Quality control of Bone Marrow derived cells using IncuCyte

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QC and assay optimization

- **Uncoated vs FN-coated plates (epithelial cells)**
- **Assay miniaturization**
    - From 6 to 96-well plates
    - Scratch wound
- **Cell labelling for Angiogenesis (CytoLight Rapid Green)**
- **Seeding density for all assays**
- **Starting point of scan:**
    - Proliferation: after media change
    - Angiogenesis: immediately after seeding cells on Matrix

MSC and ESC proliferation

Tested protocol:

Epithelial cells (from vaginal biopsy)

- Keratinocytes SFM media supplemented with rEGF and BPE + gentamycin
- 16K cells/well on 96 well plate

ESC (from bone marrow whole, centrifugation with auto stem cell kit or sedimentation on HES)

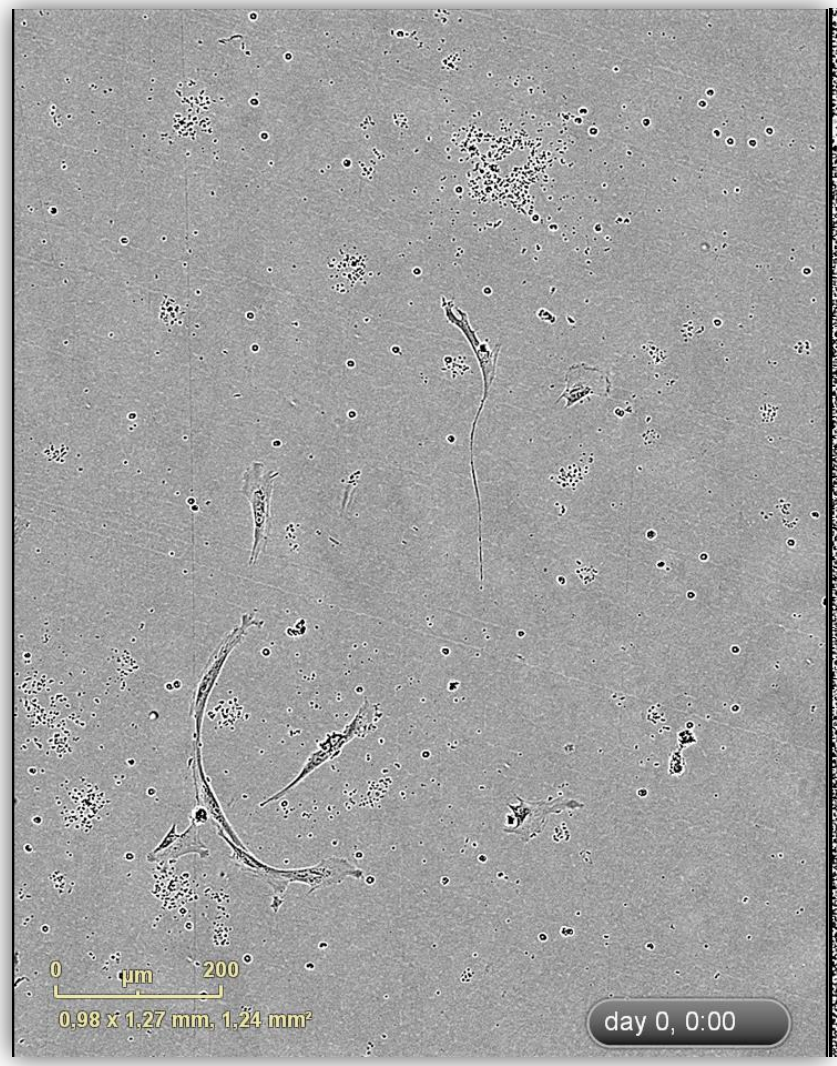
- Supplemented medium 200 with LSGS
- 15K cells/well in 96 well plate

MSC (from bone marrow whole, centrifugation with auto stem cell kit or sedimentation on HES)

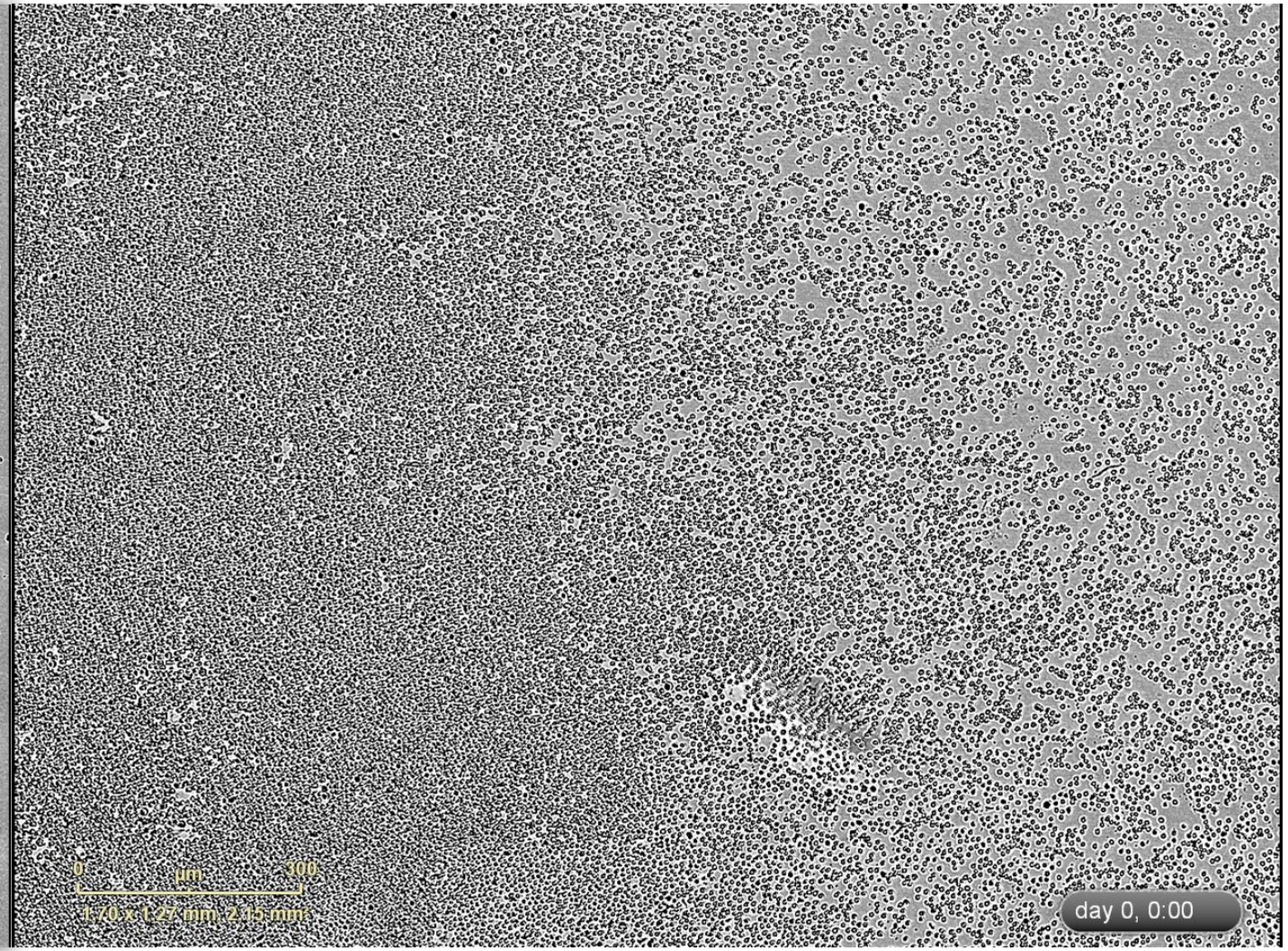
- MEMa with 10% human platelet lysate (HPL) plus Heparin (2 U/mL)
- 15K cells/well in 96 well plate

MSC proliferation

MSC in 6-well plate

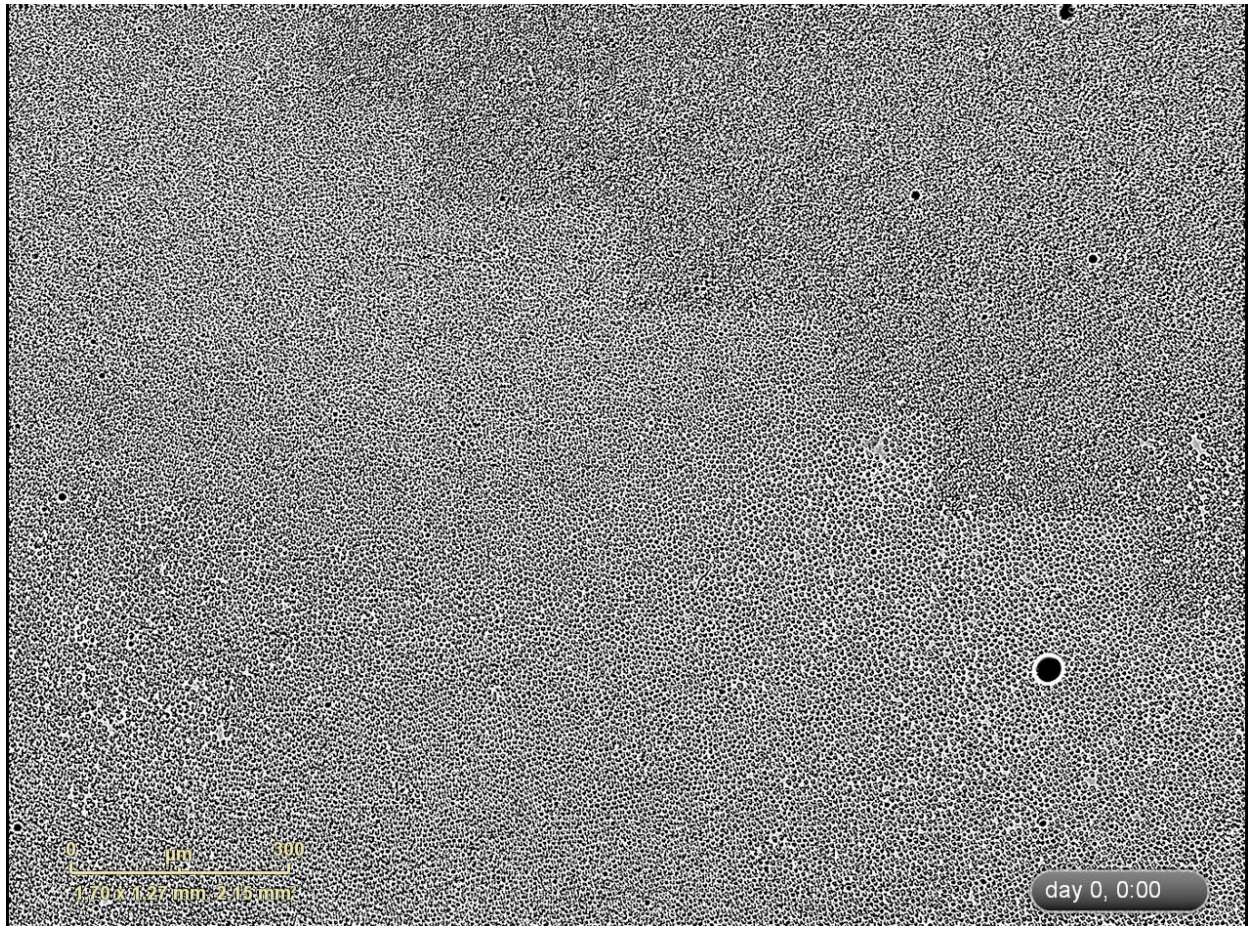


MSC in 96-well plate

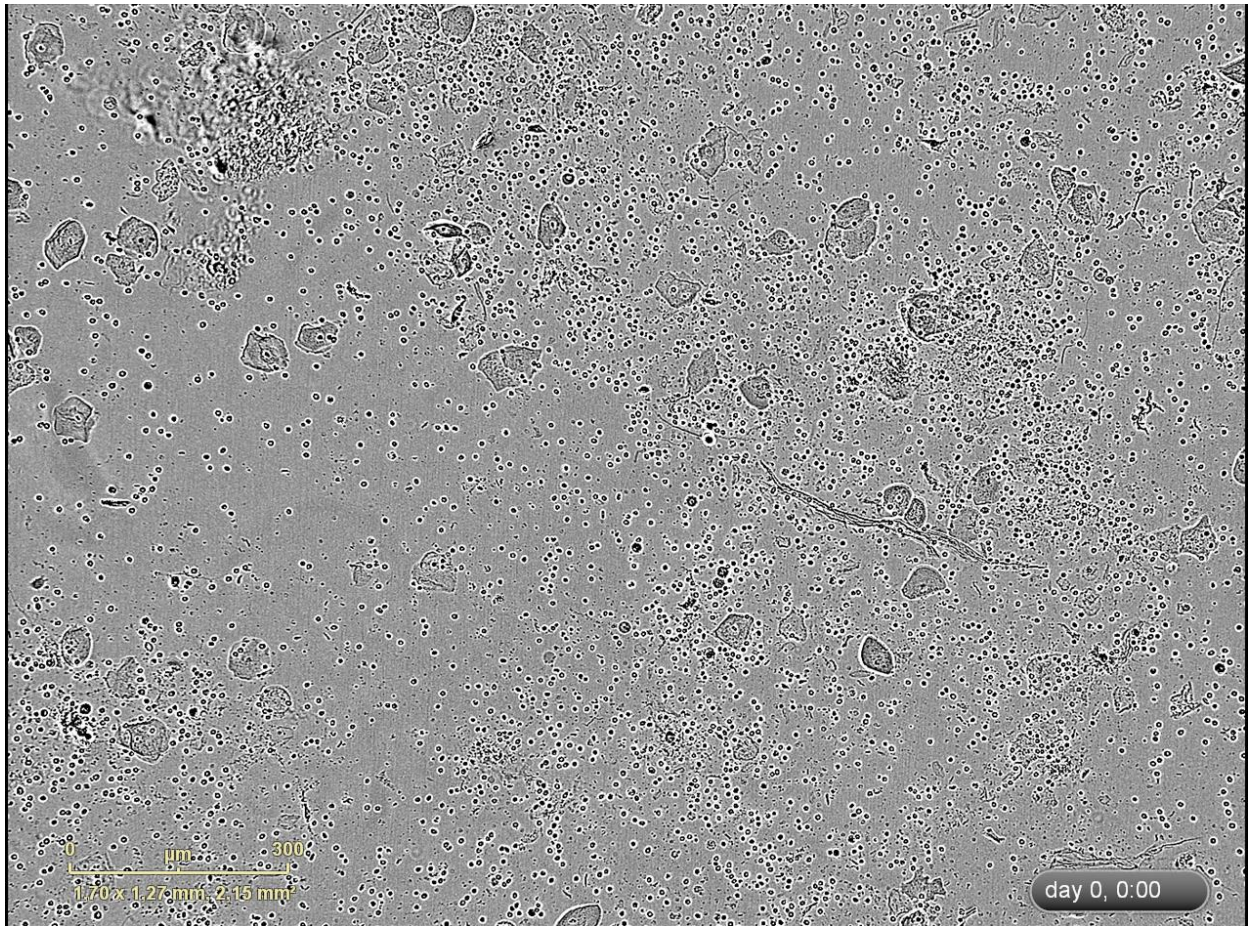


ESC and epithelial cells proliferation

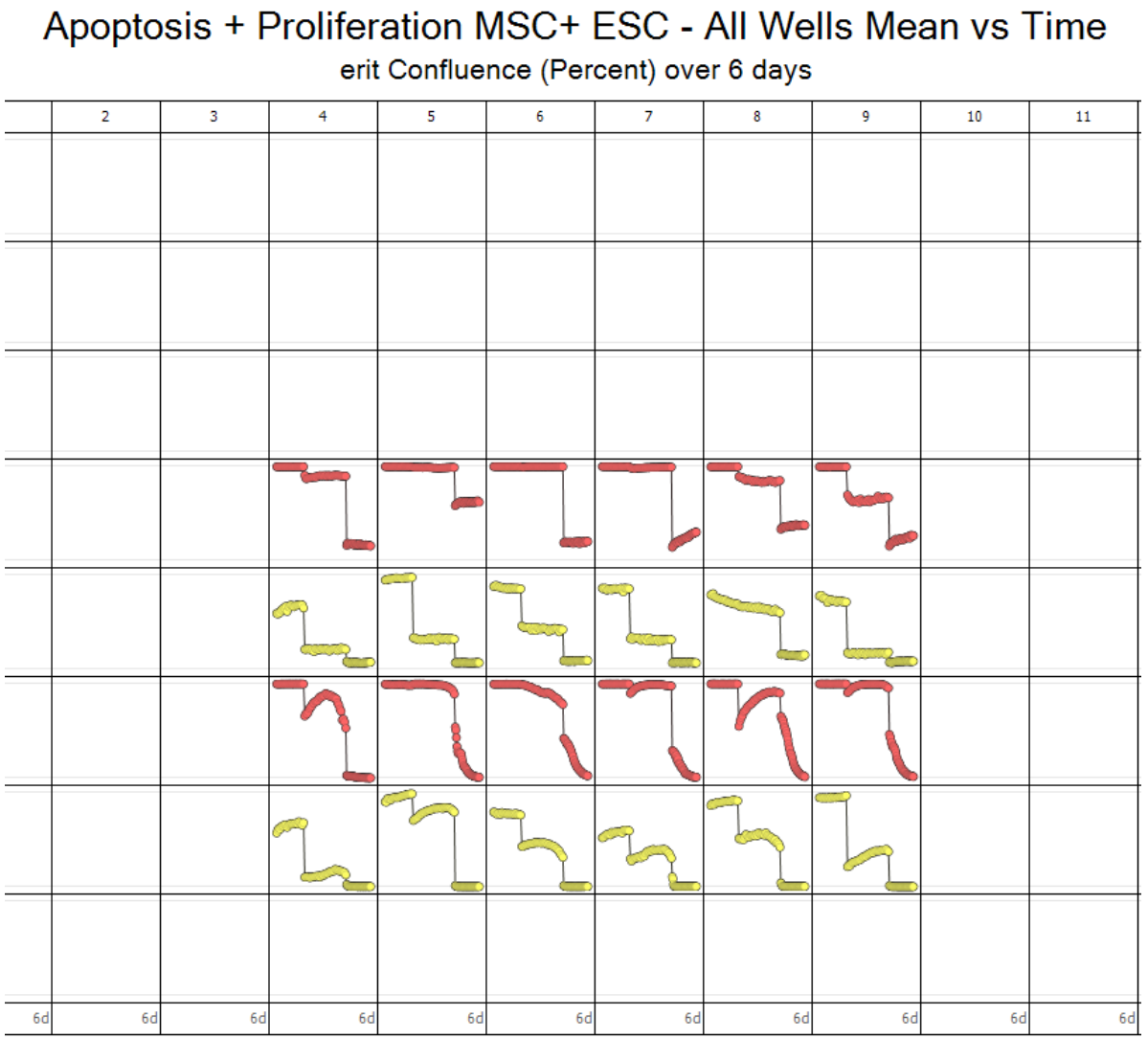
ESC in 96-well plate



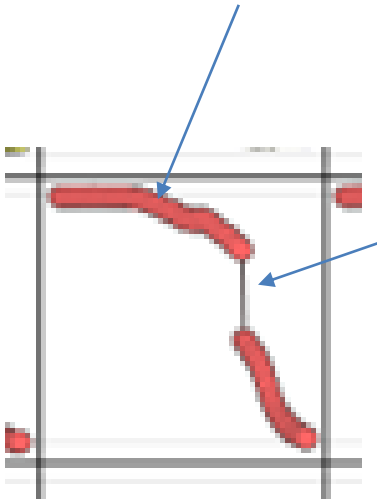
Epithelial cells in 96-well plate



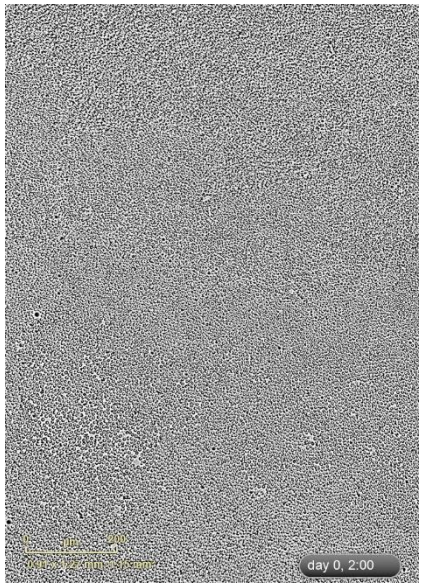
Proliferation estimated as inverted confluence

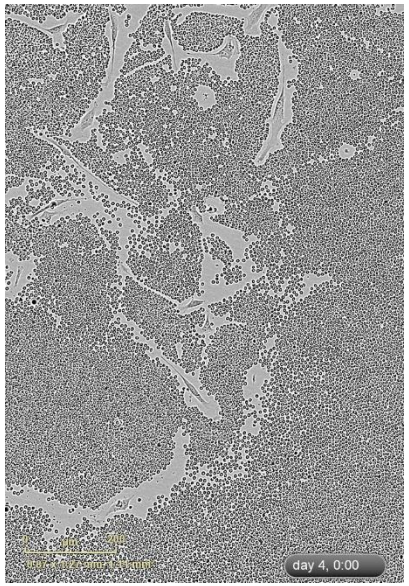


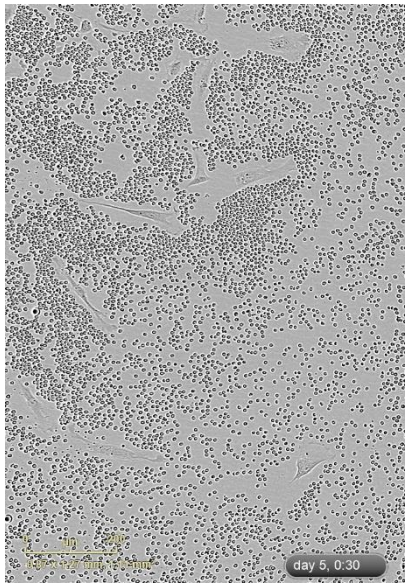
ESC appearing and proliferation



Media change







ESC cells

MSC and ESC proliferation

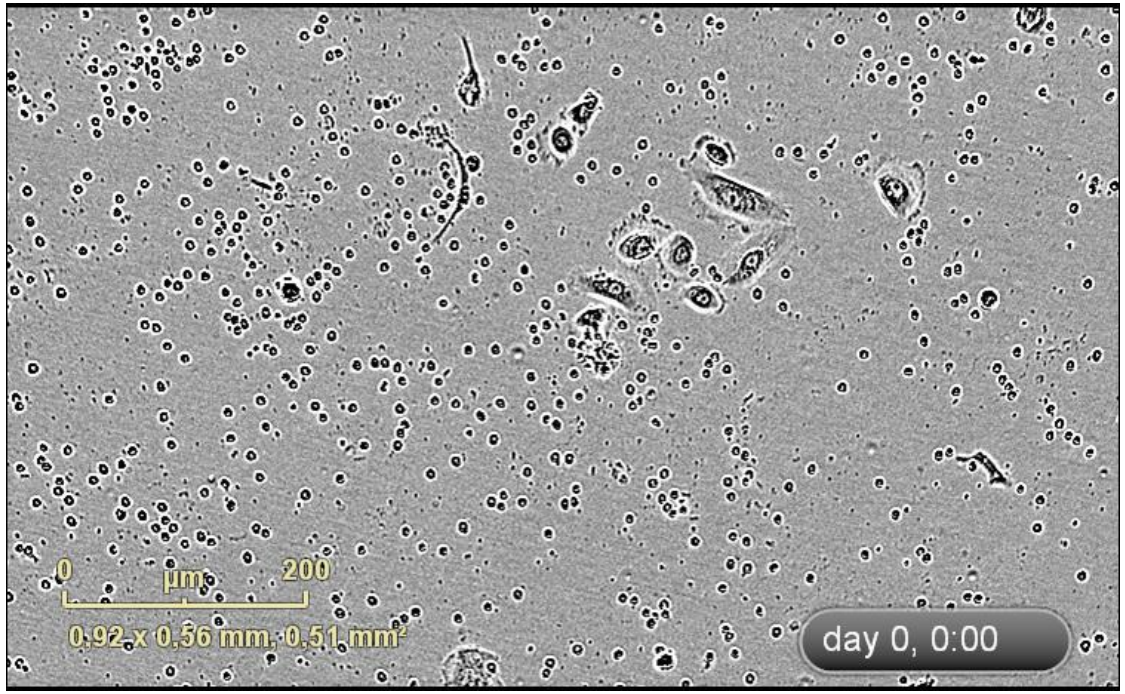
Suggested protocol for the future:

- Inverted confluence first (indirect estimation of proliferation)
- Wash cells after 3 days
- Add NucLight rapid red
- Monitor proliferation of cells (exact cell count)

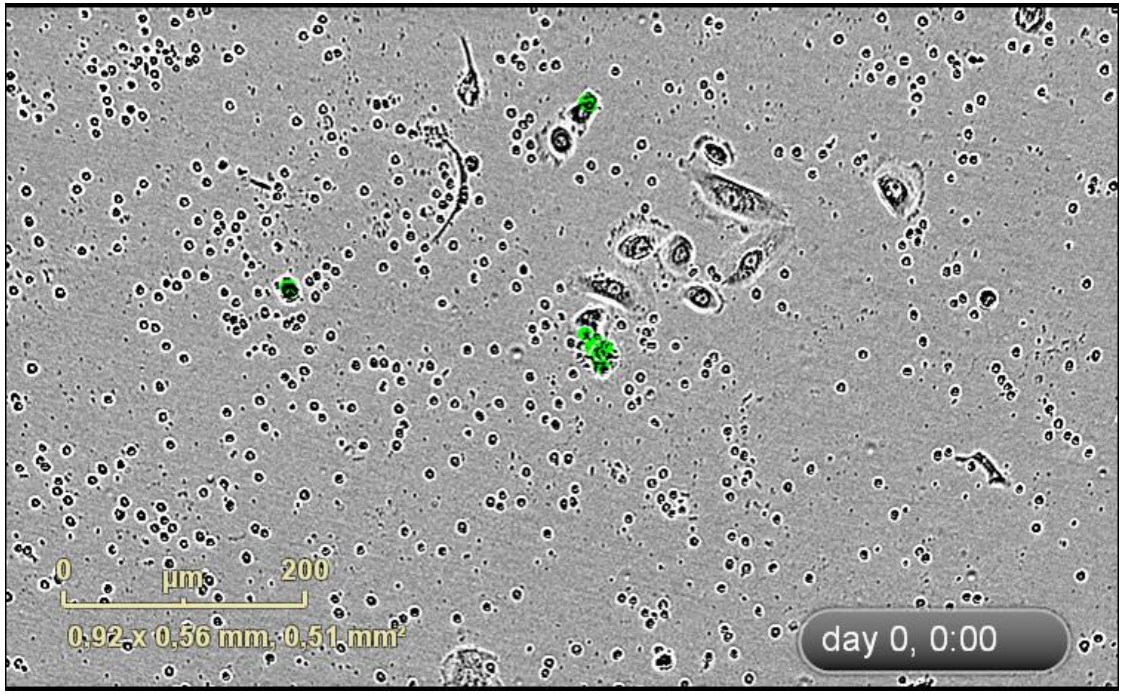
Apoptosis

Protocol

- Epithelial cells from 3 different patients
- Incubation with Caspase 3/7 substrate from time) or after media change
- IncuCyte Imaging and analysis for 6 days
- Apoptotic cells masked in Green



Phase-contrast only

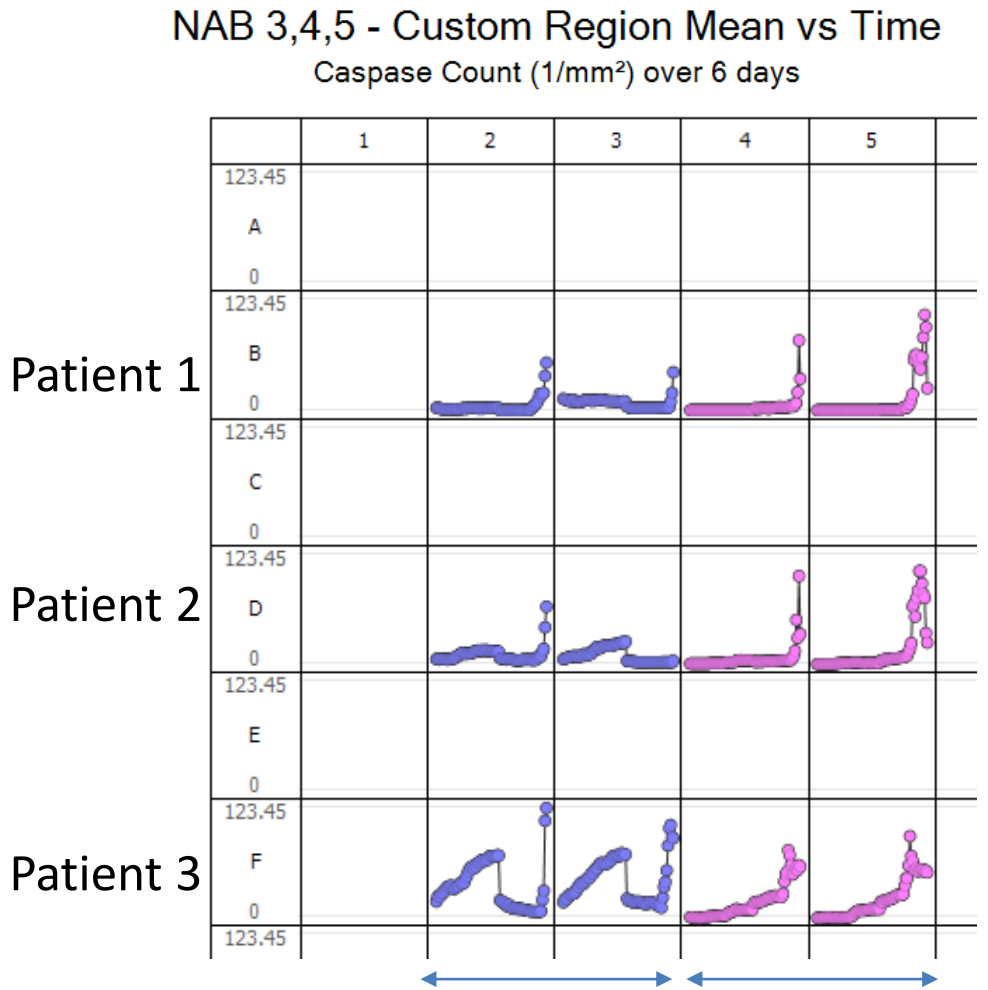


Phase-contrast + mask for apoptotic cells

Apoptosis

Epithelial cells

Different cell health in cells from different patients have different health status



Scratch wound

Optimization of cell seeding density for scratch wound experiments.

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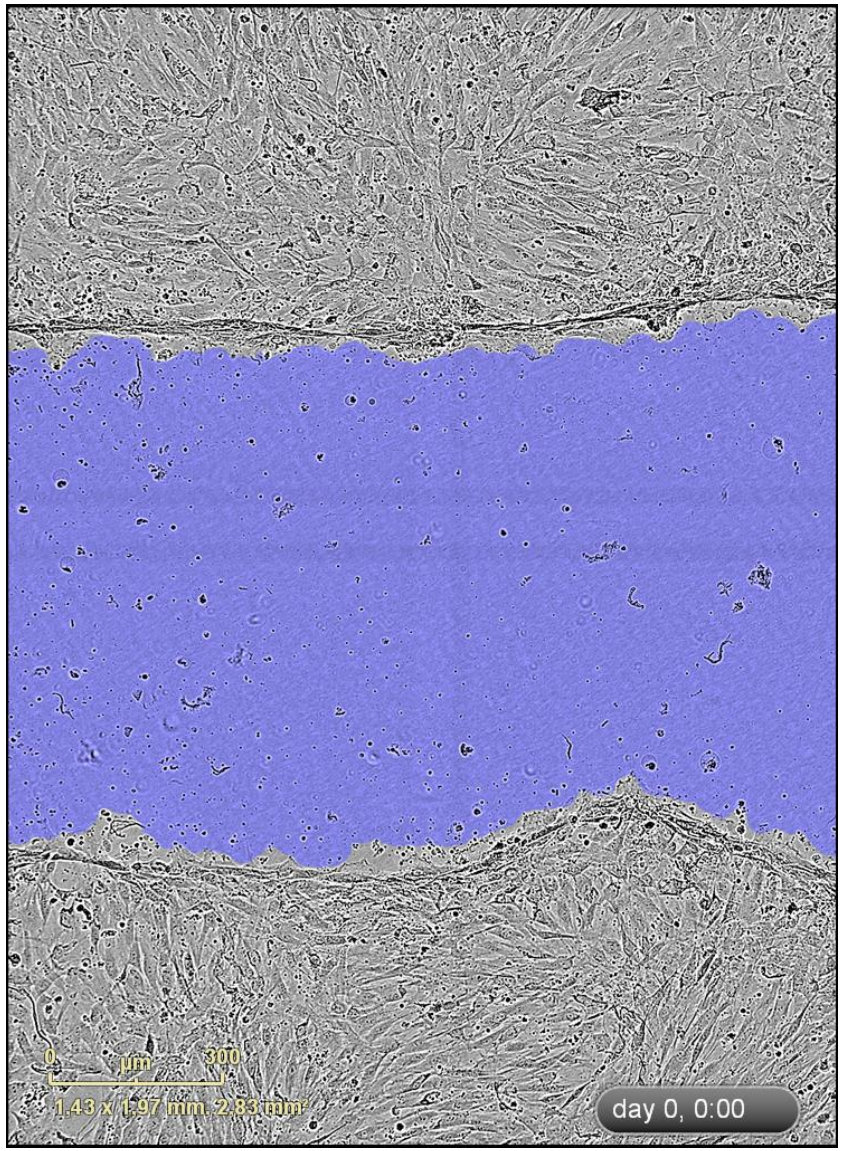
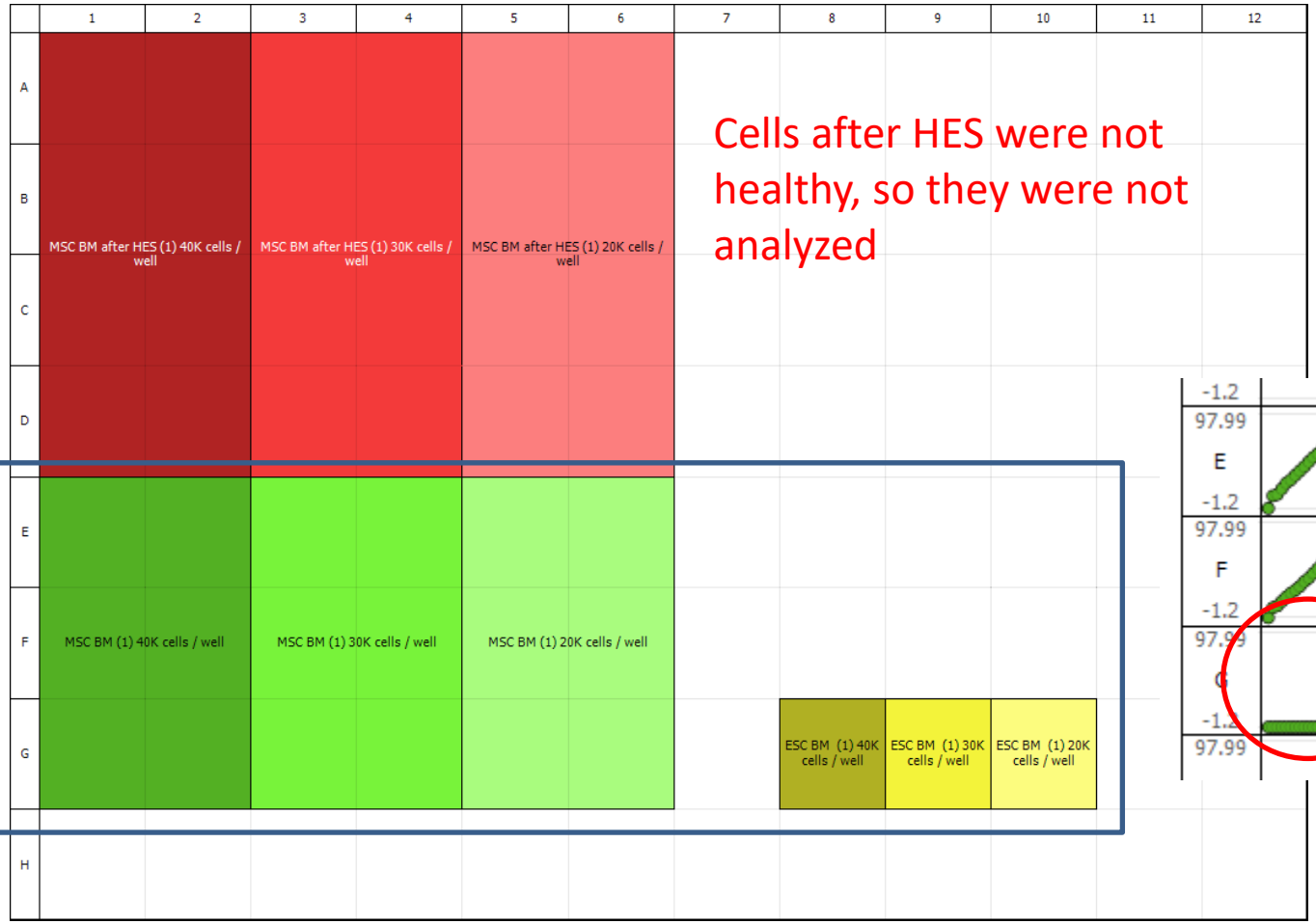
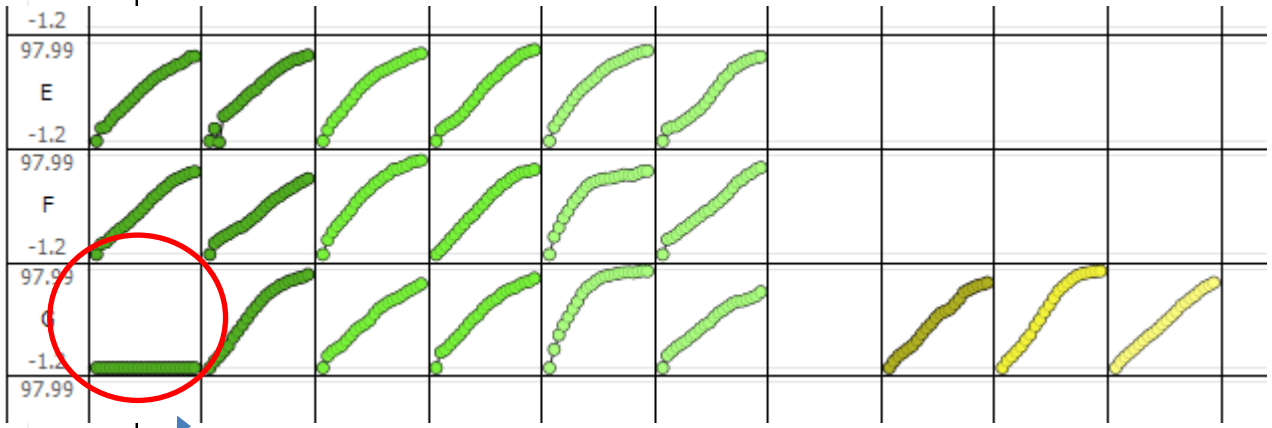


Scratch wound analysis



Cells after HES were not healthy, so they were not analyzed

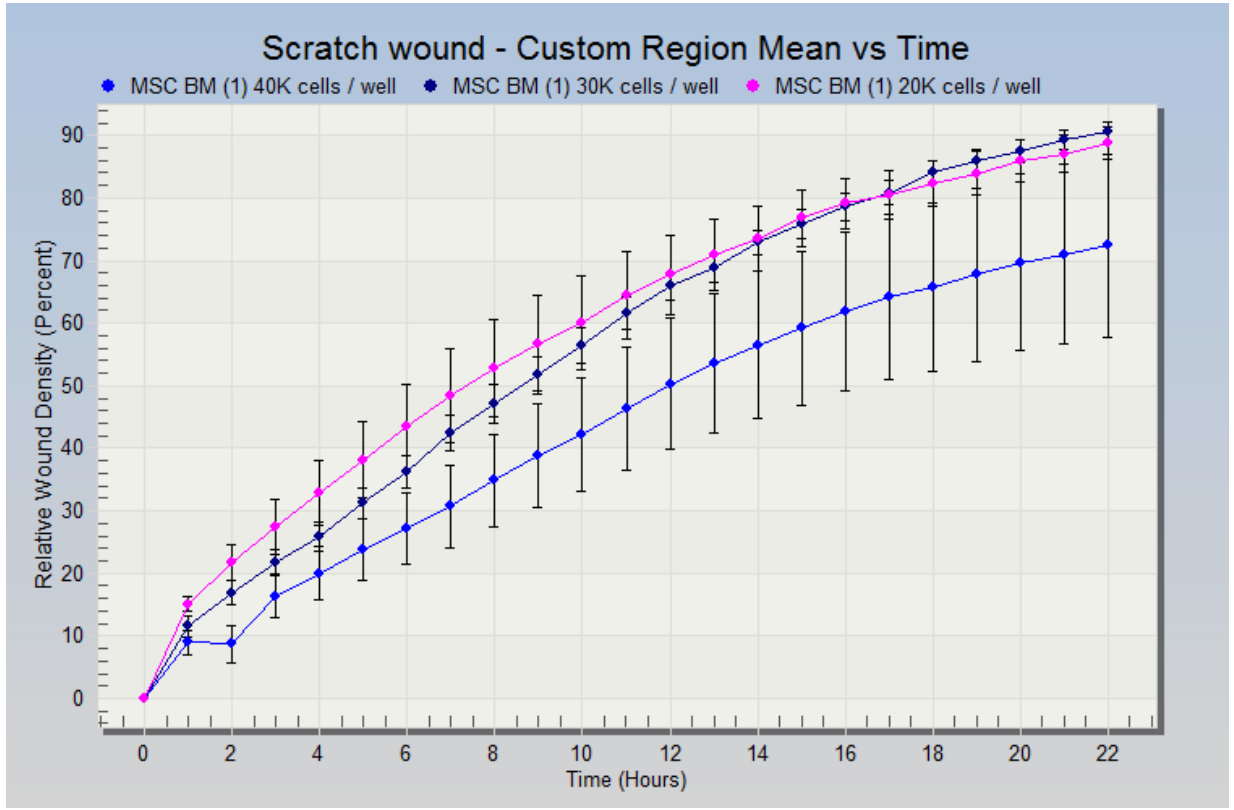
30K cells/well is the optimal seeding density for MSC
After 24 hours, all cells have closed the wound



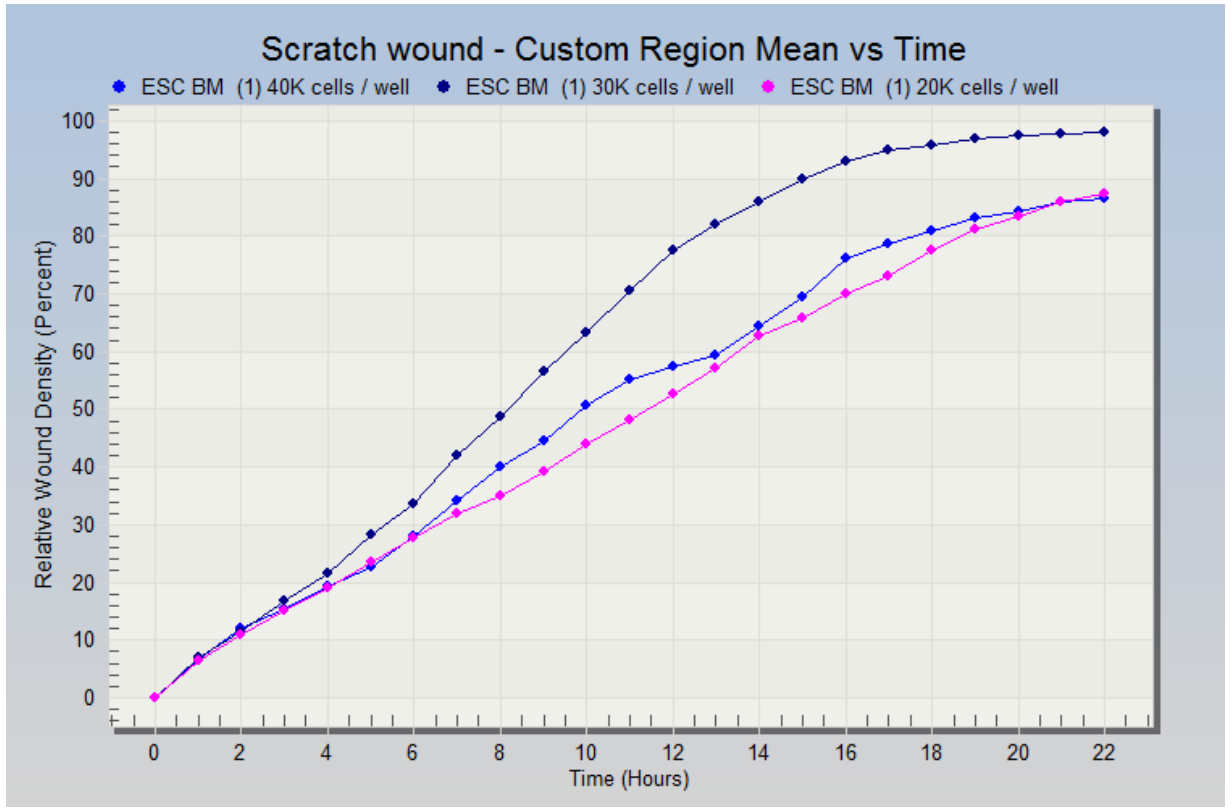
Easy to spot outliers! No wound in this well

Scratch wound

MSC



ESC



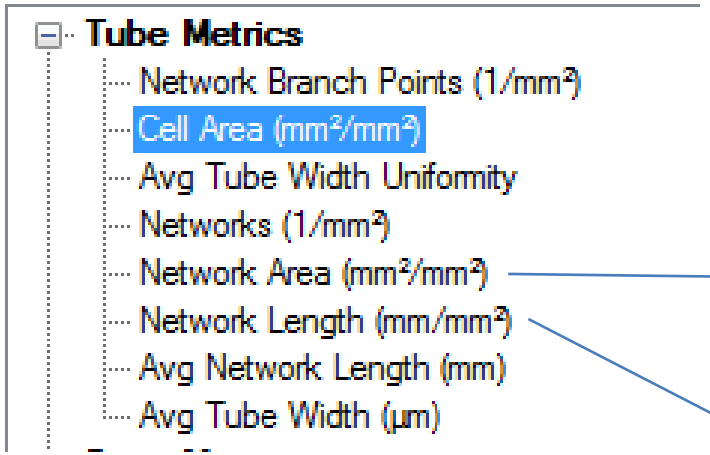
High reproducibility of results between replicates

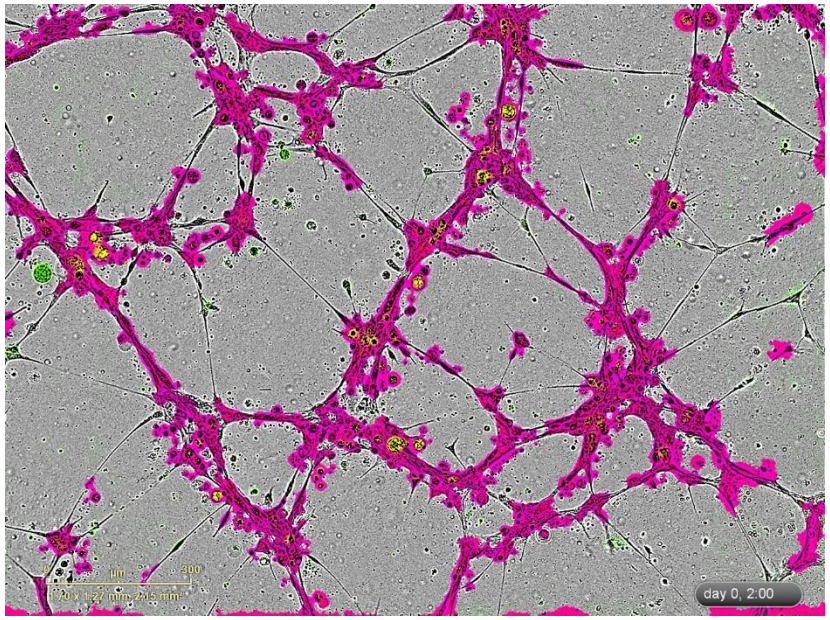
Angiogenesis

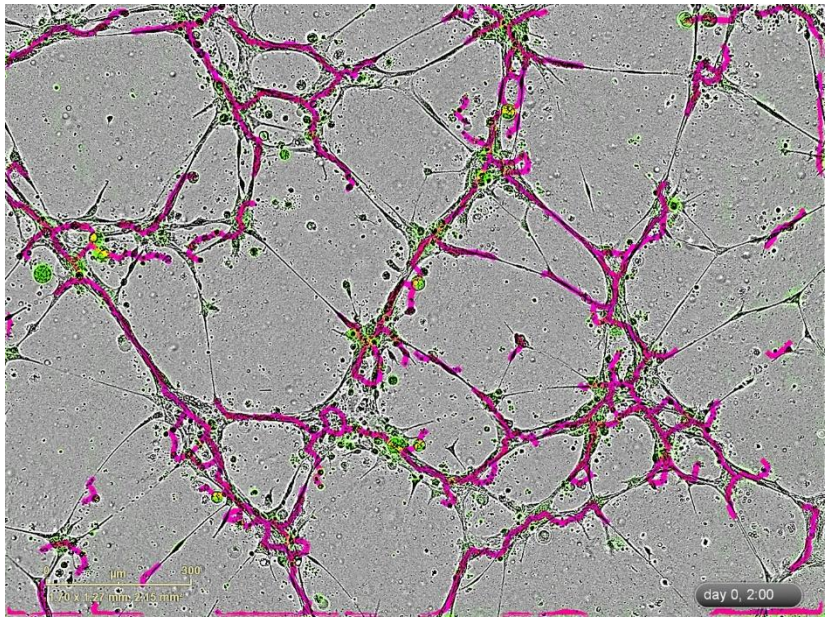
Protocol

- 96 well plate format, 40uL/well Matrigel
- Media from Angiogenesis starter kit (ThermoFisher)
- 15K cells/well after labelling with CytoLight rapid Green
- Imaging with 10X objective (imaging started 3 hours after seeding cells)

Angiogenesis analysis

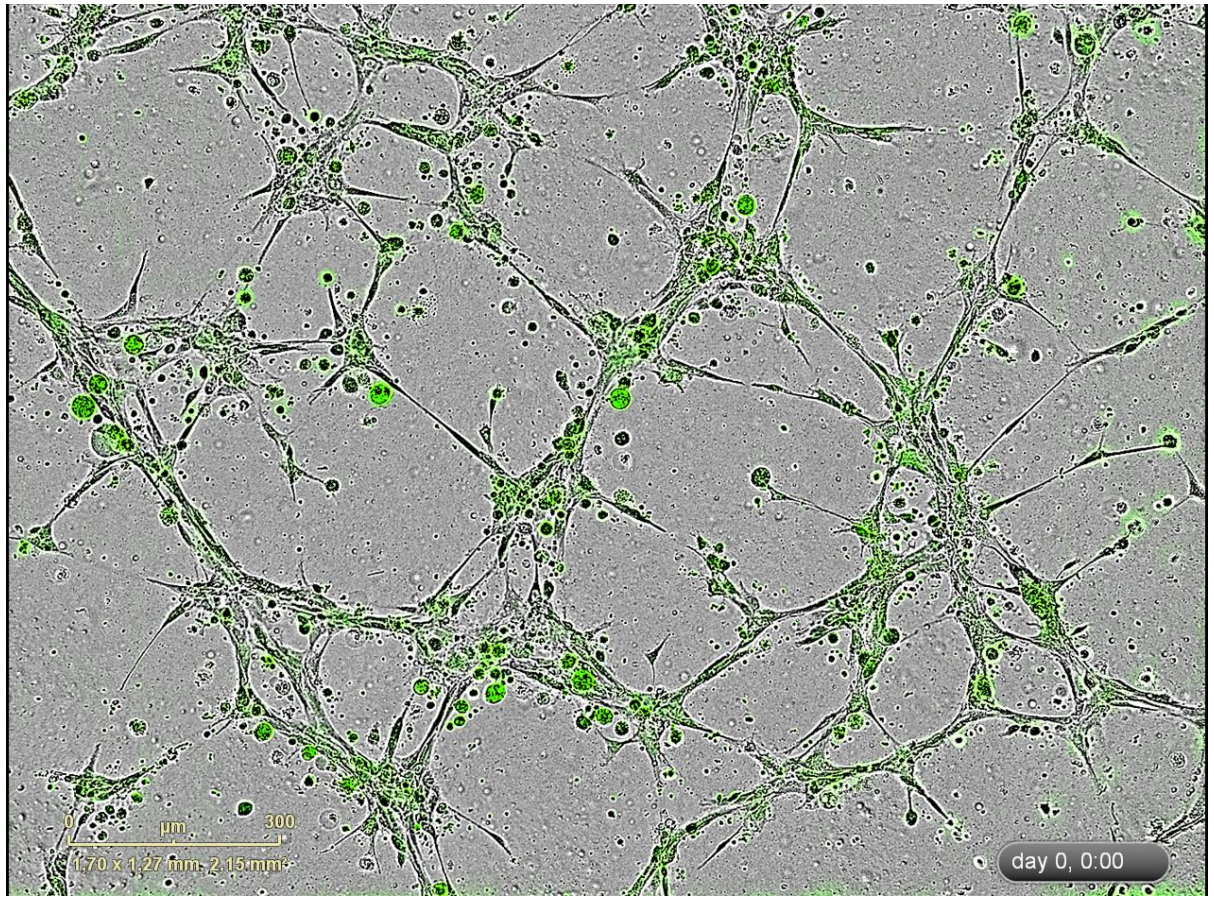




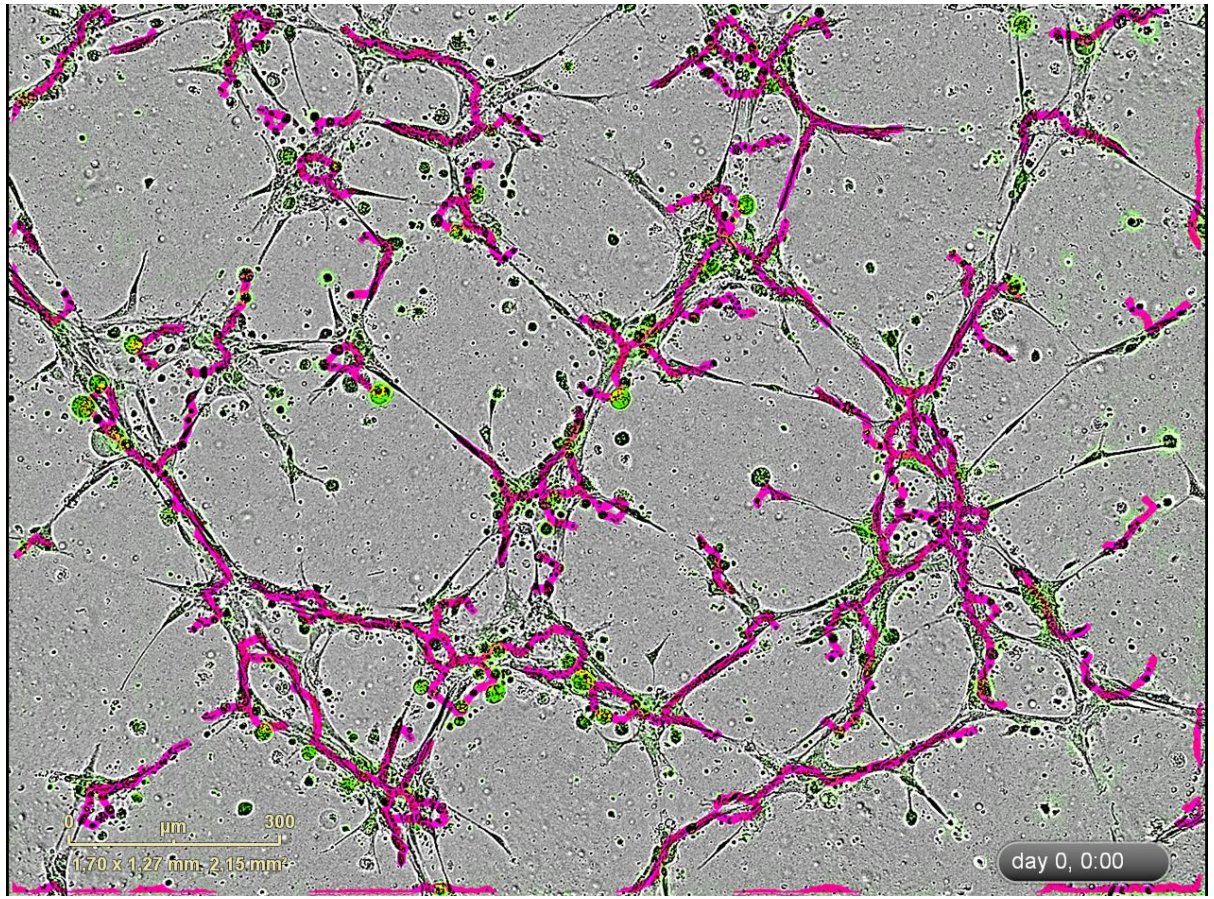


IncuCyte produces a variety of metrics to analyze network formation

Angiogenesis



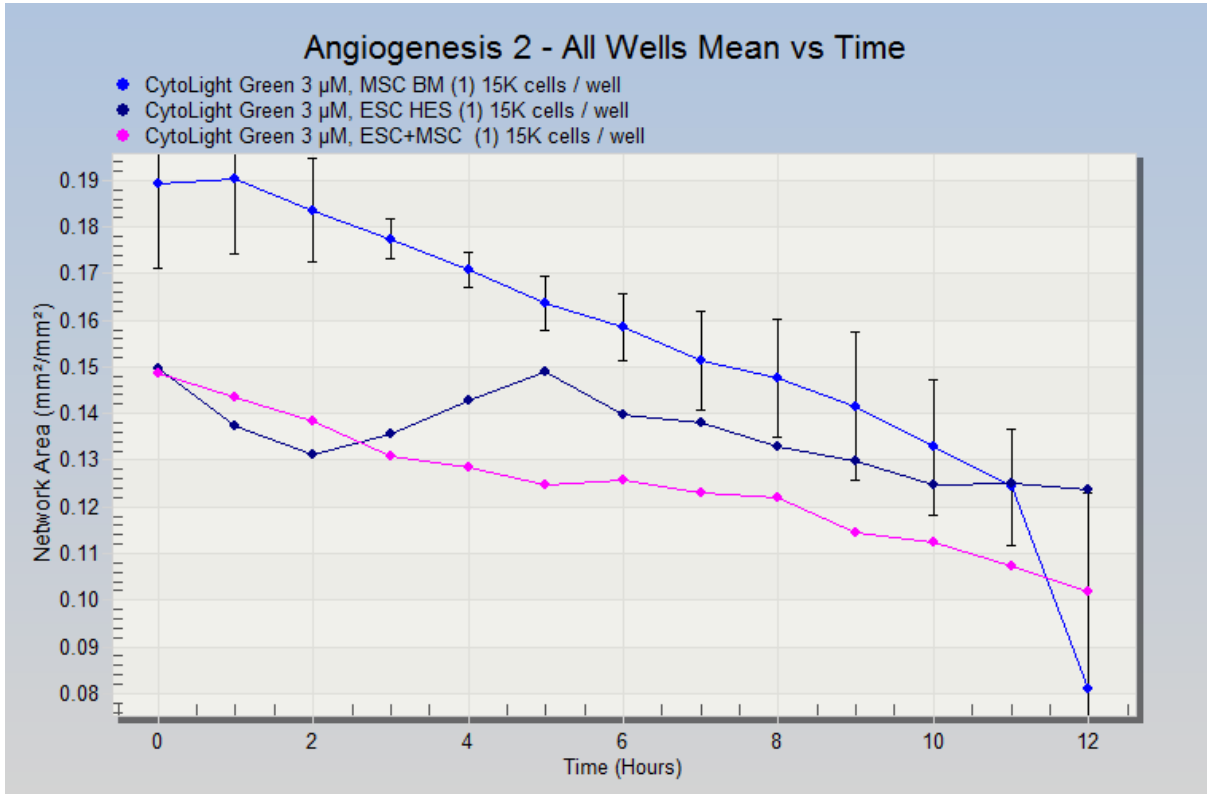
Original images



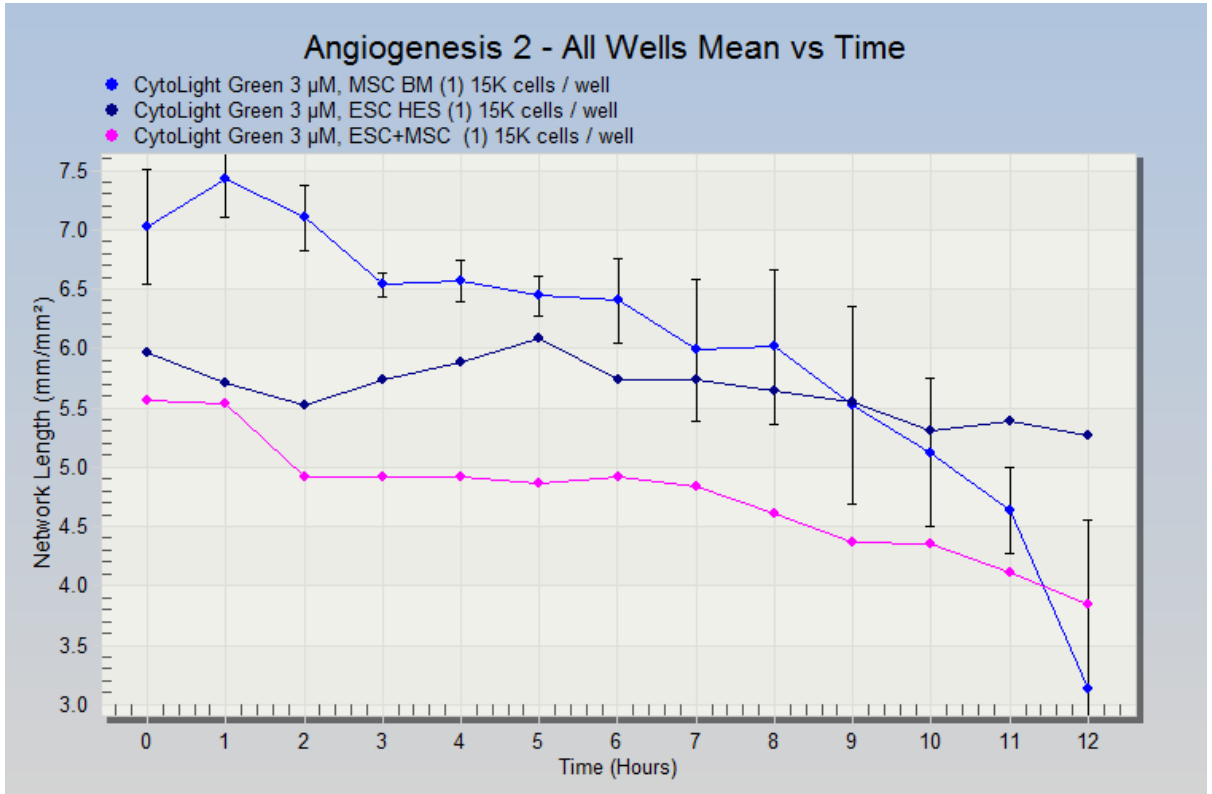
Angiogenesis network mask

Angiogenesis measurement

Network area



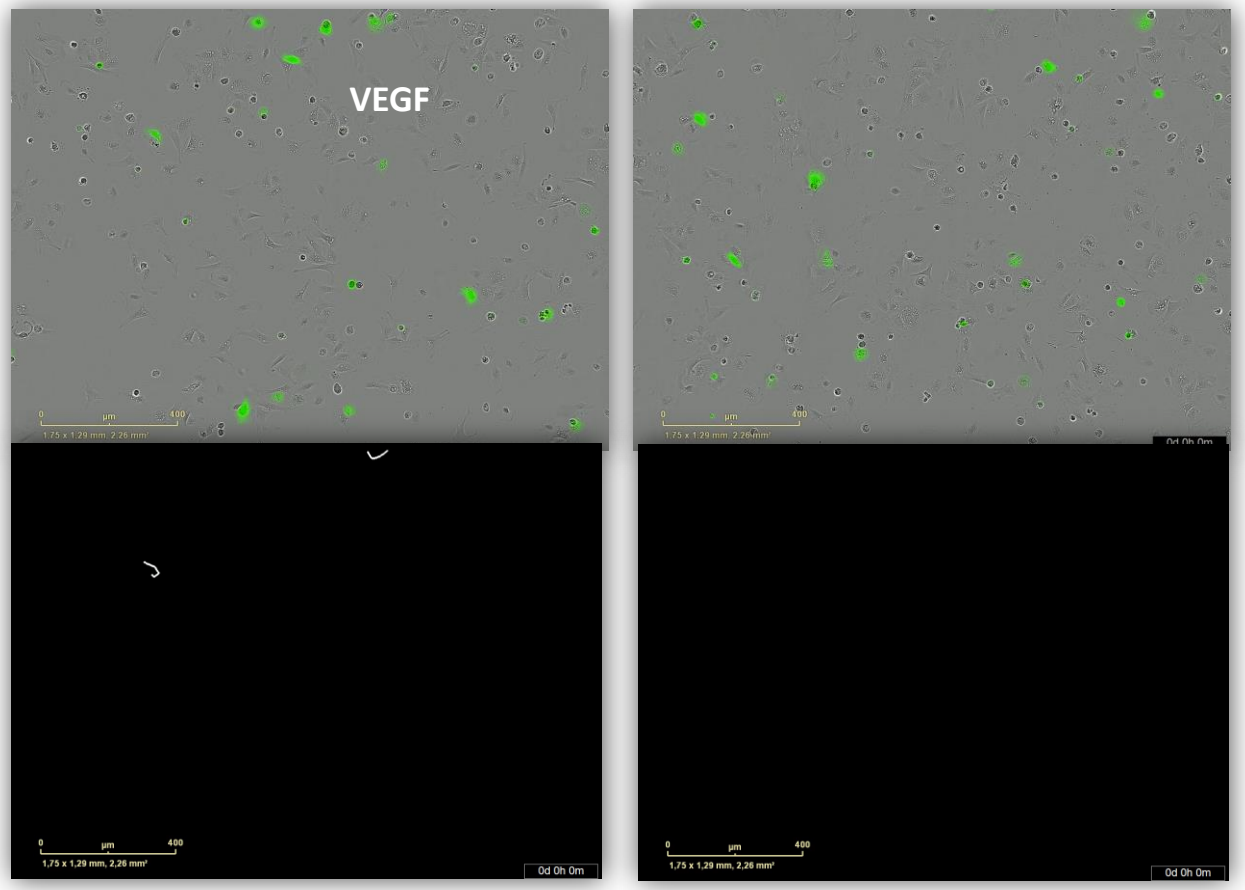
Network length



The network was already well established when the experiment started.
In the future, imaging should be started earlier and the experiment can be finished after 10-12 hours

Example of Essen kit with fibroblasts & HUVEC co-culture

ESSEN Bioscience medium



Click **here** to play movies

Application Summary

Experiment	Current method	IncuCyte ZOOM Assay Description
Quality control and experiment optimization	Not tried before	Optimization of seeding conditions, cell labelling, media, assay format and protocol (when to start scanning)
Cell proliferation	Manual microscope	Live imaging that allows to monitor cell growth and morphology over several days without cell perturbation
Apoptosis	Flow cytometry at end point	Live imaging -> kinetic studies. Count of apoptotic cells and simultaneous analysis of cell growth and morphology
Scratch wound	Not tried before	Automated scratch in 96-well plate format. Live analysis allowing to compare different samples on the same plate
Angiogenesis	Not tried before	Optimization of imaging on Matrigel. Tube formation can be measured with different parameters. Need to optimize beginning of imaging after seeding

Summary of demo data

16 experiments, lasting from 3 to 10 days

250GB of images acquired and analyzed automatically without the need for:

- Removal of cells from incubator
- Data export
- Separate software

Further reading

http://www.essenbioscience.com/en/

http://www.essenbioscience.com/en/resources/publications/