

LV200 Installation:

- 1) Place the system in a dark room if possible or avoid direct strong light!
- 2) Install system table.
- 3) Place LV200 onto table right above the prepared whole.
- 4) Don't place the PC, controllers onto the table (vibrations)
- 5) Connect transmitted light source
- 6) If you want to use filter wheels in cellM connect UCB with PC
- 7) The filter wheels are marked at the cable with FW1 and FW2. Connect them in the vice versa order into UCB (FW1 in 2. and FW2 in 1.) !
Choose "IX/51/71" in OBS config
Choose "Contrast inserts" for upper filter wheel including blockers
Choose "Filter Cubes" for lower filter wheel (empty in standard configuration)
- 8) Connect cables for temperature control (connection cable at the back of LV200)
- 9) Screw in the tube lens carefully from the bottom
- 10) Camera installation:
 - a) Fix the c-mount adaptor onto the camera
 - b) Remove the small black metal part at the front to get access to the mounting screw.
 - c) Use the long hex wrenge 3 (small whole at the front) to fix the camera. **Caution the camera may fall down !!**
- 11) The camera is used **without a trigger** cable to avoid heating of the chip
- 12) To change the objectives you have to loose the screws for the incubation chamber.
During a demo please leave this screws loose, to have the possibility of changing the objective
- 13) You can only use 35 mm Petri dishes (and with some limitations also slides). Best glass bottom Petri dishes

Important points for Software: only for cellM (until now not possible with Andor iKonM)

- 1) The integration times will be much higher as in fluorecence may be 100 000 ms or even 1 000 000 ms.
- 2) The 100 s limit of integration time is solved from version 3.1
- 3) In the standard configuration there are light blockers in every second position of the filter wheel. In bioluminescence mode use them to prevent light passing the light guide. Turn off light source during long experiments. Lowest energy level and almost closed shutter at the light source gives enough light.
- 4) Bright field. Change light blockers by hand switch or PC after you have decreased the integration time lower than 50 ms.
- 5) Acquisition using experiment manager:

don't use trigger cable. The chip will get too warm.

only use "simple acquisition" instead of image type button

use "non synchronous" mode

ad a time loop around the acquisition (is needed for nonsynchronous mode)

change the gain in live mode to the needed value.

Experiments:

- 1) The customer has to know the difference between fluorescence and luminescence. Signals are much weaker (factor 1000 to 1 000 000 !!!)
- 2) **Highest intensity with low magnifications!**
- 3) Due to 0.2 tube lens magnifications of a 20x lens means 4x in reality!
- 4) Use always glass bottom dishes for higher magnifications.
- 5) The sample has to be completely stable. During the long integration times movements or vibrations will lead to a complete loss of the signal.
- 6) Please tell customer not to open light source or changing filter wheel (blocker) during long exposure.
This can damage the camera. Keep the intensity of light source at the minimal position.
- 7) Some luminescent sources need oxygen. They will not work under a cover slip!
- 8) Focus: **The focus of transmission and luminescence is not the same!**
- 9) Focus with transmission (change position of upper filter wheel using hand switch until you see the light.
- 10) Reduce light to a minimum (lowest aperture and energy) !!
- 11) The luminescence focus is just between the transmission focus “black granules in cells” to “glass like appearance”
- 12) In most cases you will not see live luminescence!
- 13) For luminescence switch upper filter position to the next position which includes blocker or use
PC control (condenser)

The expectations of the customer will be sometimes unrealistic. They will compare it with fluorescence and confocal without thinking. Always ask them if they ever have seen a luminescence image!