CELL-IQ IMAGEN MANUAL

CELL-IQ®, FOR YOUR RESEARCH NEEDS

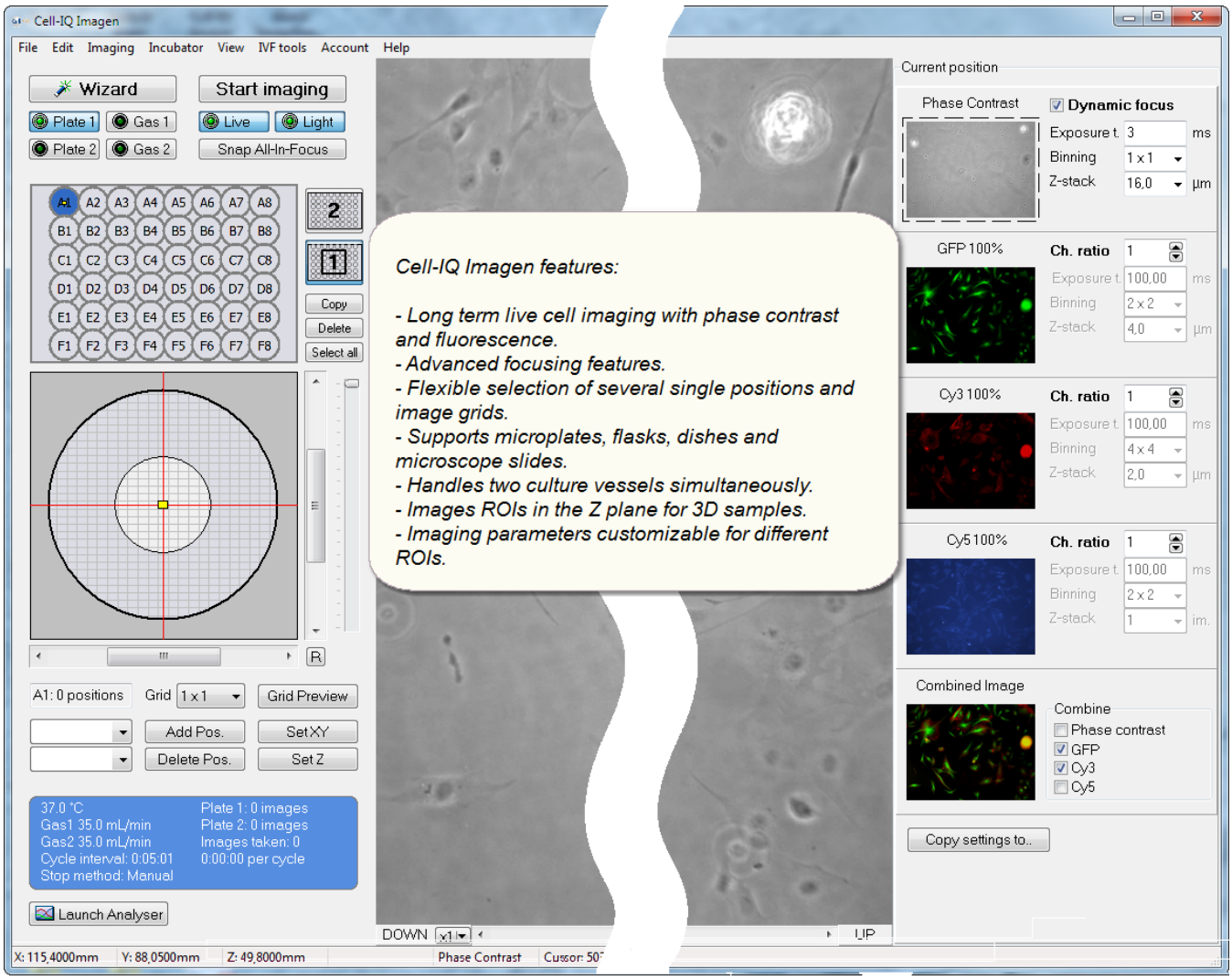
MI4.0.0.0

CM Technologies
Intelligent cell analysis

Table of Contents

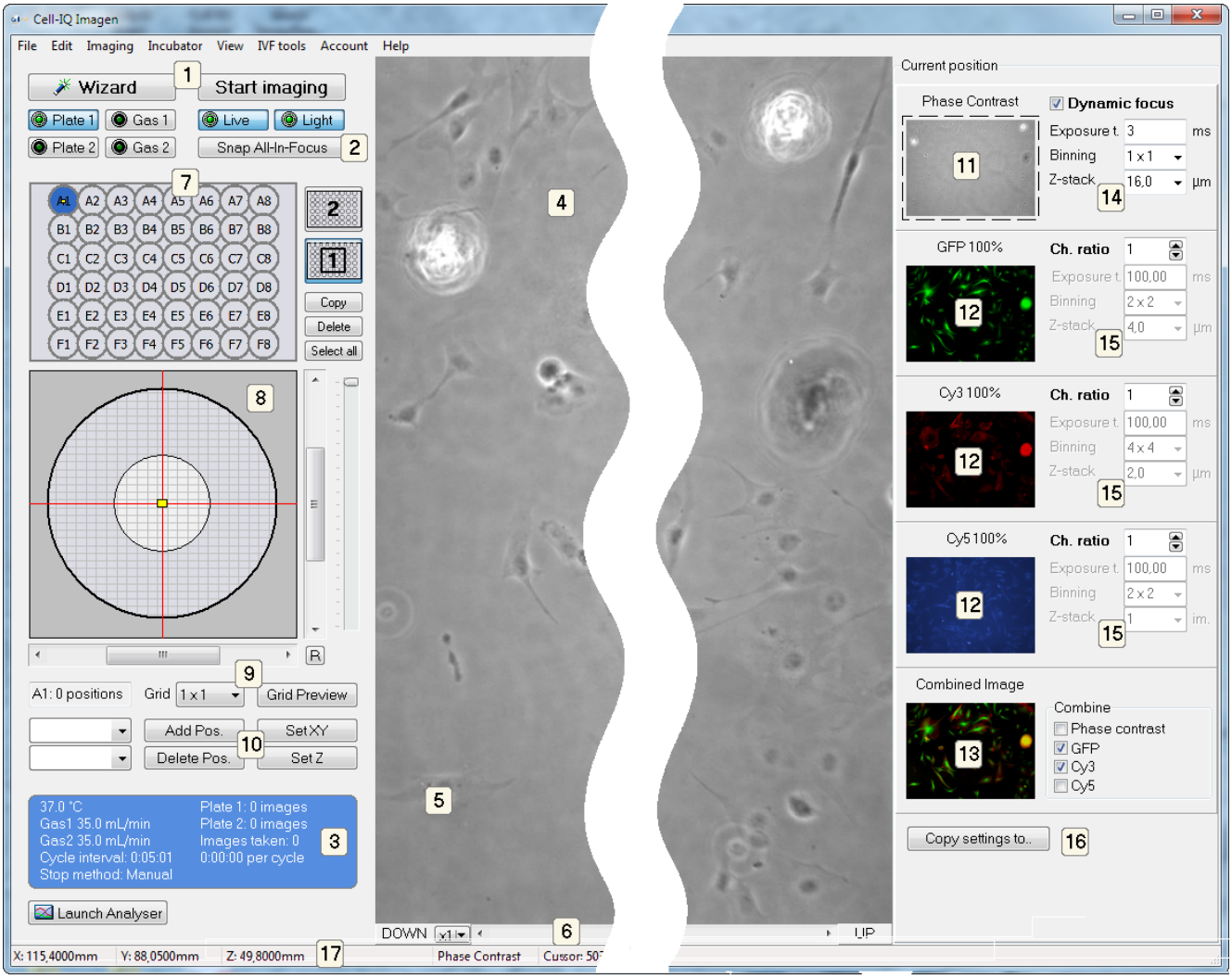
Imagen program overview

1 Imagen program overview



1.1 Program workspace

This chapter describes briefly all the buttons and fields in the main window. Please see from the later chapters the instructions for preparing the cells and the Imagen program for the different cell monitoring applications.



1. **Wizard and Start imaging -buttons**: Define the imaging settings and cycles with the wizard and start imaging. All the settings in the wizard can also be found in the drop down menus or from the main window work space. Hovering mouse over Plate 1 and Plate 2 button will show the saving directories for the plates.

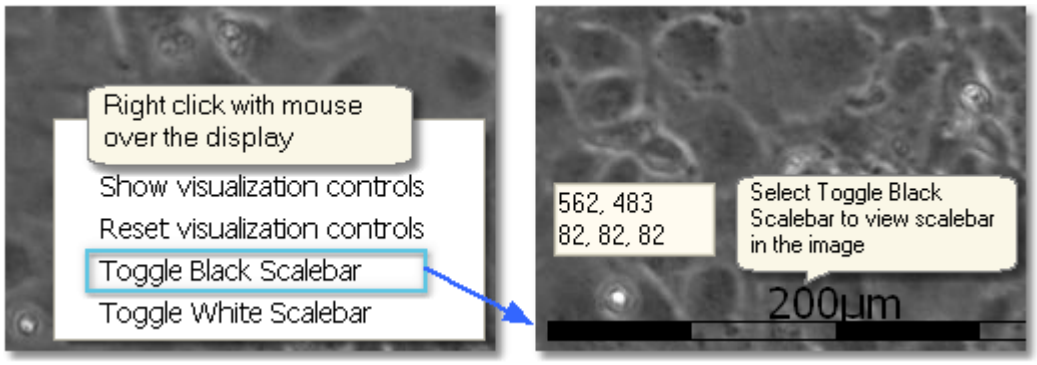
2. **Snap All-In-Focus -button**: Clicking this button down will stop the live image and grabs an all-in-focus image as defined in channel settings. Clicking the button again will resume live imaging. This is a quick way to test how thick the Z-stack should be for the current application.

3. **Cycle information. E.g. interval and images taken**: The cycle interval is set before starting a cell test in the Wizard. Interval corresponds to how often same well plate position is imaged. Inserted values are for h (hours), min (minutes) and sec (seconds). The images taken is the number of images captured so far in the cell test. Also previous cycles duration, an estimate of time until next cycle, gas flow for each plate and temperature of the incubator are displayed.

4. **Live image display**: Before starting the cycle you can select the displayed channels by clicking on the small channel cell images. During the cycle, the channel currently being imaged is always displayed in the main

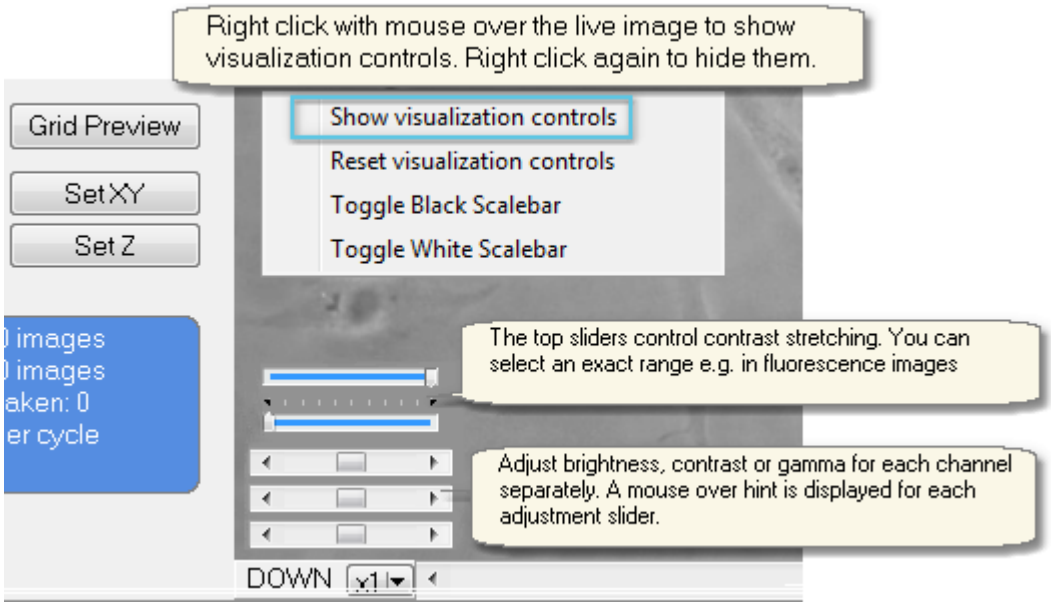
display.

- Right click by mouse to toggle the cursor information box that includes the cursor coordinates and the RGB values in that point. The pixel [R,G,B] values are the red, green and blue channel values in that pixel coordinate.
- Right click by mouse to toggle the black or white scalebar. The scalebar adjusts when the image is zoomed. Scalebar is not saved while imaging cycle is running, because it would affect the analysis, but it can be added from Analyser later on.
- Right click by mouse to edit visualization of the image: ***Show/Hide/Reset visualization controls***, the same as in 8-11. Small displays for each channel, Visualization settings in this topic.



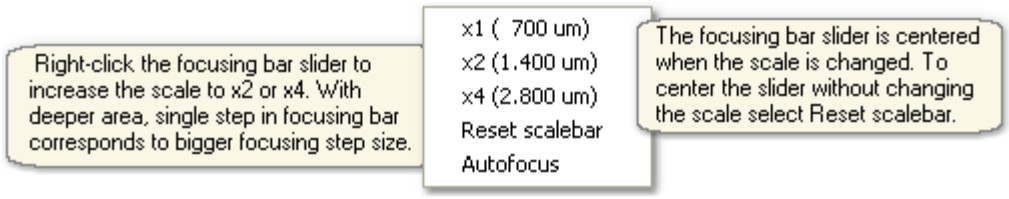
- You can move the live image area by clicking the main image display (the small channel displays don't have this feature), the image will center itself to the point clicked. You can also use the drag and drop feature on the image display to move around in the image.
- Zoom in and out in the image by scrolling with mouse wheel.

5. Visualization settings: Changing these settings helps human eye to find important structures from the image and provide more informative pictures for publications. You can save the image from the main view via the main menu, ***File -> Save Image***.

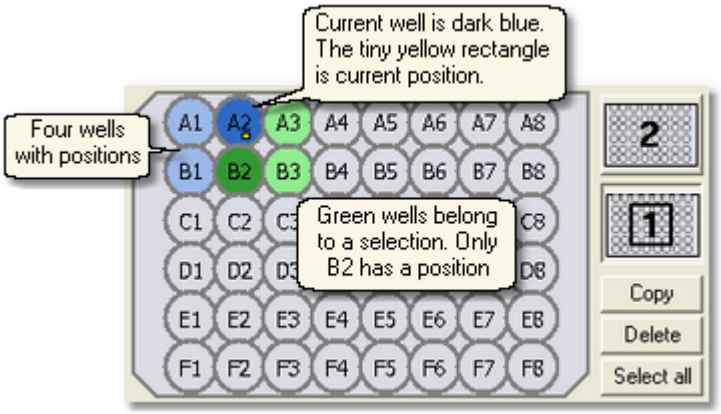


6. Focusing bar slider: Image can be focused manually using the focusing bar just below the live image. The slider moves by holding down left mouse button. Moving the slider left moves the objective down and moving

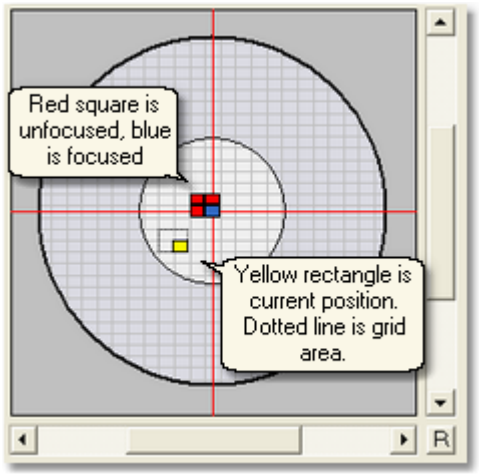
it right moves up. Image is refreshed when the slider is not moving. For more focus settings, right click by mouse over the focus bar. You can change the focusing scale, reset the focus bar and perform autofocus.



7. Well plate panel: The right side of the workspace is for selecting positions and editing their settings. On the top there is an image of the well plate. Wells are color coded by **six colors**. Blue is the main color, **dark blue** is current well, **medium blue** is wells that have positions, **light blue** are "empty" wells. Green is a selection of wells, you can copy or delete positions to a group of wells using the selection. **Dark green** (not shown in the image) is current well, **medium green** well has positions and **light green** is again "empty". You can select/unselect wells simply by holding left mouse button down and dragging the cursor. A selection rectangle is drawn to help. Left clicking a well will drive to that well. Right clicking a well will open a settings form for that well or position.



8. Well image: This is where you add positions for imaging. The lighter inner circle is where the phase contrast image quality is at its best and where most positions should reside. The **yellow** rectangle is the current view. **Blue** rectangle is a focused imaging position, **red** means that position will be focused at the beginning the cycle. You can move around by left clicking the image. The scrollbars and zoom-bar on the right change the view. ***R*** -button resets the view back to this default view.

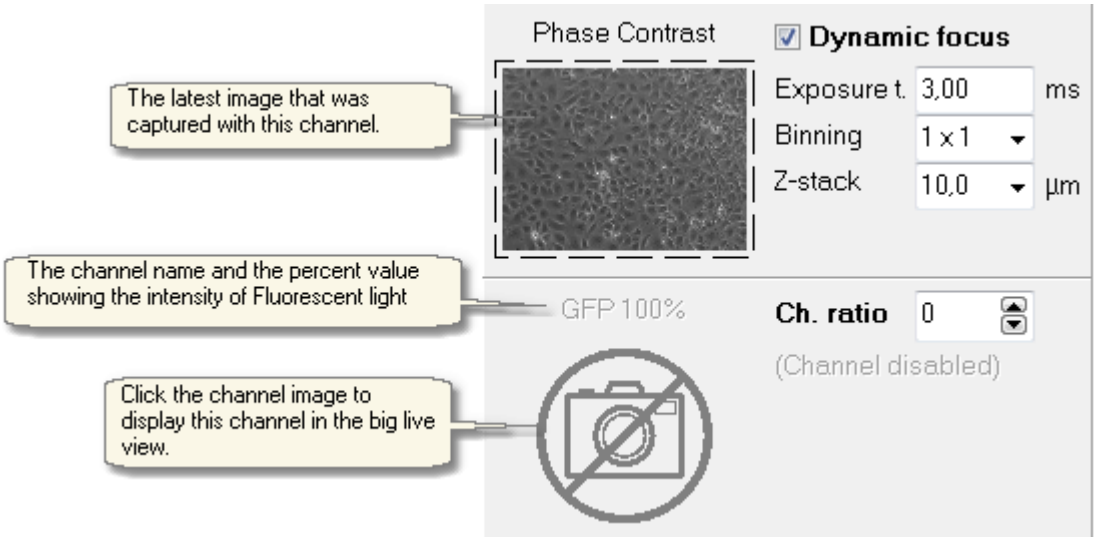


9. Grid: Here you can select a square grid as a position. There is a 2x2 grid in the well image above and there is a thin selection rectangle around the yellow rectangle to show the boundaries of the grid. ***Grid Preview*** -button opens a new form and starts imaging the whole grid.

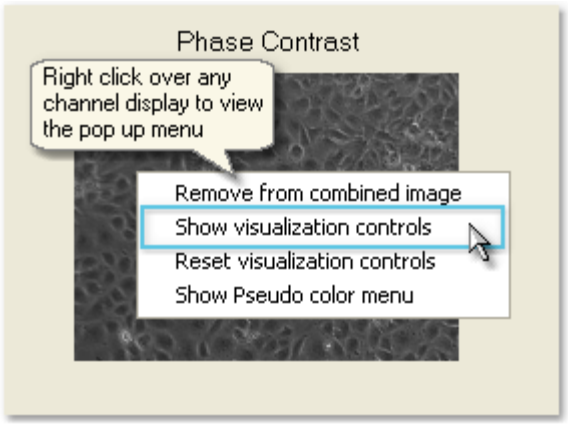
10. Position control buttons: With these buttons you can **add**, **delete**, **move and set focus**. If there is a position in the well, **Set XY** -button will move its XY-position where the yellow rectangle is. If that position is a grid, all grid images are moved. ***Set image Z*** -button applies current Z-level to selected image, it will become blue.

11 & 12. Small displays for each channel (Some of them only in Cell-IQ with fluorescence module)

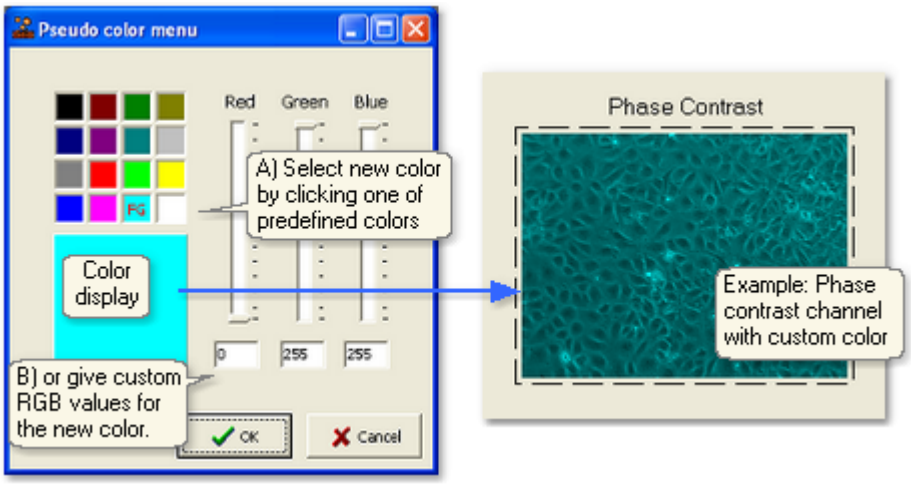
- Click any of the small channel images to select the channel for live display in the big view. If channel ration was 0 (it was disabled), it turns into 1 and the channel is added for current position. The name of the channel is above each channel image.



- **VISUALIZATION SETTINGS**: Once the channel is selected, right click over it to adjust visualization for that particular channel. See explanation of the visualization settings earlier in this chapter.

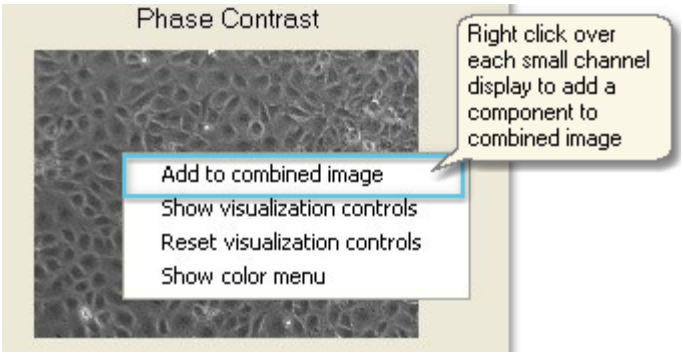


- **IMAGE COLOR**: Image color can be changed for each channel image. Set color by selecting ***Show image color menu*** in the pop-up menu. Select color from the predefined options or type in custom RGB values. The selected color is displayed in the big square. Click ***OK*** to close or ***Cancel*** to restore the previous pseudo color. The color and intensity values are saved into files ***Imagen_MFU.ini*** and ***cycle.dat.***



13. Combined image (only Cell-IQ with fluorescence module)

- **ADD COMPONENTS**: Add channels to the combined image by right clicking over each channel you want to add. Choose ***Add to combined image*** from the pop up menu. Similarly, remove a component by selecting ***Remove from combined image*** in the pop up menu:



- **VISUALIZATION SETTINGS**: Once the combined channel is selected, right click over it to adjust visualization for that particular channel. See explanation of the visualization settings earlier in this chapter.

14 & 15. Imaging settings: Image capture settings of the currently selected image position (if any selected), for each channel, is displayed next to the channel image. Changing the value automatically changes and saves that setting for the current position, and selecting a position restores the values of that position to the fields.

16. Copy settings: Copy any of the Imaging settings from current position to any other position(s).

17. Image dimensions: The dimensions of the image are displayed in the bottom information bar. The dimensions are in both in pixels and micrometers (µm).

17. (x, y, z, d) coordinates:The x, y, z -coordinate values are the offset in millimeters from the system origo. z-coordinate is objective level from bottom in millimeters. Fluorescence systems also display the filter changer position d.

1.2 Change log

Imagen program versions change log:

--- IM4.0.0 ----------
- Update: New main window, where channel images aligned vertically for increased live image size.
- Update: The image capture parameters (z-stack size etc.) added to Imagen main window.
- Update: New Wizard where it asks data step by step.
- Update: Advanced settings window replaced with the individual settings windows
- Update: When user clicks wells during imaging, it shows the last saved image.
- Update: When "Stop Imaging" clicked, ask if the current cycle is finished before stopping.
- Update: The exposure time or other selected settings can be copied to the other positions.
- Update: Launch Analyser -button added that opens the current Imagen cycle in Analyser.
- Update: User login with user specific imaging settings.
- Update: Cell-IQ type definition file added: Enabling one Imagen exe version to work in all systems.
- Update: Elapsed imaging test time displayed.
- Update: Cell test and well descriptions are written and shown in well plate table format.
- Update: Cell test and well settings are shown in well plate table format.
- Update: Well plate bottom Z coordinate saved to plate definition, for faster manual focusing.
- Update: Fluorescence channel XY offset calibration added.
- Revamp: Accurate cycle time estimate.

--- IM2.9.5 ----------
- Revamp: Axes driver automatically restarted in connection errors.
- Revamp: Autofocus fine search phase uses larger range and better algorithm.

--- IM2.9.4 ----------
- Revamp: Filter changer control accuracy improved.

--- IM2.9.3 ----------
- Bug fix: Home position calibration rounding error made bad plate mapping accuracy.

--- IM2.9.2 ----------
- Update: : Hot pixel position calibration and automatic fixing from images.
- Update: : The same cycle.dat file can be loaded to both plates in Imagen and to other Cell-IQs.
- Update: : Copy image positions from plate1 to plate2 or opposite.
- Update: Uses pdf help instead of chm html help file.
- Update: Preview image is saved in full size resolution from main window drop-down menu.
- Update: Windows 7 button styles instead Windows XP styles.
- Update: Max. values for setting test stop time increased from: 100cycles to 999cycl. and from 24 hrs. to 999hrs..
- Revamp: Grid preview does not anymore consume large amount of memory.
- Revamp: The image saving default folder in Win 7 changed to "My Documents". It was one level lower before.
- Revamp: The image saving folder is not anymore reset to default, when Start/Stop Imaging clicked.

--- IM2.9.1 ----------

- Revamp: Improvements to focusing.

--- IM2.9.0 ----------

- Update: Flask plates, slide plates made available.

Preparing for a cell test

2 Preparing for a cell test

Preparing cells

1. Warm up the Cell-IQ well plate lid to 37° C before you connect it to the well plate. For example, place it into an incubator for at least 30 minutes. This prevents moisture from condensing on the lid.
2. Cells must be in the well plate a minimum of 4h (24h recommended) before imaging cycle is started.
   - It takes time for the cells to adhere to the well plate bottom. The attachment time depends on the cell type.
   - Serum in the medium assists the cells to attach to the well plate much faster.
3. In toxicology experiments the medium is changed into serum-free medium before compounds are added.
4. Spaces between wells are filled half way with purified water to increase gas humidity and hence decrease evaporation of medium in the well.
5. Seal the Cell-IQ lid to the well plate with white tape provided by CM Technologies.
6. Connect the output connector and the filter.
7. Place the well plate into Cell-IQ incubator.
8. Connect the input connectors.

Cell number and medium volume

Cell number per well in a Nunc 48-well microplate:

- Neural cells SHSY-5Y 48 h test: 40000 cells per well
- HK2 (human kidney) cells 48h test: 20000 cells per well
- MCF-7 cells 7 day test: 3000 cells per well

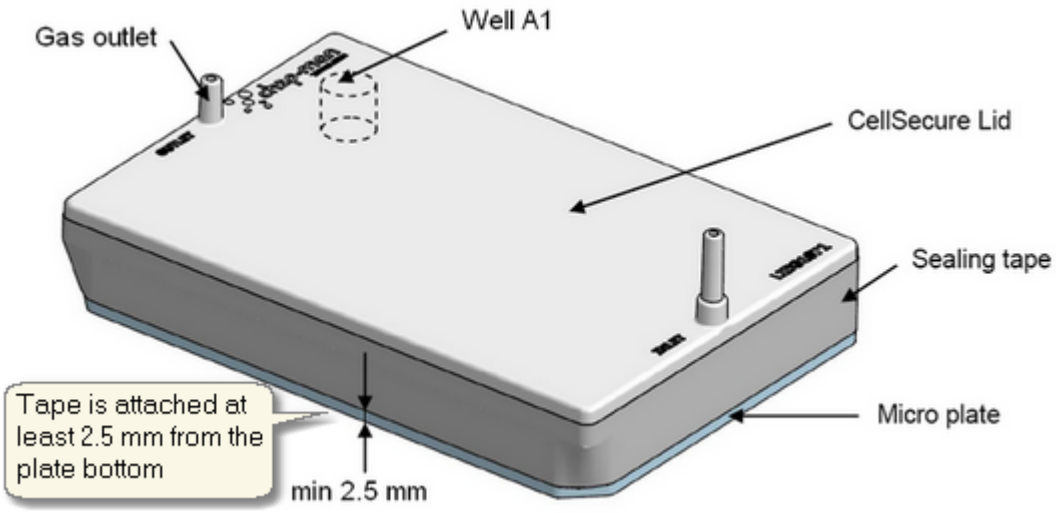
Standard cell incubation medium is used. Use 300 (to 500) µl medium in a well in Nunc 48-well plate.

Gas flow settings

- Gas flow rate is 35 ml/min
- Default gas control settings: gas On 6 min, gas Off 28 min (The first gas cycle is longer in order to fill the tubing and the entire well plate)

Sealing the well plate

Cover the well plate with CellSecure lid. Seal the plate with appropriate tape. Wrap the tape around until it overlaps.



Connecting the output connector and the filter

Connect the filter to the gas outlet (the shorter pipe on the lid) and the well plate is ready to be inserted into the incubator.

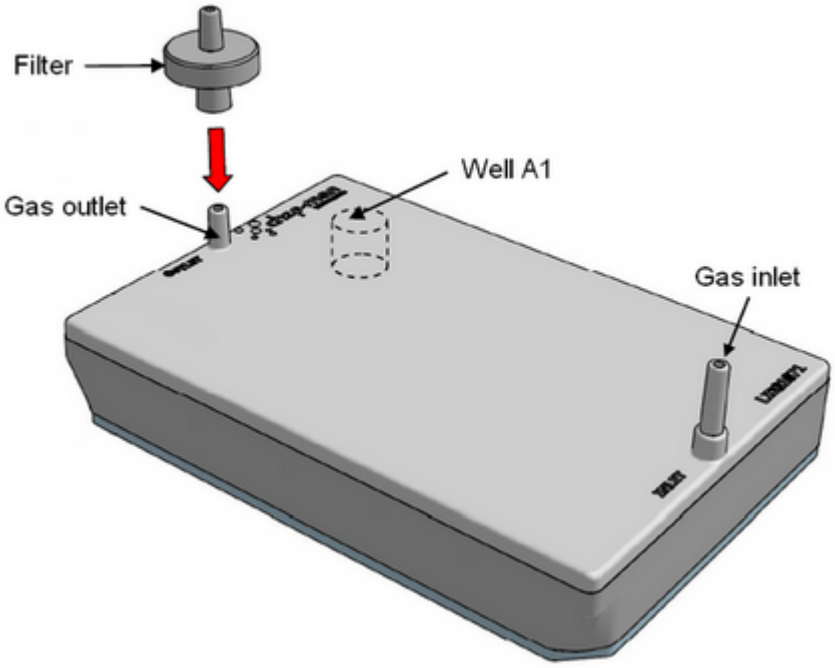
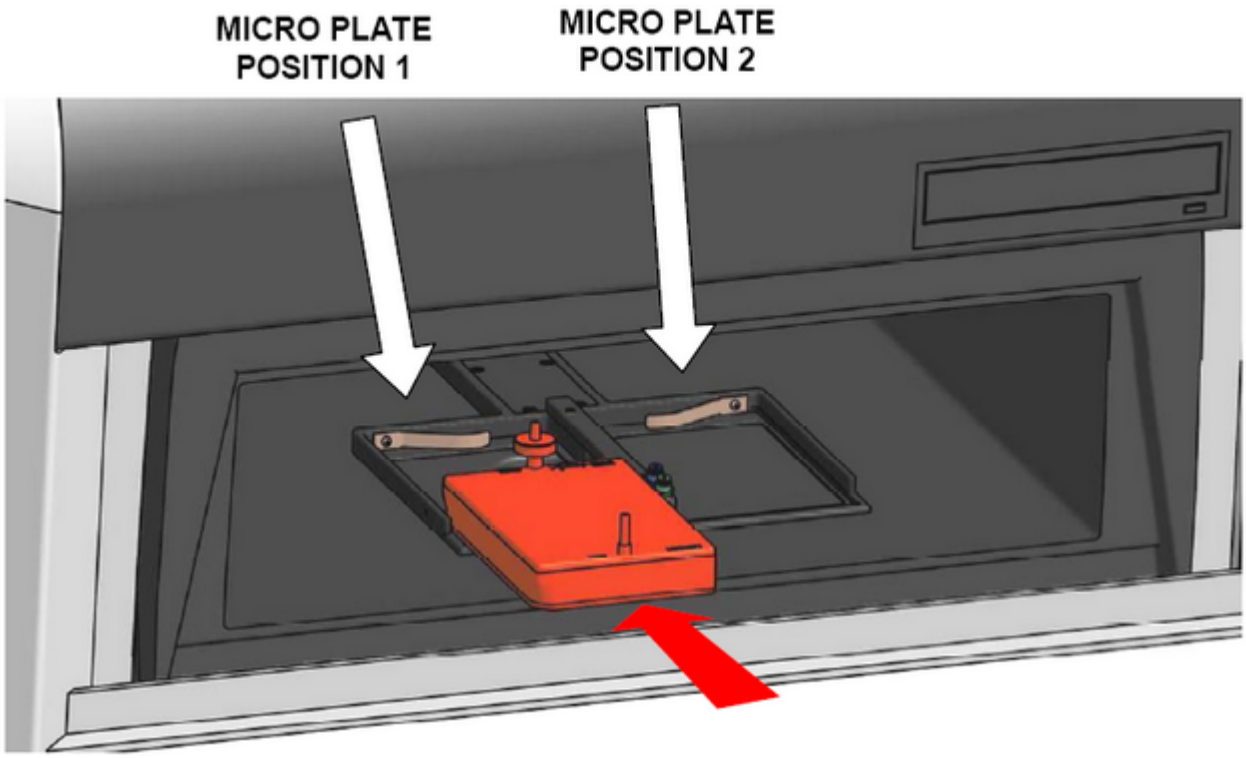


Plate locations and positioning

Insert the first plate on the left side of the holder and the second to the right side.

1. Slide the plate into the holders groove against the springs until the plate drop down to its position.
2. Pull the plate backwards against the corner that do not have the springs, to guarantee consistent plate placement.
3. Press the plate down to assure it is firmly on the bottom of the holder.

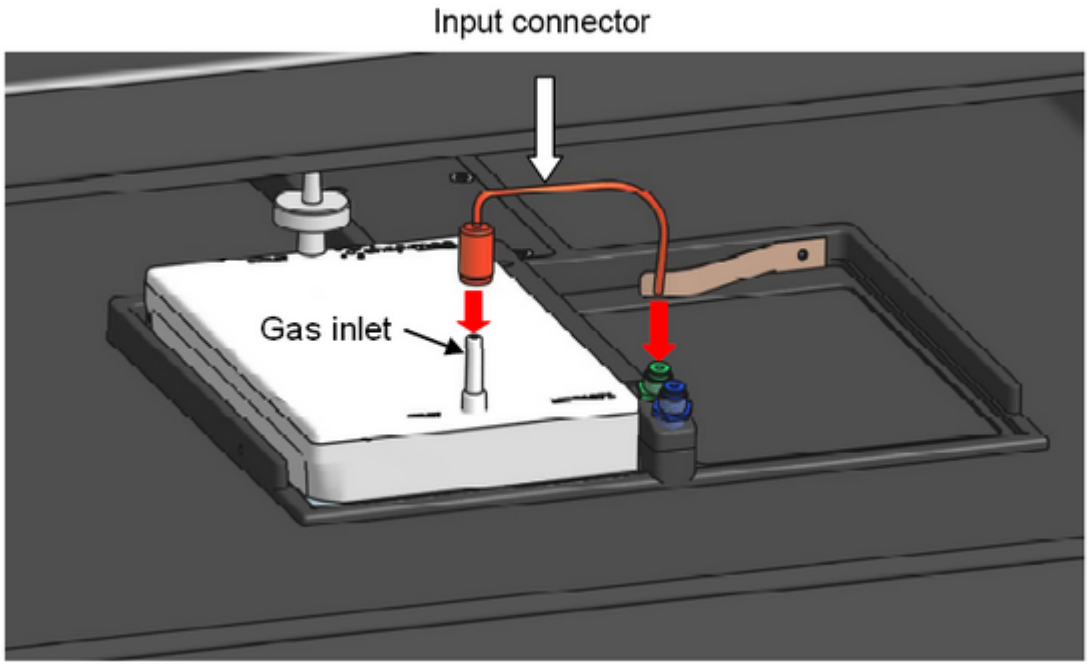
Note! Inserting the plate inside the holder can cause relocation of the second plate if two plates are used. Always check the position of the plate that has already been inserted in the holder after inserting a new plate.


MICRO PLATE
POSITION 1
MICRO PLATE
POSITION 2

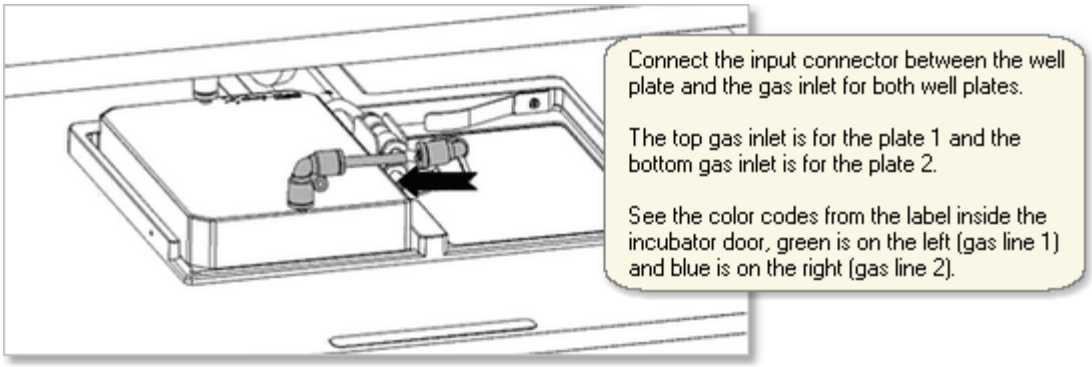
Connecting the input connectors Version 2 Cell-IQ systems

The supply for the plate 1 (left) is marked by a green colour and is located behind the first connector. Connect the tubing to the holder's connector (it is advised to push it down twice to ensure good connection). Then connect the push-on fitting to the CellSecure lid.

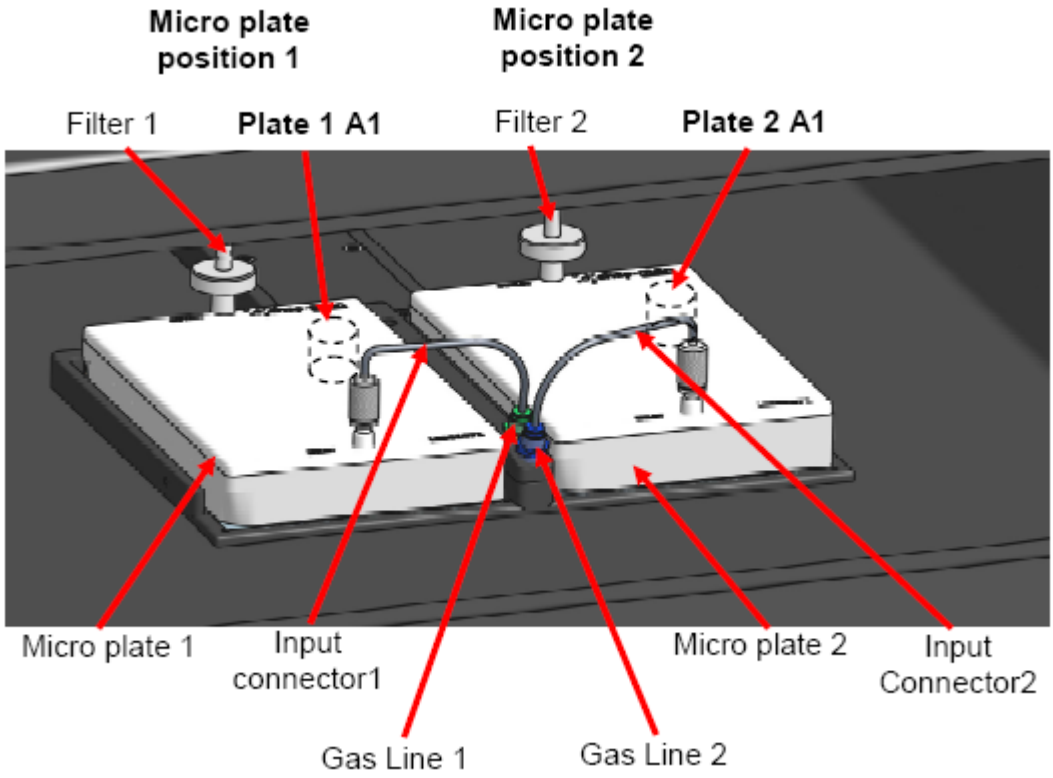
Note! When you remove well plate, also remove the connectors.



Connecting the input connectors Version 1 Cell-IQ systems



Correct placement of plates system



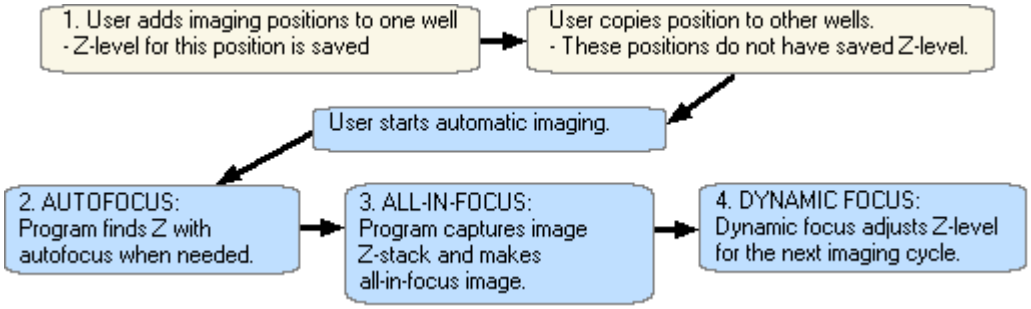
Focusing features

3 Focusing features

The high quality cell images that Imagen saves are achieved with the four focusing steps:

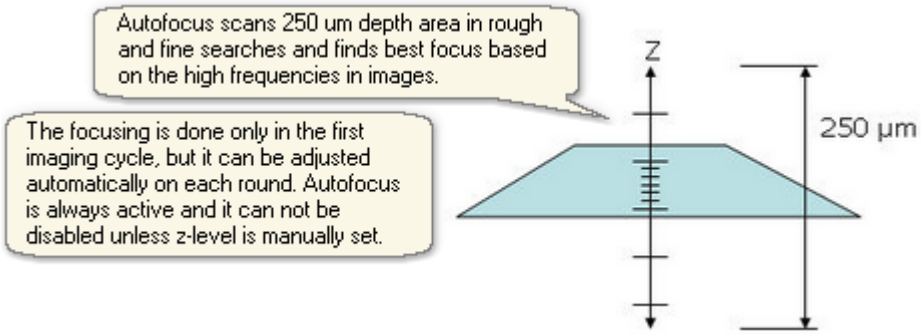
1. Manual focusing of at least one position in the well plate.
2. Autofocus.
3. All-in-focus.
4. Dynamic focus.

The four focusing steps are shown in the flow chart in below:



3.1 Autofocus

Autofocus finds the best focus Z plane. Autofocus uses only the phase contrast images, not fluorescence.



When is autofocus applied?

Autofocus never needs to be manually enabled or disabled, but the system finds focus automatically if there is no focus level previously saved for that imaging position. As you can see from the image below, it is needed to manually set Z level for at least one position in the first well that is imaged.

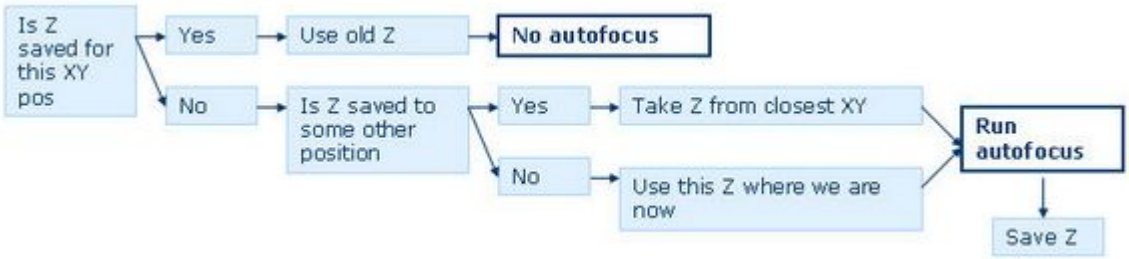
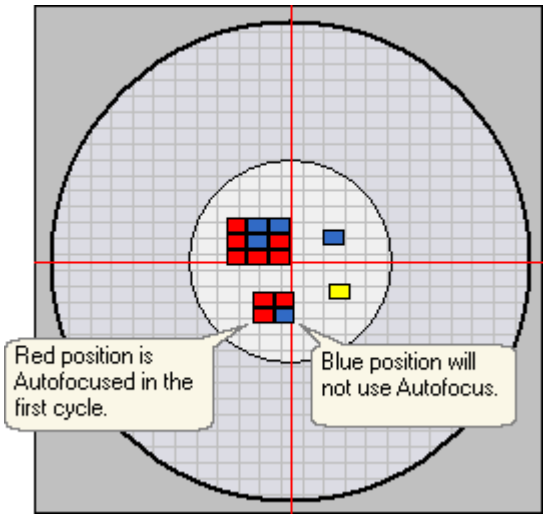


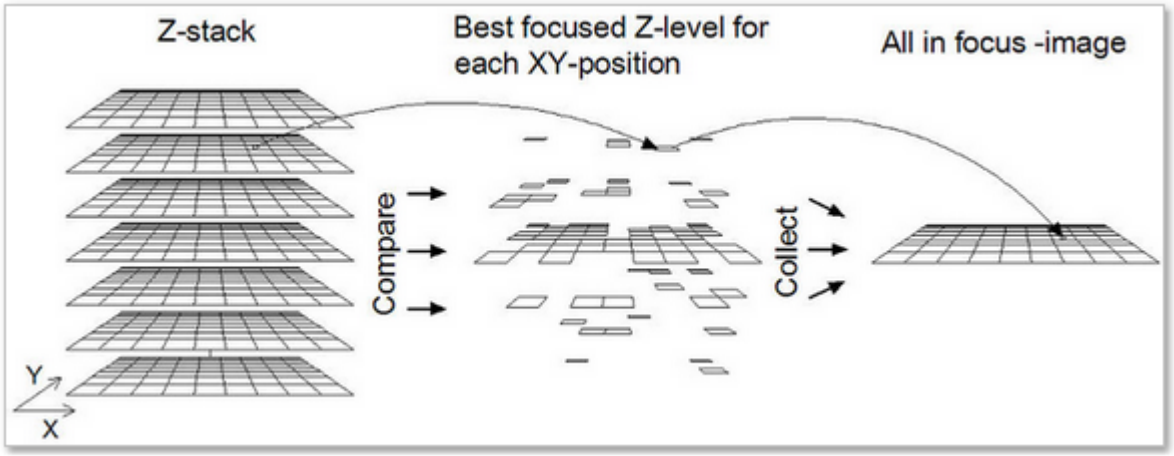
Imagen user interface shows with red color those image positions that do not have Z level saved and hence they will use autofocus in the first imaging cycle. After the first imaging cycle all the positions have Z level saved and they are displayed with blue, and no autofocus is needed.



3.2 All-in-focus image

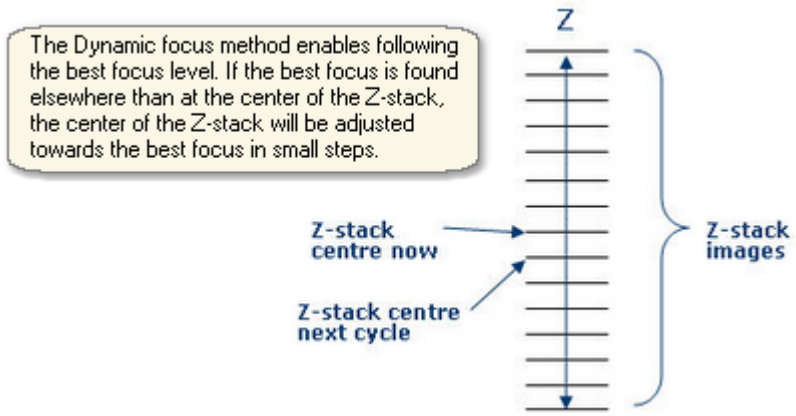
The All-in-focus image looks like normal live image, except that all the areas in image are in good focus. The Cell-IQ captures a stack of images from the same XY position, but with different Z-levels, and combines focused pixels into a single result image, which is called the all-in-focus image. Depth of focus in Nikon 10x objective is about 3 µm. Therefore it is not sufficient to take a single focused image and assume that entire imaging area is well focused.

- Normally the Z-stack step size is 1 µm (10x objective) and Z-stack thickness is 16 µm.
- The Imagen program selects the Z-stack step size automatically based on the depth of focus size of the selected objective.
- With 20x objective the same Z-stack size includes double number of images than with 10x objective.



3.3 Dynamic focus

The Dynamic focus means that the Image Z-stack center automatically follows the best focus level. The level is adjusted every time new image is saved in the automatic imaging. Dynamic focus uses only the phase contrast image Z-stack.



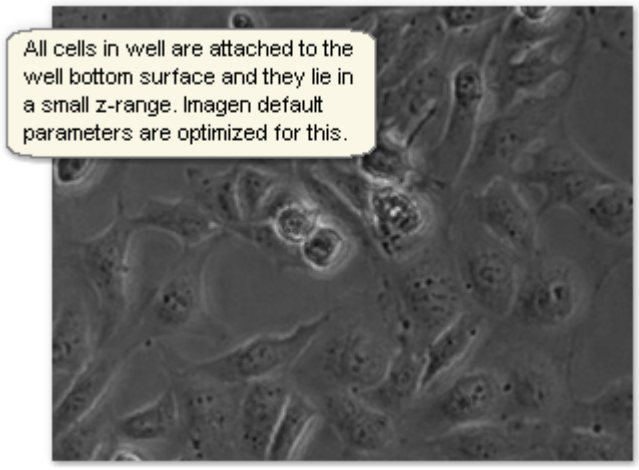
Cell-IQ applications

4 Cell-IQ applications

Imaging settings of specific cell test applications

Adherent single layer assay
Horizontal migration assay
Proliferation assay
Vertical migration assay
Cell colony assay
Embryos and oocytes assay
Fluorescence assay

4.1 Adherent single layer assay



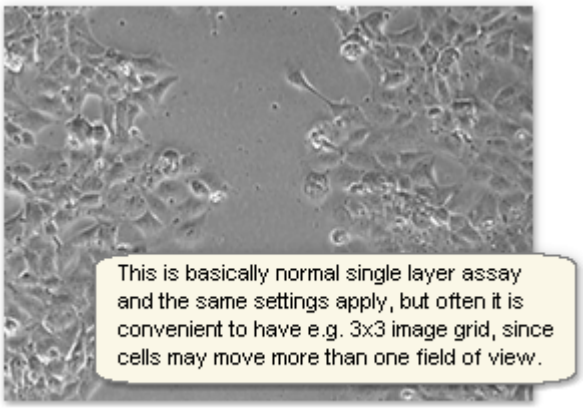
Default settings

- Z-stack: 20µm
- Dynamic focus: Yes
- Use area in and near the well center
- Focus manually the first position in first well
- Autofocus will find the right focus for other positions and wells

Cycle time

- If 48 well plate and 1 positions in each well: First cycle 10 min, next cycles 5 min.

4.2 Horizontal migration assay



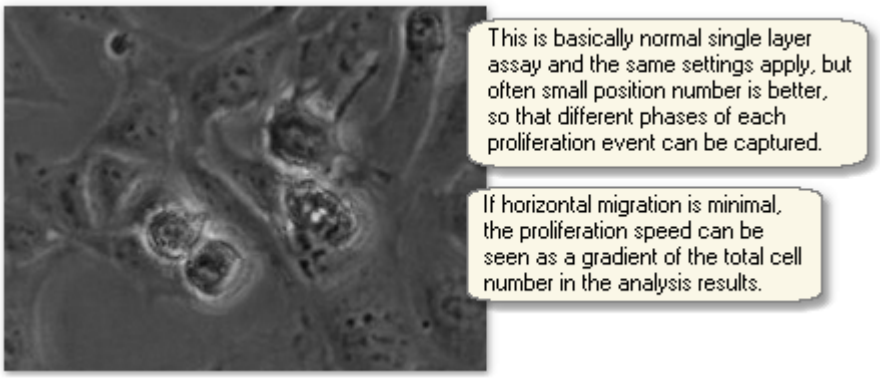
Default settings

- Z-stack 20µm
- Dynamic focus: Yes
- Use near cell center area in well.
- It is often useful to have 2x2 or 3x3 image grid.
- Focus manually the first position in first well
- Autofocus will find the right focus for other positions and wells

Cycle time

- If 48 well plate and one 3x3 grid in each well: First cycle 90 min, next cycles 45 min.

4.3 Proliferation assay



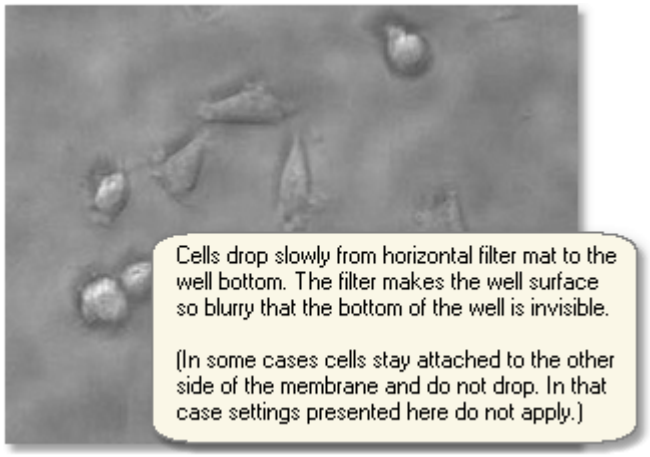
Default settings

- Z-stack: 20µm
- Dynamic focus: Yes
- Use area in and near the well center
- Focus manually the first position in first well
- Autofocus will find the right focus for other positions and wells

Cycle time

- If 24 well plate and 3 positions in each well: First cycle 15 min, next cycles 8 min.

4.4 Vertical migration assay



Focusing

Focus can be set manually if there's something visible in the well bottom to focus on, such as cells or debris. Otherwise the focus is set on the outer well bottom surface and then shifted upwards by the thickness of well bottom plastic. Focus level can be copied to the other positions in the well.

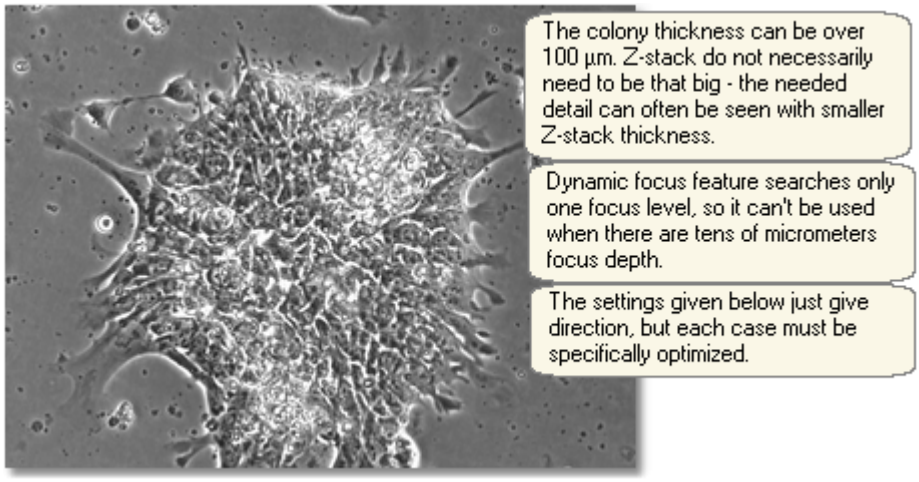
Special about this application

- Z-stack: 45µm. It should be so big that cells in well bottom stay focused.
- Dynamic focus: No. (The focus would adjust away from the cells towards the filter membrane.)
- Use an image grid, such as 3x3 or 5x5.
- Focus all wells manually. If the quality of the plate is good, z-values can be copied within a well.
- Adjust focus manually every 2 days.

Cycle time

- 1 hour with 24 well plate

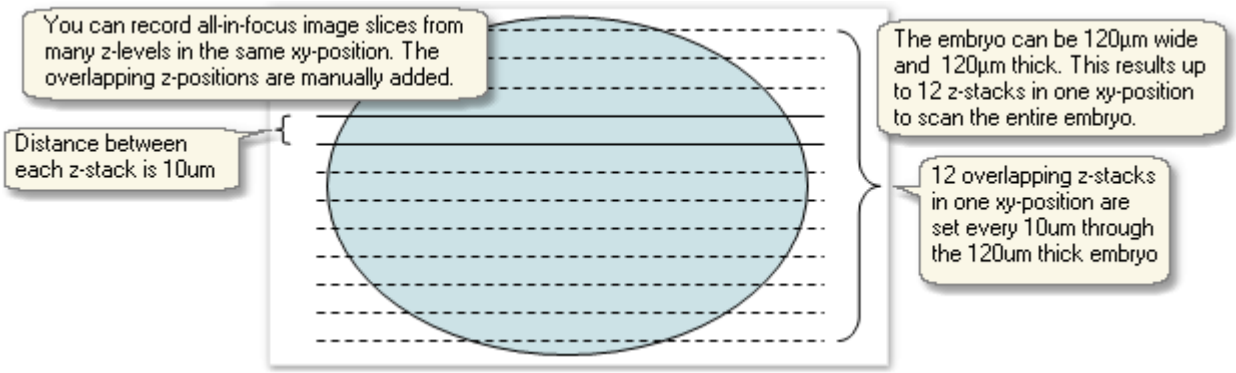
4.5 Cell colony assay



Special about this application:

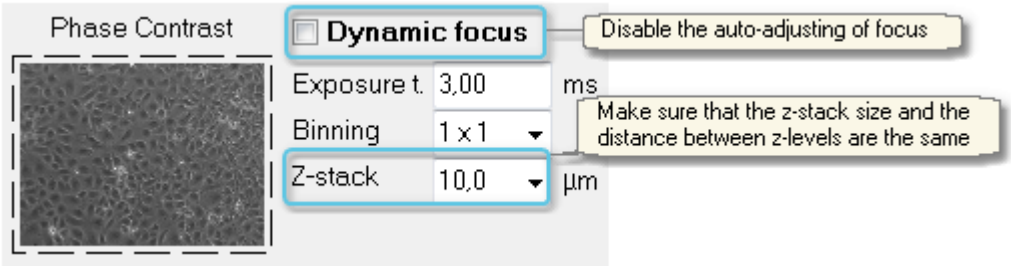
- Z-stack: 22.5-150µm, depending on the colony thickness.
- Dynamic focus: Yes if colony thickness <20µm. In thicker colonies not recommended.
- Automatic focusing works in <20µm thick colonies, in thicker colonies not recommended.

4.6 Embryos and oocytes assay



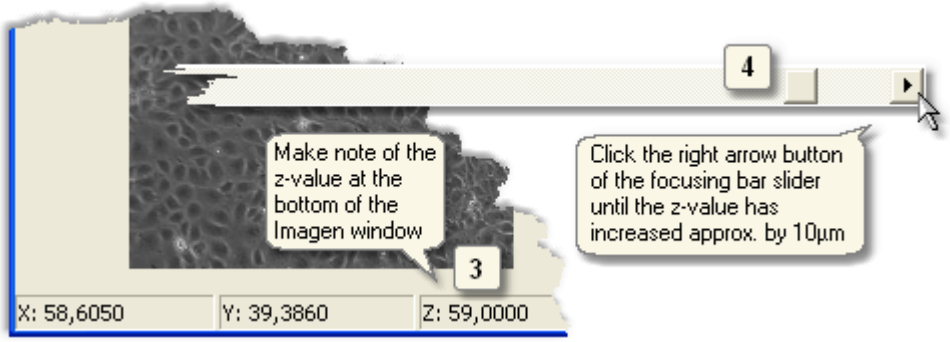
Special settings

- All-in-focus Z-range is set to 10µm. Larger stack would result into smudgy images due to too much information.
- To scan the entire embryo, preferable distance between the 12 Z-levels should be 10µm, as the embryo can be 120µm thick.
- The Z-stack size and the distance between Z-levels must be the same (10µm), otherwise some information may be lost as the stacks would not cover the entire Z-range of the embryo.
- It is important to disable the Dynamic focus during cycle –option as the 12 set Z-levels would probably drift into a bundle. Make sure the checkbox is empty!
- Grids cannot be used, 1-3 single positions in each well is acceptable. Using grids with 12 overlapping positions would create an unmanageable result folder structure.

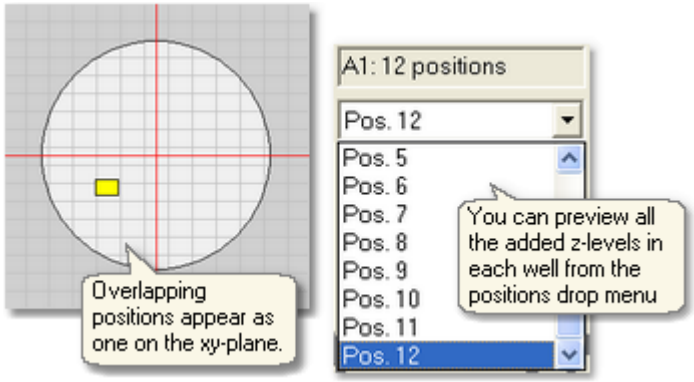


Adding the overlapping positions manually

1. Focus into the well bottom. At this focus level, no details of the embryo are visible.
2. Click ***Add Pos.*** button.
3. Check the Z-value in the lower left corner in Imagen.
4. Click the right arrow (UP) button of the focusing bar slider until the Z-value has increased approx. by 10µm.



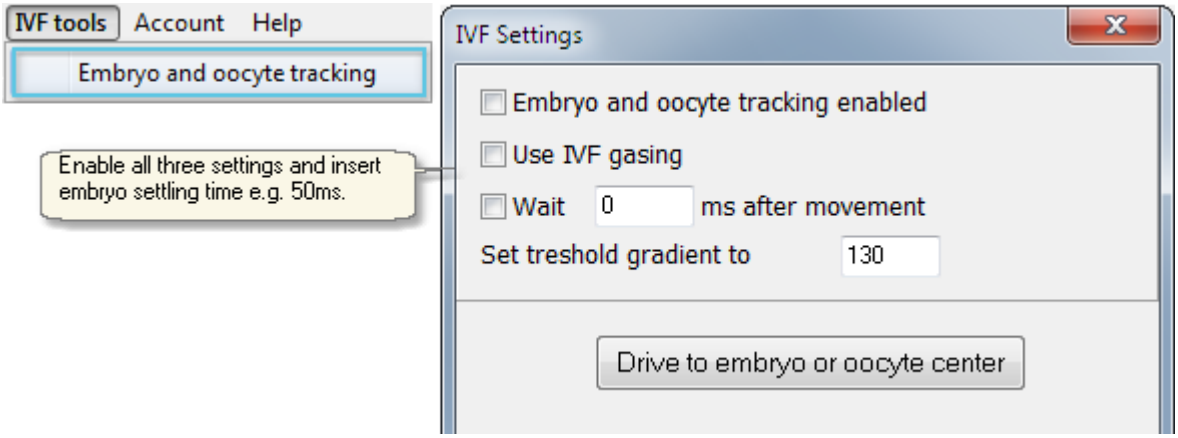
5. Click ***Add Pos.*** again.
6. Find the next Z-level 10µm up.
7. Repeat until all 12 positions are added and save the cycle.



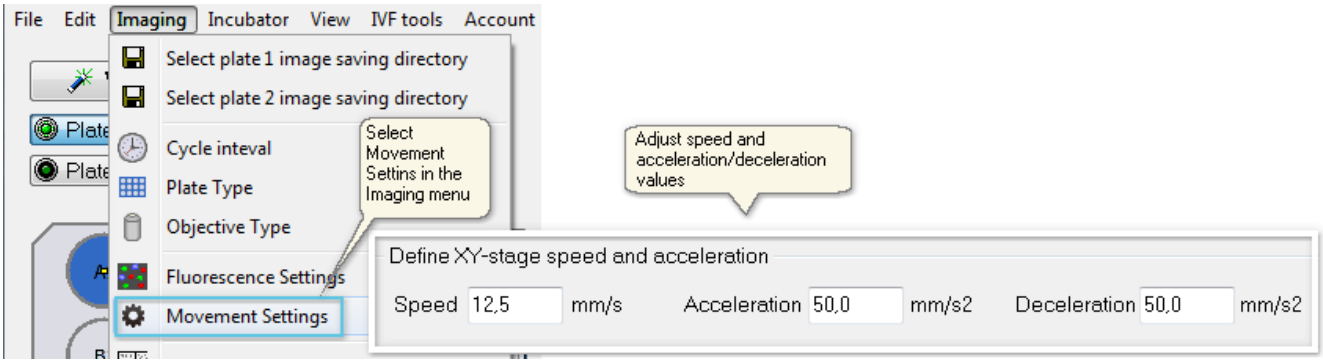
Advanced IVF settings

To apply advanced IVF settings, go to ***Embryo and oocyte tracking*** in the ***IVF tools*** menu.

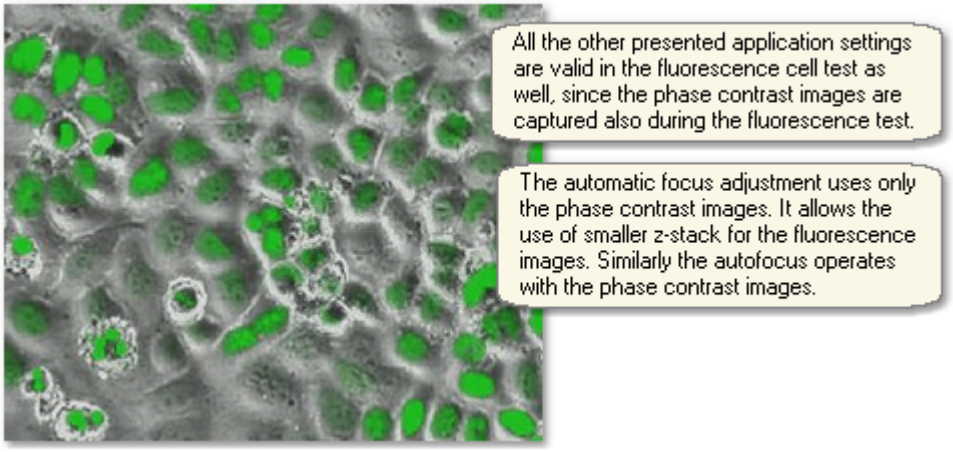
- Embryo tracking ensures the embryo is always in the image, even if it moves due growth or G-forces.
- IVF gassing changes the automatic gassing setup to full time gassing.
- The short wait after movement allows the embryo to settle down after deceleration.



- Adjust the speed and acceleration/deceleration values to assure embryo stays in view. Lower the default acceleration and deceleration value if the embryo is very mobile.



4.7 Fluorescence assay



Settings for fluorescence channels

- Z-stack: Typical 5um and 1-20um still normal. Stacks over 20um can expose cells to excessive illumination. Dynamic focus does not use fluorescence (FL), so small FL stack can be used, but if sample is thick, big stack guarantees that focused images are captured from all parts of the sample.
- The sample's exposure to the fluorescence excitation light can be minimized not only with the small Z-stack size but also with large camera pixel binning value, which on the other hand lowers the image resolution. (E.g. 2x2 binning means that 2x2 pixel group in camera CCD chip is combined before digitalization.)
- A focusing level offset can be set for each fluorescence channel, if the best focus level for fluorescence is different from the phase contrast.

Setting up cell test

5 Setting up cell test

There are two ways to setup the cell test in the Cell-IQ Imagen program:

1. The easiest way is to run Wizard, with the Wizard button in main window, and then select the well positions and start imaging.
2. The advanced user can make all selections manually: Starting gas flow, selecting imaging settings from the main window and select the image saving directories from Imaging-menu. Additionally if the main window information panel shows that image capture interval or some other setting needs adjusting, the settings can be accessed from the drop-down menus.

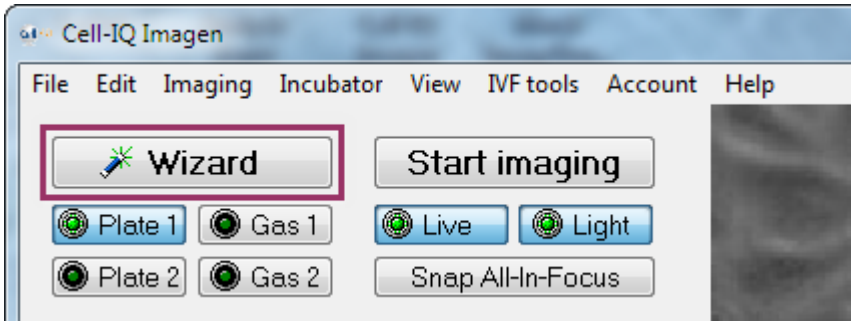
Start and stop imaging

After defining the cycles, click ***Start imaging*** button to start the automatic imaging cycle. All-in-focus images are saved to result folders selected earlier in the settings wizard, and they can be viewed with ***Cell-IQ Analyser*** program during image capture or anytime afterwards. If manual stop method was selected, stop the imaging cycle by clicking ***Stop imaging*** and wait when the image capture stops.

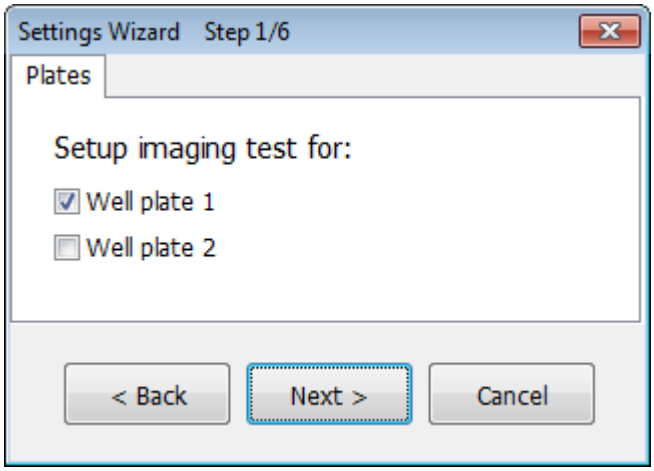
5.1 Wizard

Settings Wizard will take you through the cell test settings selection easily and fast.

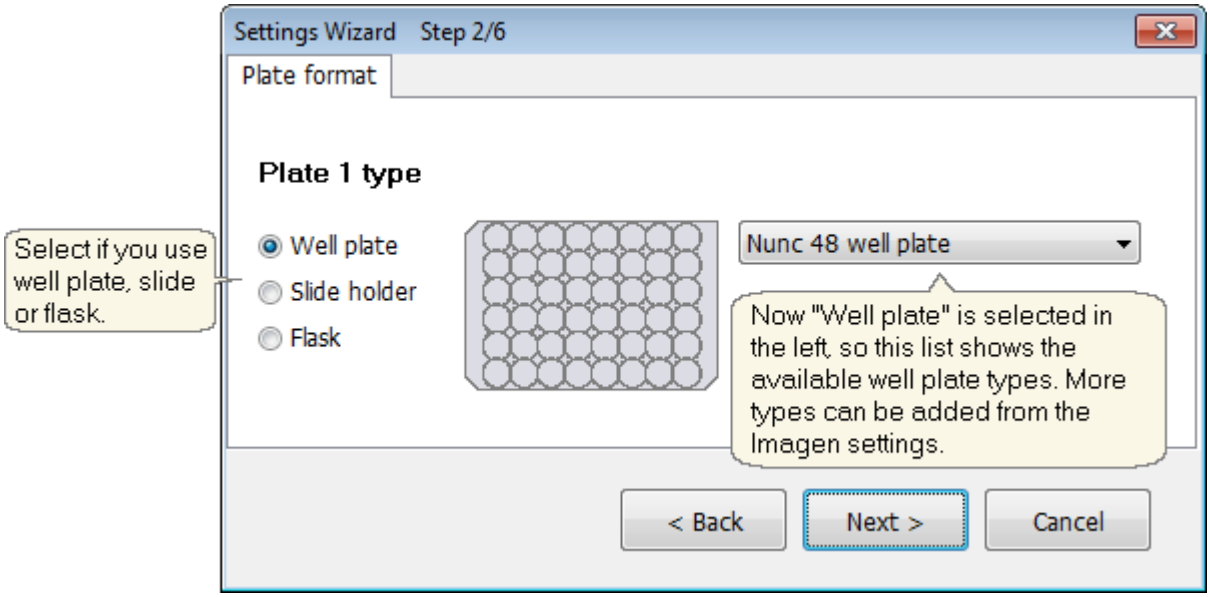
Start Wizard from the Wizard button in the main window.



Select if you have plate 1 or plate 2 or both of them in the imaging test.

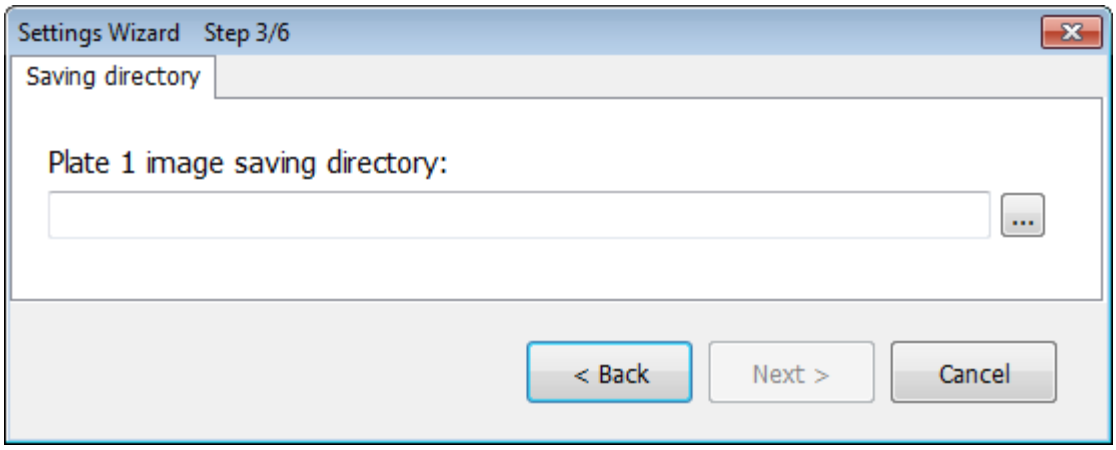


Select the type of the vessel. The dialog is shown once for the plate 1 and again for the plate 2, if both of them were selected earlier.



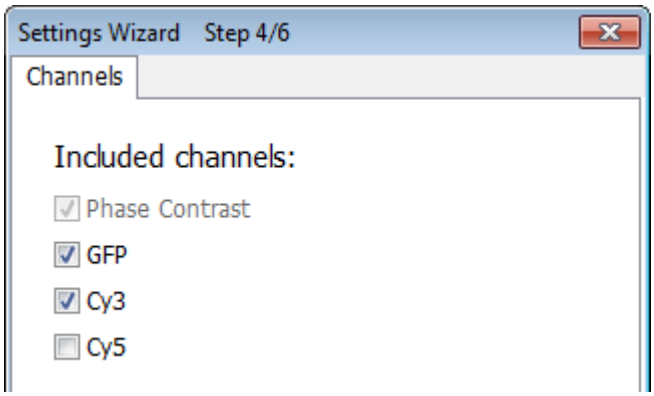
Select saving directory and the image saving format (jpeg or tiff) for the saved cell images.

- Also this dialog is shown once for the plate 1 and again for the plate 2.
- The same folder can be selected for plate 1 and plate 2, since the program automatically creates "Plate1" and "Plate2" subfolders.

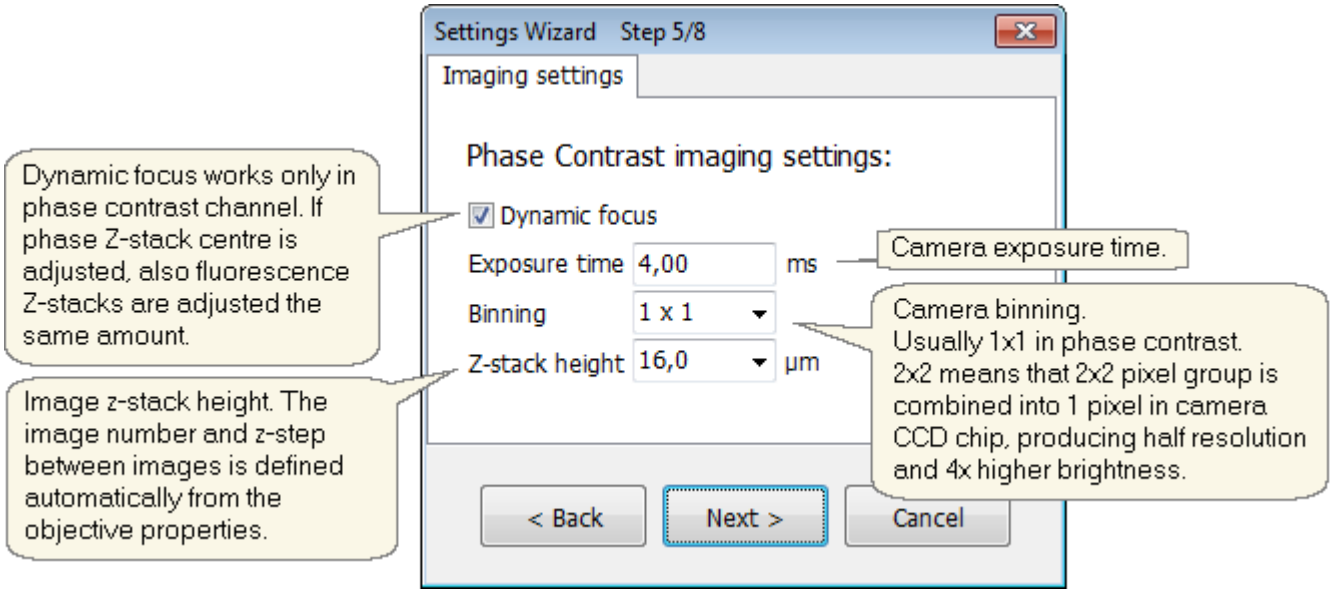


Select which fluorescence channels are included and therefore what imaging parameters will be selected later in Wizard.

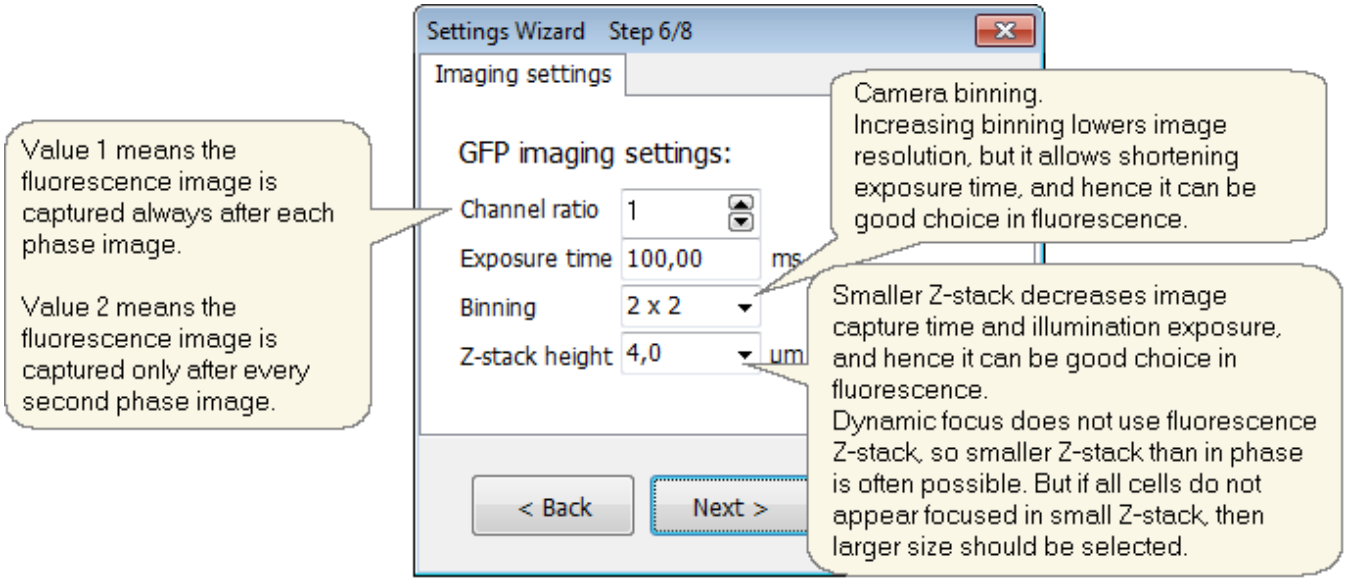
- The phase contrast is always included.
- You can change these also after the Wizard is completed.



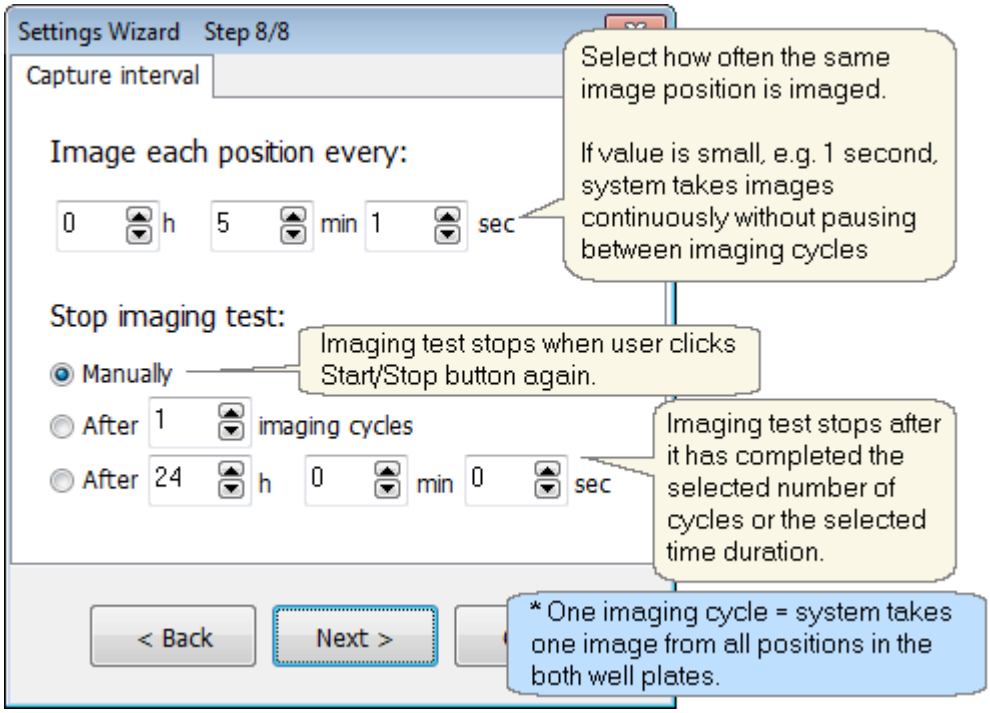
Select the imaging settings for the phase contrast image capture.



Select the imaging settings for the fluorescence image capture. This window is shown once for each fluorescence channel.



Select image capture interval and cycle stop method.

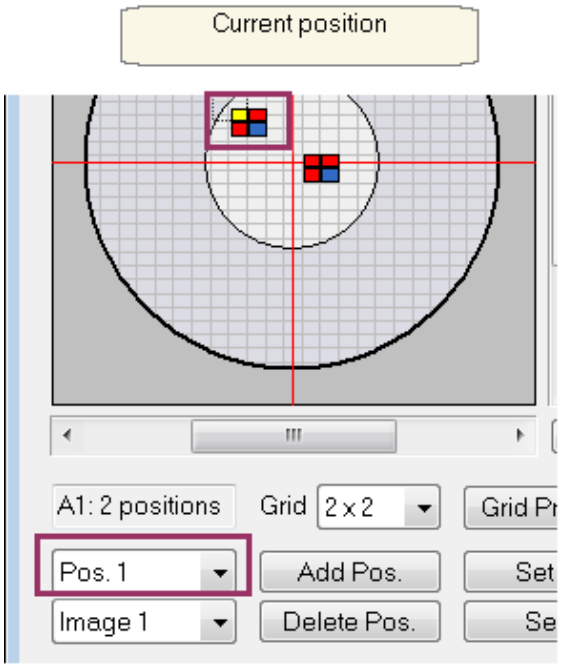


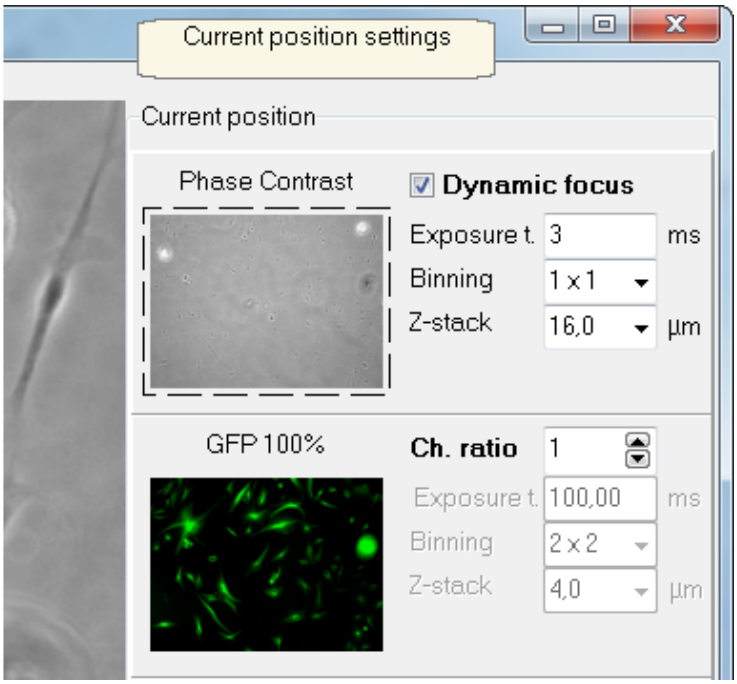
After this the Wizard closes and you need to add the image positions to the well plate 1 and 2.

5.2 Current position settings

The current image capture settings are at all times displayed in the main window.

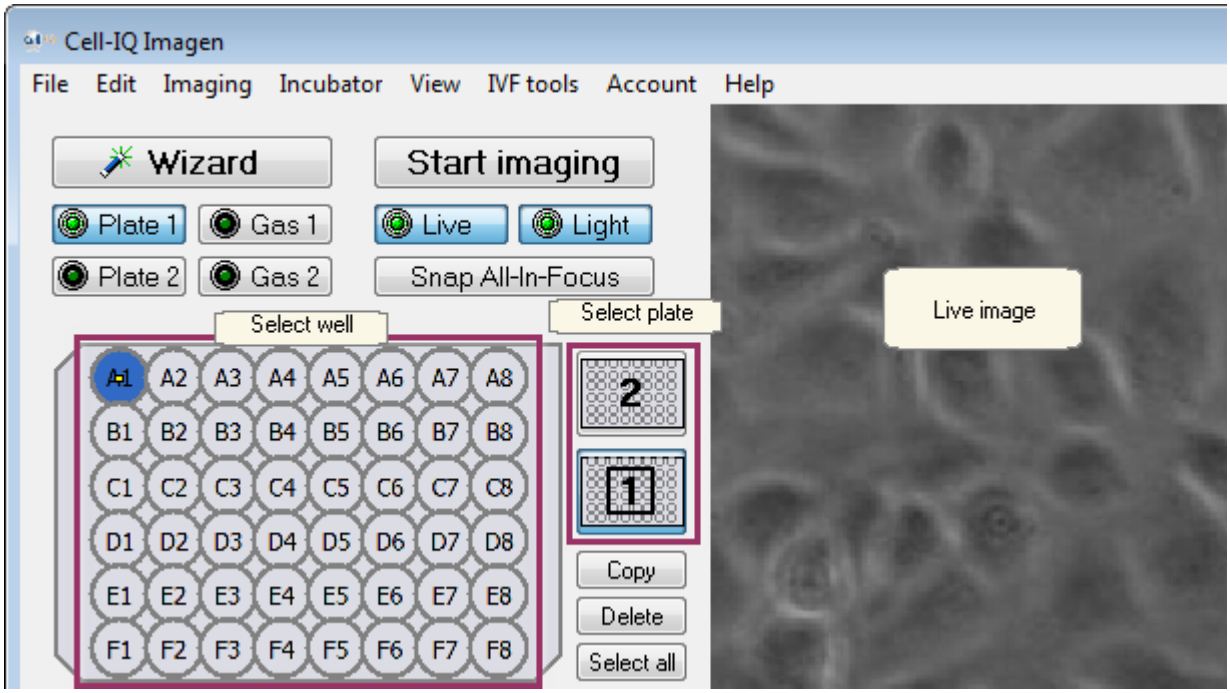
- If you select image position, the settings fields and live image are updated with the settings of that position.
- If you change the values in the settings fields, the settings are immediately saved for the currently selected position.
- If you do not have any positions in the well, the displayed settings are just for the live image and for the next position you will add.





5.3 Previewing cells in plate

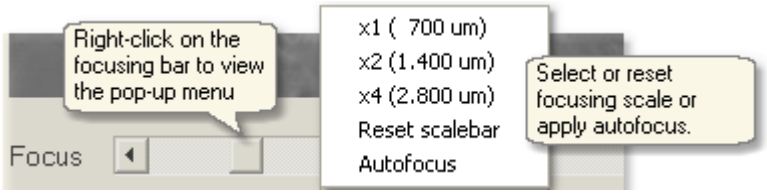
Select well plate 1 or 2 and click any well to see the cells in the center of the well



Manual focusing and Autofocus

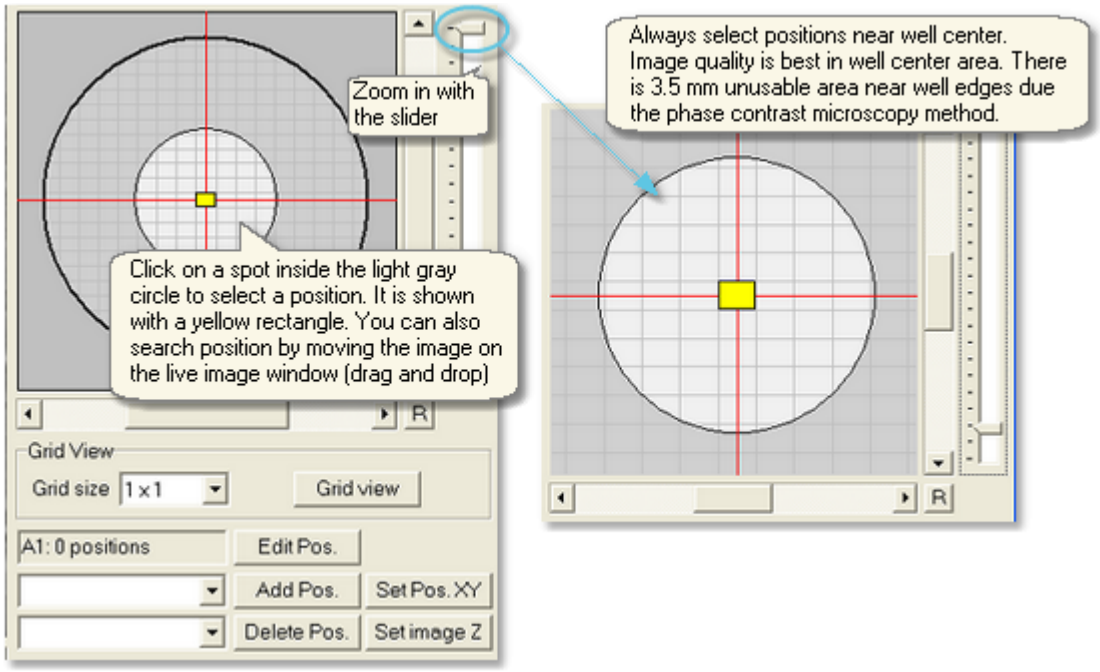
The live image can be blurry before focusing. Focus the image manually using the focusing bar. Hold down left mouse button and move the slider until the image is in focus. The image view is refreshed when you are not moving the slider.

You can select deeper focusing area using different focusing scale. Right click with mouse over the focusing bar to view the pop up menu. The options are 1, 2 or 4 times the scale of 700µm. With deeper area (2 or 4 times the scale), single step in focusing bar corresponds to bigger focusing step size. The focusing bar slider is centered when the scale is changed. You can also find the focus using Autofocus.



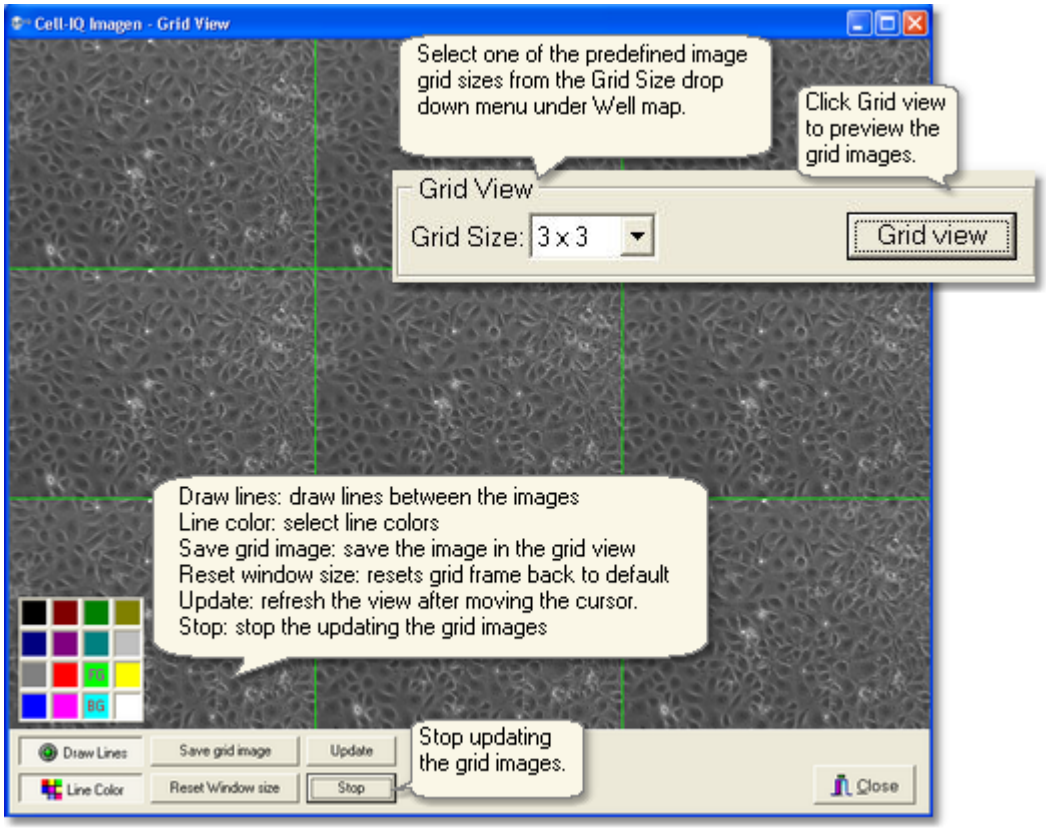
Moving around in the well

Click on a spot inside the light gray circle to choose XY-offset from well center in the well plate control panel. You can also move around with mouse using the live image display, see directions. The position is shown with a yellow rectangle.



Grid view

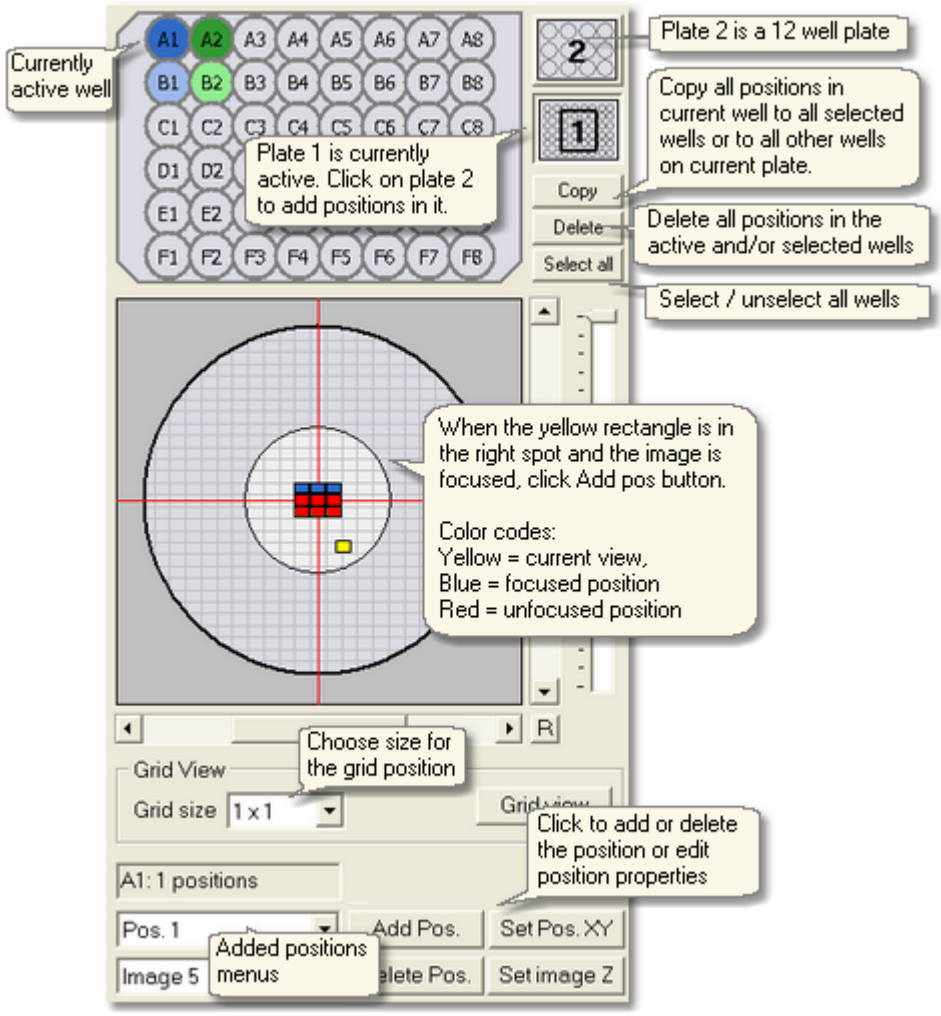
You can see larger field with the Grid view button, which is located below the Well map. E.g. 3x3 grid is formed by nine images next to each other. Clicking on the grid will drive the camera to that location.



5.4 Adding image positions

In the simplest form, you can add the image positions with these simple steps:

1. Select a well by clicking it on the plate map. Select location from well map.
2. Focus the image manually using the focusing bar slider.
3. Add the position by clicking ***Add pos***.
4. Copy the position to all other wells by clicking ***Copy***.
5. Repeat for plate 2.



Ensure good focusing

- Save the good focus Z-level manually for one position in the first imaged well, in every imaging test! Otherwise autofocus will not know where to start.

- First focus, only after that click Add pos. button!

1. Select the first well that has cells in it by mouse left-clicking the well plate map.
2. Move the focusing bar slider until the image is in focus.
3. Save the position by clicking ***Add pos.*** button (also Z-level is saved). You can still later save refined focus with the Set Z button.

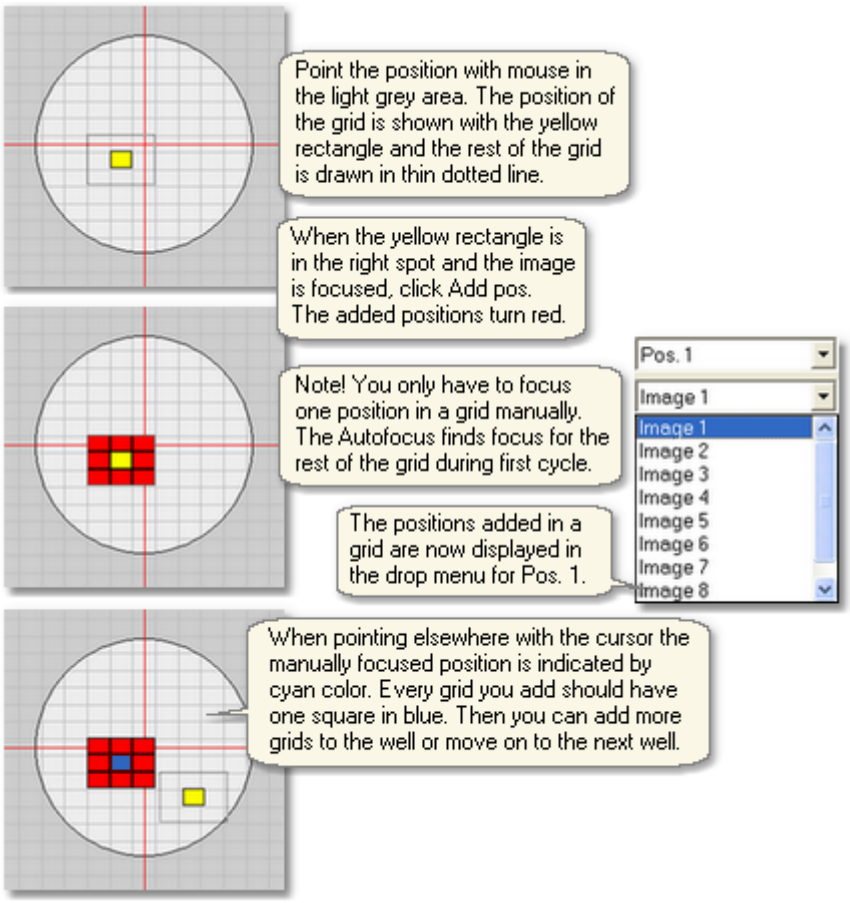
Adding custom positions in a well

For each well and each position in that well, the three steps are repeated to add the positions to be imaged:

- **Point position in the well:** Click a position inside the light gray circle or search position by moving image in the live image display to choose XY-offset from well center. The current position is shown with the yellow rectangle. You can move around in the well by left-clicking the well image or the main image display.
- **Check that first position to be added is in focus:** Focus the image manually using the focusing bar slider.
- **Save the position:** When the yellow rectangle is in the desired XY position and the image is focused, click ***Add pos.***

Repeat these three steps for all positions in a well. Move on to the next well by clicking on it in the well plate map. Always select positions near well center where the image quality is best. Parts of the well at the edges should not be used because of the lower contrast due to the phase contrast method. The width of the low contrast area near well edge is roughly 3.5 mm

Add grid positions

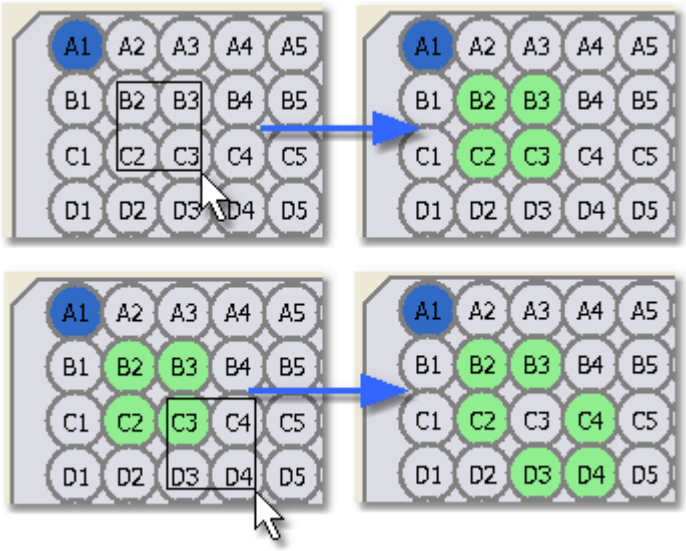


The same can be repeated for several positions in a well. You can select more positions with various grid sizes or combinations of single positions and image grids. All added positions in the source well, including grids, can be copied to other wells with ***Copy*** button. A whole grid position can be removed with ***Delete Pos.*** button.

Note! When using Nunc 6 well plates, focus one image manually in each grid you add. The well bottom z-levels can have more than 200µm differences between the wells.

Operating on a selected group of wells

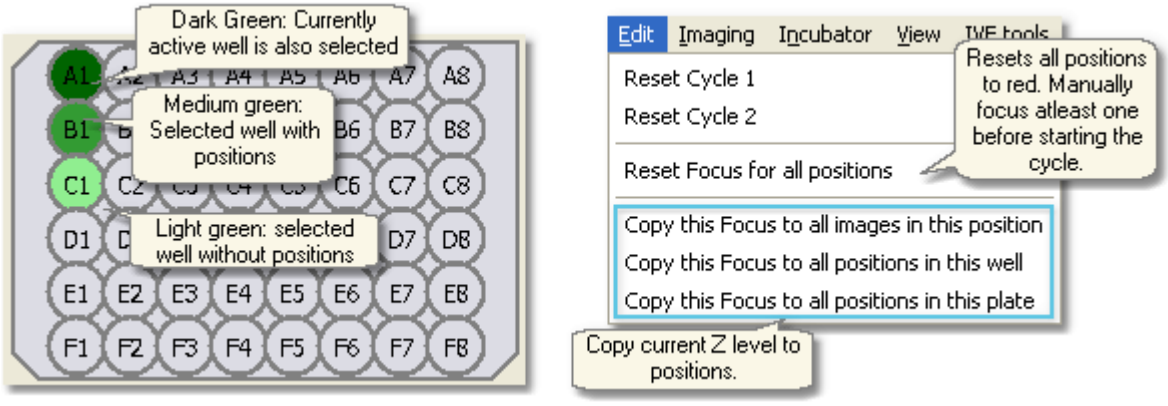
- Select or unselect wells by pressing left mouse button down and dragging over the plate image. A selection rectangle will show selected wells. Each selected well will turn green, light green if there are no positions or medium green if there are positions or dark green if current well is among selected wells
- You can select and unselect at the same time.
- The ***Copy*** and ***Delete*** functions can be applied to the selected wells in all these three cases. E.g. Click the well C2, add a position as described above. Hold down shift key and click wells C3, C4, C5 and apply Copy function. The positions in well C3 are copied only to the other three cells. To clear the positions on a plate quickly, hold down control key, click any well and apply ***Delete***. To clear only a selection of wells, hold down the shift key, click the wells to be cleared and apply ***Delete***.



Copying positions

Click ***Copy*** to copy all positions in current well to either to all selected wells or if none selected, to all other wells in the active well plate. This does not copy the focus (Z) values, only the XY-coordinates, but Imagen will automatically find focus for the copied positions during first imaging cycle. You can copy current Z level to positions via ***Main Menu - Edit***.

- The position can't be copied to wells in the other well plate. (But entire plate can be copied: Edit->Copy Plate...)
- The wells that the position is copied to, turn light blue on the well plate map.
- If there are already selected positions in other wells:
  - By selecting ***Yes*** the previously selected positions are replaced with new positions.
  - By selecting ***No*** the new positions added to the selected wells while preserving the existing positions.
- When positions are copied from one well to another, also the imaging specific settings are copied.



Deleting added wells

To remove all positions in a well or a group of wells, select the wells and click ***Delete***.

Changing position coordinates

To change the coordinates of a added position, point the new position with mouse on the well image and click ***Set XY***. The position is moved. The procedure is the same for image grids. Only an entire grid can be moved, not single images in grid.

Changing Position Z-level

To set or change current Z-level as the position's or image's focus click ***Set Z.*** Only single image focus is set. To set focus for an entire grid use the ***Edit - Copy this focus to all images in this position*** menu item mentioned above.

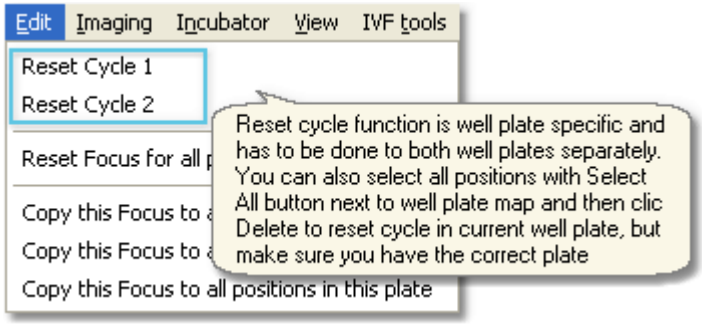
Manual focusing of all positions

Auto-focus can be disabled by selecting focus manually for all the positions in the well plate. Drive to each well and position one by one by clicking them with mouse, and adjust and save the Z-level as explained above in the "**Changing Position Z-level**".

Removing one position in a well

Point the well in the well plate map. Added positions in that well are displayed with colored squares. Select the position you want to delete by clicking it or from the drop menu under ***Delete Pos.*** button. The yellow rectangle is placed on the position. Click ***Delete Pos.*** to remove the position. Only an entire grid can be deleted, not single images in grid.

Removing all positions in a plate



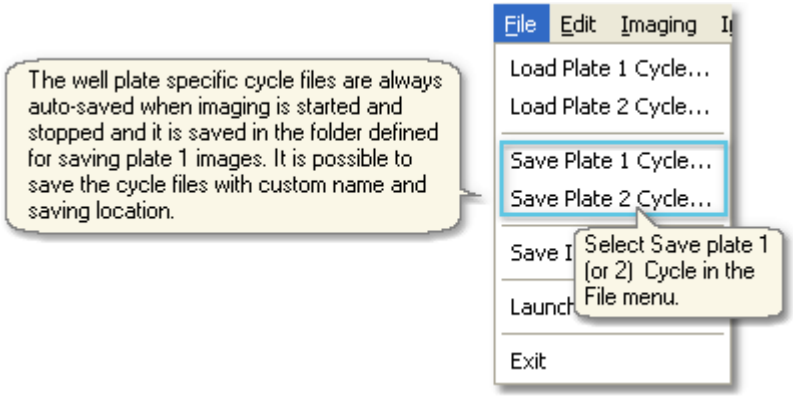
Reset cycle by selecting ***Reset Cycle 1*** or ***Reset Cycle 2*** from the ***Edit*** menu. Choosing ***Reset Cycle 1*** will delete all positions from all wells in the well plate 1. Or click ***Select all*** button to select all wells in current plate and ***Delete*** to delete all positions.

5.5 Saving and opening cycle

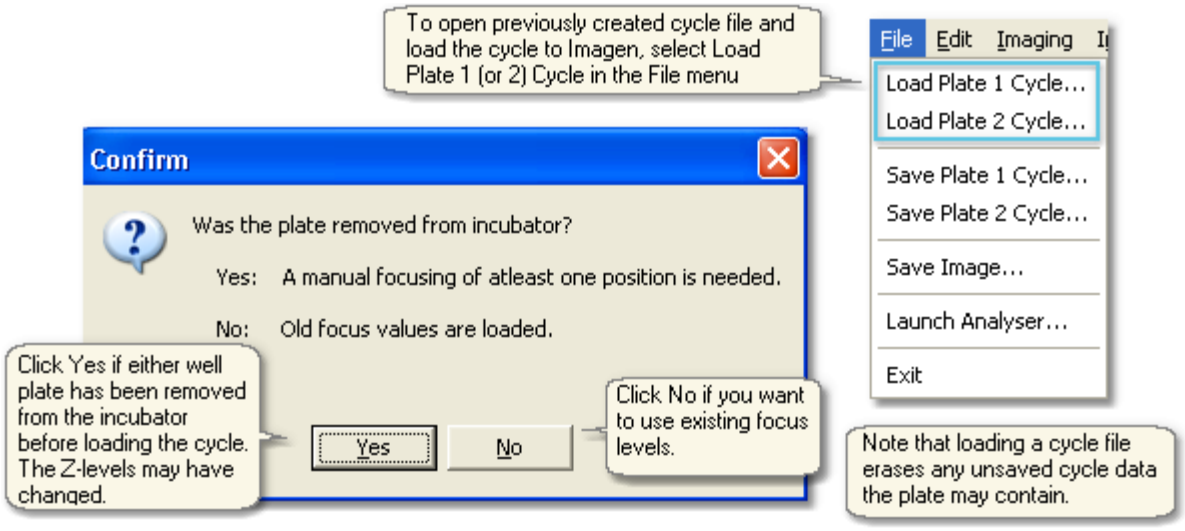
The cycle*.dat file includes the selected image locations from wells and their imaging settings.

- The imaging positions from the previous cell test can be easily restored and reused by opening the cycle file.
- The image preview in the Cell-IQ Analyser program is more convenient with the cycle file than without it, since with cycle file you can see the grids, not just number of single positions. Also the image resolution information is restored from the cycle file to the Analyser.
- The cycle*.dat file is automatically saved for each plate when the Start imaging and Stop imaging imaging button is clicked. Automatic imaging saves new Z-levels for the imaging positions, and hence the cycle is different when the Stop imaging imaging button is clicked.
- The cycle*.dat file can also be manually saved or opened, see below.

Saving cycle



Loading cycle



Open a saved cycle from ***Main Menu - File - Load Plate 1 or 2 Cycle***. Locate the ***.dat*** file you wish to load with the dialog box and click ***Open***. If the plate has positions, there will be a confirmation box to assure you want to delete current positions.

- If the well plate was not removed, click ***No***. The saved focus values (Z-levels) are loaded. Positions stay *blue*.
- If the well plate was removed, click ***Yes***. The old focus values are not loaded. Positions are displayed with red and automatic imaging will use autofocus. You need to manually set focus for the first position in the first well, so that the autofocus has the starting point.

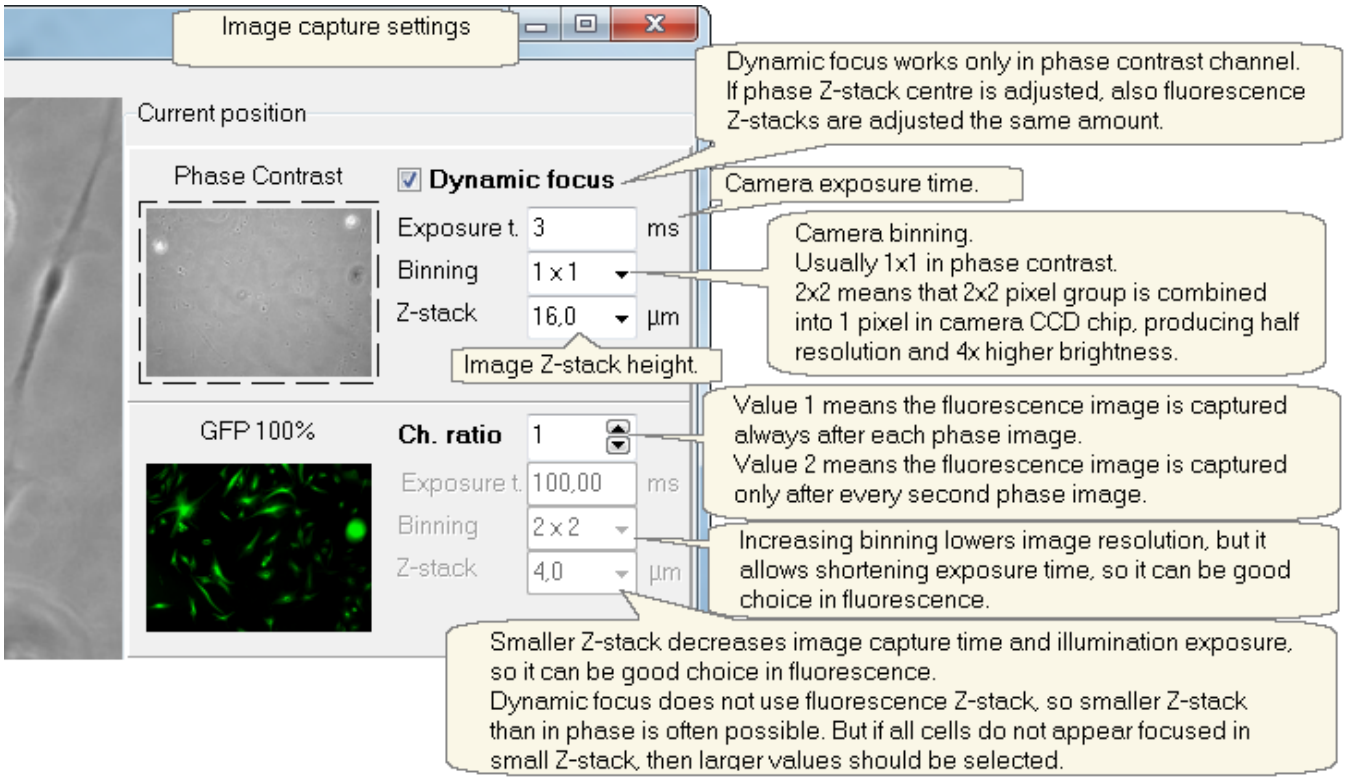
Settings

6 Settings

- Some settings are selected from the Cell-IQ Imagen main window and others can be found through the drop down menus.
- You can find more guidance for settings selection from the Cell-IQ applications and the Wizard chapters.

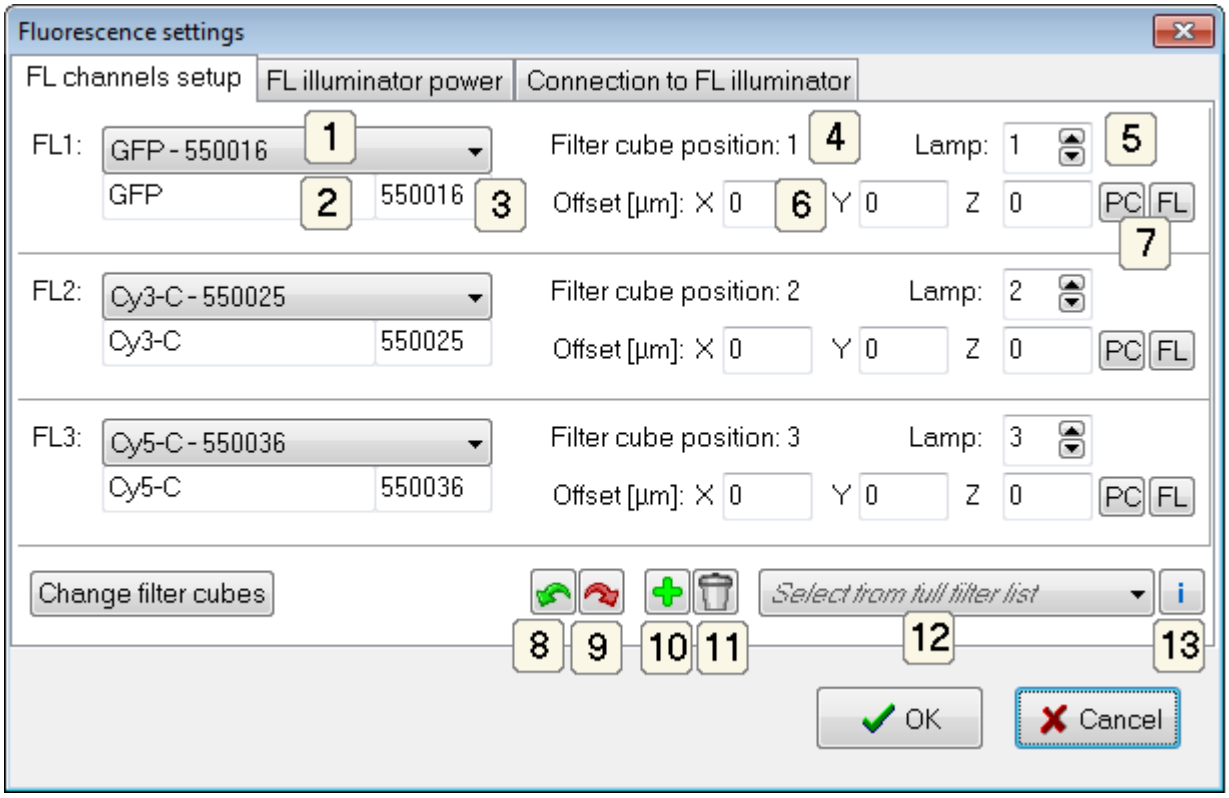
6.1 Image capture settings

The image capture settings are at all times displayed in the main window. The values are saved and applied automatically when user edits the fields.



6.2 Advanced fluorescence settings

To change the advanced fluorescence settings, click ***Fluorescence settings*** in the Imaging-menu.

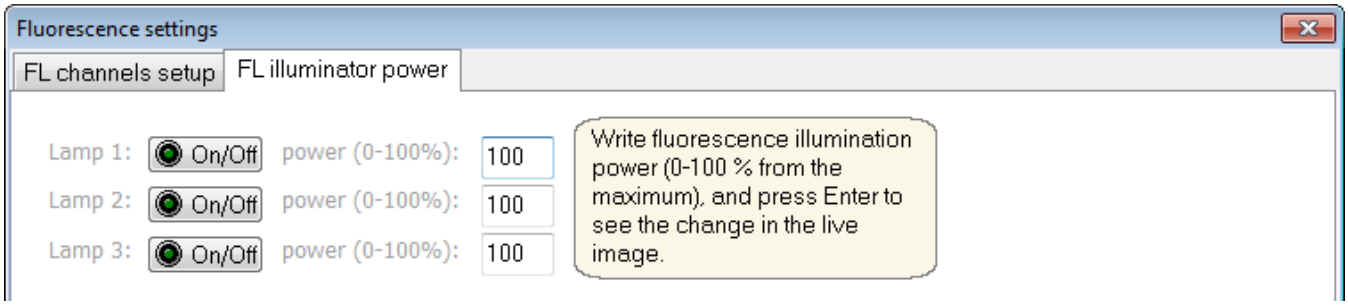


1. **Filter set selection for the channel:** The list includes the filter sets that are in use with this Cell-IQ. Select one of the sets for this FL channel. Also the phase contrast imaging uses the filter cube from channel 1, and hence the channel 1 must have GFP or other filter set selected that has the emission filter matching the green phase contrast illuminator.
2. **Filter set name:** The filter set name, which is also the channel name, comes can be here modified (and hence updated to the lists). The channel name is displayed over the little channel images on the program main window.
3. **Filter set ID:** The filter set ID number is referred when the filter set is purchased from the CM Technologies.
4. **Filter position:** The system has the three filter cube positions, counting from left to right in the filter changer. This shows where the cube is located for the channel.
5. **Light source:** The lamp from the light source that is used. Possible values are [1, 2, 3]. Usually these are 1=blue, 2=green and 3=red, but the illuminator can also have other lamp selection.
6. **X and Y offset:** The different fluorescence colors can have different XY pixels shifts in image. The phase contrast light is green, and if fluorescence emission has different color than green in channel 2 or 3, the light can shift compared to the phase contrast. The non-zero X and Y offset values make system take the image from different XY location for the channel. **Hint:** When the combined image is selected to the Imagen main window, it is updated with the new values real time when the offset values are changed.
7. **Z offset:** When the fluorescence signal is on a different Z level than phase contrast, you can define an offset. This number can be positive or negative. ***PC*** and ***FL*** -buttons can be used to set the Z offset. First use main form to find focus in phase contrast, open Fluorescence Settings and click ***PC*** button down (all three if necessary). Click ***OK*** and select a fluorescence channel and locate wanted imaging level. Open Fluorescence Settings again and click ***FL*** button down for the selected fluorescence channel. Repeat for each fluorescence channel you wish to configure. To reset the Z offset, you can click the channel's ***PC***-button or just type *0* to the edit-box.

8. **Import filter from the long list:** When new filter set is purchased, please select it from the list (12) and click this, so the filter can be selected for the channel from the shorter list (1).
9. **Hide filter from the channel selection:** Select filter from the long list (12) and click this to hide it from the short list (1). The filter still stays in the long list (12).
10. **Create and define new filter set:** When this is clicked, the new filter is added to the end of all the filter lists. Change the details by selecting it to one of the channels.
11. **Delete filter set:** Select filter from the long list (12) and click this to delete the filter from all the lists. The filter can not be restored later.
12. **List of all the filter sets available for the system:** The list has all the filter sets that can be purchased for the system from the CM Technologies.
13. **Filter set information:** Select the filter from the long list (12) and click this to display detailed information on that filter set.

Change the fluorescence illumination power

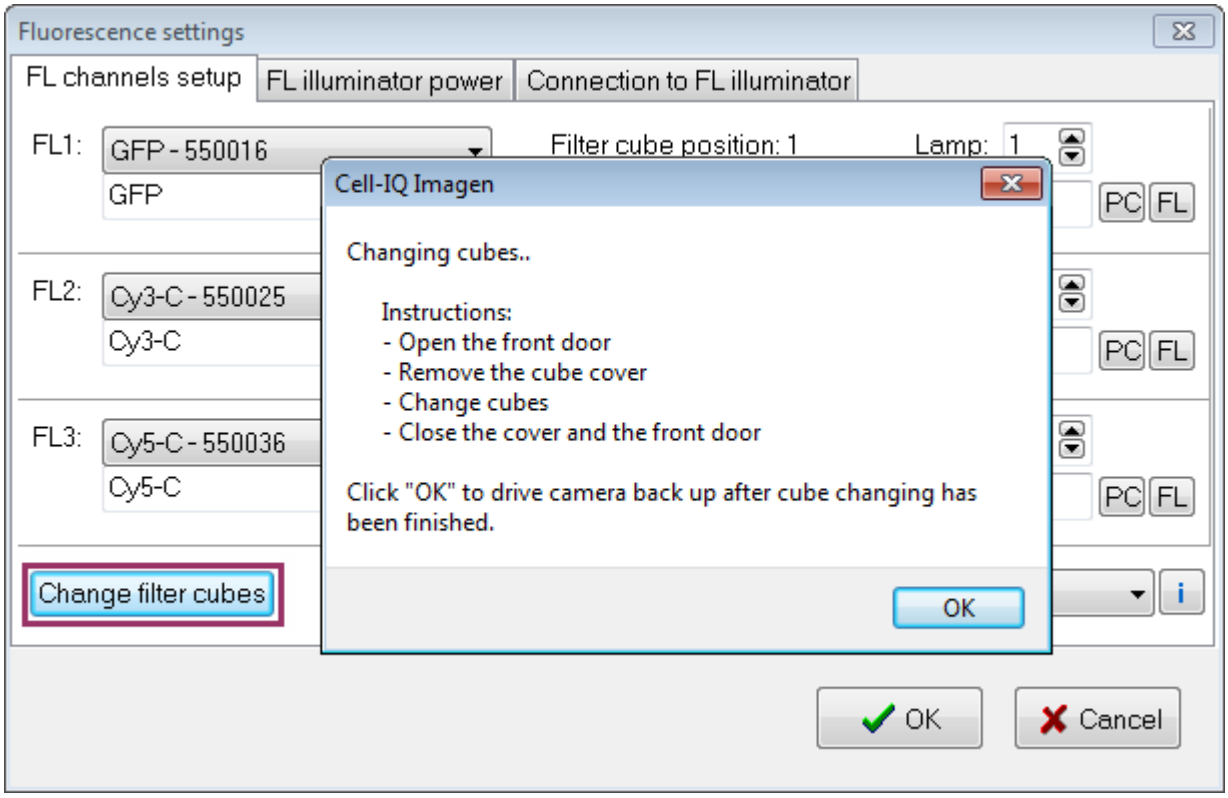
You can change the fluorescence illumination power from the second page in the *F*luorescence settings window:



6.3 Changing filter cubes

To change the filter cubes, click **Fluorescence settings** in the Imaging-menu and click ***Change filter Cubes.***

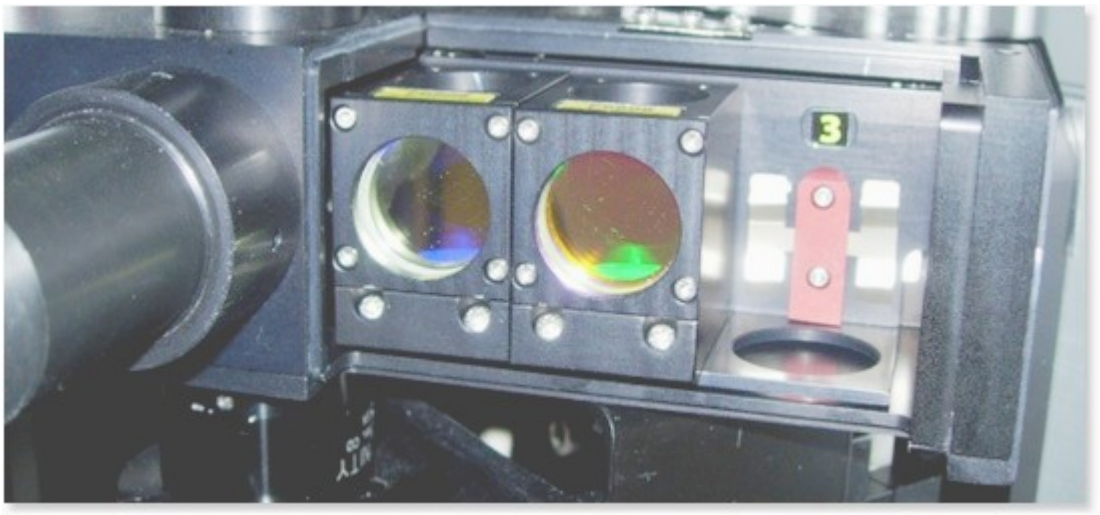
- Follow the instructions in the The Info-box and close it only after you have finished changing the cubes.
- Click ***Change filter Cubes*** drives camera down so you have better access to the filter cube changer.



1. Open the Cell-IQ system front main door to locate the cube changer

2. Remove the cover

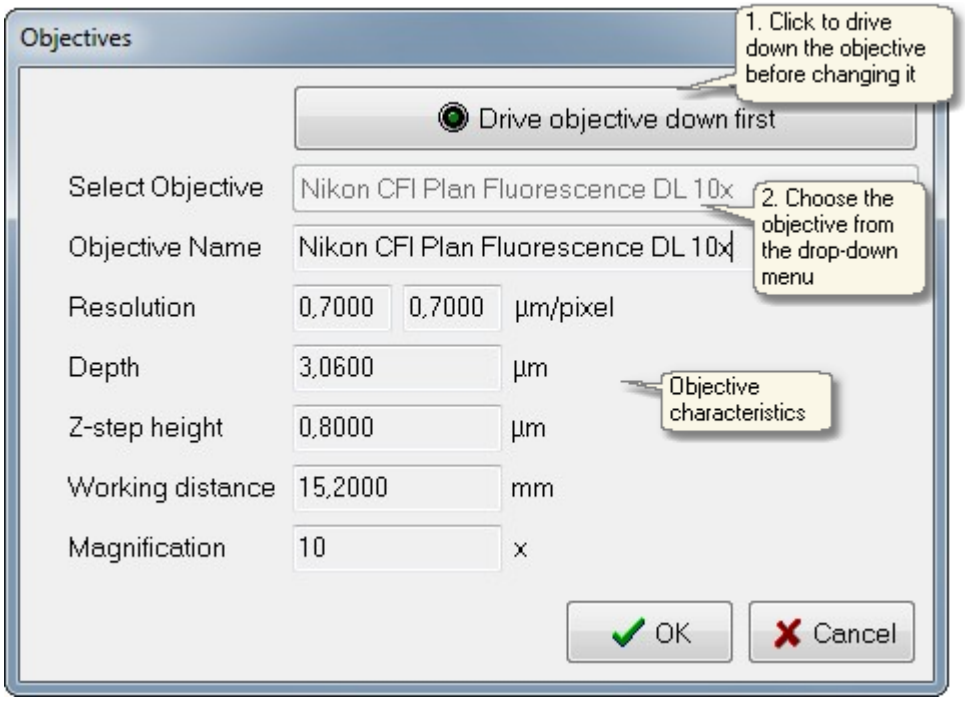
3. Carefully lift the middle or the right cube directly up. Avoid leaving fingerprints on the filters. Left cube is fixed



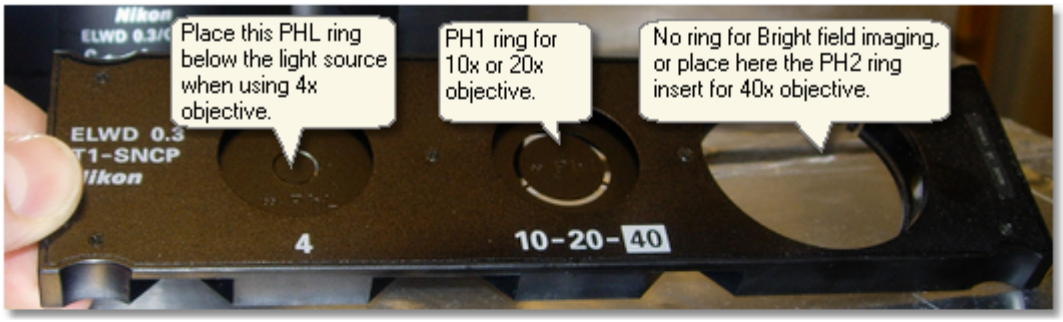
4. Insert new cube, close the cover, close the front door and click **Ok** button in the cube changing info field.

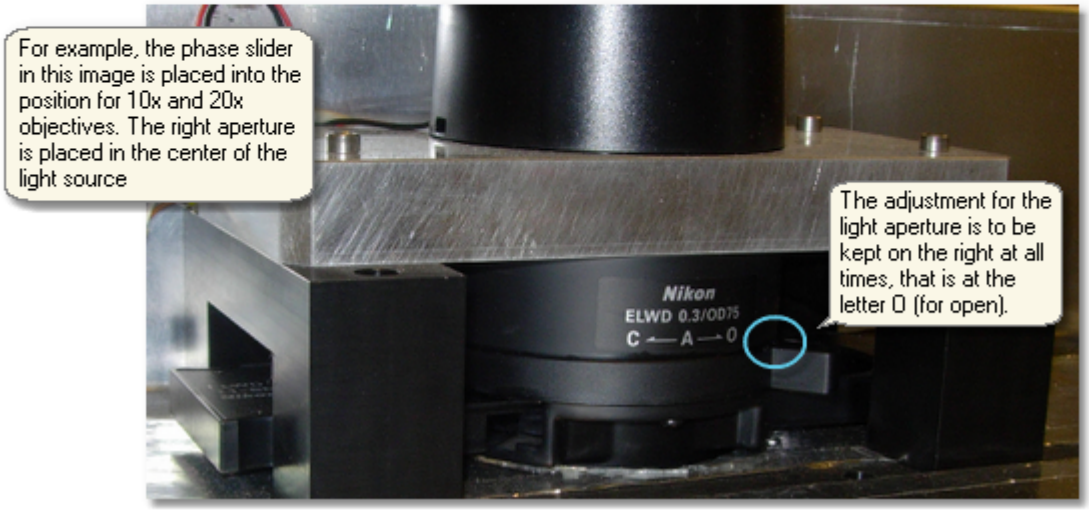
6.4 Changing objective

To change the objective, click ***Objective types*** in the Imaging-menu. Drive down the objective in order to change it. Choose an objective from predefined options in the drop-down menu:



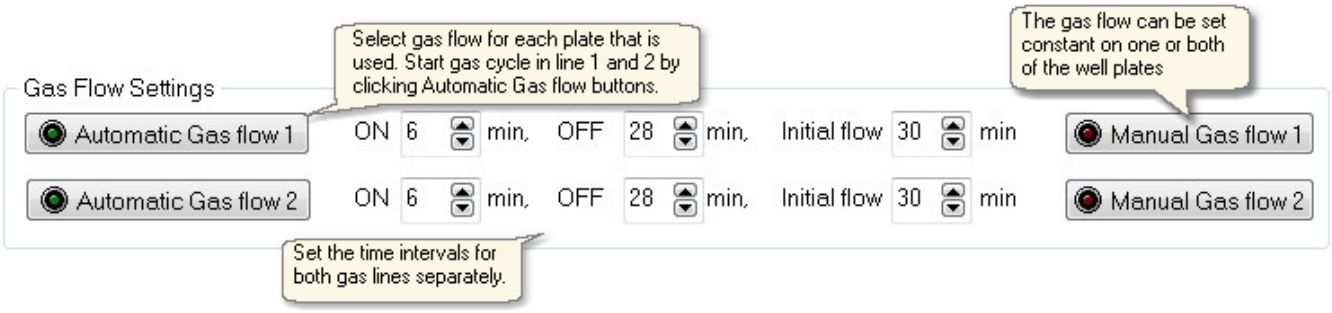
Note!: The phase slider has to positioned according to the objective. The correct position of the phase slider for 4x, 10x, 20x and 40x phase contrast objectives is shown in the image below of the detached slider. The right-most aperture is for bright field imaging and for 40x objective, which needs additional phase ring insert (PH2, not in picture).


Place this PHL ring below the light source when using 4x objective.
PH1 ring for 10x or 20x objective.
No ring for Bright field imaging, or place here the PH2 ring insert for 40x objective.
4
10-20-40


For example, the phase slider in this image is placed into the position for 10x and 20x objectives. The right aperture is placed in the center of the light source
The adjustment for the light aperture is to be kept on the right at all times, that is at the letter O (for open).
Nikon
ELWD 0.3/OD75
C — A — O

6.5 Gas flow settings

To change the gas flow settings, click ***Gas flow settings*** in the Incubator-menu.



The default gassing cycle is gas flow ON for 6 minutes and OFF for 28 minutes and the initial flow lasts for 30 minutes. Initial flow will flush the gas inside the plate at the start of the test. Edit the gas flow cycle if the cell types used require different settings that the default. The default settings ensure the correct gas control in most cases. A constant gas flow can be manually turned on for both plates separately. A red spot blinking on the settings window and in the program window indicates that constant gas flow is on.

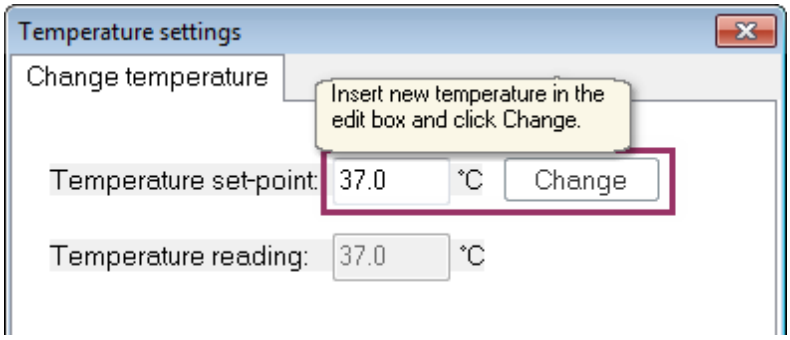
Flowmeters

The main window information field shows the real time reading of the gas flow for the plate 1 and 2.

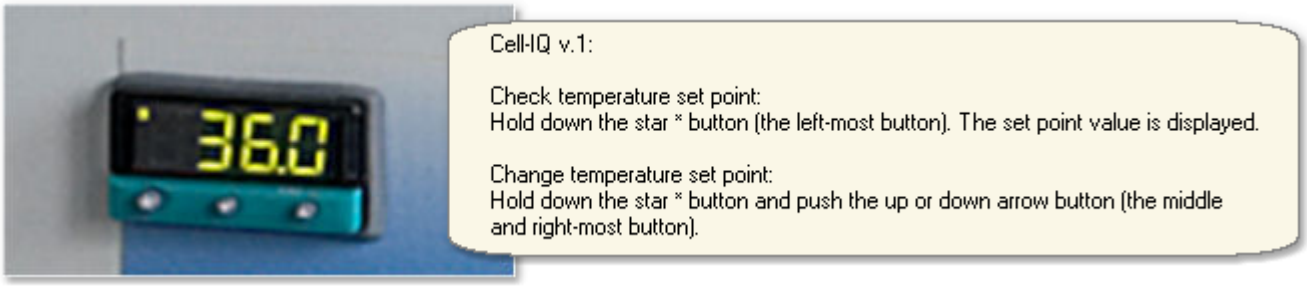
6.6 Incubator temperature

Temperature reading is displayed at all times in the main window info field. To change the temperature set point, click ***Temperature settings*** in the Incubator-menu.

- The temperature control range is 20-40ºC



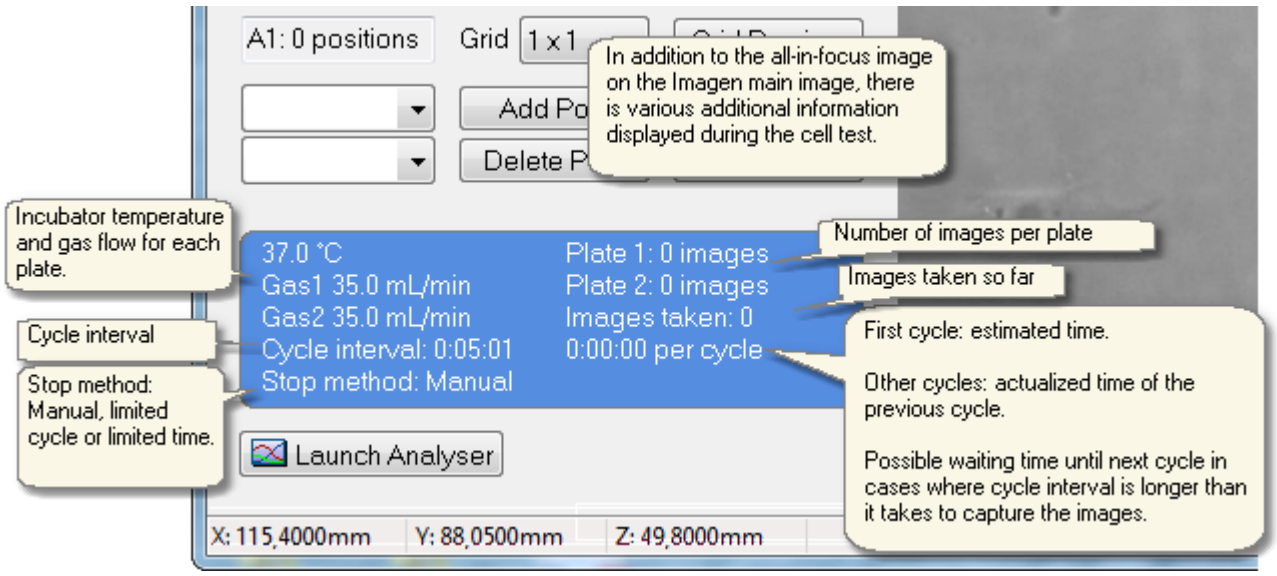
The Cell-IQ version 1. systems have direct access to the temperature controller buttons, and there you can change the temperature also directly from the controller:



Monitoring cycle

7 Monitoring cycle

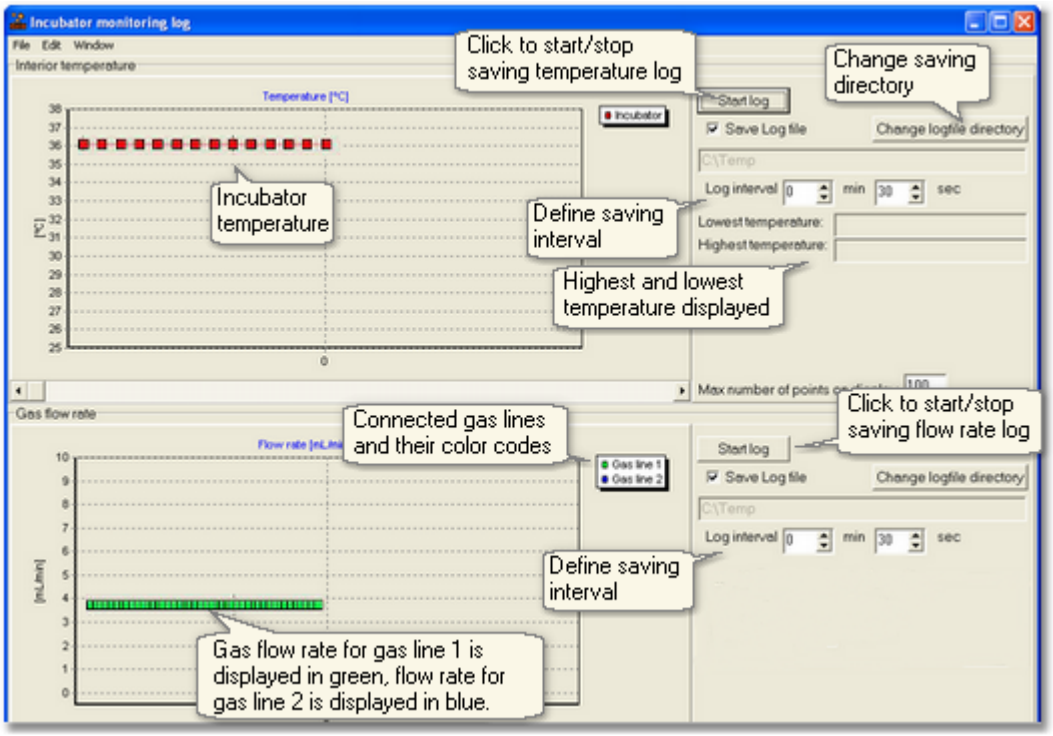
The info field displays information on selections and environment before and after the automatic imaging is started:



7.1 Environment charts

Temperature and gas flow log

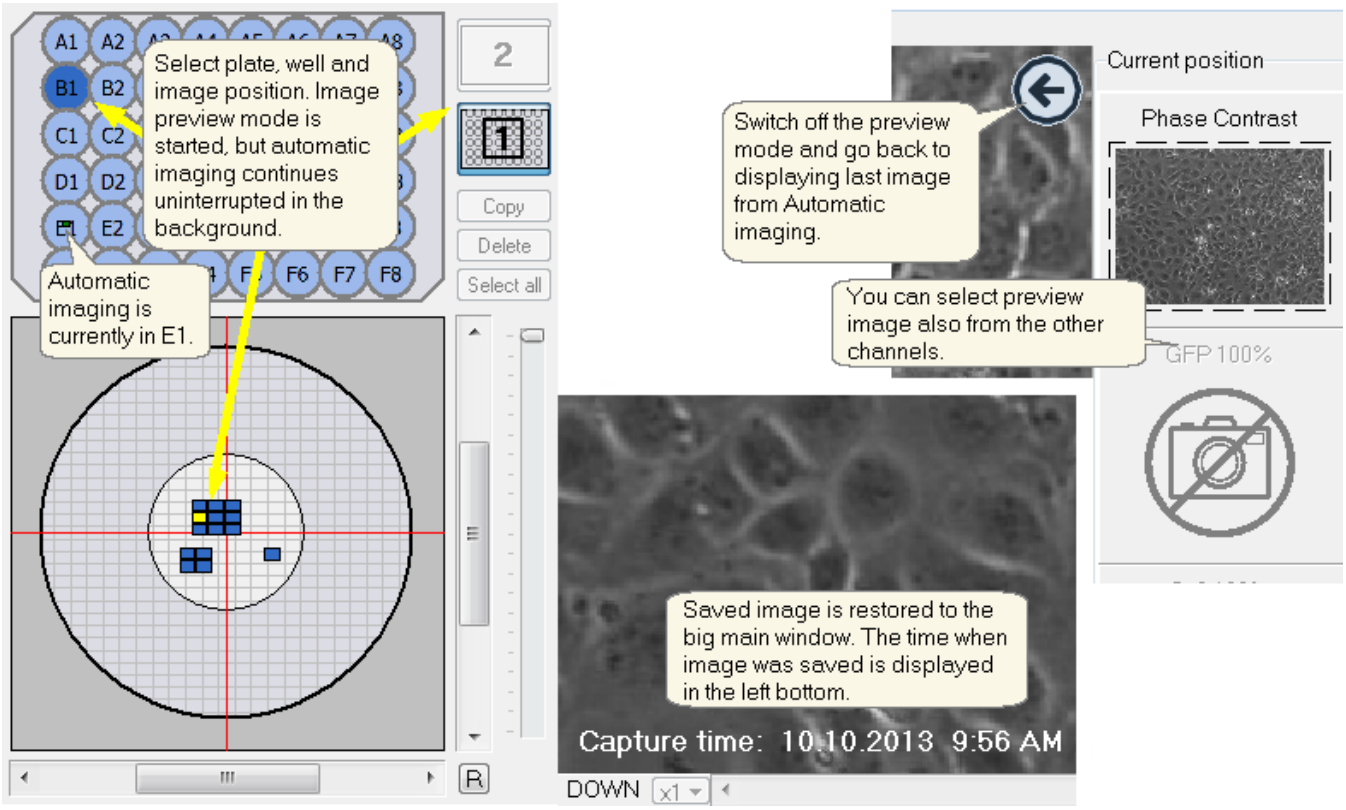
The incubator temperature and the gas flow rates are continuously displayed in the left sidebar in Imagen and can be saved into a log file. Click Incubator menu -> ***Environment charts*** to view incubator monitoring logs. Click ***Start log*** to start monitoring each variable. You can also ***Save Log*** into a file.



7.2 Image preview during cycle

The saved images can be previewed during automatic imaging

You can click the well and image position during automatic imaging to see the last image that was saved for that position.

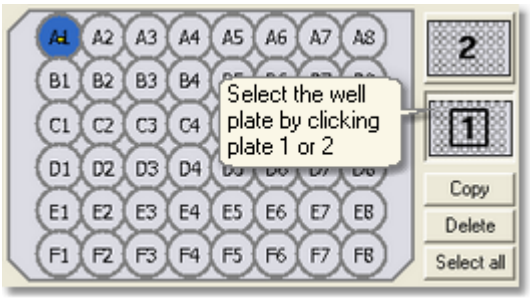


Changing medium

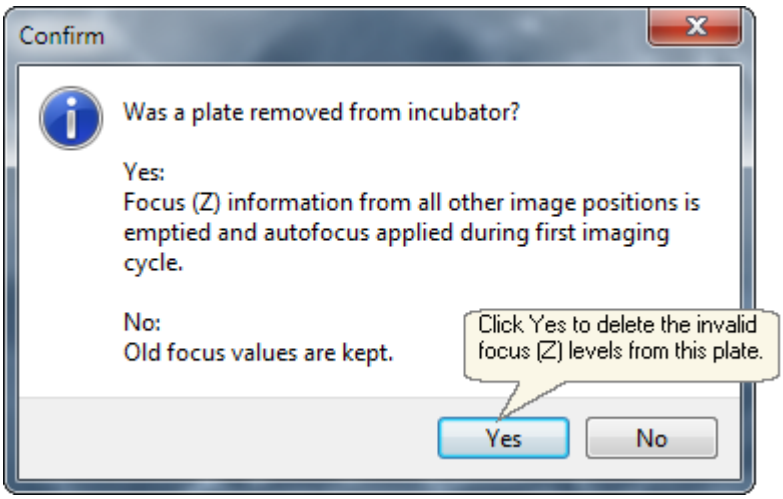
8 Changing medium

Follow these instructions to ensure that images have good focus also after the medium change.

1. Click ***Stop Imaging***.
2. Select correct well plate (1 or 2) in the well plate map.



3. Stop gas flow for that plate.
4. Remove the well plate, change medium and insert well plate back into the Cell-IQ. See the instructions for accurate plate insertion from the Preparing for a cell test chapter.
5. Re-start the gas flow.
6. Select first position in the first well.
7. **Focus the position manually and save the focus** by clicking ***Set Z***. Click yes to the Pop-up window (see below) to **delete the invalid focus (Z) levels** from the other positions from this plate - the plate was removed and hence the old Z values are not accurate anymore.



8. Repeat steps from 6 to 8 for the other plate if it is in use, even if it was not removed, since plate holder was touched.
9. Click ***Start Imaging***.

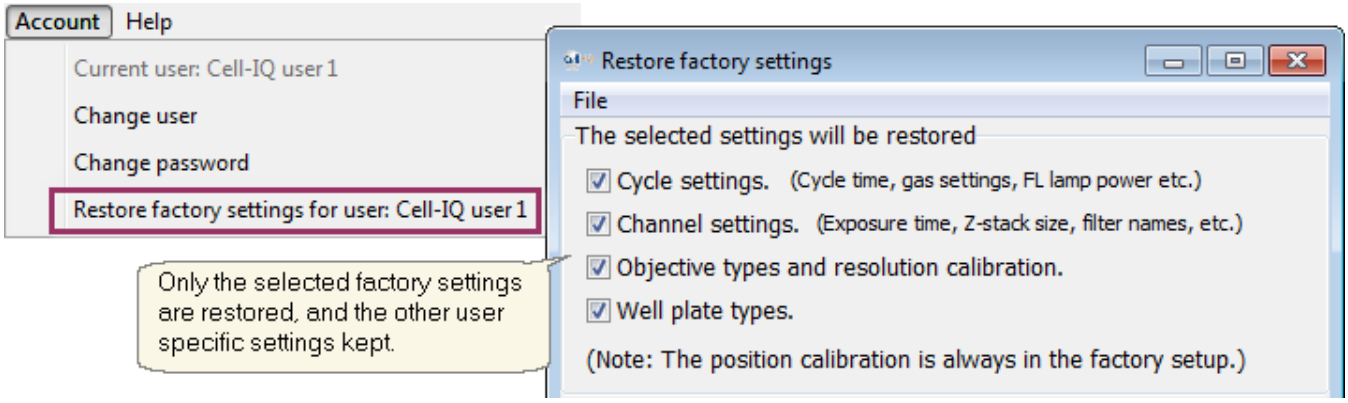
User accounts

9 User accounts

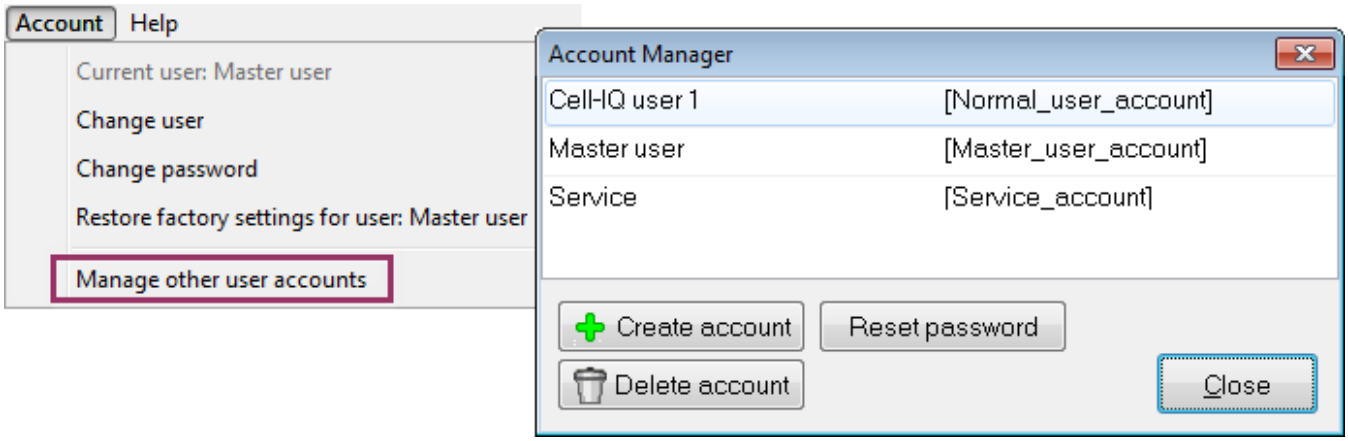
The Cell-IQ Imagen has the three different user account types:

1. Normal user:
   - User specific imaging settings are restored when user logs in to Imagen and saved in log-out.
   - The factory settings can be restored any time.
2. Master user:
   - Can create the Normal user and Master user accounts.
   - Can reset Normal user password.
   - Has all the Normal user features.
3. Service user:
   - Can calibrate the system and select the hardware connection settings.
   - Defines the factory imaging settings.
   - Can reset Master user and Normal user password and delete the Master user accounts.
   - Has all the Master user features.

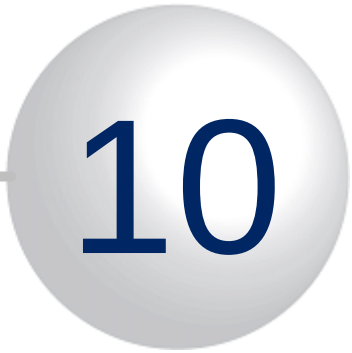
Restore the factory settings:



Manage the user accounts from the Master user or the Service user account:



Error recovery

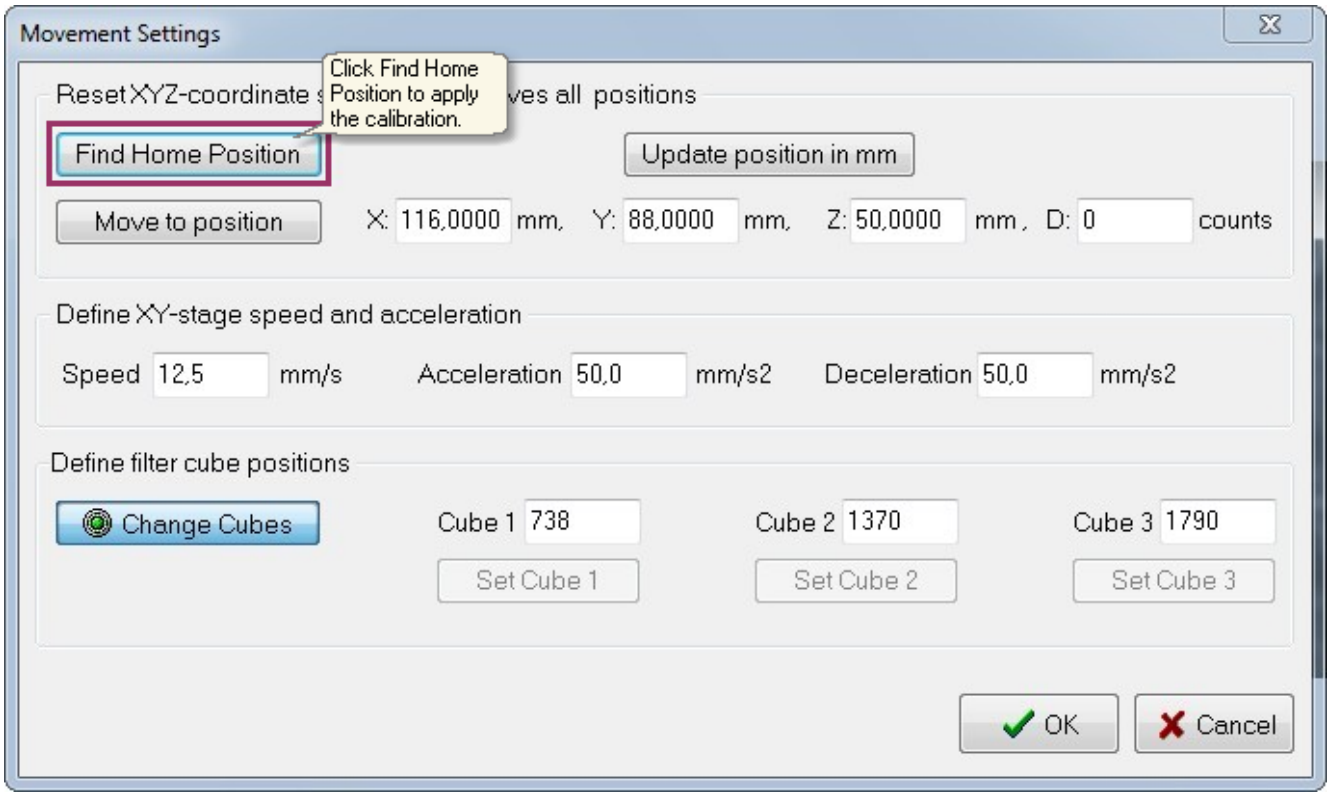


10 Error recovery

The axis calibration is done automatically after a complete system restart. It can also be done manually.

1. Open ***Movement Settings*** from Imaging menu.
2. Click Find Home Position.
3. The axes start moving and the calibration lasts for about 15-30seconds.

Note! All positions are deleted from both plates.



Glossary

11 Glossary

All-in-focus image
All pixels in image are in focus. Pixels are collected from different images in the Z-stack.

Autofocus
Method that finds the single best focus position. If depth of focus of optics is smaller than target thickness, all pixels in image are not in focus in the focused image.

Binning size
Binning size defines how many pixels of the sensor is used to collect light to one pixel in the image. The saved images are always the same size as with 1x1 binning, when other binning is used the images are scaled to that size by stretching them. By using larger binning the image resolution drops but better sensitivity is achieved and prolonged exposure times are avoided.

Cell-IQ well plate Lid
CellSecure™ Well plate lid with gas connectors that enables gas and humidity control and phase contrast imaging for the cells in the well plate.

Channel
Imaging method. Maximum of four channels for each position can be used simultaneously. These are the phase contrast and three different fluorescence channels.

Cycle
A set of defined XYZ-positions in the wells of one or two well plates.

Connector
Piece that connects two gas line components hermetically together.

Dynamic focus
Image Z-stack center automatically follows the best focus level between the imaging cycles.

Fluorescence offset
Difference in focus-level between phase contrast and each fluorescence channel.

Focus measure

Measures single pixel focusing accuracy. This represents normally the strength of high frequency component in the image position.

Horizontal migration
Movement of adherent cells along the well plate bottom.

Image grid
NxN set of non-overlapping consecutive images.

Imaging interval
The period of time between two consecutive images taken from the same XY-position during imaging cycle.

Incubator
Temperature, gas and humidity controlled space.

Medium
Nutrition rich solution for cell cultivation.

Position
Location in XYZ-space.

Vertical migration
Cells drop from horizontal migration filter towards the well bottom.

Well plate
Synonym for microtiter plate. They are intended for long term cell cultivation and they typically enable phase contrast and fluorescence imaging.

x-direction
Horizontal direction in well plate row direction.

y-direction
Horizontal direction in well plate column direction.

z-direction
Vertical direction.

Z-stack
Several images from the same XY-position are combined by focusing to different depths with even Z-spacing.

12 Contact

12 Contact

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Index

N

O

P

R

S

T

U

V

X

Y

Z