

2015-10-29

PROTOCOL TL1 Wound Healing 20140121.docx

## Wound healing @ TL1 with Axiovision

- Use plastic or glass bottom 12 well dish. No more than 8 wells per experiment, as setup is extended (>1h) and the experiment is progressing before sampling starts.
- Use special wound healing inserts, for example Ibidi (NBT).

Cat. No.	Description	Pcs./Box
80206	Culture-Insert in $\mu$ -Dish 35 mm, low, ibiTreat: ready to use, tissue culture treated, sterilized	30
81176	Culture-Insert in $\mu$ -Dish 35 mm, high, ibiTreat: ready to use, tissue culture treated, sterilized	30
80241	Culture-Insert 24, ibiTreat: a $\mu$ -Plate 24 Well with 24 ready to use Culture-Inserts, tissue culture treated, sterilized	3
80209	25 Culture-Inserts for self-insertion: in a 10 cm transport dish, sterilized	25

- Workspace  $\rightarrow$  microscope  $\rightarrow$  2.8V-3V.
- Workspace  $\rightarrow$  camera  $\rightarrow$  check full frame, no binning, high quality.
- Tools  $\rightarrow$  options  $\rightarrow$  acquisition  $\rightarrow$  auto save.
- Tools  $\rightarrow$  options  $\rightarrow$  display  $\rightarrow$  constant update.
- X10 objective, if removal of insert is perfect. You should see the two fronts simultaneously, otherwise use X5.
- Set experiment parameters: image name, channels, time interval 10 minutes, duration, dish calibration.
- Remove dish from system and aseptically (in laminar flow hood) remove the insert and add medium/required factors.
- Return dish, set up Kohler illumination.
- Uncheck "include focus in move".
- Set 5-7 frames from beginning to end of wound. If X5  $\approx$  3 frames will suffice.
- Check "include focus in move".
- Set exposure time.
- Set "autofocus from current position".
- START
- If autofocus is not successful use "current focus position".
- Monitor after one or two hours.
- Leave overnight on "autofocus from current position".