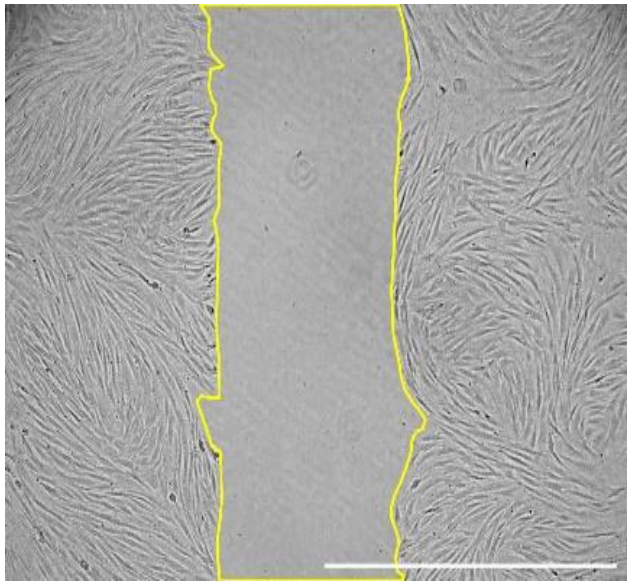
- Your task is to perform cell segmentation
- Morphological descriptors, such as shape factor ($4\pi A/P^2$, where A is the area of the object and P is the perimeter), aspect ratio (long axis length/short axis length), and eccentricity, will need to be assessed under the different treatment conditions
- We are interested in single cell analysis



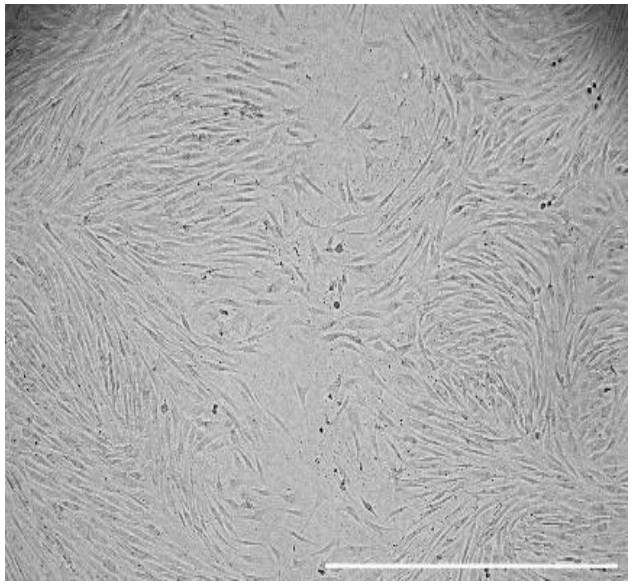
Effect of antifibrotic drug TOP N53 on the migratory properties of patient derived cells

- As mentioned in slide 6: Myofibroblasts have less migratory properties than fibroblast
- By inserting a scratch in the middle of a monolayer cell on can monitor over time the gap closure and evaluate the migratory properties of cells

Wound confluence 0%



Wound confluence 100%

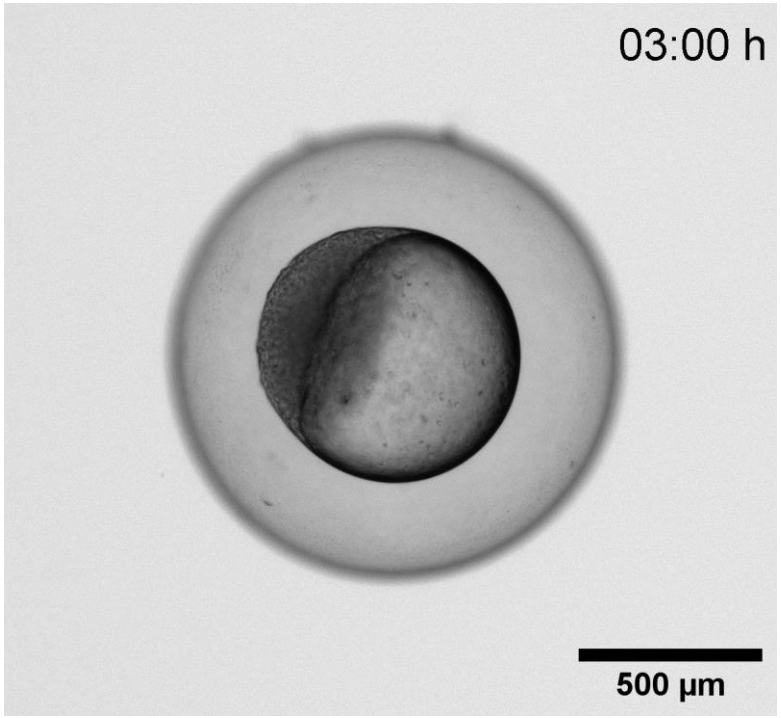


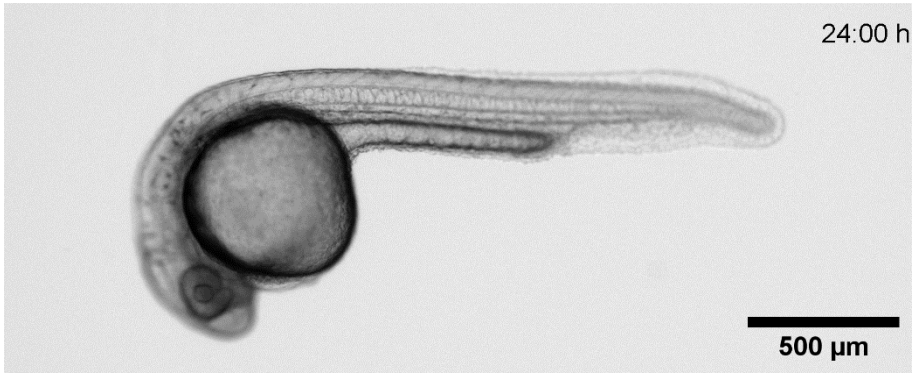
Effect of antifibrotic drug TOP N53 on the migratory properties of patient derived cells

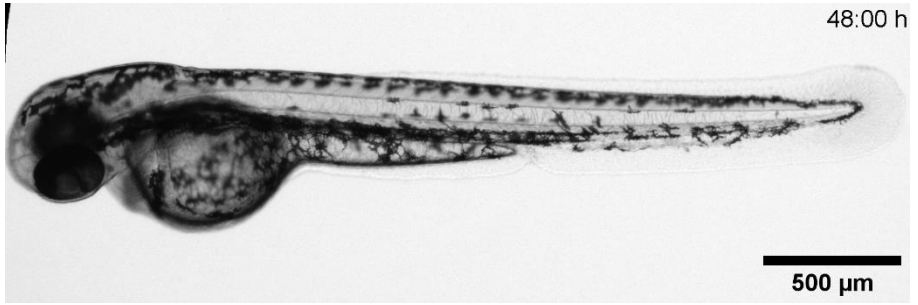
- In this experiment patient derived cells were seeded in a 2D culture and incubated with TGF-β1 for 24 hours
- After incubation, a scratch was mechanically inserted in the center of the cell layer using an automated scratch device and cells were incubated with antifibrotic drug TOP N53
- Bright-field images of the cells were captured over a 24-72-hour period
- The aim is to evaluate the effect of TOP N53 with/out the presence of TGF-β1

Zebrafish embryo model for the screening of antimicrobial drugs

- Zebrafish embryos are an excellent model for performing in vivo pharmacology experiments
- Transparent, cost effective and share many homologous genes with humans

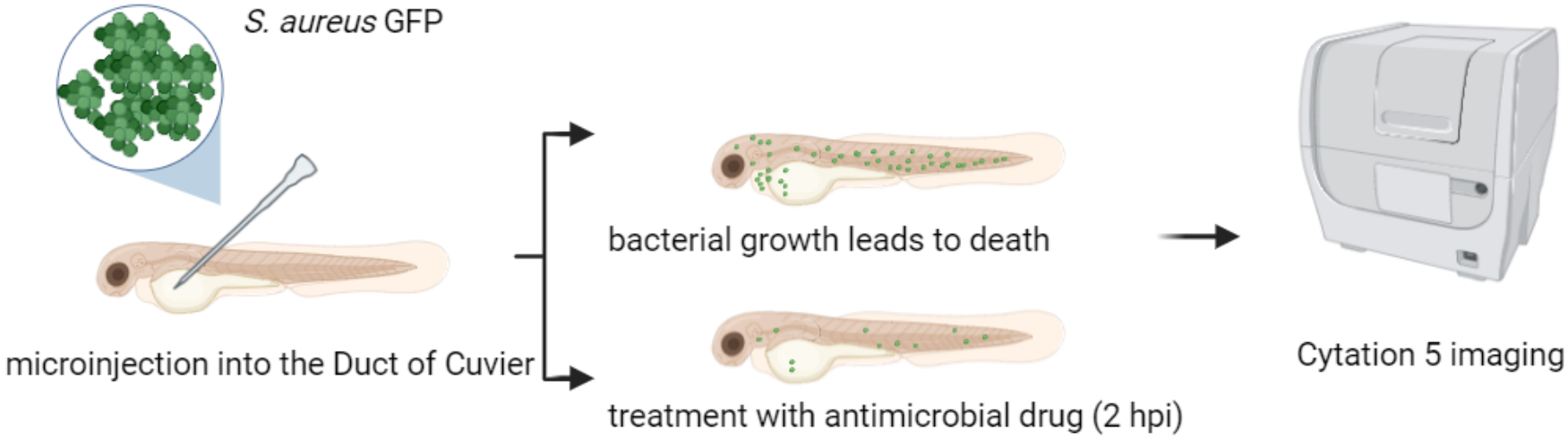




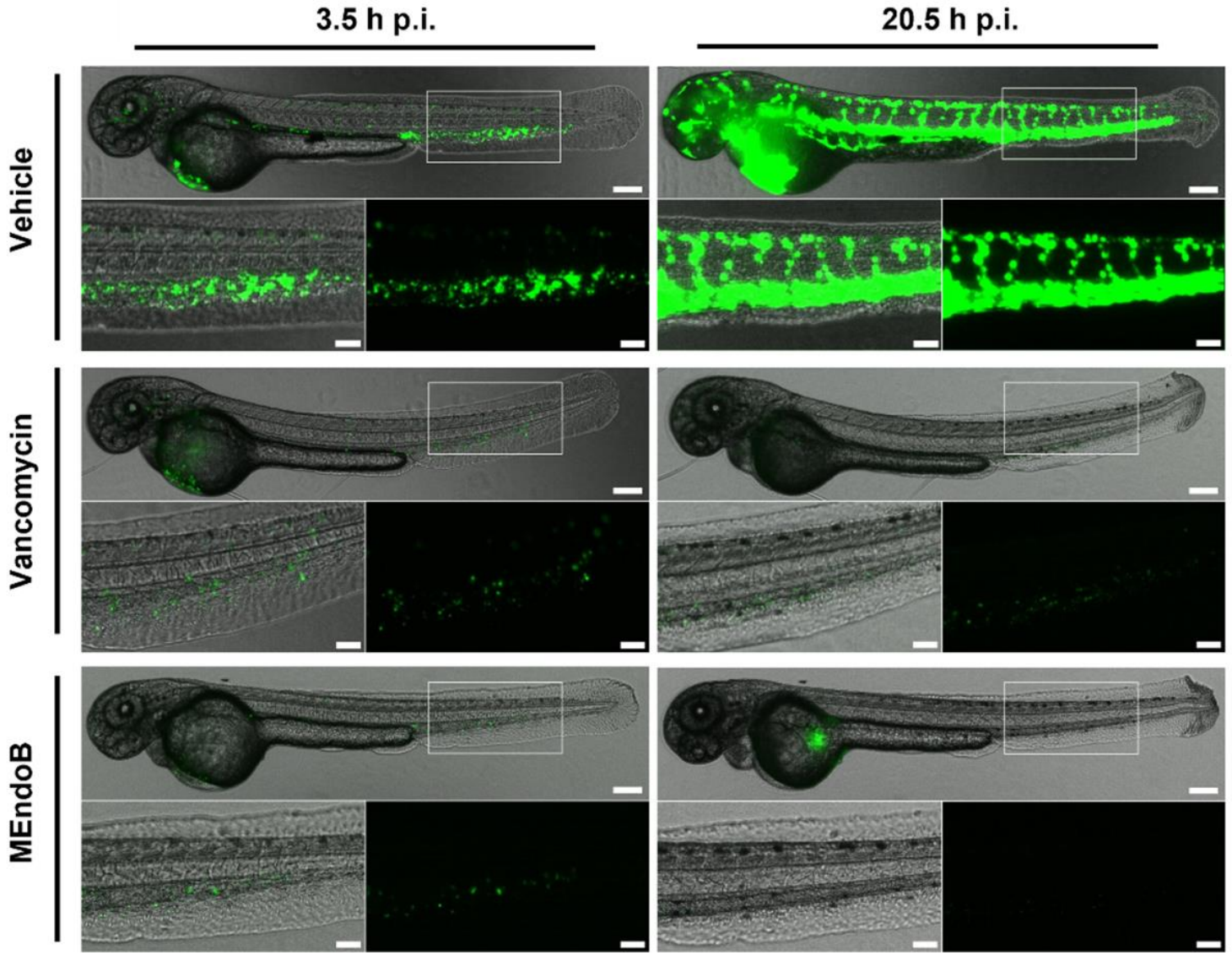


Zebrafish embryo model for the screening of antimicrobial drugs

- In this experiment we want to evaluate the effectiveness of antimicrobial drugs against Staphylococcus aureus using zebrafish embryos
- Zebrafish embryos (48 hours post-fertilization) were systemically injected with GFP-expressing *S. aureus*. Two hours after bacterial injection, the embryos were systemically injected with an antimicrobial drug (for example Vancomycin or MEndoB). The embryos were imaged over time
- Your task will be to quantify the GFP signal of the bacteria in the zebrafish embryo over time to assess if and to which extent the drug influences the bacteria growth



Zebrafish embryo model for the screening of antimicrobial drugs



How are the images organized

Questions?