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Documentation

Introduction

Contemporary microscopy is a common technology to image cells in life science. The experimental setup consists of a microscope, cameras, light sources, mirrors, filters, x-y stages, focus devices, shutters, as well as a computer connected to the microscope to control the image acquisition. For this application we wrote a new program, called **YouScope**, to expand the functions and to improve the comfortability of imaging. Additional to the functions and features provided by conventional microscope control software, in **YouScope** (i) the setup of a measurement is orientated in a user-friendly way: one only tells the microscope what it should do without knowing the technical details. That means, if e.g. several wells under the same conditions have to be measured, the microscopist just has to select the wells of the microplate in **YouScope**, while in other conventional microscope programs the exact distances between the wells have to be known to move the microscope from one position to the other, (ii) complex measurement procedures combined with a variety of properties can be automated more easily, like imaging several positions with identical parameters in the selected wells of a plate, without changing the position and parameters manually between different wells. (iii) The experiment can be controlled remotely from any computer in the network, and (iv) the functionality of the program can be easily customized, either by scripting (e.g. Java Script, Matlab) or by programming (Java).

To get a first impression about the opportunities of YouScope, look at our Tutorials**.**

YouScope is an open source software that runs on Windows, Linux, MacOS X and other platforms.

Installation

Installation and Running

Windows 7 (32bit), Windows Vista, XP, ...

Installation Process

1. If not done, yet: Install the current version of the Java Runtime Environment (JRE), version 1.6 or higher:http://www.oracle.com/technetwork/java/javase/downloads/index.html
2. Download and install Manager: http://www.micro-manager.org/ , version 1.3 or higher.
3. Add Manager main folder (e.g. "C:\Program Files\Micro-Manager-X") to path (e.g. System Properties -> Advanced -> Environmental Variables -> System variables. Double-click "Path". Add a semicolon add the end of the existing path list and add "C:\Program Files\Micro-Manager-X").
4. Run the YouScope installer (YouScope Setup.exe). On other systems, unpack the achieve.

Running

Open the explorer and go to the YouScope installation directory. Double click YouScope.

Windows 7 (64bit)

Installation Process

1. Install the 32bit version of the Java Runtime Environment (JRE), version 1.6 or higher:http://www.oracle.com/technetwork/java/javase/downloads/index.html

Explanation: The current version (1.3) of Micro-Manager ships only with 32bit DLLs. Running 32bit DLLs is only possible with a 32bit JRE. This might change in the future.

2. Download and install Manager: http://www.micro-manager.org/ , version 1.3 or higher.
3. Add Manager main folder (e.g. "C:\Program Files\Micro-Manager-X") to path (e.g. System Properties -> Advanced -> Environmental Variables -> System variables. Double-click "Path". Add a semicolon add the end of the existing path list and add "C:\Program Files\Micro-Manager-X").
4. Run the YouScope installer (YouScope Setup.exe). On other systems, unpack the achieve.

Running

Open the explorer and go to the YouScope installation directory. Double click YouScope.

MacOS X

Installation Process

1. Download and install Manager: http://www.micro-manager.org/ , version 1.3 or higher.
2. Unzip YouScope.zip to your favorite directory.

Running

1. Open a new shell.
2. Go to the installation directory of YouScope
3. Type

```
DYLD_LIBRARY_PATH=<Manager>
export DYLD_LIBRARY_PATH
java -jar youscope-starter.jar
```

where <Manager> is the directory where you installed Manager.

Linux

I guess similar to MacOS X, except that you replace DYLD_LIBRARY_PATH with LD_LIBRARY_PATH in the most distributions.

Tutorials

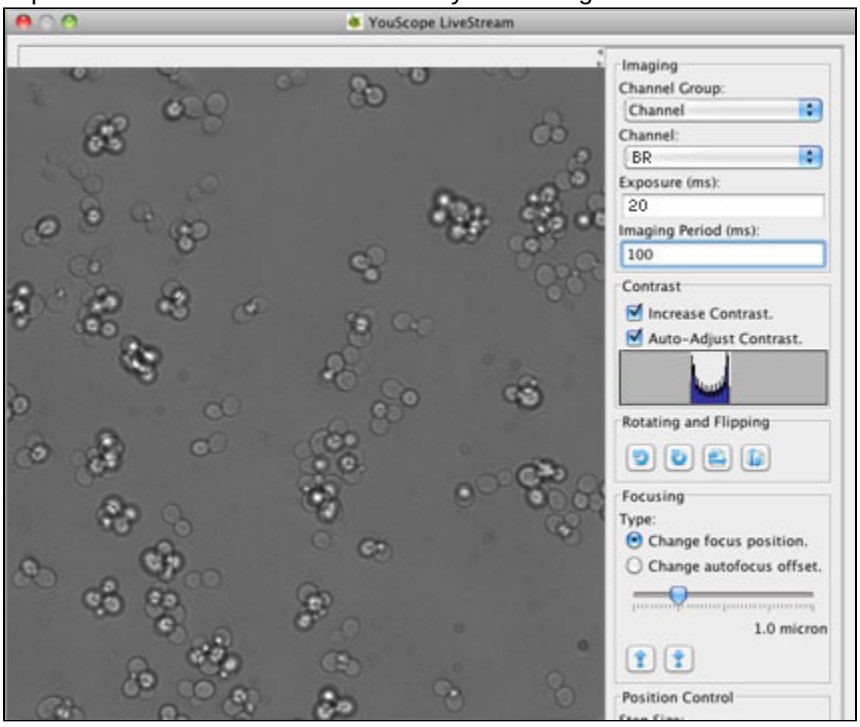
Tutorials

These tutorials contain a collection of guidelines for configuring the commonly used measurement procedures in **YouScope**. The examples should help to learn all characteristics of **YouScope** and to configure your own novel measurement procedures.

Preparation of the Measurement

Before starting a measurement several parameters have to be adjusted, like (i) the cells in the well have to be localized, (ii) the focus has to be adjusted, (iii) the exposure time has to be determined. For visualization of the changes in the adjustment process the **YouScope LiveStream** window in **Tools** has to be opened. The option *Increase Contrast* has to be selected to have optimal visualization conditions. The microplate can be moved manually in the xy-plane till some cells are visible and the focus has to be adjusted manually. If the autofocus on the microscope

is switched on, the offset has to be corrected. Finally, the option Increase Contrast has to be deselected and the exposure time has to be determined by increasing the latter till the cells start being visible.



Example 1 - Microplate Measurement

Example 1 - Microplate Measurement

Your Browser cannot display the video. Please visit http://www.youtube.com/watch?feature=player_embedded&v=mulRvAVExSc to view the video.

Video 1: Dummy video which has to be replaced by real video. Just upload your video to youtube, and then change the corresponding link.

A Measurement Procedure for the following Purpose

Every 5 min three different kinds of channels should be imaged for the cells in 6 wells of a microplate: (A) The cells should be imaged in bright field light. (B) The emitted blue light of the fluorescent proteins in these cells should be visualized. (C) Out-off focus bright field images of the cells should be taken. All three jobs should be repeated twice for all 6 wells in the microplate.

Set up of the Measurement

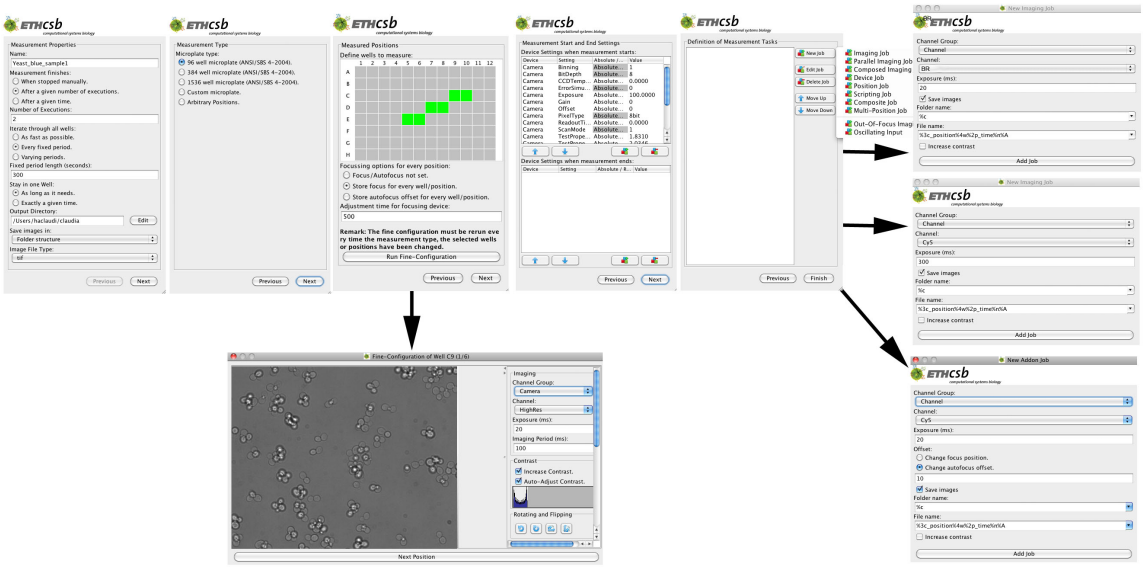
(1) In *Measurement Properties* the name of the sample for identification as well as the Output Directory and the file-type dependent on the software used later for image processing should be given. It is advisable to save them in *Folder Structure* for a better organization. In this case for *Measurement Finishes* the option *After a given number of executions* has to be selected and in the field upcoming below the number 2 has to be tipped. The three jobs for the 6 wells are executed twice. For the *iteration through all wells Every fixed period* has to be selected and in the upcoming field below 300 s has to be tipped in the field for the time period. One execution/iteration is 300 s long. Last, for *Stay in one well* the option *As long as it needs* has to be selected. After the images are performed for a well, it immediately goes on with the next well. If the period of the task is longer than the time, that is needed for the imaging jobs in the wells, the microscope waits till it enters the second cycle.

(2) In *Measurement Type* the kind of microplate, in our case the 96-well microplate has to be marked.

(3) Now in *Measured Positions* the wells containing the sample have to be highlighted and the option *focus for each well has to be stored* should be marked. Now the fine configuration must be rerun for every well with *Run Fine-Configuration*. A new live stream window appears, where the optimal imaging position in the well and the focus have to be adjusted manually and controlled on the screen using the option *Increase Contrast* for better visibility.

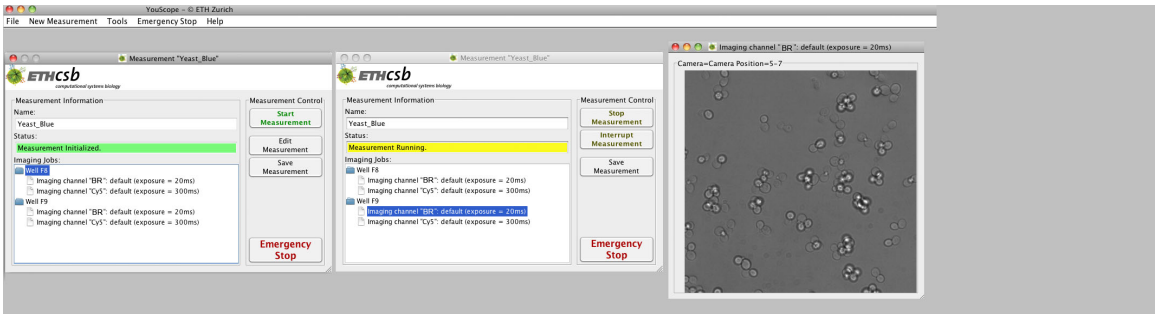
(4) In *Measurement Start and End Settings* usually nothing has to be changed.

(5) In *Definition of Measurement Tasks* the kind of jobs have to be selected. By clicking on *New job* a list of jobs appears and *Imaging job* has to be selected. A new window turns up containing the parameters for this imaging job. (i) For our microscope we grouped the channels for measuring red-, green-, yellow- images, or the bright field image in one group and called the channel for bright field measurement BR and for the blue image Cy5. Therefore, we select the Channel Group the BF channel with an exposure time of around 20 ms for our microscope as well as sample and added the job to the task. (ii) An additional imaging job with the Cy5 channel and the exposure time of 300 ms for our microscope and sample has to be selected for a blue image and also to be added to the task. Finally the job type Out-Off-Focus has to be selected with the option *Change Autofocus*, if the autofocus on the microscope is switched on. A good value for the autofocus offset for our microscope is 10, and the channel for bright field in the Channel group Channel with an exposure time of 20 ms. The optimal exposure times are determined as describe in Preparation of a Measurement in Coarse adjustment of the Microscope. At the end clicking *Finished* opens the window for controlling the measurement, labeled with Measurement and the name of the measurement. It is important that the *Save images* option is selected.



Execution by the Microscope

The action of the microscope can be followed in the window for controlling the measurement. Each well-folder contains an image-name, that has to be clicked to open a window, in that the images are visualized. The microscope executes all three imaging jobs for a well, before it moves to the next well. The imaging in the wells is performed without a break and therefore the microscope waits at the end of the first cycle till rest of the 300 seconds of the task period are over and continues with the second execution of the task.



Example 2 - Advanced Measurement

Example 2 - Advanced Measurement

A Measurement Method for the following Purpose

One measurement example is, to take every 10 seconds a blue fluorescent image of the sample and to make as many bright field images as possible between the blue images until the measurement is finished by the microscopist.

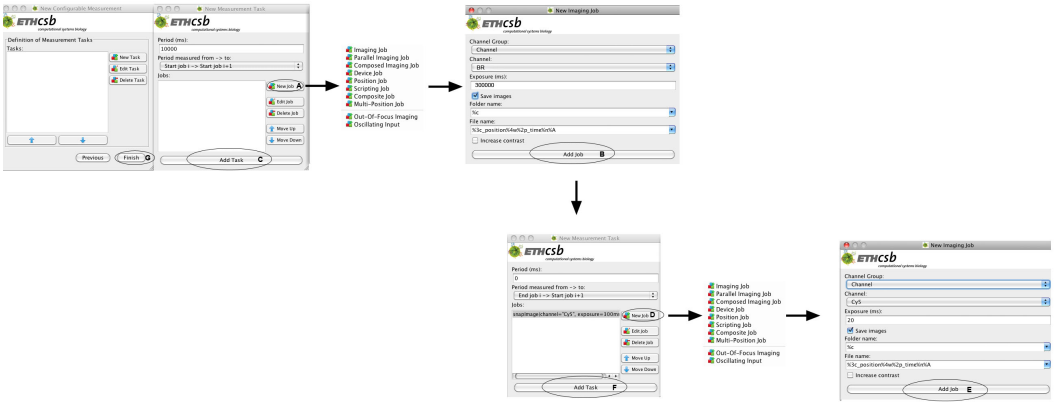
Set-up for the Measurement

(1) In *Measurement Properties* the name of the sample, the Output Directory and the image file type has to be given as well as how the pictures should be saved. For this measurement it doesn't make any difference if *Folder Structure* or *All in one folder* is chosen. In this case for *Measurement finishes* the option *When stopped manually/after tasks finished* has to be selected, that means that the imaging has to be stopped with clicking on *Stop Measurement*.

(2) In the next window, called *Measurement Start and End Settings,* nothing has to be changed.

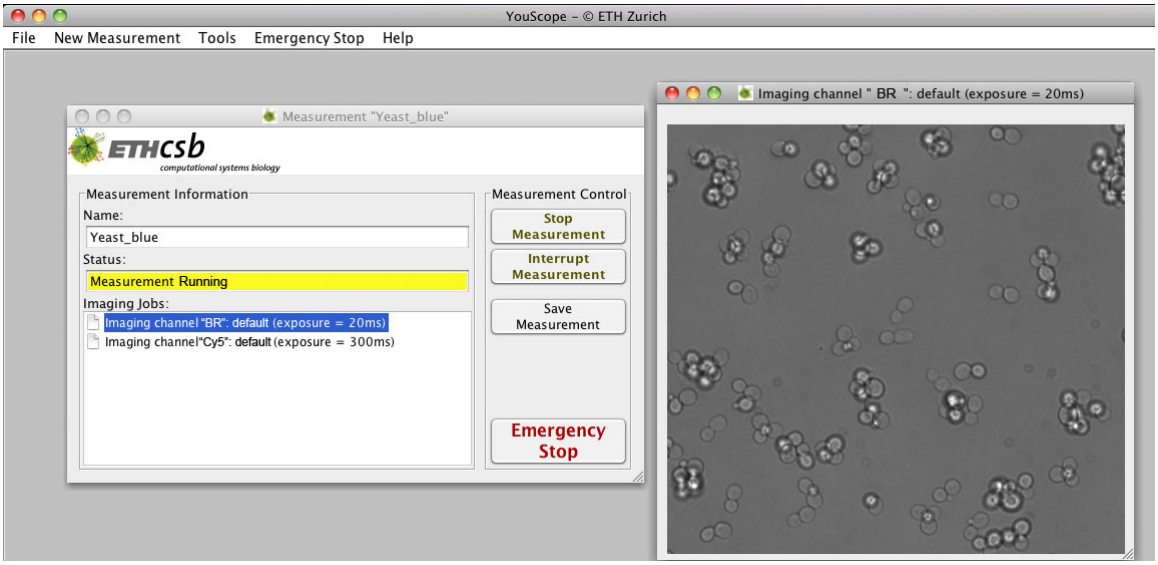
(3) In the last window, called *Definition of Measurement tasks*, 2 tasks have to be created: For our microscope we grouped the channels for measuring red-, green-, yellow- images, or the bright field image in one group. We called the channel for bright field measurement BR and for the blue image Cy5. The optimal exposure times for our microscope and sample are determined as describe in Preparation of a Measurement in the section Coarse Adjustment of the Measurement. For the first task an imaging job with the option Cy5 in the Channel Group Channel and an exposure time of 300 ms have to be selected for blue images (A) and added to the task 1 (B). The period of the task 1 should be 10 s and fixed. This is defined by the option *from start job i to start job i+1*, where the period of the performance of the blue images by the microscope as well as the sending of the blue image jobs to the queue by task1 is equal and constant. Finally the task 1 is added to the task list (C). For the task 2 a job with the option BF in Channel Group Channel and an exposure time of 20 ms period should be selected for bright field images (D) and added to task 2 (E).

The variable period of the task 2 is 0 sec and is defined by the option from *end job i -> start job i+1*, where the time distance between the end of the BR imaging job and the sending of the next BR imaging job to the queue is constant, but the time period between sending of two BR image jobs to the queue is variable. The length of the period of task 2 is 0 sec, so that immediately after finishing every BR image, the BR image job can be send to the queue and later to the microscope, if the latter is not busy with executing the Cy5 imaging job. In addition the optimal exposure time has to be given for both jobs and the option *Save images* has to be selected. This task 2 is added to the task list (F). Now the measurement window is opened by clicking the option *Finished* (G), where the imaging can be started and stopped.



Execution by the Microscope

The imaging process and also the image quality can be monitored in the windows that are turned up by double-clicking on the image names in the field imaging jobs. At the end the microscope takes every 10 sec an blue-image and between the blue images as many bright field pictures as possible and can be stopped with *Stop Measurement*.



Starting YouScope

Starting YouScope

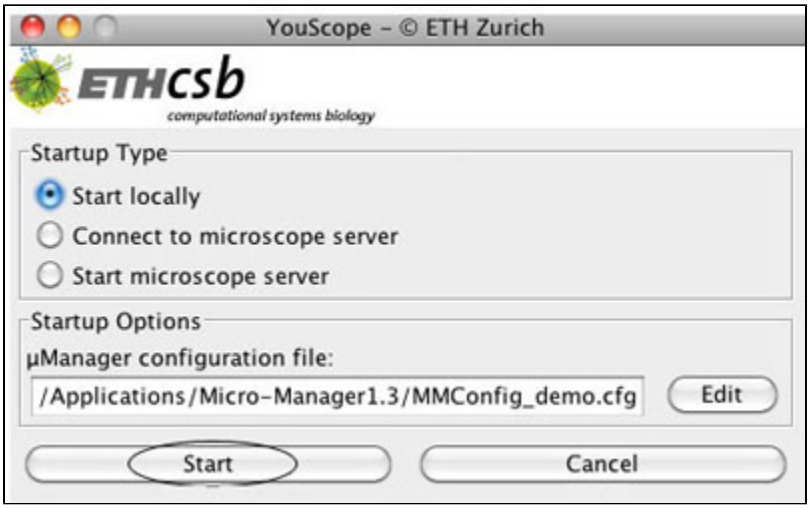
- Windows: YouScope is localized under **Start->Programs->YouScope**.
- MacOSX: YouScope is localized in **Applications->YouScope**.
- Linux: The localization of YouScope depends on the distribution.

When YouScope is started a window appears, where the startup parameters have to be defined. In *Startup Type* an d *Startup Options,* have to be defined.

Startup Options

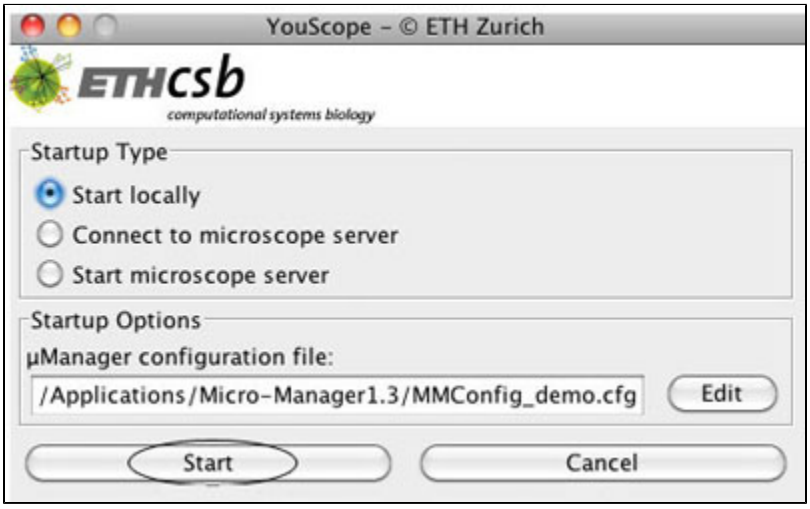
- In the field *Startup Options* the path and name of a configuration file has to be chosen, which is created before the first usage of the microscope. The configuration file contains the information which hardware is a part of the microscope, such that **YouScope** can load and initialize the drivers and configuration

presets. The configuration file has to be created specific for each microscope before the first usage. By default the configuration file of the last session is loaded.

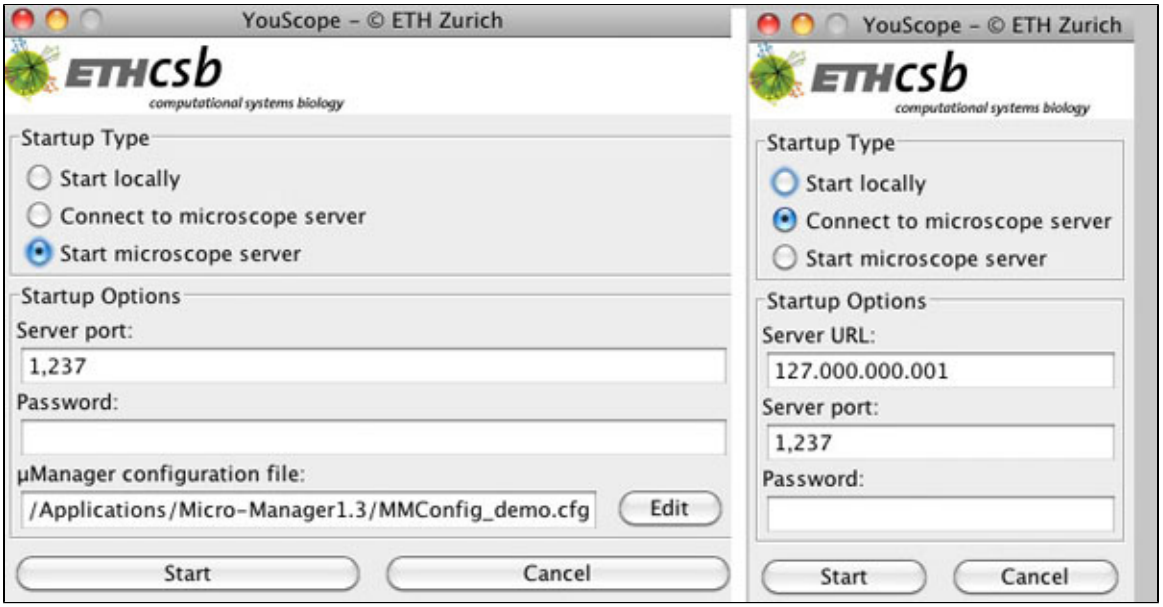


Startup Type

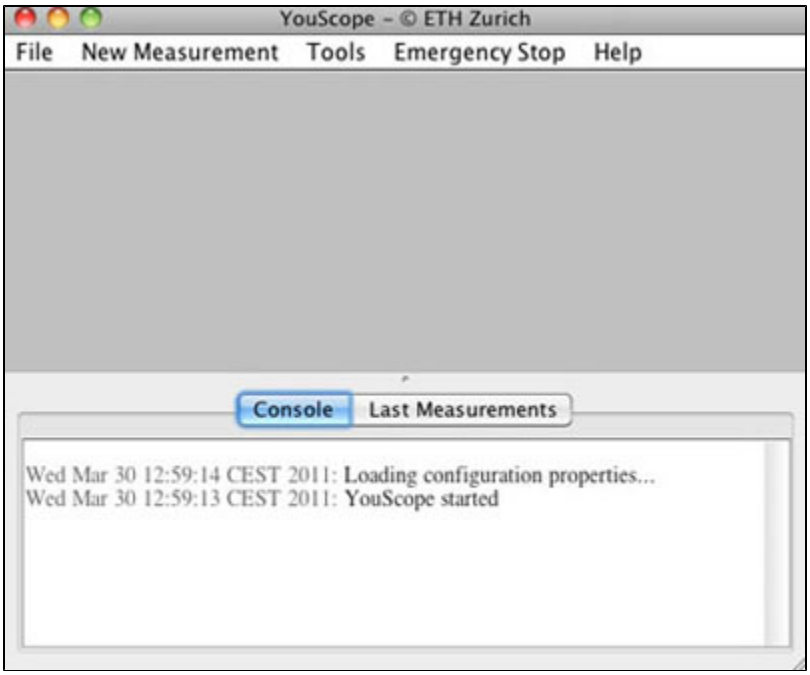
- **Local connection**: If the microscope should be controlled by the computer directly connected to the microscope, the option *Start locally* has to be selected in *Startup Type*.



- **Remote connection:** To contol the microscope remotely from another computer in the network or the internet, the **YouScope** server has first to be started on the computer connected to the microscope. The option *Start microscope server* in *Startup Type,* the port number, as well as a freely choosable password and the location of the *.cfg file in the corresponding fields have to be selected. The **YouScope** client can then be connected to the server from any computer in the network by starting **YouScope** on this computer and by selecting the option *Connect to microscope server* in *Startup Type.* The port, password and the IP-adress of the microscope computer have to be typed in the corresponding fields.



By clicking on *start*, the main window appears on the screen. The upper part displays the **YouScope** interface between the microscopist and the microscope. The lower part can be switched between *console* with the history of all actions in the current **YouScope** session, and *latest measurements* with the history of previous measurements. **YouScope** is ready to be used.



Setting up a Microplate Measurement

Setting up a Microplate Measurement

General Informations

The option microplate measurement found in **New Measurement->Microplate Measurement** helps to perform an identical imaging protocol for several wells and/or positions in a microplate. The wells have to be selected and combined with the imaging protocol consisting of several subelements, called jobs. The set of jobs form a task, which sends these jobs in a given order into a queue from where they are executed one by one for each well by the

microscope. First the stage has to move to its location, and the focus/autofocus has to be adjusted. Then the microscope starts imaging and maybe pause for a certain time. Both, the movement of the plate, the focus, as well as the waiting are done automatically for every well, such that only the imaging procedure has to be defined. The imaging procedure is the same for each well. The Flowchart of a microplate measurement is described in Figure 1. In the following chapters we will explain, how thw single parts of this flowchart can be configured.

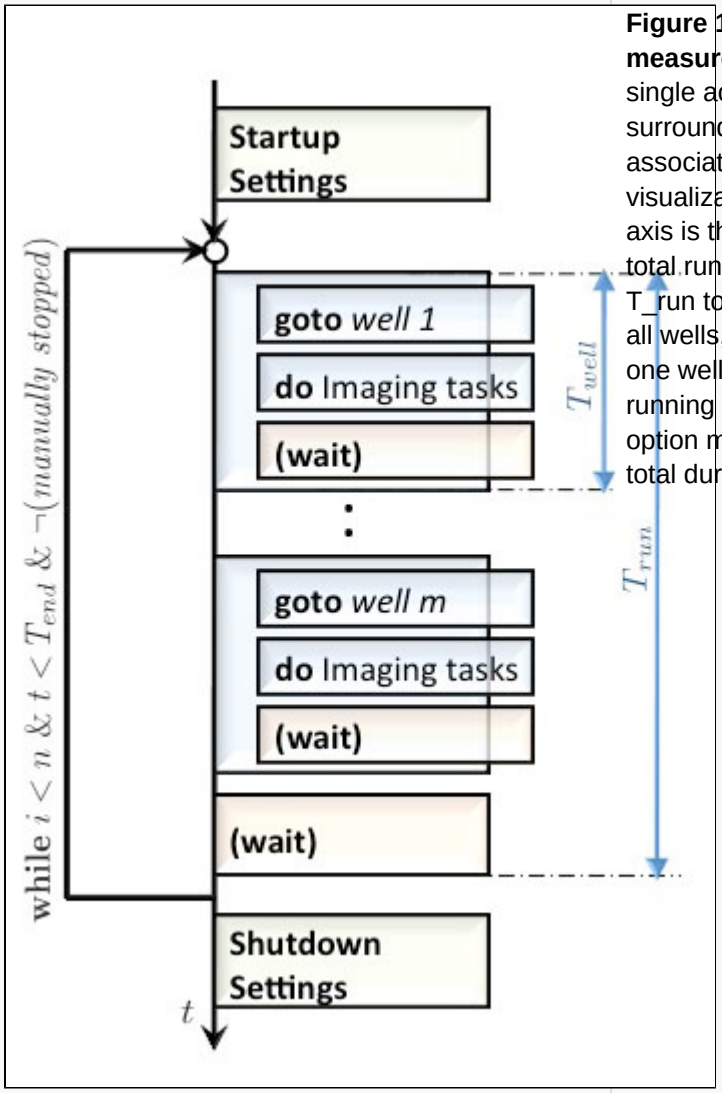
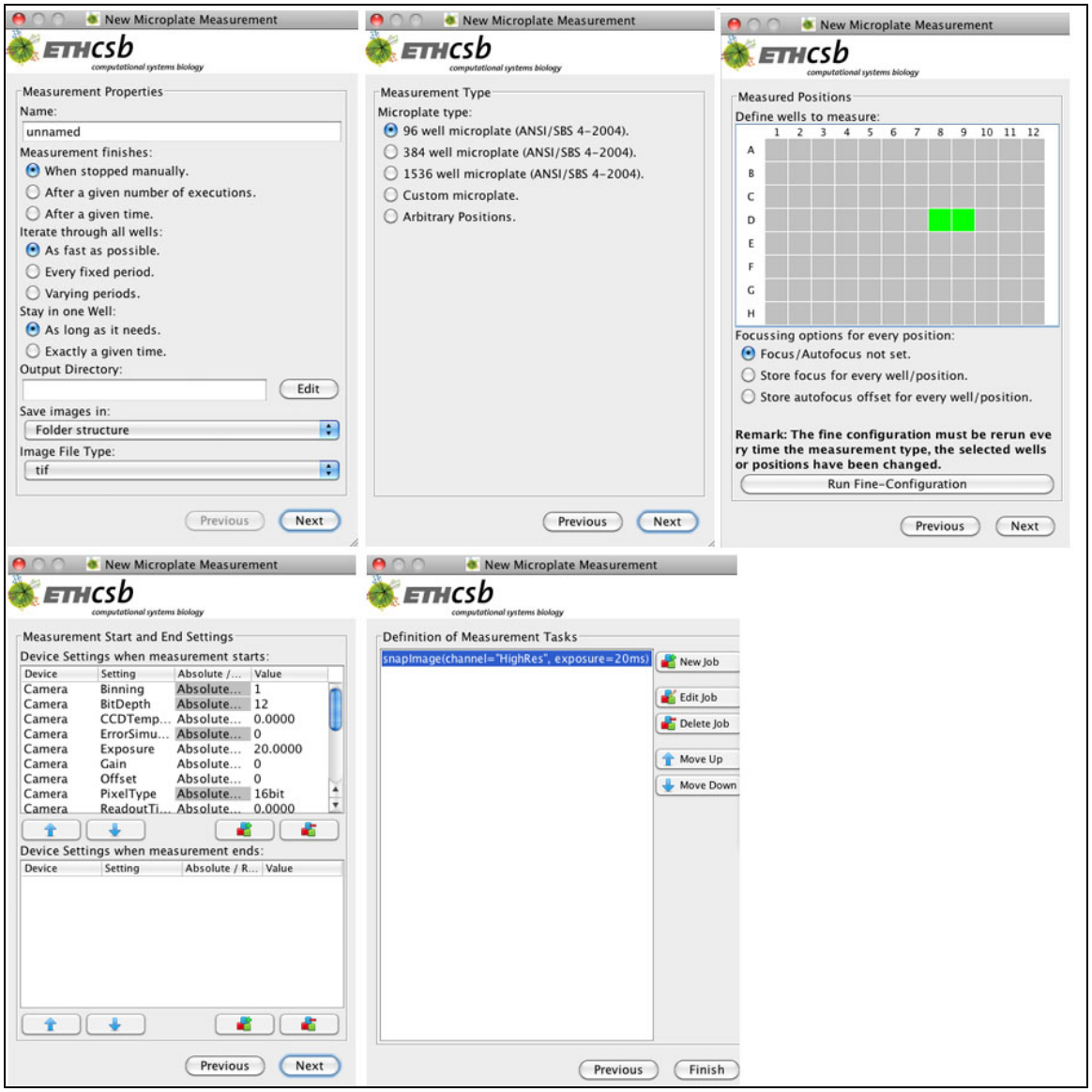


Figure 1: Flowchart of a microplate measurement. The small rectangles represent single actions of the microscope. The large surrounding rectangles group the actions associated to one well together, and are for visualization proposes only. The perpendicular axis is the time axis, T_end corresponds to the total running time of the whole measurement, T_run to the running time of one iteration through all wells, and T_well to the time needed to image one well. The number of executions n, the total running time of the measurement T_end, or the option manually stop the experiment defines the total duration of the measurement.

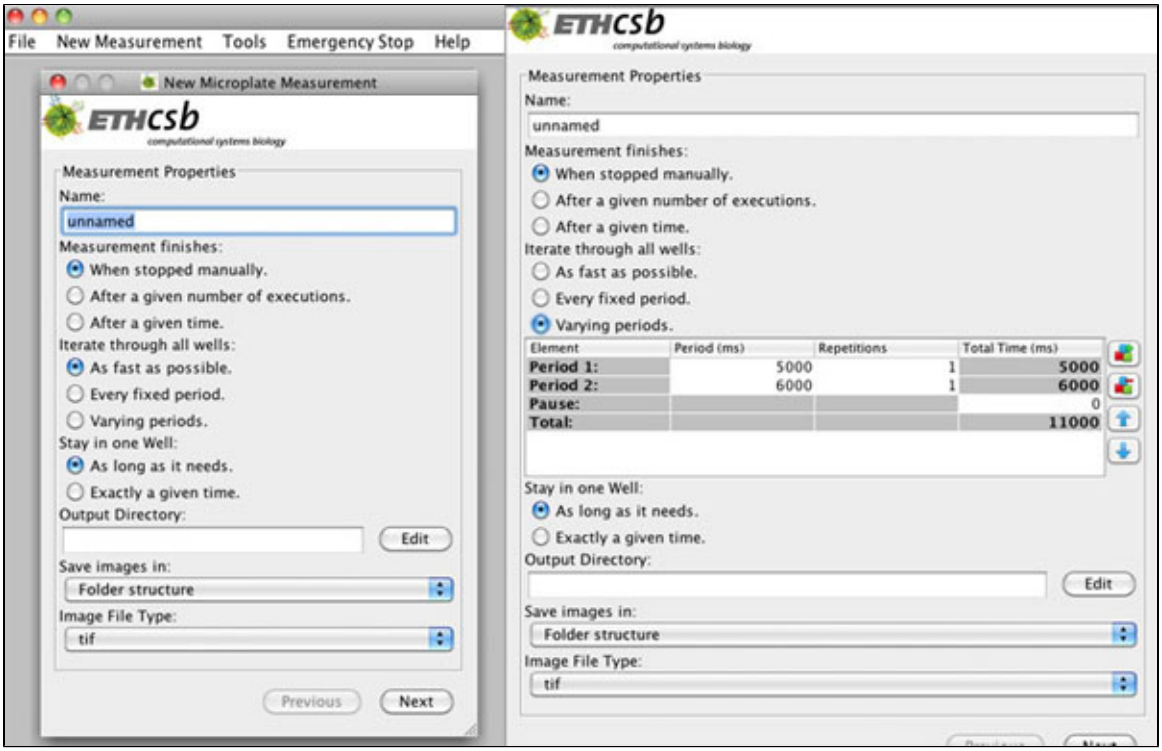
General Properties for an Experiment

The parameters for each microplate measurement are grouped in four classes and are displayed in four windows, called **Measurement Properties**, **Measurement Type**, **Measured Positions**, **Measurement Start and End Settings and Imaging Procedure**. The *Next* and *Previous* buttons switch between the different windows. After defining all the parameters and clicking the *Finish*-button a window, called *Measurement control* window, opens.



Measurement Properties for a Microplate Measurement

Measurement Properties



Properties for Identification and Organization of the Measured Images

- *Image Filename*: A unique name has to be given for each measurement for identification purpose. In *Image Filename* three types of a name are choosable. The name is composed out of several parameters. The exact description of the parameters in the selected filename can be read, if the mouse is moved to the field containing the filename. The parameters contain a name, information about measurement details like position in a well, number of the well, etc.
- *Output Directory*: In addition the folder, where the images should be stored, has to be given. If many microscopists are using the microscope, each user can save his images in a separate folder.
- *Save images in*: This is another helpful feature to organize the data storage. The data for each well can be stored in a separate folder or all images can be stored in one folder.
- *Image File Type*: In general, using a non-compressing image format, like tif, is recommended. The file format can be chosen depending on the program used for data post-processing.

Timing Parameters

- *Measurement finishes* - Time settings for the total running time of the measurement. There are three possibilities to finish a measurement. (i) The measurement can continue running until it is stopped manually, by choosing the option *When finished manually*. This is used, if e.g. a change in the development of the cells has to be analyzed and the microscopist does not know, when the steady-state is reached. The other two possibilities define (ii) a specific number of executions of the task (*After a given number of executions*), or (iii) a time period (*After a given time period*) as conditions for a measurement stop. The timing parameters, which define the duration of the measurement are highlighted with an ellipse in Figure 1a.

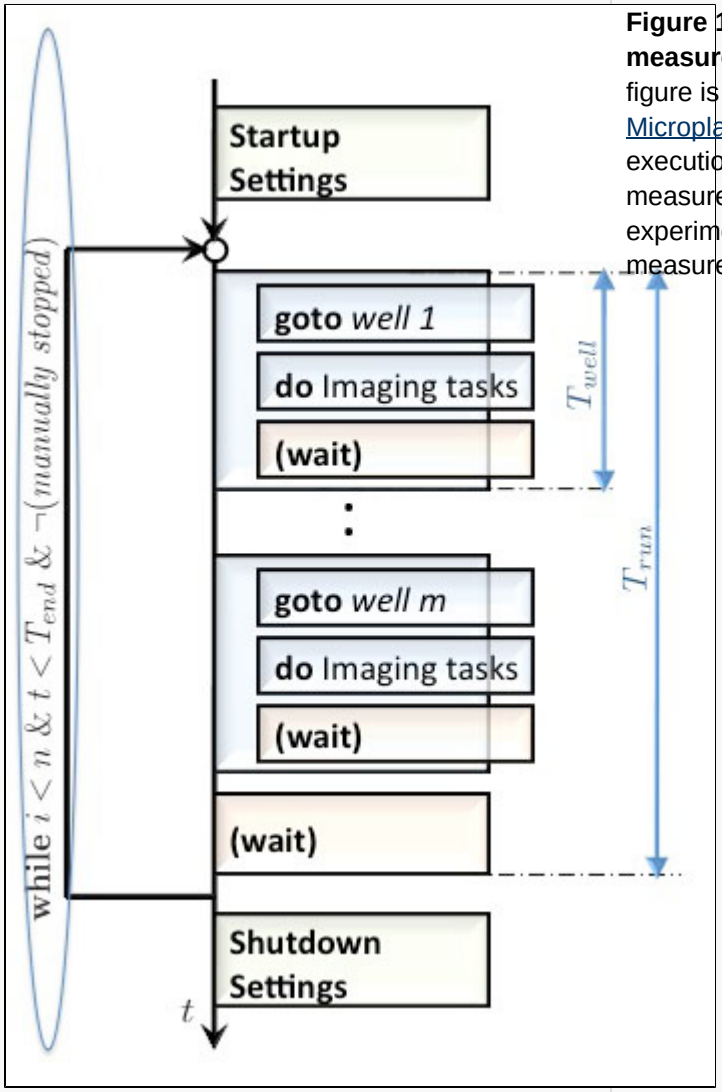


Figure 1a: Flowchart of a microplate measurement. The general description of this figure is located in Figure 1 in Setting up a Microplate Measurement. The number of executions n, the total running time of the measurement T_end as well as stopping the experiment manually defines the duration of the measurement and are highlighted by a ellipse.

- *Iterate through all the wells* - Time settings for the repetition of the imaging jobs. The time parameter, which defines the length of an iteration is highlighted with an ellipse in Figure 1b. There are also three possibilities: (i) *Iterate as fast as possible*. The microscope iterates through all selected wells and starts without a break the second iteration. (ii) *Every fixed period* - The iteration through all wells will be repeated after a fixed time period. If the imaging jobs in all selected wells are finished before the period for the task is over, the microscope waits until the period time is over and then starts with the next iteration. (iii) *Varying periods*. With this option several period lengths can be defined. Every period is defined by the period length and the number of executions.

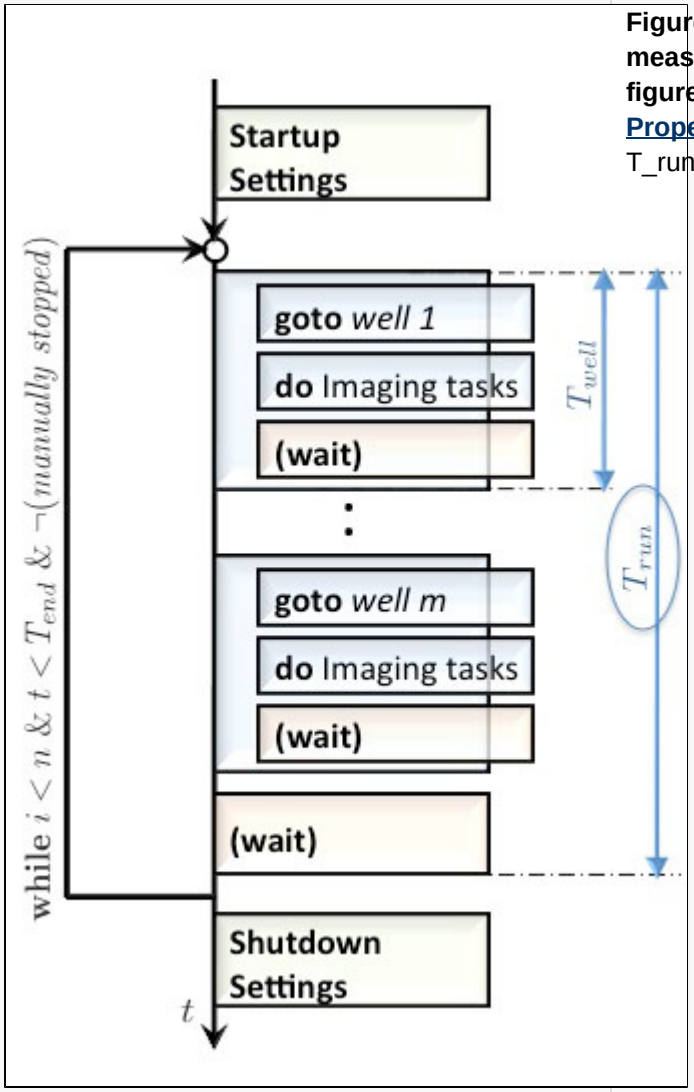


Figure 1b: Flowchart of a microplate measurement. The general description of this figure is located in Figure 1 in Measurement Properties for a Microplate Measurement. T_run is highlighted by an ellipse.

- *Stay in one well* - Time settings for one well. The time parameter, which defines the time for one well, is highlighted by an ellipse in Figure 1c. (i) *As long as it needs* - Here, the microscope executes the imaging jobs and immediately goes to the next well. (ii) *Exactly at a given time* - Here, the exact time for one well is defined. If the microscope finishes earlier, it waits.

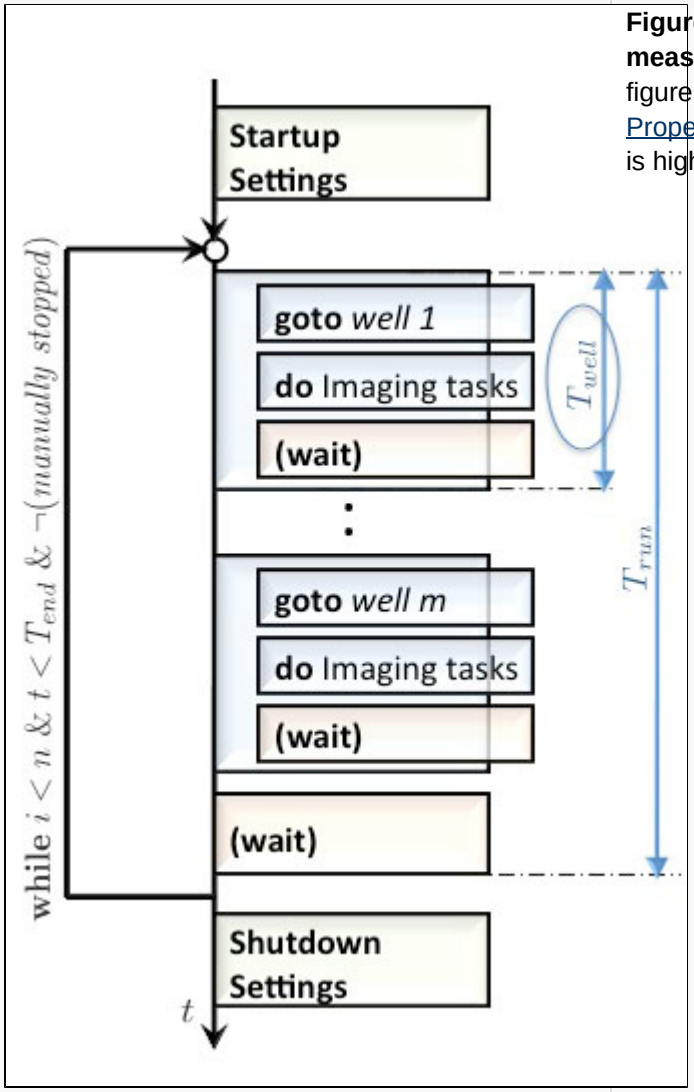


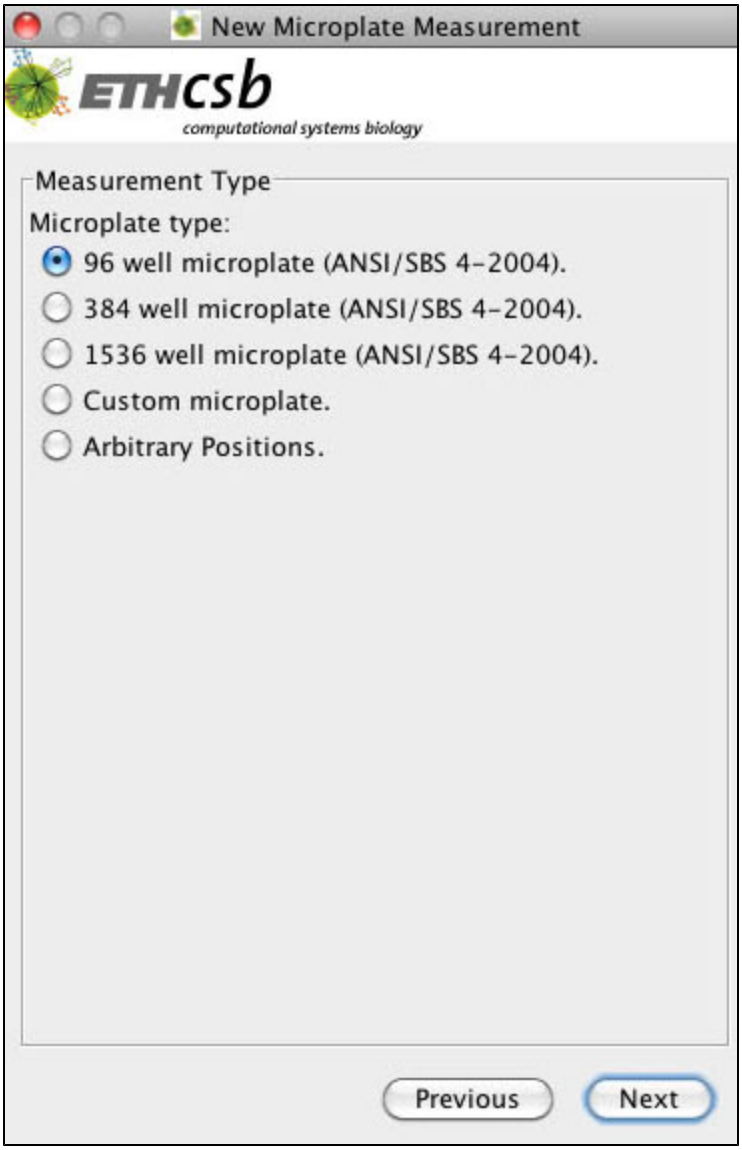
Figure 1c: Flowchart of a microplate measurement. The general description of this figure is located in Figure 1 in Measurement Properties for a Microplate Measurement. T_well is highlighted by an ellipse.

All settings define a period length and include the execution time of the corresponding jobs and waiting times. If e.g. the running time T_run is 60 s, and the iteration through all wells takes already 40 s, the microscope pauses for 20 s, before it continues with the next iteration.

Measurement Type for a Microplate Measurement

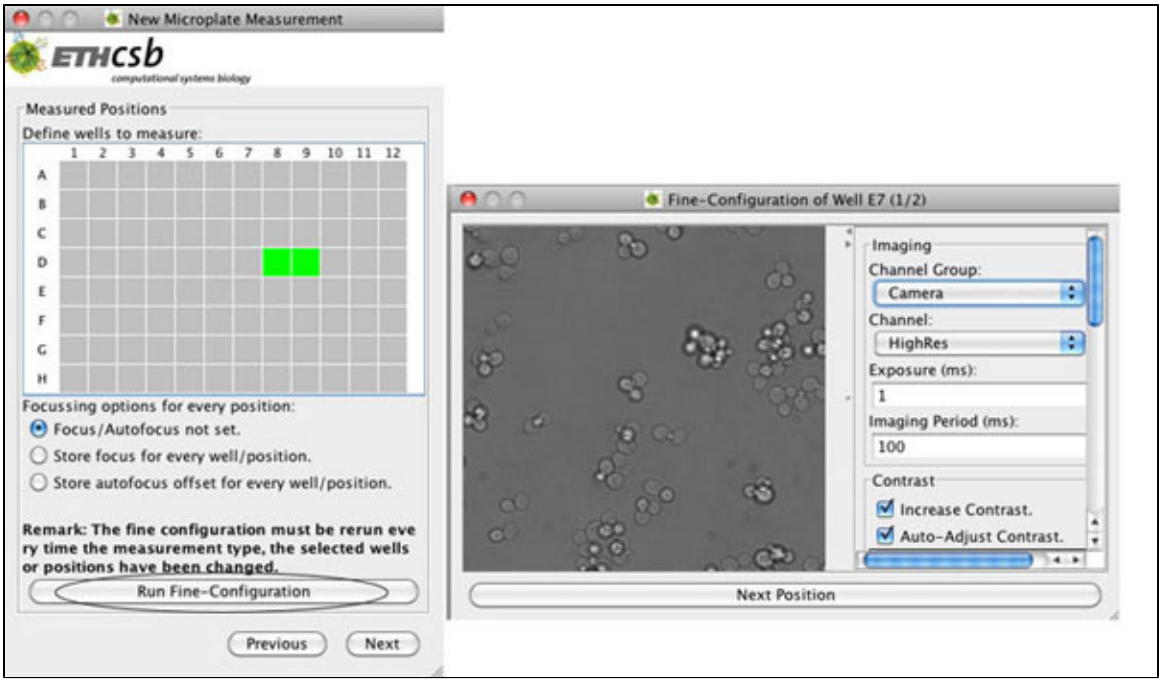
Measurement Type

The dimensions of the microplate have to be defined here. The parameters for commonly used microplate types (e.g. formats defined by ANSI/SBS 1-2004 through ANSI/SBS 4-2004) are preconfigured in YouScope. There is also the possibility to use any custom microplate, but then the microscopist has to provide the dimensions of the microplate. In the last option arbitrary positions can be chosen, if e.g. microscope slides are used instead of a microplate. This feature of YouScope makes the creation of a measurement much easier for the microscopist, because the dimensions of the wells in the microplate do not need to be manually defined.



Measured Positions for a Microplate Measurement

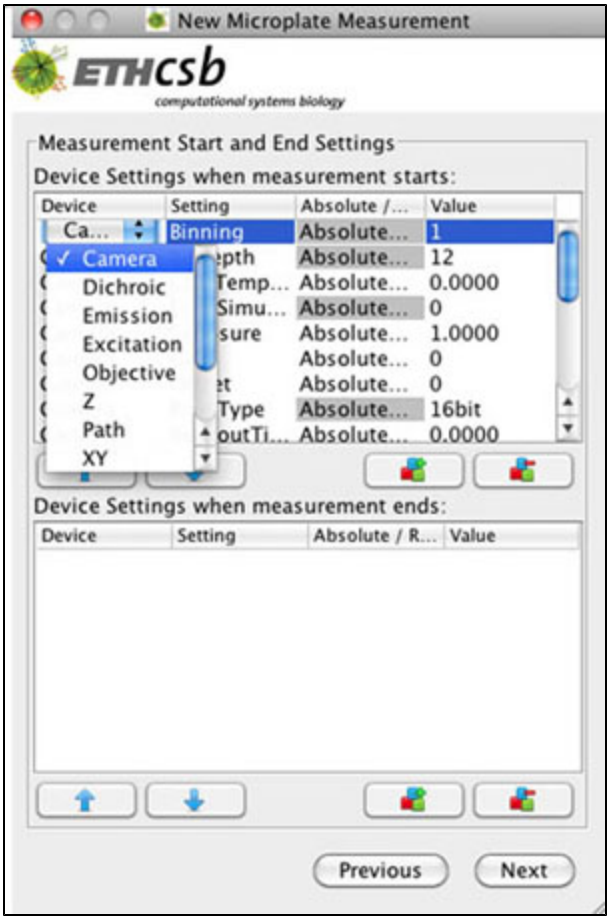
Measured Positions



- The wells of a microplate can be selected to be measured/highlighted by clicking on the fields corresponding to the respective wells in *Define a well to measure.* Then the focus and the imaging area can be optimized manually for each well by running the *Run Fine-Configuration* using typically bright-field imaging. After the image area as well as the focus is optimized, the well to fine configure can be changed with the respective arrow button. When changing the well, the position and the focus settings are stored for the previous well automatically. After all wells are configures, the *Fine-Configuration* can be closed with *ok*. An existing fine configuration can be edited with *Edit Fine-Configuration*. For a description of the *Image Options* in the fine configuration window, go to Imaging Procedure in each Well or Position.

Measurement Start and End Settings for a Microplate Measurement

Measurement Start and End Settings



In ***Measurement Start and End Settings*** **the** window is splitted into two parts. These two parts are labeled with *De vice settings when measurement starts* and *Device Settings when measurement ends.* They contain the device settings before and after the measurement. For each device different settings can be chosen, like binning, gain etc. for the device camera. For the settings an absolute or relative value can be given. In most measurements the values for the camera settings are changed during the preparation of the measurement, e.g. if the imaging has to be done with lower exposure time to avoid sample damage, the gain can be increased. Thus by default, the current camera settings are pre-initialized in the field *Device settings when measurement starts*. The pre-initialization of the camera settings in *Devise settings when measurement starts* can be activated or deactivated in the *Configuration Settings*, localized in **File->Configuration->Compartibility**. However other device settings can be easily added and set at the start or end of a measurement.

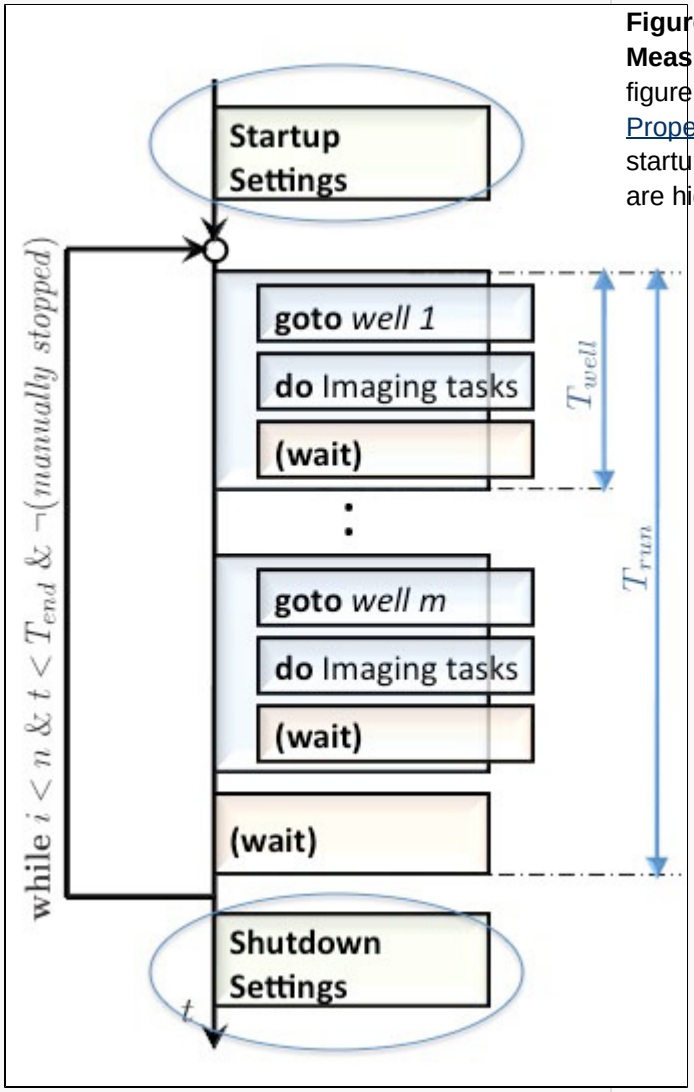


Figure 2a:Flowchart of a Microplate Measurement: The general description of this figure is located in Figure 1 in Measurement Properties for a Microplate Measurement. The startup settings and the shutdown settings and are highlighted by a ellipse.

Imaging Procedure in each Well or Position

Imaging Procedure in each Well or Position

An imaging procedure can be build up by selecting imaging jobs for the procedure. The jobs are send in the given order to a queue, which is sequentially processed on the microscope as shown in the flowchart in Figure 3. To perform the imaging procedure for a special well several single actions are necessary, like going to a well, making images. These actions, except the imaging procedure, don't have to be specially programmed or selected by the microscopist. By clicking on *Add*, a popup opens, where the imaging procedure job has to be selected. A job can be deleted with *Delete Job*, edited with *Edit Job* or the execution order of the job can be modified with *Move Up* or *Mov e Down*. By clicking on *Finished* a new window *Measurement Control* is created where the measurement can be started.

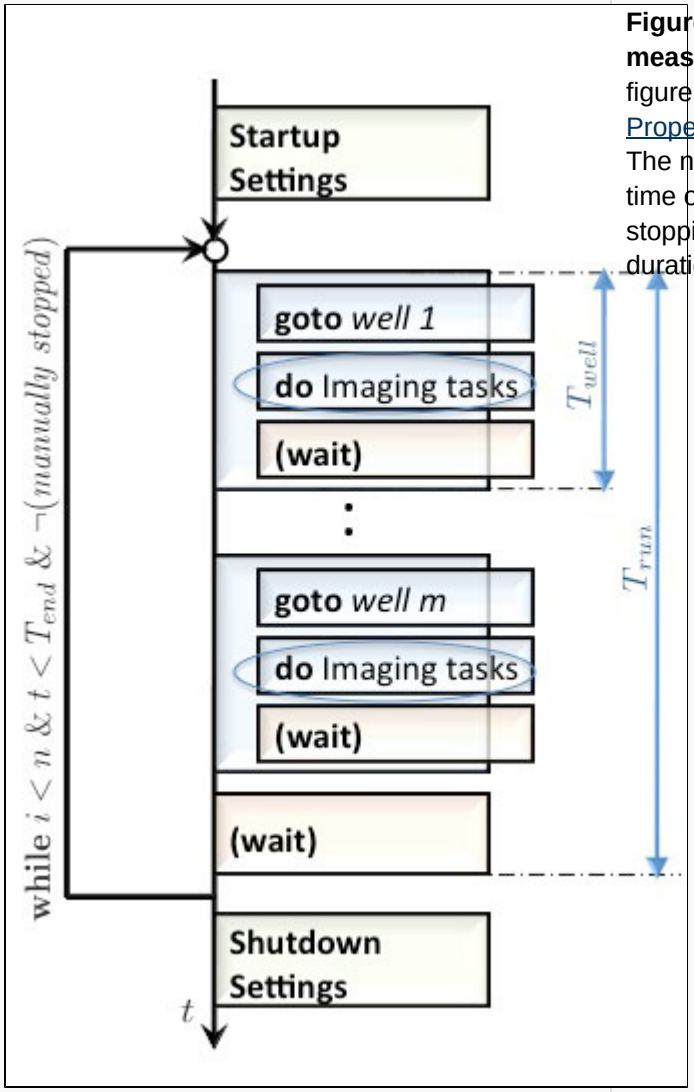


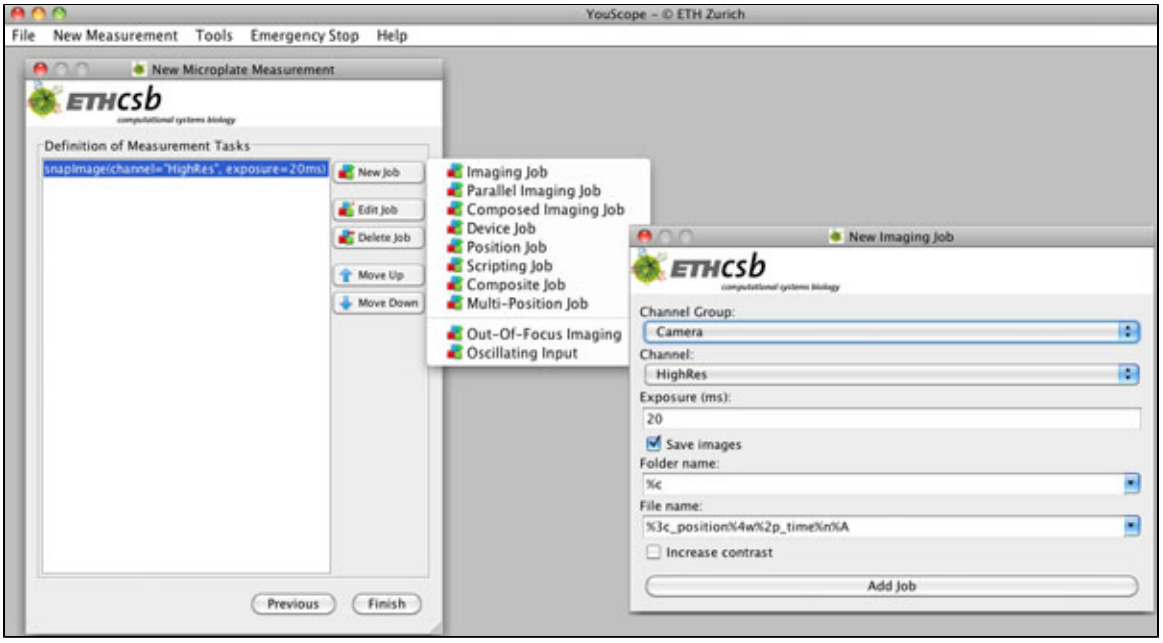
Figure 3: Flowchart of a microplate measurement. The general description of this figure is located in Figure 1 in Measurement Properties for a Microplate Measurement. The number of executions n, the total running time of the measurement T_end as well as stopping the experiment manually defines the duration of the measurement.

In Definition of Measurement Tasks the following Jobs can be selected

Imaging Job

This job is takes an image in a special channel in the selected wells. After the Job is selected, a new window appears, in which the channel and exposure time can be defined:

- *Channel group*: Go to Preparation of a Measurement.
- *Channel*: Go to Preparation of a Measurement.
- *Exposure*: Go to Preparation of a Measurement.



Parallel Imaging Job

several cameras are necessary, not yet available!!!

Composed Imaging Job

In this job several adjacent areas in a well can be imaged with an overlap, if a large image with high resolution should be composed out of several smaller images. The images can be build together with an external image processing program. For this job informations on the area and channel have to be defined:

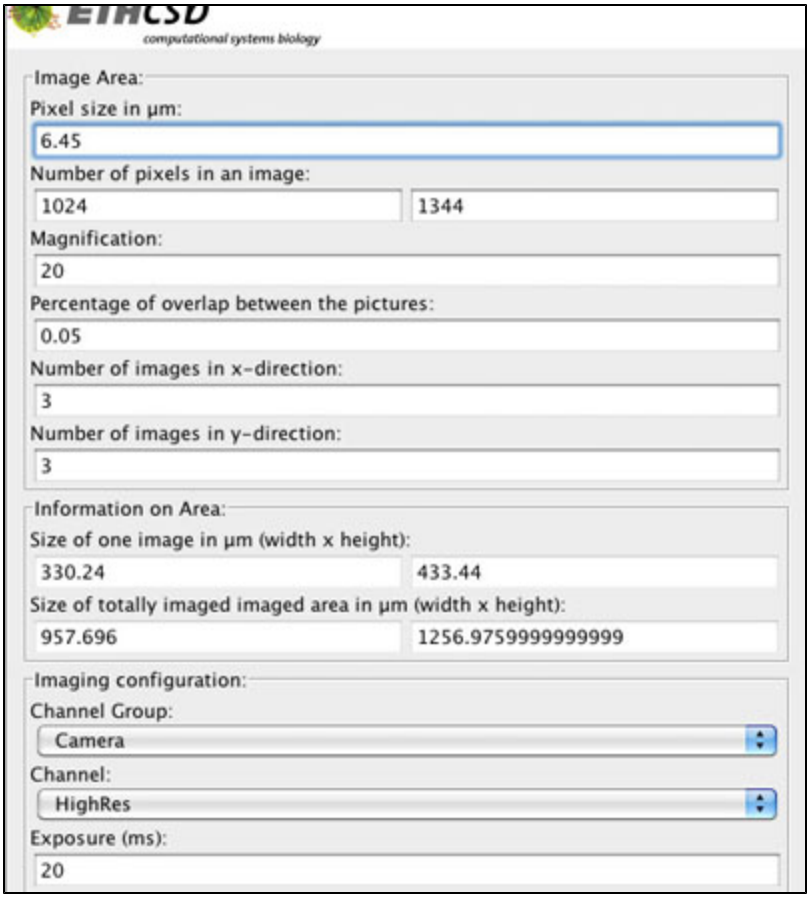
- *Pixel Size*: The pixel size is camera specific and is given by the manufacturer of the camera.
- *Number of pixels in an image:* These values in pixel-units for the x and y-axis describe the resolution of the camera and are given by the manufacturer or easily obtained by making an image and looking at this position..
- *Magnification*: The current magnification of the microscope has to be profiled here.
- *Percentage of overlap between the pictures*: To overlay the pictures correctly and to build up the whole picture, commonly an overlap between the single picturesis necessary. Usually 10%-30 % is a good value.

The next fields are output fields, which display the dimensions of the area from above in m-units:

- *Size of one image in m*: The size of x or y can be calculated with the formula
  `x_area=(l_pixel * nx / magnification)`
  The value of y is calculated in an equivalent way.
- *Size of totally imaged area in m*: Here the total size in the x- and y- direction of the assembled picture are displayed in the corresponding fields. They are calculated by,
  `x=(l_pixel * nx / magnification)*(1-overlapp) * mx`

with l_pixel as the size of a pixel, nx the number of pixels in the x-direction and mx the number of images to be made in the x-direction.
The value of y is calculated in an equivalent way

- Channel Group: Go to Preparation of a Measurement.
- *Channel*: Go to Preparation of a Measurement.

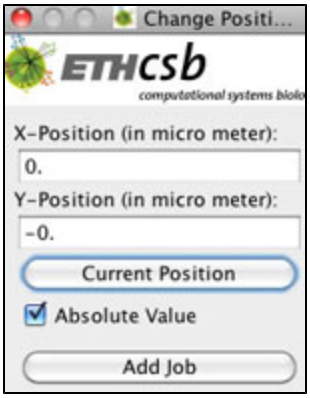


Device Job

If some devices, like binning of the camera or light intensity, have to be changed between the imaging jobs, this can be done by adding a Device Job to the task.

Position Job

If the position of the stage between two jobs has to be changed, a Position Job can be added between these jobs, where the values of the new position are entered. In the upcoming window the x- and y-value, relative or absolute in m have to be fed in. If the option *Absolute Value* is selected, the values in the fields x and y define the position corresponding to the origin used by the microscope. If the option Absolute Value is deselected, the values in the fields x and y are the changes of x and y relative to the current position.

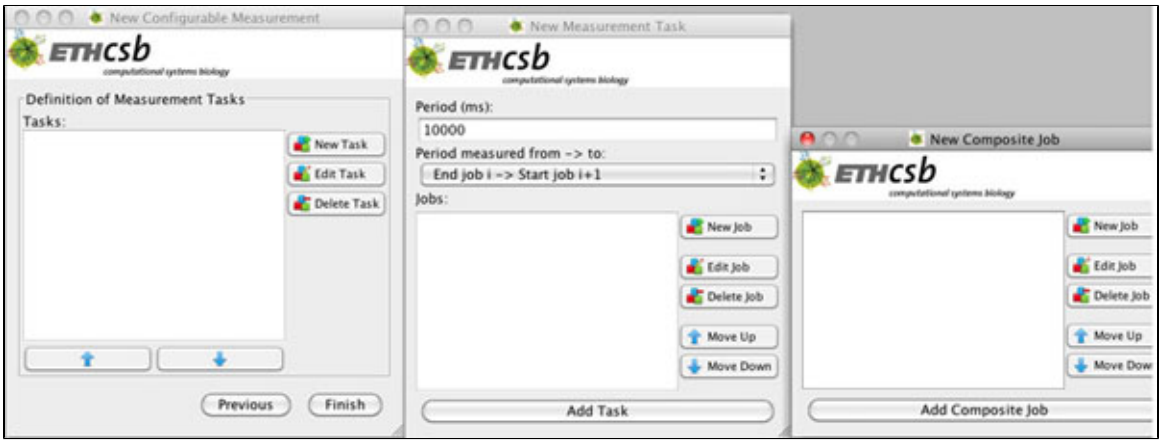


Scripting Job

Moritz!!

Composite Job

In composite job it is possible to group related jobs. A parent job (composite job) can contain several children-jobs h. For most purposes a composite job has only visualization proposes.

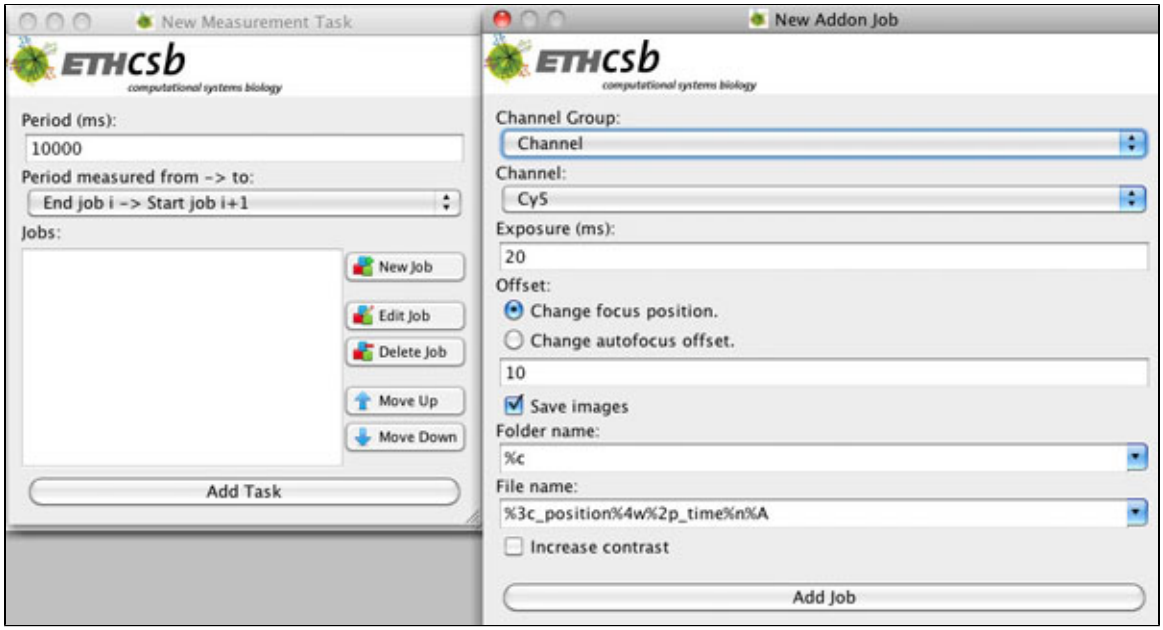


Multi-Position Job

!!Max!!

Out-off Focus Imaging

Images at different focuses of one position are taken to make the detection of the cells in the sample easier. There are two possibilities to change the focus, one to change the focus position in z-direction or to change the autofocus offset. If the autofocus button on the microscope is pressed, the *Change Autofocus* option has to be selected.The value for the autofocus change is dependent on the microscope, the objective and the object. The rest of parameters are identical with the ones from Imaging Job.



Oscillating Input

In this job any device parameter can oscillate during imaging. In the up-coming window the parameters for the oscillation can be defined.

Running and Stopping a Measurement

Running and Stopping a Measurement

After all parameters are adjusted, the measurement can be started in the measurement window identified with the name of the measurement given in the *Measurement Properties*. The measurement window consists of two parts, *M easurement Information* and *Measurement Control*.

Measurement Information

- *Name*: The name given in Measurement Properties can only be read.
- *Status*: This field informs about the Status of the System. The message *Measurement Initialized* means that the system is ready to start the imaging and the measurement can be started with *Start Measurement* in *Mea surement Control*. The message changes into *Measurement Running*, followed by *Measurement Finished*.
- *Imaging Jobs*: In the field imaging jobs the names of images are displayed, for each well or position. They can be double clicked and a image window turns up, where the images are instantly displayed during measurement.

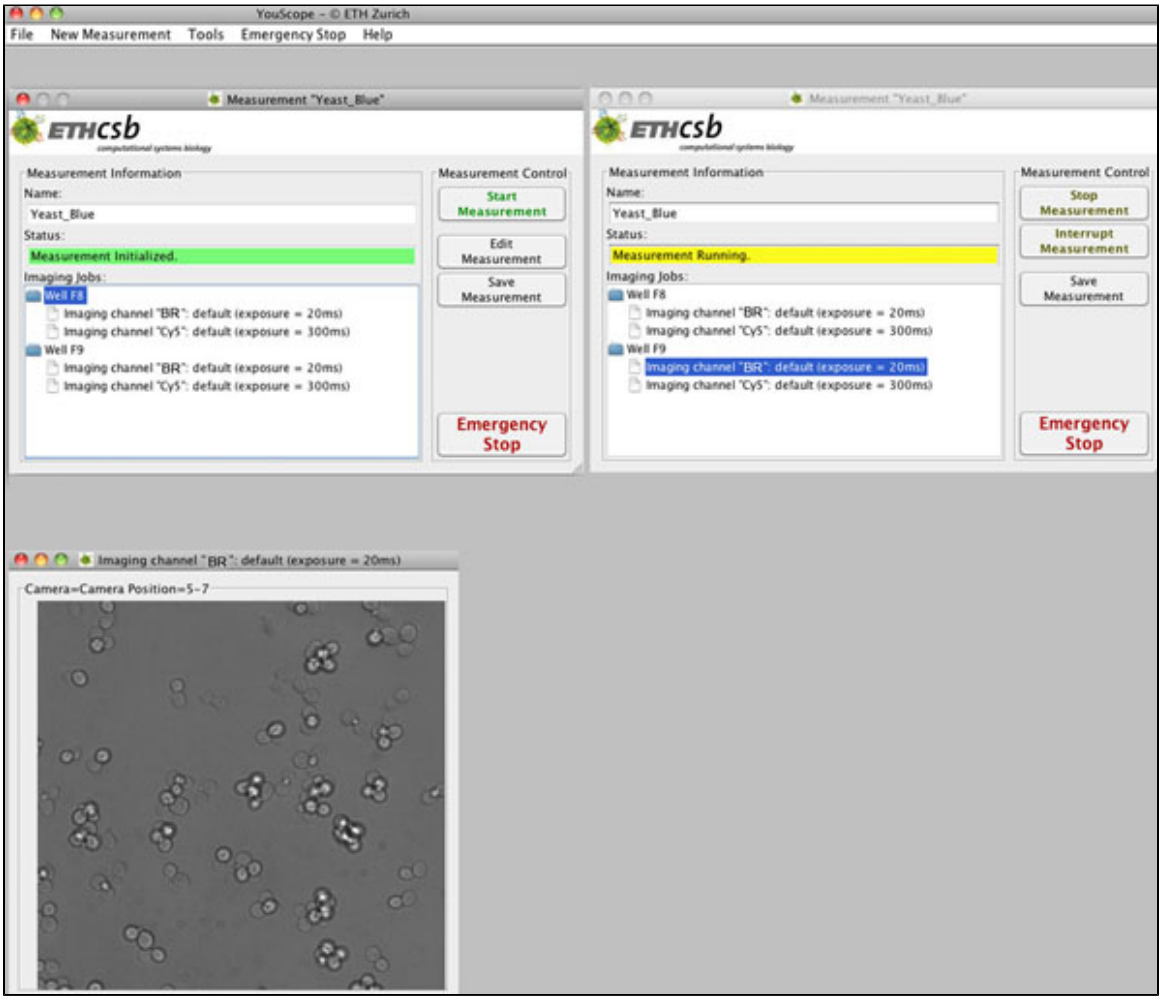
Measurement Control

- Edit Measurement: This option gives the possibility to go back to the four windows containing the measurement parameters to control or to edit them.
- Save Measurement: After the measurement is configured, it can be saved to use it later again.
- *Stop Measurement:* Stops a running measurement after the task is completed.
- *Interrupt Measurement*. The measurement will be stopped immediately, the job or task will not be completed, and the measurement end settings are not set.
- *Emergency Stop:* The system is switched-off as fast as possible and the microscope is blocked to get any other call from YouScope until emergency state is reseted.

Emergency Stop

We highly recommend to have always a hardware emergency stop button in your proximity during a measurement (e.g. the power supply button of the microscope) instead of relying on this feature in **YouScope**. It could be that in such a case the computer is crashed and then its useless to use this option in the program! After usage of the Emergency Stop the system has to be reseted by **Tools->Reset Emergency**.

The owner of this site shall not be responsible does not guaranty that the Emergency Stop will stop the measurement in case of emergency.



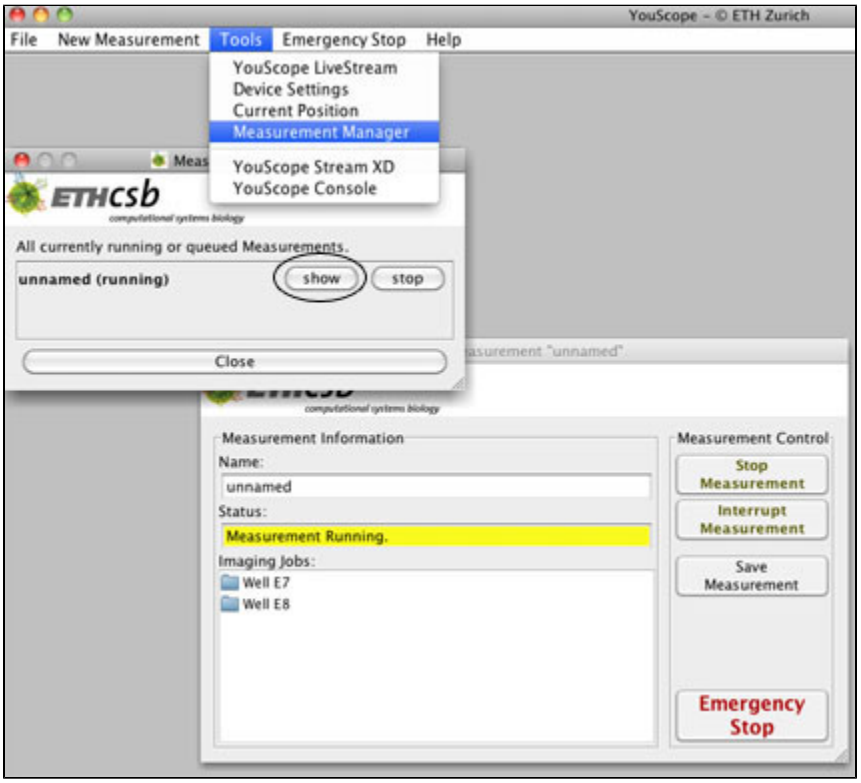
Additional Tools

Additional Tools

Open a new Measurement Window

If by accident the measurement window was closed, there is a second possibility to open the ***Measurement* windo w** with **Tools->Measurement Manager** in the toolbar of the **YouScope** window. Or if a microscopist wants to control his running measurement from any computer in the network, he has to open the measurement window after

he started **YouScope** on the remotely connected computer. A new window called *Measurement Manager* appears, where the running or queued jobs are displayed. Clicking on the *Show* opens the *Measurement* window again.



Emergency Stop

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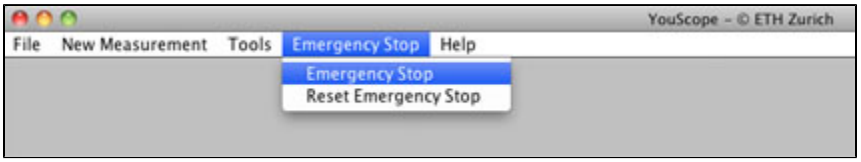
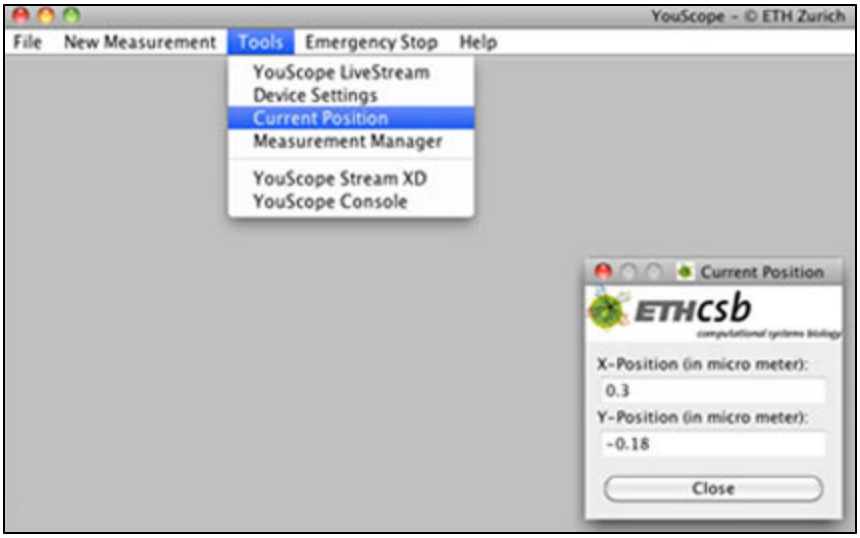


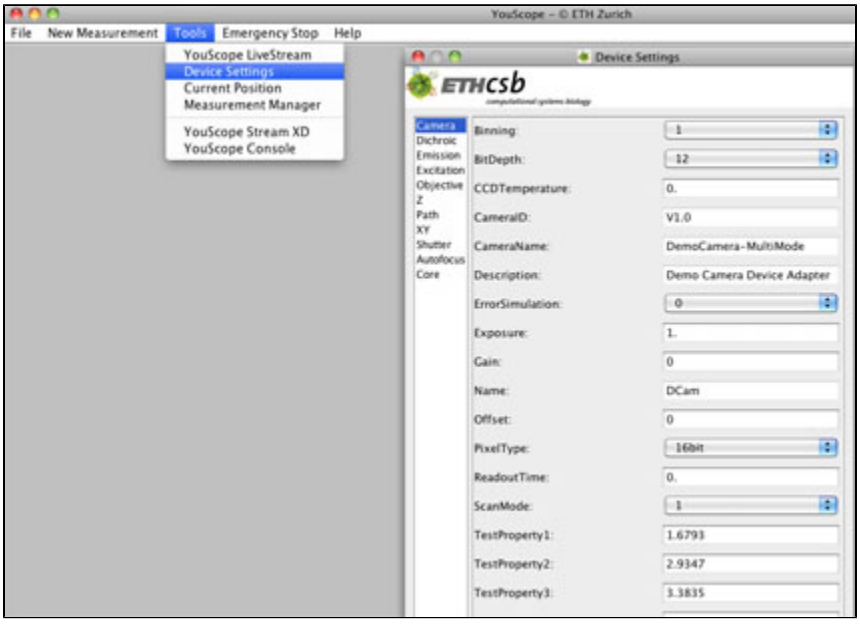
Display the Position of the Position of the Stage

There is a possibility to display the position of the beam on the sample. This is important, if an old position has to be found again, or to check, if in the configuration the right coordinate system is chosen. The position cannot be changed in this option, just displayed. The position can be changed manually. The option is under **Tools->Current Position**.



Device Settings

The device settings can be recalled with **Tools->Device Settings** for controlling and -if necessary - manipulating the current settings of the devices of a microscope. Common settings should however be rather made in the System.Startup configuration group in the *.cfg file.



Configuration of YouScope

Configuration of YouScope

An important step in the configuration of **YouScope** is to define the coordinate system in **File->Configuration** depe nding on the microscope. Most microscope manufacturers use a right handed coordinate system, with the y- and y-axis defining the stage position and the z-axis defining the focus position. Usually an increasing z-position corresponds to a decreasing distance between the objective and the object. There exist two different standards how the direction of the x- and y-axis are defined:

Case 1: Moving the microplate with increasing y-value results in movement of the imaging position from well A1 to H1, and moving the microplate with increasing x-value results in movement of the imaging position from well A12 to

A1. That means that the statement *y-value increases from A1 to H1* is true (upper right-handed coordinate system in Figure 1) and the statement x-*value increases from A1 to A12* is false.

Case 2: Moving the microplate with increasing y-values results in movement of the imaging position from well H1 to A1, and moving the plate with increasing x-values results in movement of the imaging position from A1 to A12. That means only the statement x-*value increases from A1 to A12* is true (lower right-handed coordinate system in Figure 1) and the statement *y-value increases from A1 to H1* is false.

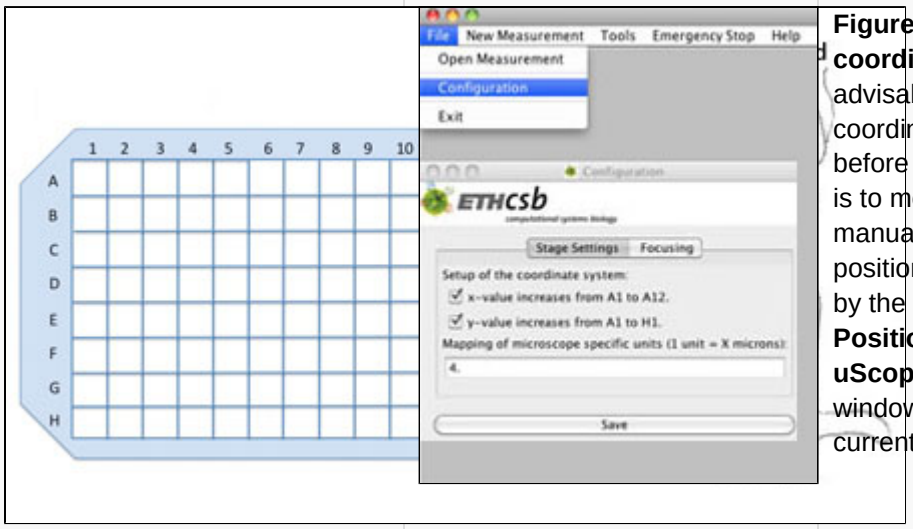
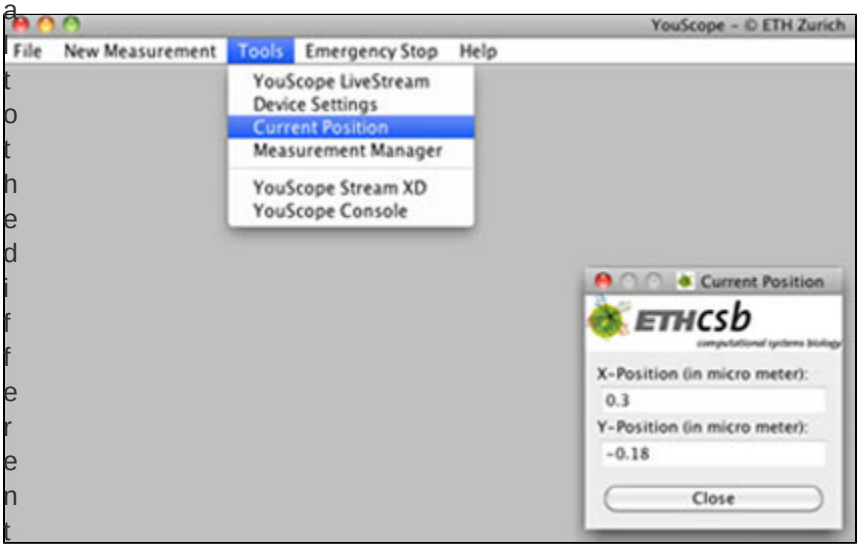


Figure 1: Right-handed coordinate system. It's now advisable to check if the chosen coordinate system is correct before continuing. The best way is to move the microplate manually in x-y-direction. The position can be followed easily by the option **Tools->Current Position** in the toolbar of the **YouScope** window. A small window appears, where the current position in displayed. |

> In machine constructions a right-handed coordinate system is used.
> But, YouScope internally transforms each coordinated system to a left-handed one. In this left-handed coordinate system an increasing x-position corresponds to a movement from A1 to A12 and an increasing y-position corresponds to a movement from A1 to H1. As typical a, increasing z-position corresponds to an increasing focus value. We have made the experience that this definition seems to be more intuitive for the experimentalists.

Additional to the different orientations of the axis some microscopes measure distances in microscope specific units. This units can be transformed in m. Since YouScope is measuring all distances m, the conversion factor between the microscope



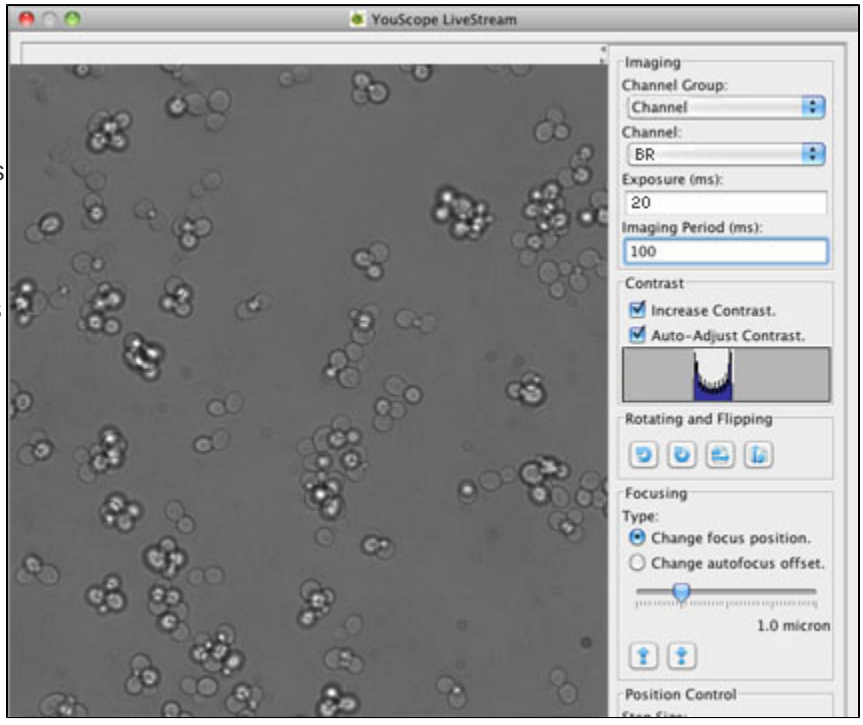
internal units and the units used by YouScope has to be determined. For many microscopes the conversions factor is 1, and for the other ones it is typically an integer. To determine the conversion factor for a given microscope the beam has to be moved to the center of two wells and the values for x1 and x2 of the x-axis in units have to be read out. THe current position can be read out with the help of Tools->Current Position. The size of a well, called a, has to be known (e.g. 900 m for a 96-well microplate, 4500 m for a 384 well plate, 2250 m for a 1536 well plate). The value which has to be inserted in "Mapping of microscope specific units" can be calculated by the formular mapping=|a|/|x2-x1|.

Preparation of a Measurement

Preparation of a Measurement

Coarse Parameter Settings (LiveStream)

Before starting a measurement, the position of the cells have to be found, the focus has to be adjusted and the optimal exposure time for bright field or fluorescent imaging has to be determined. For this purpose a *LiveSteam* mo de can be used. The LiveStream can be found in **Tools->YouScope LiveStream**. To increase the visibility of the cells in a rather dark image exposure time can be adjusted, or the option *Increase Contrast* can be selected. The latter makes it easier to localize the cells, to adjust the focus and to get an impression about the viability of the cells and their fluorescence properties. The LiveStream is used in the following way: First, the microplate is moved within the xy-plane in a position, where the beam illuminates the sample. Second, the focus is adjusted by moving the microplate along the z-axis, if the microscope has a hardware focus system. Finally the autofocus on the microscope is switched on and its offset is corrected. Then the option Increase Contrast is deselected to determine a good exposure time: The exposure time is increased or decreased until the cells are clearly visible in the live stream. The determination of a good exposure time is further described by the histogram, which should have its maximum in the middle.



Channel group: In each channel group different channels are grouped in sets for more clarity. The groups are defined in the configuration file loaded at the startup of YouScope. For example, the different colors of the emitted wavelengths for red-, blue-, green- and yellow-fluorescent proteins, or the bright field light could form a group. Ano ther group could contain the same channels with different values for the binning of the camera.

Channel: Depending on the selection of the *Channel Group,* one *Channel* from this group can be chosen, representing e.g. the filter settings for imaging the wavelengths of red-, blue-, green-, yellow light.

Imaging Period: In the *LiveStream* the image is refreshed in a defined time interval. This is done automatically and there is no need to refresh it manually. The size of interval can be defined in *Imaging Period*. By default it is set to 100 ms. However, the real period highly depends on the hardware, as well as on the chosen exposure time. Thus, the given value rather corresponds to the minimal refresh-period.

Contrast and Histogram: The histogram of the intensity distribution is helpful for the determination of a good exposure time as described below in the section *Exposure*. The option *Increase Contrast* optimizes the contrast between the cells and the background in the image. This option is activated for the adjustment of the focus or the localization of the cells in the well. The option *Auto-Adjust Contrast* will adjust the upper and lower clipping boarders, based on the pixel with the highest and lowest intensity in the range. If *Auto Adjust Contrast* is activated, the boarders cannot be manually adjusted by the user.

Exposure: To illuminate the imaging area properly, the exposure time has to be determined for each channel. After a position containing cells is found and the focus is adjusted, the exposure time has to be determined. The option *Incr ease Contrast* has to be deselected first. Then the exposure time has to be increased till the cells start being visible in the live stream and the histogram fills the area maximal.

Setting up an Advanced Measurement

Setting up an Advanced Measurement

General Information

An advanced measurement can be created with the option **New Measurement->New Advanced Measurement** in the toolbar of the **YouScope** window. This measurement type has the advantage of more flexibility in setting up the measurement procedure and the possibility to run several tasks with different periods in parallel compared to a microplate measurement. Figure 1 shows the flowchart of an advanced measurement.

In general a task is defined as a set of program instructions. The single instructions are called jobs. The task sends its jobs into a queue, which is processed by the microscope executes one by one. The order of the jobs in the queue depends on the arrival of the jobs (first in - first out).

While in microplate measurement the jobs for one big task are selected, in advanced measurement several tasks with fixed or variable period containing several jobs can be specified. The tasks are executed in parallel (multi-threading)and send their jobs to the queue, like demonstrated in Figure 1. Job 1 and job 2 of task 1 are sent with a fixed period to the queue. Job 1 is immediately executed by the microscope and job 2 waits in the queue until job 1 is finished. The fixed period of task 1 is measured between the starts of two following jobs (start job i to start job i+1) and named as T1 in Figure 1. The period length of the task is identical to the period length between the executions by the microscope.

Task 2 sends its job 3 to the queue or directly to the microscope, when the microscope is not busy. After job 3 is executed by the microscope a response is send back to task2, that sends then again a job 3 to the queue or microscope. T2 is measured between the end and start of two following jobs (end of job i to start of job i+1). With the result that task 2 sends its job 3 with a variable time period to the queue, but the time T2 between the feedback at end of executed job 3 and send of the following job 3 is constant.

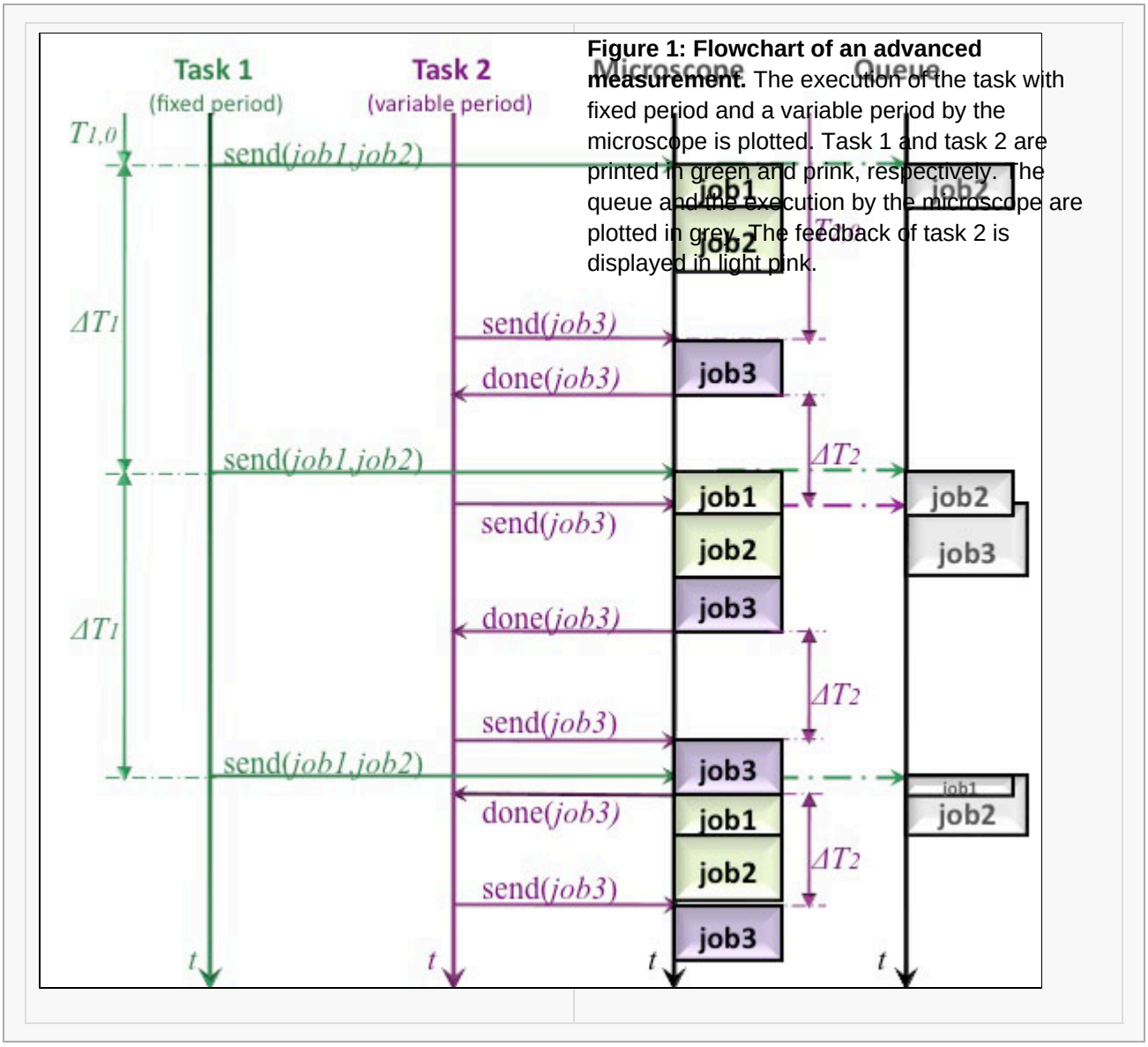
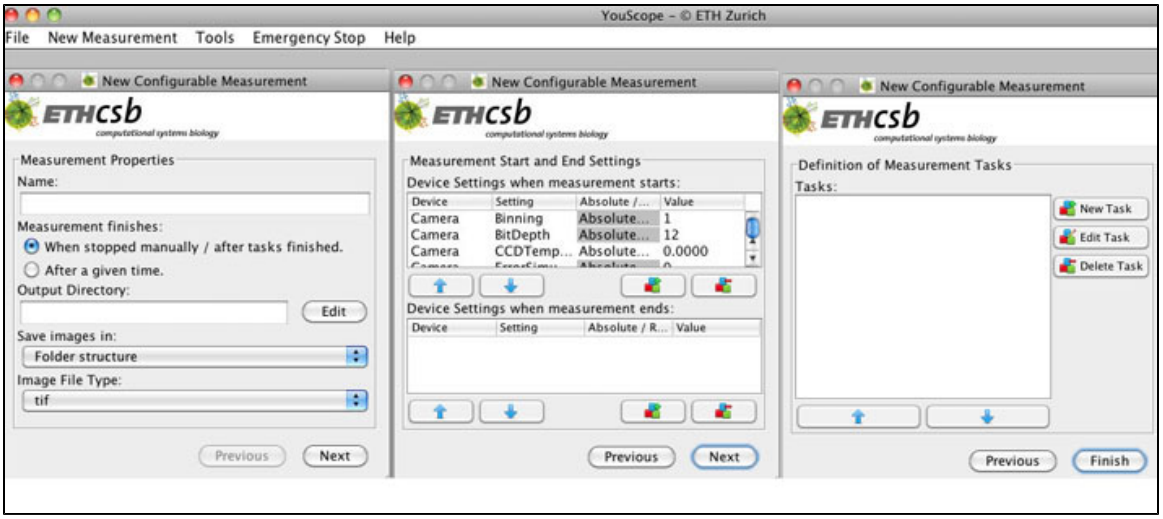


Figure 1: Flowchart of an advanced measurement. The execution of the task with fixed period and a variable period by the microscope is plotted. Task 1 and task 2 are printed in green and prink, respectively. The queue and the execution by the microscope are plotted in grey. The feedback of task 2 is displayed in light pink.

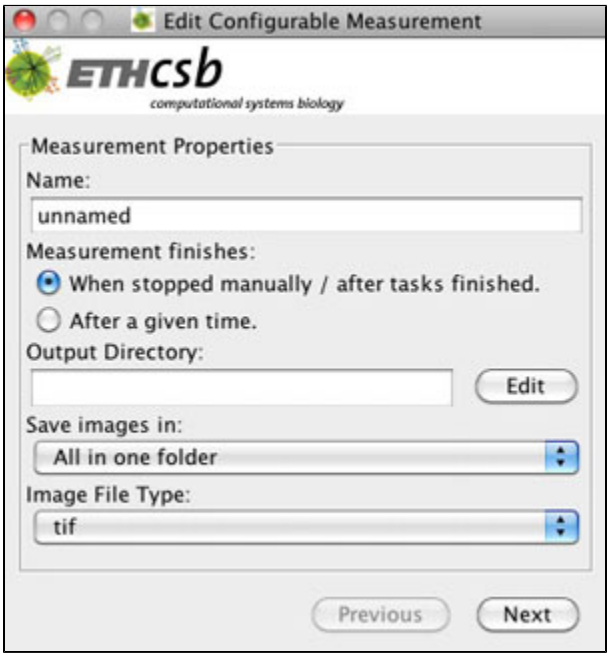
General Properties

In case of New Advanced Measurement a window, called *New Configurable Measurement*, appears with a *Next* and *Previous* on the bottom for switching between different windows. These windows display parameters grouped in three classes, like ***Measurement Properties***, ***Measurement Start and End settings and Definition of Measurement Tasks***.



Measurement Properties of an Advanced Measurement

Measurement Properties



Properties for identification and organization of the measured images

- *Name*: Measurement Properties for a Microplate Measurement
- *Output Directory*: Measurement Properties for a Microplate Measurement
- *Save images in*: Measurement Properties for a Microplate Measurement
- *Image File Type*: Measurement Properties for a Microplate Measurement

Timing parameters

- *Measurement finishes* - Time settings for the total running time of the imaging process: There are two possibilities to finish the measurement. It can be stopped manually, by choosing the option *When finished manually/after tasks finished*. The microscope repeats the imaging jobs of the tasks until the user stops the measurement. This is used, if maybe a change in the development of the cells has to be observed and the microscopist does not know, when the steady-state status is achieved. The other possibility is to define a time period, in which the tasks are repeated n-times (*After a given time period*).

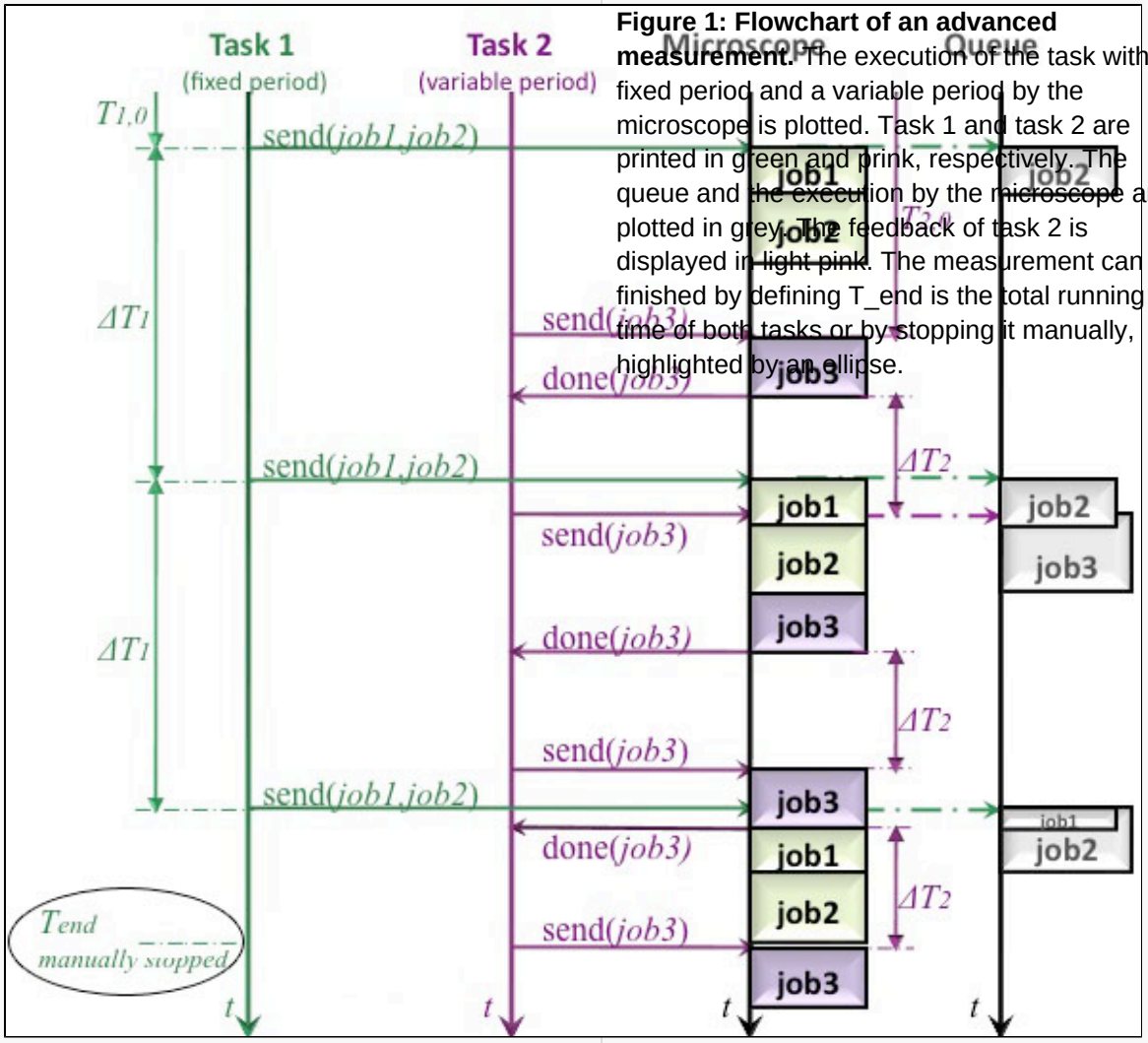


Figure 1: Flowchart of an advanced measurement. The execution of the task with fixed period and a variable period by the microscope is plotted. Task 1 and task 2 are printed in green and prink, respectively. The queue and the execution by the microscope are plotted in grey. The feedback of task 2 is displayed in light pink. The measurement can be finished by defining T_end is the total running time of both tasks or by stopping it manually, highlighted by an ellipse.

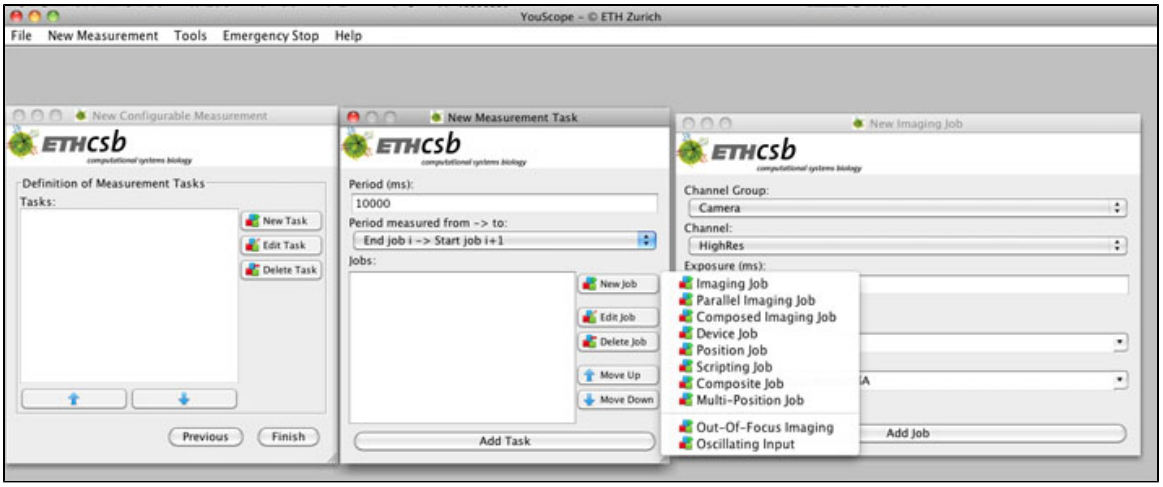
Measurement Start and End Settings of an Advanced Measurement

Measurement Start and End Settings

The detailed description of the Measurement Start and End Settings for a Microplate Measurement is identical to the Measurement Start and End Settings of the microplate measurement.

Definition of Measurement Tasks for an Advanced Measurement

Definition of Measurement Tasks



While in a microplate measurement the jobs for one task have to be selected in the *Definition of the Measurement Tasks*, in advanced measurement several tasks containing several jobs can be combined. The advantage is that several tasks can run in parallel and send their jobs to the queue. The microscope executes the jobs in the queue. T he button *New Task* opens a Task window. This task can be filled with the same imaging jobs like in microplate measurement. But in addition there is the possibility to measure the p*eriod measured from* (i) *End job i->Start job i+1* or (ii) *Start job i ->Start job i+1*, which correspond to variable or fixed period of a task, respectively. The period length of the task has to be defined and a set of jobs have to be selected for each task.

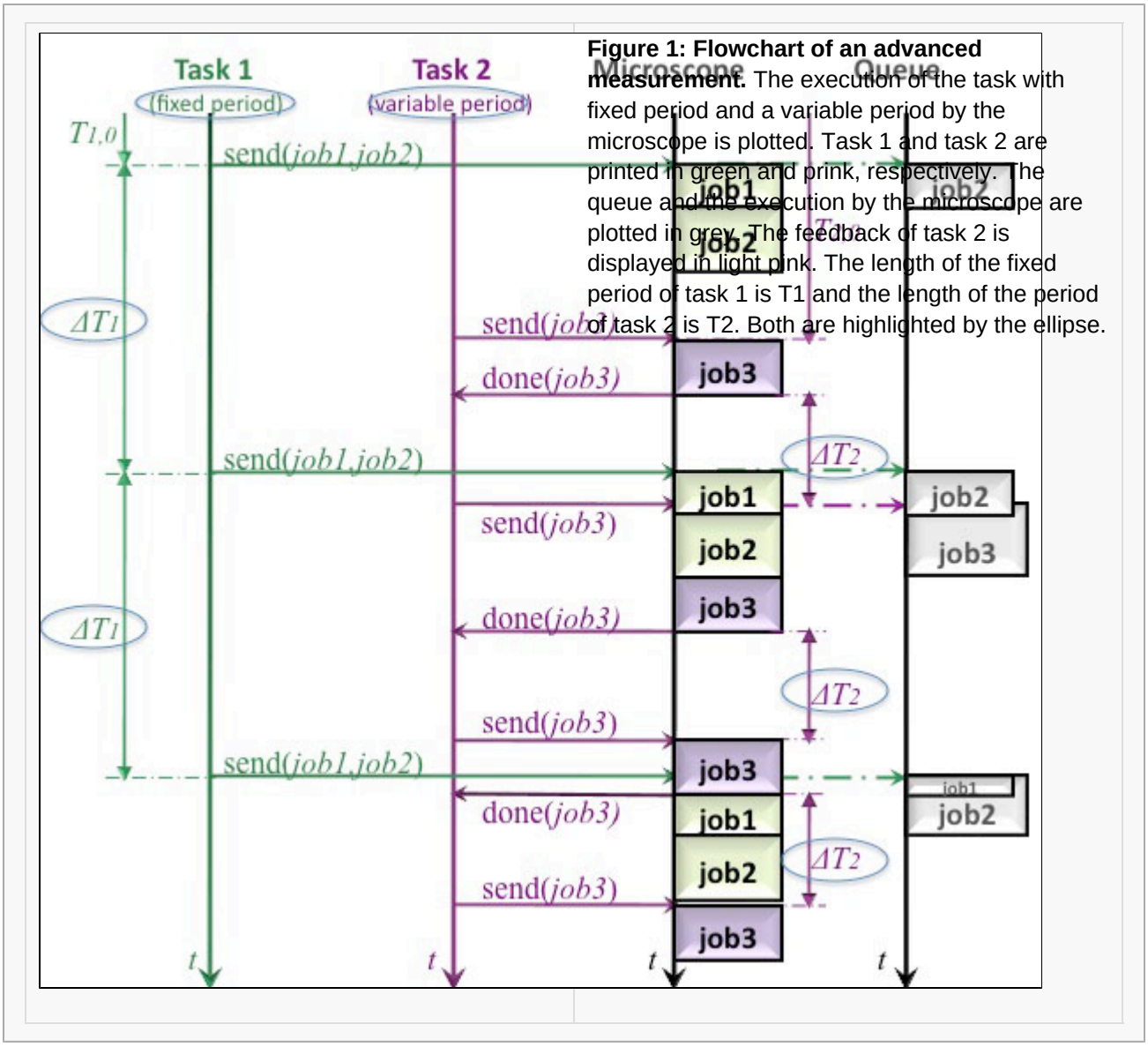


Figure 1: Flowchart of an advanced measurement. The execution of the task with fixed period and a variable period by the microscope is plotted. Task 1 and task 2 are printed in green and prink, respectively. The queue and the execution by the microscope are plotted in grey. The feedback of task 2 is displayed in light pink. The length of the fixed period of task 1 is T1 and the length of the period of task 2 is T2. Both are highlighted by the ellipse.

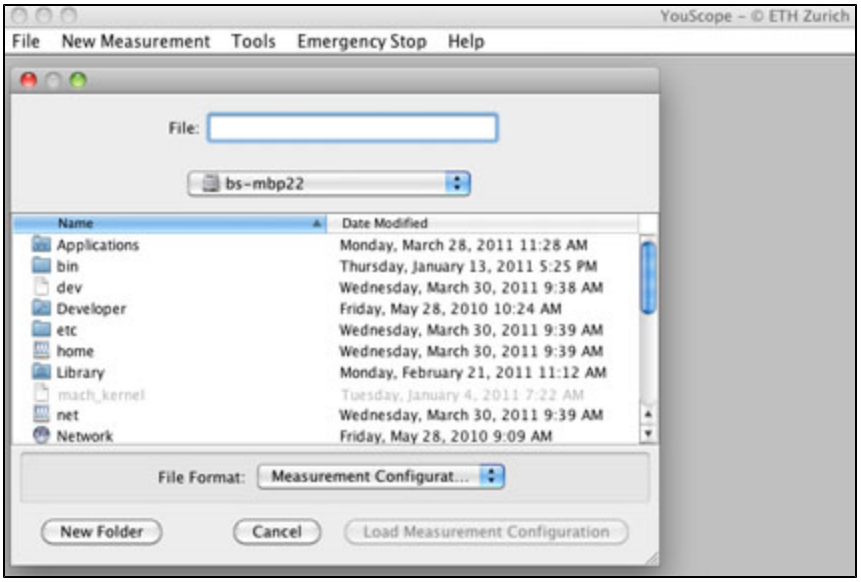
In Definition of Measurement tasks the jobs can be selected

The jobs are identical to the jobs in microplate measurement and are detailed described in the section Imaging Procedure in each Well or Position**.**

Open a Saved Measurement

Open a saved Measurement

A saved measurement can be reloaded by choosing the option **File ->Open Measurement** in the tool bar of the **YouScope** window. This gives the possibility to open and modify a previous configured measurement. Remark that the configuration of every measurement, that was executed, is saved in a separate folder.



Understanding YouScope

Understanding YouScope

This section of the documentation gives detailed information on how YouScope works. It is not strictly necessary to have this detailed background knowledge if one only wants to use YouScope as it is. However, if one plans to use YouScope for more sophisticated applications, if one wants to extend the functionality of YouScope writing plugins, or even if one plans to incorporate (parts of) the functionality of YouScope into other software products, it can be helpfull to get a deeper knowledge on how YouScope works.

Microscope Connection

Microscope Connection

To connect to the microscope, YouScope uses parts of the functionality provided by the open source project Micro-Manager. Micro-Manager is a microscope control software similar to YouScope, but with a different scope. Compared to YouScope, it might be described as a lower-level, which in this context means that one can control the microscope more direct. Similar to YouScope, Micro-Manager is split up into a server (mainly providing the functionality) and a client (mainly providing the user interface). The server part of Micro-Manager can be described as a large set of drivers for different microscope devices, and a small layer of interfaces allowing any client application to choose, load and control the drivers for the hardware the microscope consists of. YouScope uses these interfaces of the Micro-Manager server to connect to the microscope, but has no relation to the Micor-Manager client. Thus, YouScope profits from the large amount of drivers already available for Micro-Manager, and it is easily possible to switch between the Micro-Manager client and YouScope without the need to do the configuration of the microscope twice.

When the YouScope server is started, it is loading the Micro-Manager server in its process. In a first step, it is then hiding all interfaces of Micro-Manager by its own, more abstract interfaces. The main modules of the YouScope server, all plugins and the clients connected to the YouScope server can only access the microscope using this additional layer of interfaces, but do not have access to the lower level layer provided by Micro-Manager.

Server-Client Concept

Server-Client Concept

YouScope is implemented strictly separating the functionality from the user interface. This separation is done to

ease the evolution of YouScope, and to simplify reusing the same functionality in related workflows. The separation is implemented by splitting YouScope into two modules, the server -implementing the functionality- and the client -implementing the user interface. The separation between client and server might be misleading. In deed, the server and the client can be started on two different computers/in two different processes. This method allows to control the microscope from any computer in the same network/connected to the internet (see Figure 1); however, the access requires an authentification, i.e. is password protected. Thus, it is e.g. possible to start a long-time measurement at work and to control its progress from home, increasing the experimentalist's free-time. However, it is also possible to start the client and the server in the same process on the computer directly connected to the micrscope (see Figure 2). Although, then, it is not possible anymore to control the microscope from other computers, this method reduces network traffic, increases speed (especially for fast imaging), and naturally increases security.

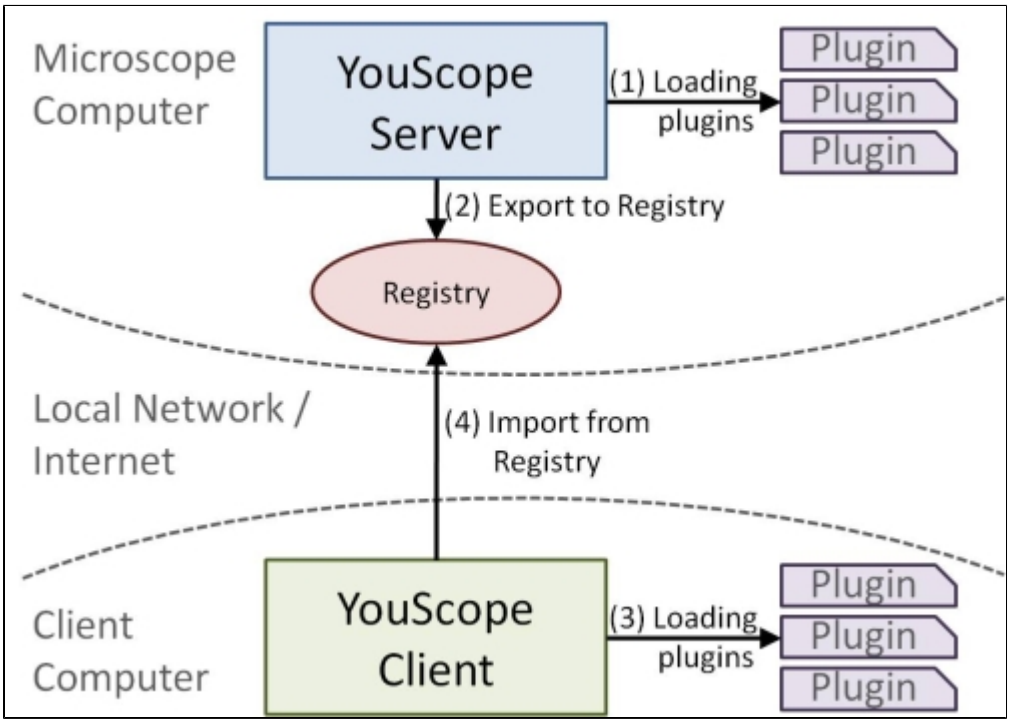


Figure 1: YouScope server and client run in two different processes, which might (or might not) be on two different computers connected via a local network or the internet. First, the YouScope server is started on the computer connected to the microscope, which (1) loads dynamically all server related plugins, and (2) exports itself into a registry, where it can be found by client applications. Second, the YouScope client is started on either the same or any other computer, which (3) loads all client related plugins, and (4) connects to the microscope computer. The client authentificates itself and querries from the registry an interface with which it can control the server and therewith the microscope.

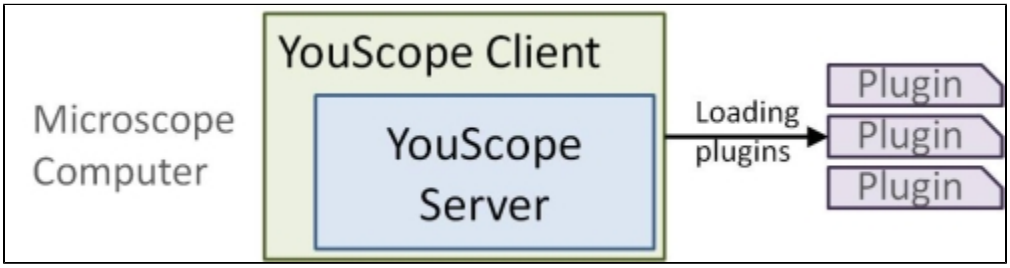


Figure 2: Alternatively to the workflow described in Figure 1, the client and the server can be loaded into the same process on the microscope computer. The client is then communicating directly to the server and to therewith to the microscope, which reduces network traffic and increases the speed.

Which of the two methods to run YouScope is chosen only depends on the use case and the user preferences. However, it is important to understand that YouScope is splitted up into the two functional modules "client" and

"server". This splitting was done such that neither the client has any information about how the functionality is implemented on the server side, nor that the server knows which type of client is connected to it (client and server only communicate through well defined interfaces). This allows to separatly continue to develop the client and the server, or to even replace the YouScope client with an own application, which just uses the functionality of the server.

Compatibility

Please add here if you tested YouScope with a new system configuration.

OS

OS	compatibility	remarks
Windows XP (32bit)	compatible	
Windows 7 (64bit)	compatible	Used 32bit JRE due to the 32bit Manager DLLs

Manager Versions

Manager Version	compatibility	remarks
1.3	compatible	

I (Moritz) had some older versions installed. On the older versions there were no compatibility problems, however I cannot remember the exact versions. Thus I guess we have to test them again.

Matlab Versions

This is only important for the MatlabScript plugin.

Matlab Version	OS	compatibility	remarks
7.10.0 (R2010a)	Windows 7, 64bit	compatible	

I (Moritz) had some older versions of Matlab installed on my previous Windows XP system (I think some R2008 or even earlier). On the older versions there were no compatibility problems, however I cannot remember the exact versions. Thus I guess we have to test them again.

Microscopes

In principal YouScope should work for all microscope for which Manager works (see http://valelab.ucsf.edu/~nico/MMwiki/index.php/Device_Support), since we do access all microscope devices over the Manager server. However, testing is better than guessing...

Microscope	Location	Owner/contact person	compatibility	remarks
ECLIPSE Ti from Nikon	ETH Zurich, D-BSSE, CSB, Yeast Lab	Fabian Rudolf, Moritz Lang	compatible	

Scripting Languages

YouScope supports the use of arbitrary scripting languages through the Scripting Interface in the JFC. New scripting languages can be added by just moving the respective JAR files in the plugins directory or in the respective directory in the JRE. However, if a given scripting language is really working, can not be guaranteed due to the nearly arbitrary possibilities of different syntaxes which can be implemented as scripting languages.

Language	available at	compatibility	remarks
JavaScript	part of the JFC	compatible	
Matlab Script	part of YouScope	compatible	

Microscope Devices

Every microscope can be extended with several devices. Even if the core microscope is compatible, these devices not necessarily have to...

Device	Type	Vendor	Microscope	compatibility	remarks
pE-2	4-wavelength excitation system	CoolLED	ECLIPSE Ti from Nikon	compatible	Fully tested with Oscillatory Input Job.

ToDos

Please visit https://jira-bsse.ethz.ch/secure/IssueNavigator.jspa to get information about the tasks and issues.

Errors

Errors

Please visit https://jira-bsse.ethz.ch/secure/IssueNavigator.jspa to get information about the tasks and issues.

Projects and Publication

Please contact us if you used YouScope for your project/for your publication.

- E. lemming: Following chemotaxis of E. coli, controlling chemotaxis by light-responsive receptors / light induced localization.

Creation of JAR files and whole project (BUILD process)

Introduction

The build process is done automatically by a dedicated build server.

However, it can also be done by hand. Below some notes about how the result should look like, and the steps to get them...

Folder structure

/.

+ youscope-starter.jar

+ YouScope.exe

+ client/

  + fugue-icons-2.6.4.jar

  + jsyntaxpane-0.9.5-b29

  + youscope-client.jar

  + youscope-client-addon.jar

  + youscope-client-uielements.jar

+ server/

  + youscope-server.jar

  + youscope-server-addon.jar

+ plugins/

  + jai-imageio.jar

  + matlab-scripting.jar

+ youscope-microscopeaccess14.jar

+ <other plug-ins (only some of the most important listed above)>

+ shared/

+ xmlpull-1.1.3.1.jar

+ xpp3_min-1.1.4c.jar

+ xstream-1.4.2.jar

+ youscope-shared.jar

+ drivers/

+ MMCoreJ_wrap.dll (or corresponding)

+ MMCoreJ.jar

+ <All drivers and dependent system libraries>

+ scripts/

+ JavaScript/

+ jobs/

+ oscillatingInput.js

+ Matlab/

+ jobs/

+ oscillatingInput.m

+ documentation

<export documentation from wiki, then run "youscope-documentation-converter" to arrive at the version which has to go here.>

Remarks:

- Do also NOT add "jmi.jar". This file is from MathWorks and gets shipped with every Matlab installation, e.g. in the folder "C:/Program Files/MATLAB/R2010a/java/jar/". The functions provided by the JAR file are only needed by these parts of the program which is started in Matlab, where the JAR file is automatically loaded anyway. Thus, this JAR file is for Compiling only. We probably have no license to redistribute it, too...

JAR FILES

General

In each repository which corresponds to a JAR file there is a manifest file (MANIFEST.MF) in the root folder. Use this one for the JAR file. Additionally, in the projects which correspond to plugins, there are folders named "META-INF". These folders represent the services which are supported by the JAR file. The final folder structure for

the JAR file should be as follows:

/.

+ META-INF

  + MANIFEST.MF

  + services

    + <full class name of supported interface>

where <full class name of supported interface> is a file, e.g. "javax.script.ScriptEngineFactory".

youscope-starter.jar

Packages

+ ch.ethz.csb.youscope.starter

+ ch.ethz.csb.youscope.starter.images

Remarks

Do not add packages of the client or of the server. They are loaded dynamically.

youscope-client.jar

Packages

+ ch.ethz.csb.youscope.client

+ ch.ethz.csb.youscope.client.addon.job

+ ch.ethz.csb.youscope.client.images

+ ch.ethz.csb.youscope.client.uielements

+ ch.ethz.csb.youscope.shared

+ ch.ethz.csb.youscope.shared.configuration

+ ch.ethz.csb.youscope.shared.scripting

youscope-server.jar

Packages

+ ch.ethz.csb.youscope.server

+ ch.ethz.csb.youscope.server.images

+ ch.ethz.csb.youscope.server.microscopeaccess

+ ch.ethz.csb.youscope.shared

+ ch.ethz.csb.youscope.shared.configuration

+ ch.ethz.csb.youscope.shared.scripting

matlab-scripting.jar

Packages:

+ ch.ethz.csb.matlabscripting

+ ch.ethz.csb.matlabscripting.util

Simple Building:

Add "ch_ethz_csb_matlabscripting_util_LongFileNameToShort.dll" file in JAR root (this file is only needed for a workaround which is only necessary for Windows systems (The people at Mathwork cannot program). For simplicity, also add it to Linux, ... versions, it will just not be called there.

Rebuilding ch_ethz_csb_matlabscripting_util_LongFileNameToShort.dll

Normally rebuilding the DLL should not be necessary, since the functionality provided is extremely simple (and there is no reason to extend or modify it). However, if you e.g. change the name of the respective JMI function in Java, rebuilding the file might become necessary.

The project was created using the IDE Dev-C++(http://www.bloodshed.net/dev/devcpp.html), the respective project file has the ending ".dev". The project consists of two files, a header and a source file. The header can be automatically created using the "generateHeader.bat" file or by typing

```
javah -jni -classpath ../bin/ ch.ethz.csb.matlabscripting.util.LongFileNameToShort
```

or similar (classpath might have to be adjusted depending on settings). The project depend on some header files of java, which can be found in the "include" and "include/win32" directories in the JDK installation. The paths to these folders have to be adjusted in the project configuration.

youscope-oscillating-device-job.jar

Packages

+ ch.ethz.csb.youscope.client.addon.job.oscillatingdevice

Remarks

Do NOT add "ch.ethz.csb.youscope.client.addon.job" or "ch.ethz.csb.youscope.client.uielements".

youscope-out-of-focus-job.jar

Packages

+ ch.ethz.csb.youscope.client.addon.job.outoffocus

Remarks

Do NOT add "ch.ethz.csb.youscope.client.addon.job" or "ch.ethz.csb.youscope.client.uielements".

Documentation Tree

Installation

Tutorials

- Preparation of the Measurement
- Example 1 - Microplate Measurement
- Example 2 - Advanced Measurement

Starting YouScope

Setting up a Microplate Measurement

- Measurement Properties for a Microplate Measurement
- Measurement Type for a Microplate Measurement
- Measured Positions for a Microplate Measurement
- Measurement Start and End Settings for a Microplate Measurement
- Imaging Procedure in each Well or Position

Running and Stopping a Measurement

Additional Tools

Configuration of YouScope

Preparation of a Measurement

Setting up an Advanced Measurement

- Measurement Properties of an Advanced Measurement
- Measurement Start and End Settings of an Advanced Measurement
- Definition of Measurement Tasks for an Advanced Measurement

Open a Saved Measurement

Understanding YouScope

- Microscope Connection
- Server-Client Concept