

## 1. CAMERA INSTALLATION

**What you will need:** microscope, microscope camera incl. adapters, USB-cable, and a computing device (computer, iPad or Android device).

### Option 1: C-MOUNT

If your microscope has a c-mount, please use it.

**Step 1:** unscrew the protective cap from the camera

**Step 2:** screw the camera onto the C-mount thread.

Make sure that the camera is sitting straight.

**Step 3:** connect the camera to the computing device via the connection cable

### Option 2: EYEPIECE

If your microscope doesn't have a c-mount you can also attach the camera to the eyepiece.

**Step 1:** unscrew the protective cap of the camera

**Step 2:** remove one eyepiece from your microscope

**Step 3:** attach the camera. Note that depending on your microscope model a different adapter (included) might be needed. Make sure the camera sits straight after the installation.

**Step 5:** connect the camera to the chosen computing device via the supplied cable.



## 2. CALIBRATION

**What you will need:** microscope, mounted camera, USB-cable, and your device of choice logged into our Android App or WebApp respectively

In order to be able to correctly calculate the concentration, the camera must be calibrated for the used microscope and magnification. If there is no change in the magnification and the same microscope is used, this only has to be done the first time.

Otherwise, the calibration must be repeated because the saved calibration factor is no longer correct.

For a video tutorial on how to calibrate the microscope camera please scan the QR-code below.



**Step 1:** set your microscope to "brightfield"

**Step 2:** open the chosen app and go to "Settings"

**Step 3:** pick "Microscope Camera" in the setting dropdown menu for "Camera" options.

**Step 4:** click on "Calibrate Camera" and start the calibration process

**Step 5:** position the counting chamber without any sample and without the cover slip in the microscope.

Make sure the center of the counting grid with its smallest squares is shown sharply in the picture.

If needed, adjust the illumination intensity on the microscope to ensure the lines are clearly visible.

**Step 6:** follow the instructions in the app step-by-step and save the calibration once the app has recognized a correct square. If a different square or no square is recognized, you can also manually draw in the square (WebApp only).

### 3. DILUTION

**What you will need:** measuring cylinder, water, pasteur pipette, a yeast sample from fermenter or propagator

Depending on the density of your yeast sample, more or less dilution will be needed. Here is an **example**

**1:100 dilution:**

**Step 1:** fill your measuring cylinder with 99 ml of water (example dilution for inoculation)

**Step 2:** fill your pasteur pipette with 1 ml of yeast and empty it into the measuring cylinder

**Step 3:** run the solution in and out of the pipette three times to make sure it is completely empty

**Step 4:** take the pasteur pipette and stir vigorously; now it's diluted!

### 4. STAINING (only required when measuring viability)

**What you will need:** diluted yeast sample, pasteur pipette, reaction tube, methylene violet

**Step 1:** take the 0.5 ml of the diluted yeast sample and put it into the reaction tube with the pipette

**Step 2:** take 0.5 ml of the methylene violet solution and put it into the reaction tube

**Step 3:** run the mixture through the pipette a few times

### 5. LOADING THE CHAMBER

**What you will need:** diluted (and stained) yeast sample, pasteur pipette, hemocytometer, ethanol

**Step 1:** moisten the two support structures with ethanol

**Step 2:** push the cover slip over the two support structures and wait until the ethanol evaporates. The coverslip is placed correctly when ring-shaped interference patterns called "Newton rings" can be observed in the areas where the coverslip touches the support structures.

**Step 3:** pipette the sample next to the coverslip

**Step 4:** let the capillary forces pull the sample through the chamber. Make sure not to overload it

**Step 5:** if you stained the sample, leave it for approx. 5 minutes to let the staining react

**Very important:** Never pipette the liquid directly onto the chamber and then add the coverslip on top, as this will not lead to a well-defined volume in the counting area.

### 6. MEASURING

**What you will need:** microscope, mounted camera, USB-cable, computing device, chamber loaded with diluted (and stained) yeast sample

#### Taking the images

**Step 1:** connect the microscope via cable to your computer or mobile device and open the Oculyze app

**Step 2:** choose in the app whether you want to conduct a single measurement or track an entire fermentation and if you want to perform an analysis with or without viability

**Step 3:** put the chamber into the microscope and find a spot outside the grid lines

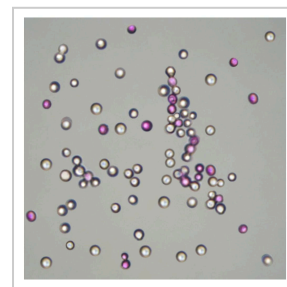
**Step 4:** now adjust the focus of the microscope until you see a sharp image in the app

**Step 5:** take the picture and click "keep" to add the image to the analysis

**Step 6:** move the hemocytometer to a new position

**Step 7:** repeat the steps above to take 5 images

Find an example image below for optimal focus and a good brightness for best image analysis results:



#### Performing the analysis

**Step 1:** after you took 5 images, enter a name for your sample (date & time are filled automatically)

**Step 2:** enter the ratio for dilution and staining (in this example this would be 1:99 and 1:1)

**Step 3:** add a comment if you like, i.e. sample origin, yeast type, generation of yeast, etc.

**Step 4:** click "next" to perform the analysis and to review your results