PerkinElmer Operetta Tutorial
1) Switch on the Operetta CLS. The instrument is initialized (status light blinking green). As soon as the status light stops blinking (constantly green), the Operetta CLS is ready and can be used.

2) Switch on the Harmony PC and log in.

3) Check the available space in the Hard Disc Drive C.

4) Double-click the Harmony icon on the desktop. Harmony is started and a login dialog appears.

5) Log into Harmony.
TCO (temperature and CO\textsubscript{2})

Switch on temperature and/or CO\textsubscript{2} if needed.

1) Select **Settings**

2) Click on **TCO Settings**
TCO (temperature and CO$_2$)

1) Switch **On** the temperature and choose a target.

The system takes 2h to reach the temperature !!

2) Switch **On** the CO$_2$ and choose a concentration.

3) Turn on the CO$_2$ valve.

The system takes 10min to reach the correct CO$_2$ concentration !!
If you use water immersion objectives then **Flush** the objective.

**!!If you use a water immersion objective you wet the plate bottom!!**

**If you need to switch to an air immersion objective you have to dry the bottom (ejecting the plate)!!**

1) Select the water immersion objective.

2) Select **Settings**.

3) Click on **Operetta CLS**.
Flush objective

4) Click on **Flush Water Objective**. (it takes 30sec)

5) Repeat the Flush for a total of **3 times**.

6) During the flush, open the side lid and check that drops are falling in the waste bottle.
1) Clean the plate bottom. Avoid dust and condensation.

2) Select an air immersion objective (optional). Remember water immersion objectives wet the plate!!

3) Click on **Eject**.

4) Place the well plate on the holder. Pay attention that all the marks are outside the plate.

5) Click on **Load Plate**.
1) Select **Setup**.

2) **Choose the Plate Type**.
   (Naming = N of wells + company + plate name).
   If the plate is not in the list ask the BIOP to create one.
1) Choose the objective.
   Remember water immersion objectives wet the plate!!

2) Choose the observation mode.
   Confocal requires higher exposure time and led power.

3) Choose the Binning

4) Activate live preview.
   (It displays images when acquired)

Symbols for Temperature and CO₂.

- **Start-up**: current value is still significantly lower than target value.
- **Regulating**: target value nearly reached, but not stabilized yet. In this phase temperature is at maximum 4 °C below target value and CO₂ is at maximum one percentage point below target value. Transiently slightly higher values for temperature and CO₂ may also occur in this phase, please check the Messages window.
- **Ready**: target value reached (target temperature ±1 °C, target concentration ±0.5 percentage points).
Define focal position

Strategy

1) Define where to do the test

2) Create a channel

3) Adjust the channel (just to see the sample)

4) Acquire a Z-stack to individuate the correct focal position
Define focal position

1/4 Define where to do the test

On the navigation panel:

1) Select a well with left click
   The well appears in orange (the test will be done here).

   (If you click on “Select” the well becomes gray.
    The gray wells will be used for the measurements.)

2) By default the center of the well is selected (in gray).
   If you need to change the position:
   left click on a different position > the position becomes orange
   click select > the position becomes gray
   Select only one gray position.
Create a channel

1) On the Channels Selection panel click on the small black triangle.

2) Click on the “+” that appears.
Define focal position

2/4 Create a channel just to see the sample

Create a channel

3) In the Load Channel select the folder: Channel>PKI Service

4) In the list select the dye.

If there is a problem with the dye selection ask BIOP for help.

5) Click on OK.
Adjust the channel acquisition parameters

1) On Channel Selection click **Snapshot**.

The system acquires an temporary image.

2) On the Image Control panel, click on the small black triangle to display the histogram.

3) Check the maximum value.

If it is above 65535 in the Channel Selection decrease:
Exposure **Time** and/or led **Power**.
1) On layout selection click on black triangle:

2) On the Stack window define:
   a) First plane
   b) Number of planes
   c) Distance

3) Double-check that the “Use in Test” option is selected.

4) Hit Test.

The system acquires a temporary z-stack.
5) Select **Test Images** on the Navigation panel.

6) Click on the well.

7) Click on the position.

8) Click on one plane.

9) Use the keyboard arrows Up and Down to move between the stack.

10) Individuate the plane with the correct focus looking at the image. Mark down the focal position.

11) If you need to do an experiment with z-stack. Individuate the **beginning** and the **end** of your future z-stack and mark them down.
1) Add or remove channels with “+” or “−” in the Channel Selection panel.

2) Type the **Height** defined in the previous step (define focal position).

3) Adjust the Exposure **Time** and/or led **Power**. Check them doing a **Snapshot**.
For tiling acquisition define the overlap percentage.

On the Layout Selection panel go on Well window and insert the percentage desired.

To set the tile dimensions see: “Define wells and positions”.
For the z-stack use the values (begin and end) obtained in the previous step (Define focal position).

1) Type your **begin** value in “First Plane at” on the Stack window.

2) Define the **Number of planes** and the **Distance** in order to match the desired **end** position (displayed in “Last Plane at”).
1) For the time lapse go on Time series panel.

2) Select Sequence 1.

3) Deactivate as Fast as possible.

4) Define the Fixed Interval.

5) Define the Number of Timepoints.

Let the plate warm for 30min before to start the acquisition otherwise there will be a drift in XY!!
Define wells and positions

1) Go on **Define Layout** on the Navigation panel.

2) On Plate window: right click on the wells to acquire (they appear in **orange**).

3) Click on **Select** (the wells turn grey).

4) On Well window: right click on the positions to acquire (they appear in **orange**).

5) Click on **Select** (the positions turn grey).
Start Measurements

1) Go on **Run Experiment**.
2) Give a name to the plate
3) Hit Start

No display available. Please select a single well, field
See full protocol on the “Operetta workflow Data Management” (printed or in c4science).

1) Backup you data > Write archive.

2) If you need the TIFF > Export Data (as Tiff)

3) Delete your data. Your data can stay for 24h in the system

Keep in mind that it is possible to do these steps with the BIOP PCs.
1) Remove the plate.

2) Click on **Load Plate** to close the plate door.

1) Switch **Off** the temperature.

2) Switch **Off** the CO₂.

3) Turn Off the CO₂ valve.

4) Close the Harmony software.

5) **Log off** from the PC.

Please keep the PC on. Other users may need to remotely access the data!!