

Observing Life As It Happens

QUICKSTART GUIDE:
WIDFIELD 1
ZEISS AXIOVERT 200 / VOLOCITY
(SAFB 409)



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Startup procedure

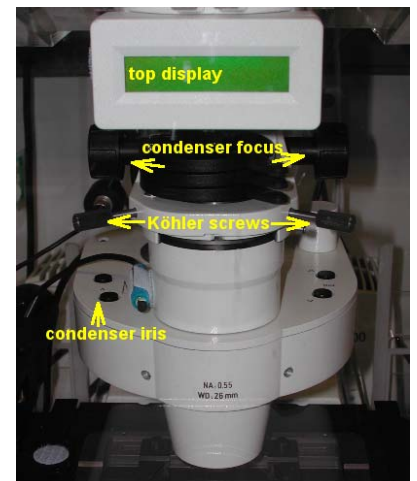
Turn on (in this order):

- if required: **heater unit** and **heater**, 4 hours before live imaging to allow focus to stabilise:
 - ① plate holder
 - ② chamber
- if required: CO₂ supply
- switch on the mercury **fluorescence lamp**
- MAIN switch on the wall, on the right side of the microscope
- computer

Log in with your local account (not your IC login) and start the Volocity software.

Adjusting brightfield Köhler illumination

- make sure the LIGHT PATH (button on the left / bottom) is switched to 100% BINOCULAR: press button until displayed on the microscope top display
- switch to 10x objective with the objective buttons on the right side of the microscope
- you can move your specimen in xy-direction with the joystick, holding the button on top of the joystick makes the stage moving faster
- focus on your sample
- if focus beeps, override the top limit by pushing the top focus button on the right side of the microscope while turning the focus wheel (when you hear double beeps, the mechanical limit of the focus is reached which cannot be overrun)
- open brightfield shutter with S1 (labelled 'BF' on the filter wheel pad)
- adjust brightness with large black button on front / bottom of microscope)
- make sure condenser iris is not completely closed (two buttons on top of illumination filter wheel)
- completely close field iris (top silver lever) (if the image turns completely black, reopen until you see some light, proceed to next step and close again after you have focussed the iris)
- focus iris (black condenser focus wheel)
- centre iris (2 silver Köhler screws)
- reopen field iris until the edge is just not visible anymore



Observing fluorescence through the eyepiece

There are 2 options to observe fluorescence: the common way would be to use the filter cubes in the cube revolver; for some applications the use of a dichroic mirror in the filter cube position together with the filters in the filter wheels is necessary. The latter setup will only work with the camera, not with the eyepiece.

- make sure the excitation / emission filter wheels are in the open position, unless needed:
 - on the filter wheel pad, select FW1 and then HOME
 - repeat for FW2
- with the REFLECTOR buttons choose the illumination filter (microscope, right / bottom behind focus wheel)
- switch between brightfield and fluorescence with FL ON / OFF (left / front / top button on microscope)
- → turn fluorescence immediately off if not needed, to avoid bleaching!
- find your sample



Acquiring images with the Improvion Volocity software

General informations

All microscope parts can be controlled by the software (shutters, filters, focus, ...), except the light path (BINOCULAR/ FRONT PORT / BASE PORT) and the objectives

Acquiring the image

- start the VOLOCITY software (make sure the dongle is attached to a USB port)
- click on the camera icon to activate the live view and microscope controls (for more information about the Video Toolbar in the upcoming window see Additional Information section)
- on the microscope stand, change the light path to BASEPORT
- refocus/ re-centre (if needed, see "refocussing/ re-centering")
- adjust the channel presets of the channels you need (see "adjusting channel presets")
- adjust other acquisition parameters (see "multidimensional acquisition setup")
- with a click on the camera icon on the video toolbar you capture a single frame with the settings actually displayed in the video toolbar
- with a click on the red button icon on the video toolbar you start your recording as defined in "Acquisition Setup"

Useful: by clicking the "Freeze" button in the software you can keep your actual live image frozen on the screen; the acquisition stops and the shutters will close, preventing your sample from bleaching.

refocussing/ re-centring:

- choose the brightfield preset (preset button 1)

- refocus/ re-centre

or

- choose a fluorescent preset
- if there is only a faint signal switch "Auto Contrast" on
- refocus/ re-centre
- switch off "Auto Contrast"

adjusting channel presets:

- choose a preset button for your channel settings by clicking on one
 - standard presets for filter cubes are
 - 1 = Brightfield
 - 2 = DAPI/ Hoechst
 - 3 = rhodamine / TRITC/ Alexa543
 - 4 = GFP / FITC/ Alexa488
- adjust
 - exposure time, binning, gain and offset (make sure "Auto Contrast" is off!!!)
 - bit depth
- right click on the preset button > Overwrite

multidimensional acquisition setup:

- set **multiple channels**
 - open "Acquisition Setup" dialogue
 - tick "Change channels"
 - add as many channels as you need with the "+" button
 - from the appearing drop down menus choose the desired channel presets
 - if you don't want to do a stack, time series or multiple positions set timepoint to 1, choose no focus and no xy
- set **stack**
 - before any other adjustments: click on Z position button and set "Top" and "Bottom" to the mechanical limits
 - using defined focus positions
 - move focus to mid focus plane
 - click on Z position button, choose "Set Zero"
 - move focus to top position
 - click on Z position button, choose "Set Top"
 - move focus to bottom position
 - click on Z position button, choose "Set Bottom"
 - in "Acquisition Setup" dialogue
 - set "Change focus using" to "Zeiss Focus Drive"
 - choose and set Z-spacing or number of slices
 - using Autofocus

- in "Acquisition Setup" dialogue
 - set "Change focus using" to "Zeiss Focus Drive"
 - choose and set Z-spacing or number of slices
 - set "Auto-focus using" to "Zeiss Focus Drive"
 - choose the lightpath where the Autofocus should take its cues from
 - set **time series**
 - in "Acquisition Setup" dialogue: set duration
 - in video toolbar: set intervals
 - set **multiple positions**
 - stage needs to be calibrated for this
 - remove sample!!!
 - switch to shortest objective (10x, 2,5x or empty position)!!!
 - move objective turret to the lowest position!!!
 - choose "Calibrate Stage" from the "Stage" menu
 - in the "Acquisition Setup" dialogue set "Change XY using" to "Ludl XY Stage"
 - "handmade" positioning
 - "Video" menu > "Live Video"
 - "Stage" menu > "Clear All Points"
 - move to desired point
 - "Stage" menu > "Add Point"
 - with "Stage" menu > "Review Points" you can adjust the focus for each of your points separately
 - automated positioning
 - "Video" > "XY Stage"
 - "Stage" > "Make Well Overlay"
 - "Stage" > "Create Points from Well Overlay"
 - follow the instructions

Shutdown procedure

- check if anyone is booked after you within 2 hours

If nobody is booked within two hours:

- remove specimen
- clean oil objective lenses with lens tissue
- Important: close incubation chamber completely (→ protects from dust)
- update time in Sharepoint
- secure your data (e.g. copy them to the server)
- shut down hardware in reverse order from startup

If someone's booked within two hours:

- update usage in Sharepoint
- remove your samples
- clean objective lenses with fresh lens tissue and close incubation chamber
- clear up the desk
- save files onto the server or on a mobile harddrive
- log off

Important additional information

Volocity Video Toolbar

The screenshot shows the Volocity Video Toolbar with various controls and their functions explained by arrows and text:

- 18:17:36**: * Single capture
- Series capture icon**: * Series capture
- Pause icon**: * Pause series
- Intensity histogram**: Intensity histogram of actual image
- Maximum Speed dropdown**: [] Time delay between captures in a series
- Freeze icon**: * Freeze capture to preserve sample
- Acquisition Setup icon**: ** Go to Acquisition Setup dialogue
- Presets 1-9**: * Presets
- Exposure time display**: [] Exposure time
- Binning display (x1)**: [] Binning
- Auto Contrast checkbox**: * Auto Contrast on/ off
- Gain slider**: [] Gain
- Offset slider**: [] Offset
- Illumination conditions dropdown (Low Light)**: [] Illumination conditions
- Bit depth dropdown (8 Bits Per Channel)**: [] Bit depth
- Fluorescence lamp shutter**: * Fluorescence lamp shutter
- Filter cube buttons (1-5)**: * Filter cube
- Z position display (-7232.70 µm)**: # Set Z positions (top, bottom, zero, etc.)
- Lamp brightness slider**: [] Lamp brightness
- Brightfield shutter**: * Brightfield shutter
- Excitation filter wheel position (1: empty)**: * Excitation filter wheel position (1: empty)
- Emission filter wheel position (1: empty)**: * Emmission filter wheel position (1: empty)
- X-Position display (12.109 mm)**: X-Position from top left corner
- Y-Position display (2.169 mm)**: Y-Position from top left corner

* > click; ** > double-click; # > right-click;
 [] > fill in/ choose from drop-down menu/ move slider

Auto Exposure

- sets max pixels to 80% (not 100%) (can also be applied to a selected area)

Auto Contrast

- unlike Gain **only** affects appearance of the image on the screen(!!!)

Keypad controls:

- top level button 1 = transmitted light on/off (#2-4 = empty)
- FW button switches between control of:
 - FW1 = excitation filter wheel (orbit excitation filters, only used for FRET or CFP+YFP)
 - FW2 = emission filter wheel in front of camera, underneath microscope
→ *the orbit excitation filters must be in HOME position for use of the emission filters; if they aren't, press the HOME button on the external keyboard*

Control buttons on the lefthand side of the microscope:

- lowest button:
 - switches between frontport (empty) <> eyepiece <> baseport (camera)
- middle button:
 - switches between sideport 100% (empty) <> 50/50 <> eye/ front/ base 100%
- top: Optovar in / out



File formats

- The original Volocity libraries are stored as "mvd2" files together with a folder containing the image data in "aisf" files
- TIFF: loses all enhancements but keeps original ("raw") data
 - all stacks/ channels are stored in 1 multipage TIFF with the original bit-depth (8 or 12 bit)
- TIFF for publication
 - screen version of image, including colours, merge, enhancements, on-screen image resolution, it is always 8-bit → *loss of original ("raw") data!!!*

merge colours:

- CREATE NEW IMAGE SEQUENCE > MULTIPLE CHANNELS
- drag single channel (or z / timepoint) images onto new image sequence window