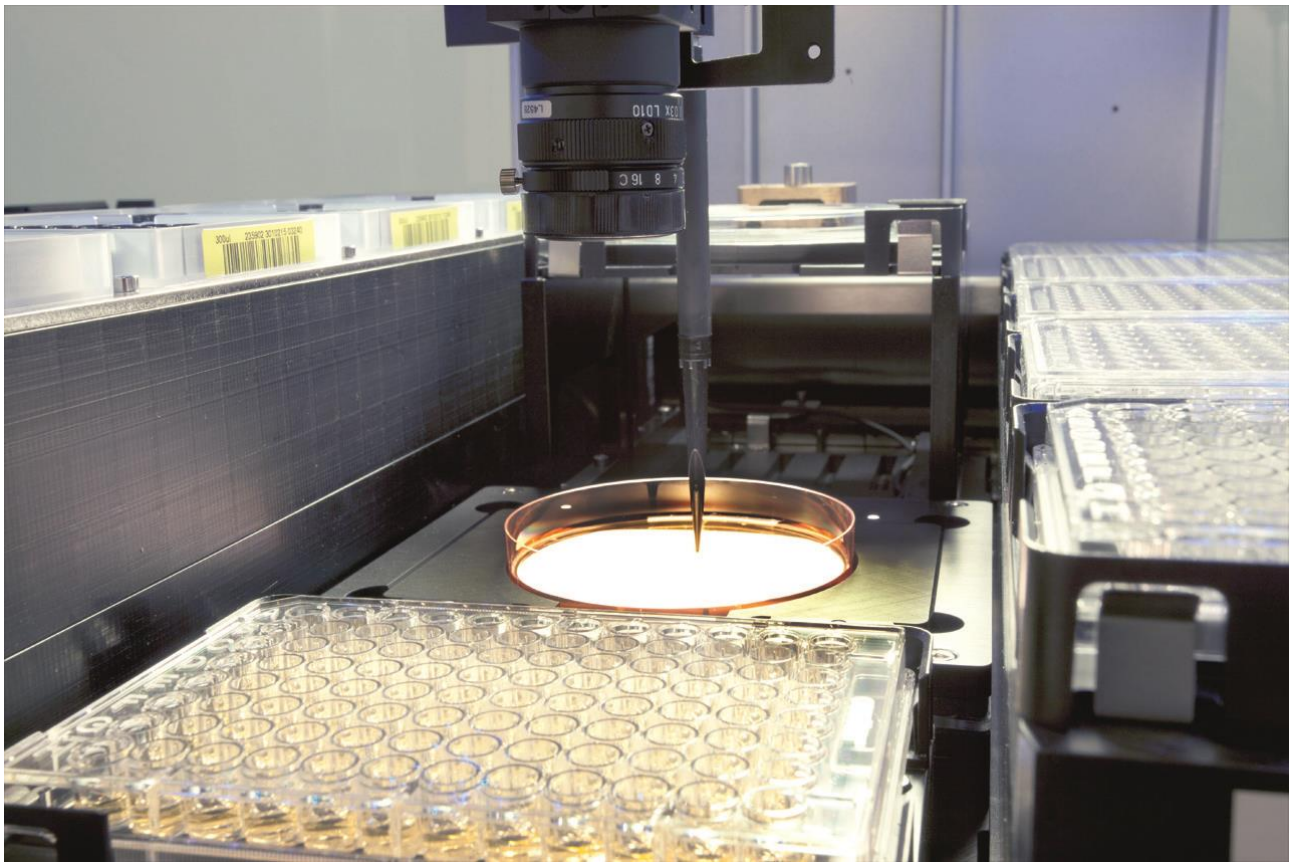
easyPickII

Operator’s Manual for Microlab® STAR



CE

HAMILTON

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1 General Information

easyPickII is an add-on to Hamilton's Microlab STAR Venus software and is designed to work with a camera for the detection and picking of bacterial colonies.
The easyPick light table is a carrier that can be mounted on the deck of the Hamilton Microlab STAR and contains a backlight.

This user manual is designed to help you to get the most out of this product.

1.1 About This Manual

The purpose of this manual is to help the user to get familiar with the easyPick software and to get the best performance out of this product.

The manual describes the installation, the functionality and how to find the best settings. It refers to the easyPickII release V1.1.0. for the Microlab® STAR.

ATTENTION or ***NOTE*** emphasizes important and critical instructions. They are printed in italic and appear as follows:

ATTENTION: *Any special issues, warning or important information will be accompanied by this symbol. Read these items carefully.*

NOTE: *Information is given to the operator that is useful but not essential to the task at hand.*

___ References to Manuals, Figures, Sections, etc. are Underlined.

1.2 Additional Manuals

Additional information can be found in the manuals for the Microlab® STAR; the Operator's Manual and also in the Programmer's Manual. A detailed software reference for the Microlab® STAR can also be found in the On-Line Help of the Microlab® STAR VENUS Software.

1.3 Intended Use

easyPick is designed to find the positions of bacterial colonies by image processing. The images to be processed will be captured by a camera, which is mounted on an additional channel on the arm of the MICROLAB® STAR. The positions found can then be used to pick the colonies with disposable tips on pipetting channels.

The installation must be executed by a trained Service Engineer. Operation and Maintenance are intended to be performed by the Operator.

1.4 Operation

Operators of the Microlab® STAR must have attended an appropriate training course. The procedures described in this manual are tested by the manufacturer and are deemed fully functional. Any deviation from the procedures given here could lead to erroneous results or malfunction.

Training courses will be held by your Hamilton Representative. Please feel free to contact your local dealer to arrange Operator Training.

1.5 Safety Precautions and Hazards

ATTENTION: *All items in this section are of importance for a safe and reliable operation of your equipment.*

The following section describes the main safety considerations to follow and the hazards involved when operating this product.

When using the Microlab® STAR, Good Laboratory Practices (GLP) should be observed. Suitable protective clothing, safety glasses and protective gloves should be worn.

1.5.1 General Precautions

Refer to the Microlab® STAR Operator's Manual.

1.5.2 Computer Precautions

Guard against software viruses. Use only manufacturer's original installation CD-ROM sets, the original Hamilton Microlab® STAR VENUS Software and the original easyPickII Software as well as the computer operating system.

1.5.3 easyPick Specific Precautions

ATTENTION: *Direct exposure of the Instrument's front towards light sources (artificial or natural) must be avoided.*

The optical path must not be obstructed by the operator, specifically the glass plate of the easyPick carrier must be free of dust and dirt.

1.5.4 Hazards

ATTENTION: *easyPick Light table carrier is breakable!*

Handle with care!

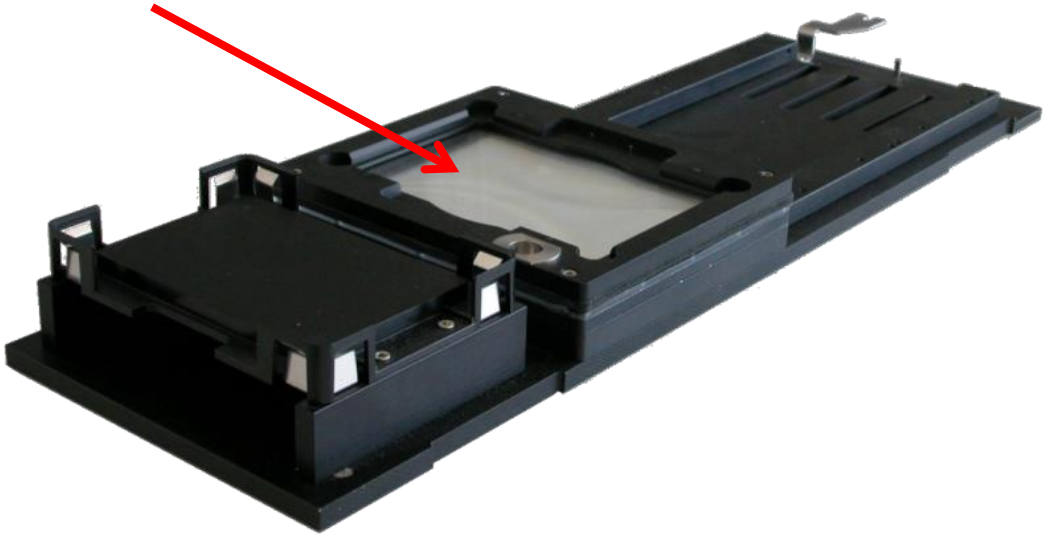


Figure 1: easyPick carrier

2 Description of easyPick

2.1 Overview

easyPick is designed to find the positions of bacterial colonies by image processing. The images to be processed will be captured by a camera, which is mounted on an additional channel on the arm of the MICROLAB® STAR. The bacterial medium (agar) is placed on a light table on the MICROLAB® STAR deck. The positions found by the easyPick software can then be used to pick colonies with the pipetting channels using disposable CO-RE tips.

Using as small as possible tips offer better picking precision results. 10µl, 50µl and 300µl CO-RE tips deliver suitable precision results.

ATTENTION

It is NOT recommended to use 1000µl tips!

2.2 Optical System

The following figure shows the mechanical assembly of the imaging system:
Camera TopEye: 2560x1920 (5 megapixels)
Camera Channel: 2048x1536 (3 megapixels)

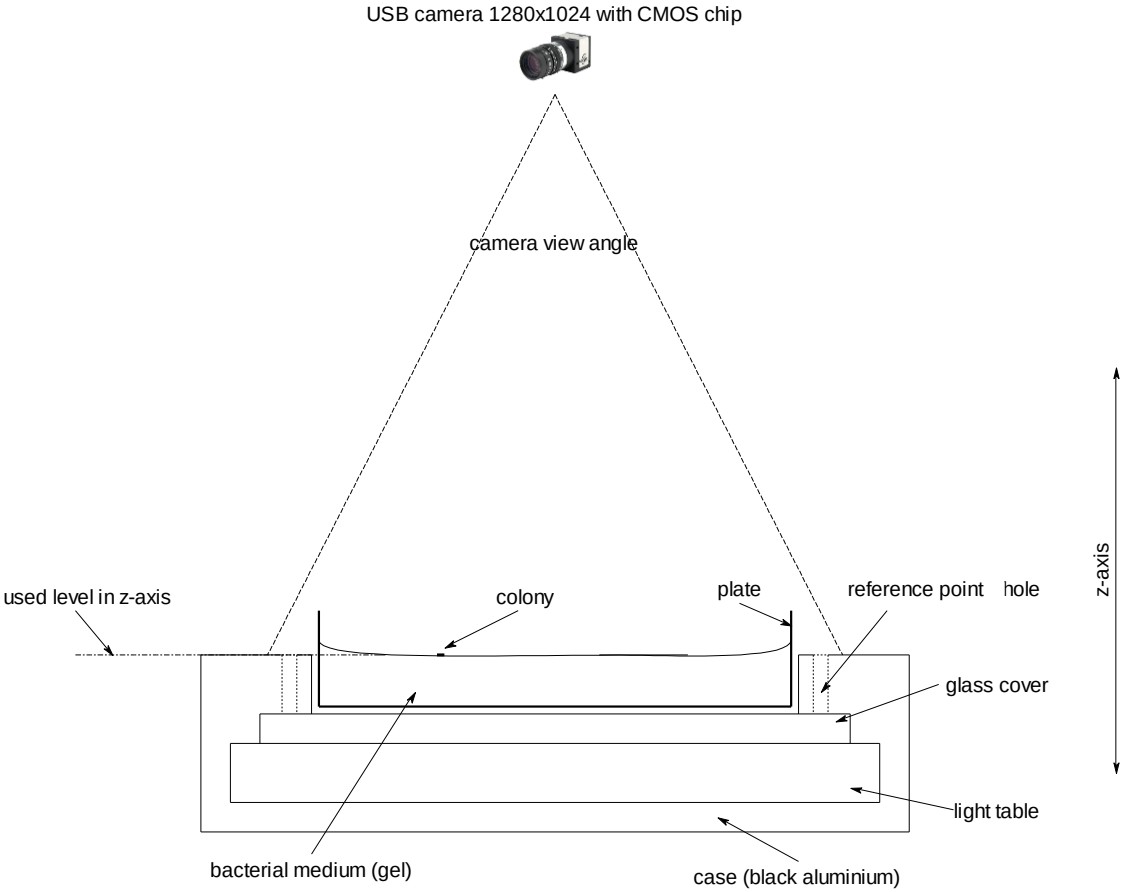


Figure 3: Colony picking mechanical assembly

The dashed lines depict the viewing angle of the camera. By means of four reference points the camera's coordinate system with respect to its pixels will be computed into the millimeter based coordinate system on the MICROLAB® STAR deck. This calculation is only possible for one level in Z-direction.
Whole

2.3 Coordinate systems

There are two different coordinate systems:

- Coordinate system of the MICROLAB® STAR (measured in [mm] with front-left origin)
- Coordinate system of the camera (measured in [pixels] with the origin at the far-left of the camera plane)

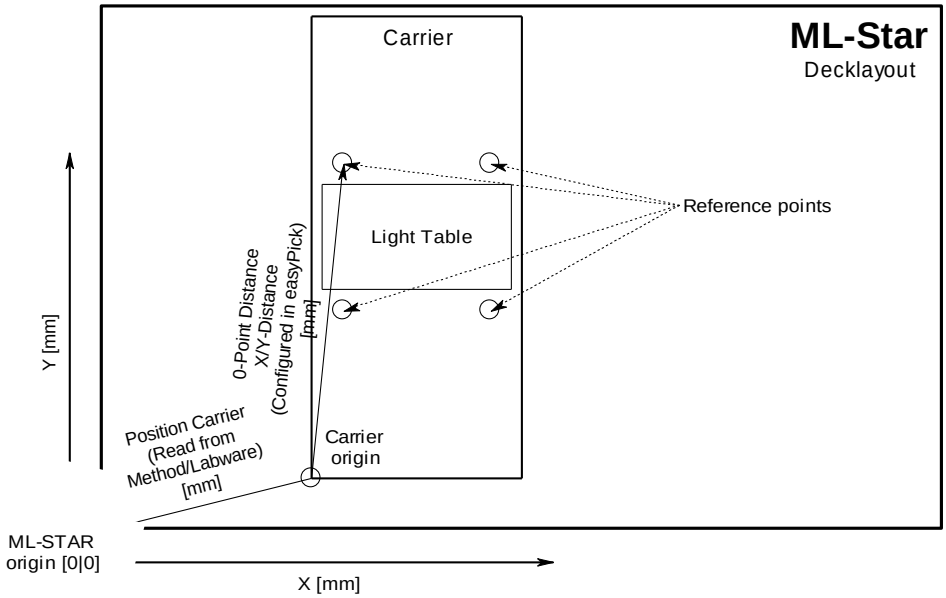


Figure 4: Coordinate system of the MICROLAB® STAR

The coordinate system of the camera picture will be computed into the mm-based MICROLAB® STAR coordinate system with respect to the well-known distances of the reference points.

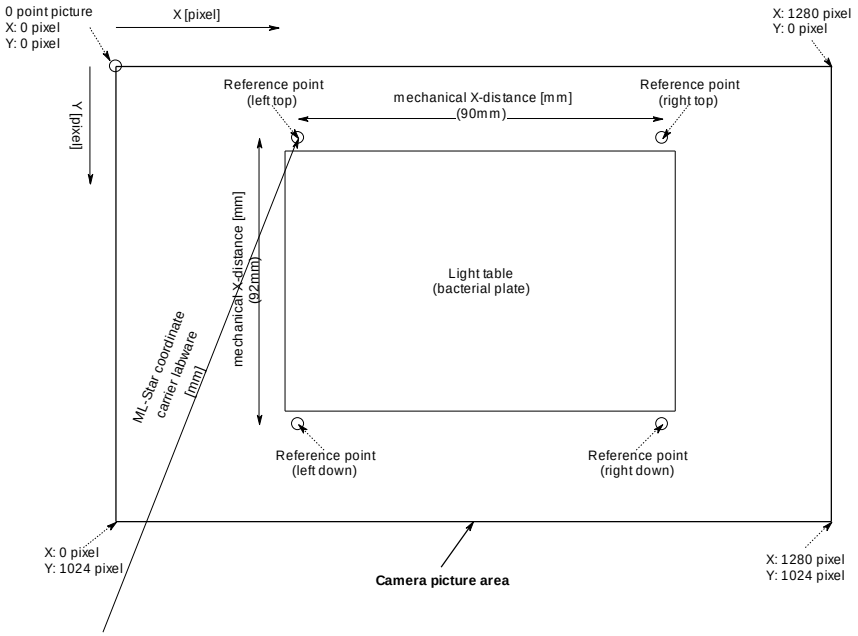


Figure 5: Camera coordinate system

The computation of pixel coordinates into mm coordinates will be done with respect to the fixed distances of the reference points.
The easyPick application delivers all coordinates in [mm] with respect to the top left reference point. The methods read the offset of the carrier to the MICROLAB® STAR origin from the carrier's labware.
If the plate is too big, easyPickII allows to work with two different types of easyPick carrier, the easyPick Light Table and the easyPick Light table XL for bigger Q-Trays. These trays are too big for one image and therefor have to be stitched out of six pictures with overlapping reference points. It is important that the images contain the corresponding reference points of the neighboring picture. The camera will take six pictures and the easyPickII software will combine them as shown below. For further information, please refer to section: 5 Five Steps to successful Colony Picking

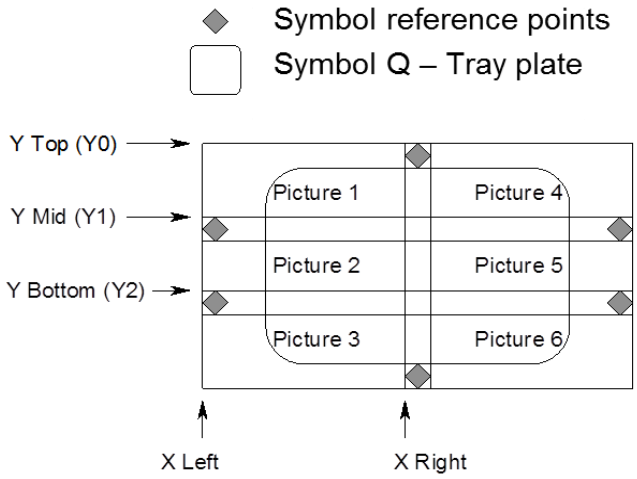


Figure 6: Positioning of pictures

2.4 Data flow

The camera takes a color picture and computes the gray-scaled image from the color image. Non-color characteristics will be evaluated from the gray-scaled image, only color characteristics will be extracted from the color image. The characteristics will then be divided into 'good' and 'bad' colonies. From the weighted list, the coordinates will be read and then used to pick the colony with the MICROLAB®STAR.

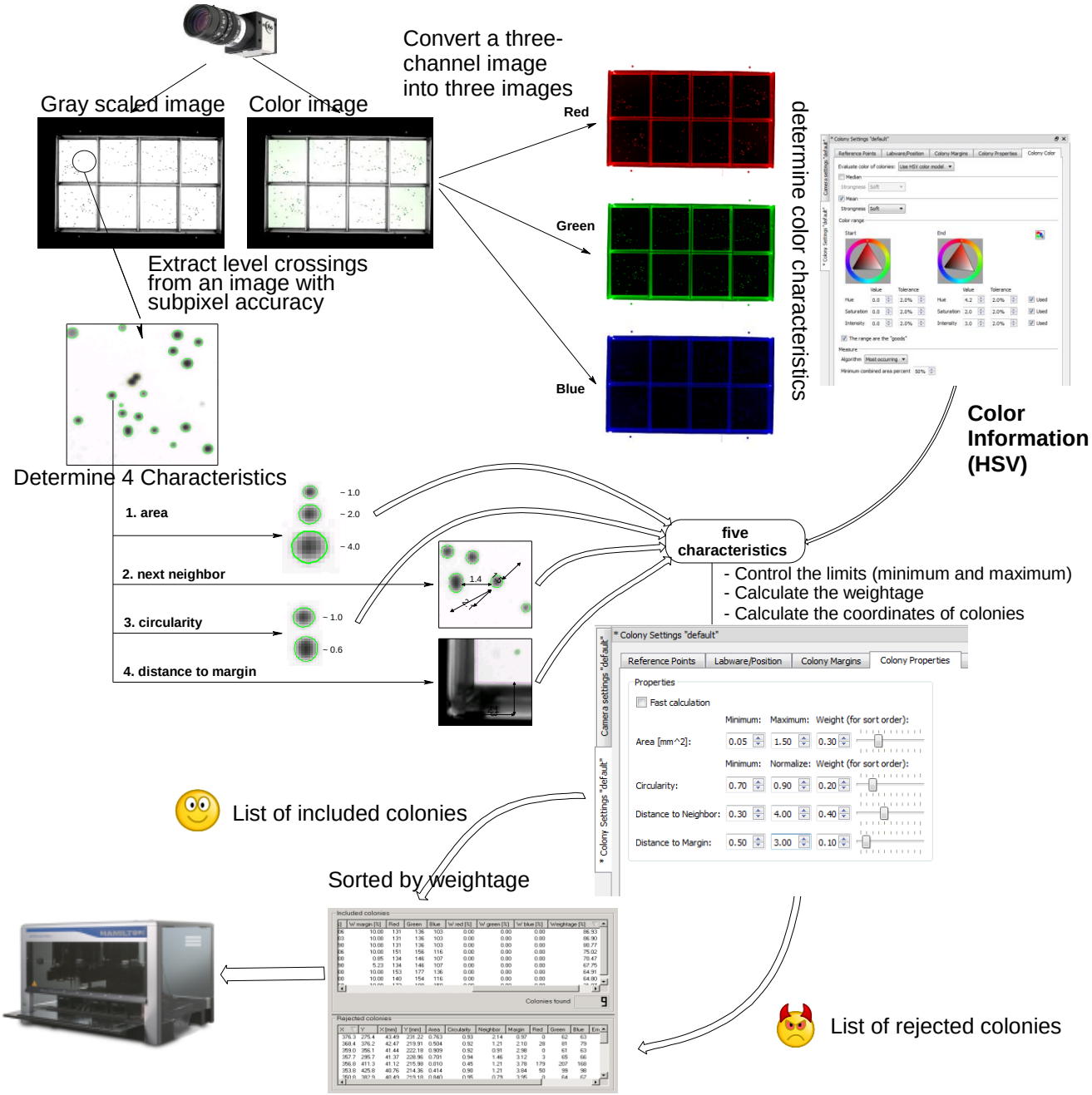


Figure 7: Data flow of easyPick system from camera to the MICROLAB® STAR

2.5 Installation of the easyPick Carrier

The easyPick Carrier has to be installed or relocated by a Hamilton trained Service Engineer. Installations of instrument options and accessories are also done by a Hamilton trained Service Engineer.

Tighten the fixing clip to the light table carrier.

Mount the light table carrier on the desired place on the MICROLAB® STAR deck and fix it on the docking station at the back of the instrument using the fixing clip.

ATTENTION

Move the light table carrier to the hard stop and tighten the screw at the fixing clip!

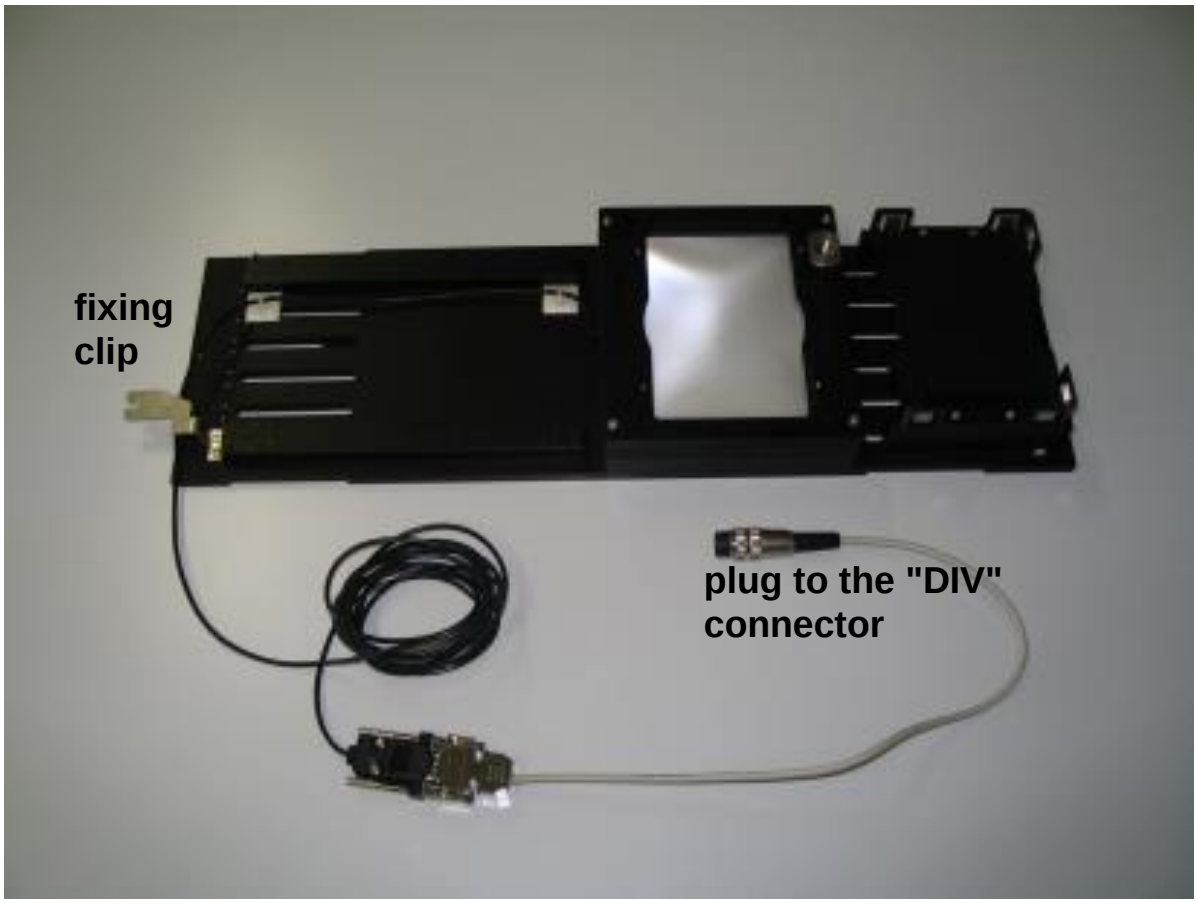


Figure 8: Light table mounted on a 7T Multiflex carrier base

For Q-trays the 13T Light table XL has to be installed in the same way.

Put the cable into the docking station leading to the left, connect the light table with the packaged converter cable, and connect the converter cable's round plug to the "DIV " connector on the

MICROLAB® STAR's left side connector plate. The connector can be set in the method (see Light_On Command in the easyPickII Library). In the Demo Method, connector DIV2 is preset.

connector plate on left side of the ML-STAR

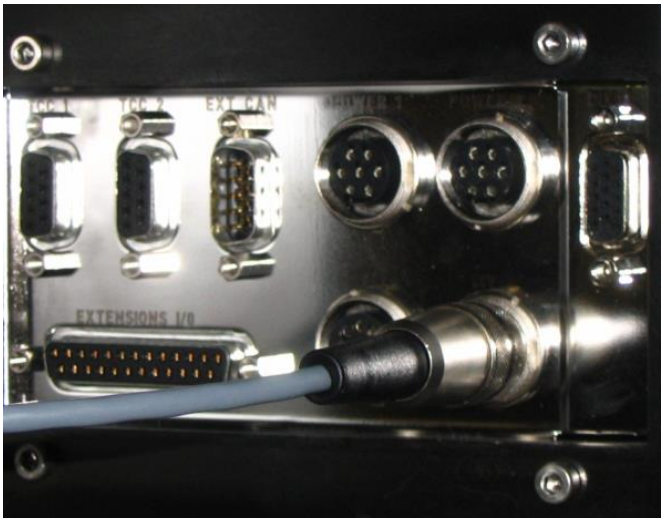


Figure 9: Connecting light table to Microlab® STAR DIV connector

ATTENTION
Do NOT connect the light table directly to the TCC1- or the TCC2-DB9 plug! The light table may be damaged because of the 40V used by TCCs.

2.6 Installation of the easyPickII Software

For information regarding installation of the easyPickII Software, please see Section 3.4 Installation of easyPickII Software.

2.7 Maintenance

Generally, refer to the Microlab® STAR Operator's Manual for the Microlab® STAR Line Maintenance Concept and Procedures and for Ordering Information e.g. Disinfectant Solvents.

Carry out the following tasks for Daily Maintenance while the instrument is switched OFF:

- Check the easyPick Carrier for dust or dried liquids. If necessary, remove dried liquids or dust from the carrier.

ATTENTION: *If the dust cannot be removed, use Deconex Solarsept Detergent and a soft lint free cloth to clean the glass/ mirror. For further information on cleaning tools, consult the Microlab® STAR Operator's Manual.*

ATTENTION: *Make sure not to scratch the surface of the easyPick Carrier.*

NOTE: *If the glass is broken or damaged, the easyPick Carrier is considered damaged. Please contact your Hamilton representative to get a replacement.*

2.8 Disposal

Please refer to the Microlab® STAR Operator's Manual for further information.

3 easyPickII Software

3.1 Introduction

An image processing camera takes an image of an area of interest. The image processing software then searches for colonies within this area.

easyPickII can be controlled from Hamilton's Microlab® STAR VENUS Software by means of the easyPickII libraries.

During installation, a database file is installed on the controlling PC hard disk. The results of the evaluation, the link to the image file and the settings are saved to this database.

3.2 Compatibility

The easyPickII Software is compatible with Microlab® VENUS two Software or higher.

NOTE: *The easyPickII Software is optimized for the Windows7, 64bit Operating System. Older operating systems are not supported..*

3.3 Computer Requirements

The easyPickII software is meant to be used with the Microlab® STAR VENUS Software, which controls all functions for daily work routine, method programming, running methods and other services.

The computer requirements are Windows7, 64bit Operating System (see Section 3.2 Compatibility), therefore at least running under the Microlab® STAR VENUS two Software or higher. A free USB Port 2.0 (or higher) is needed for the USB Camera of the camera channel.

ATTENTION: *It is necessary to use USB 2.0 or higher! Older USB versions are not supported. Using USB Hubs or USB cable lengths over 5 meters may cause loss of signal and this will limit functionality.*

NOTE: *The file path <<Program Files>> that are mentioned in this manual varies depending on the Windows Operating System and the local language of Windows.*

3.4 Installation of easyPickII Software

3.4.1 Components

The complete installation comprises the following components:

- easyPickII Software
- easyPickII Libraries for Microlab® STAR VENUS Software
- Demo Methods for Microlab® STAR VENUS Software
- Labware Files for the Microlab® STAR VENUS Software
- uEye Camera Driver
- easyPickII Software and Operator's Manual
- Camera Settings: (the number refers to the exposure time in milliseconds)

3.4.2 Installation Procedure

ATTENTION: *The Microlab® STAR VENUS Software has to be installed prior to the easyPickII Software.*

ATTENTION: *The easyPickII Software has to be installed prior to the USB Cable connection between the camera and the Controlling PC.*

1. Insert the easyPickII Installation CD in the CD Drive.
2. Install the uEye-Camera Driver: Start the program

   uEye64_47100_WHQL.exe

   For a 64bit Windows installation from the easyPickII Installation CD. The files will extract themselves. Afterwards, choose "Install driver" and later choose "Complete setup".

   If a 32bit Windows installation is used, start the program: **uEye32_47100_WHQL.exe**

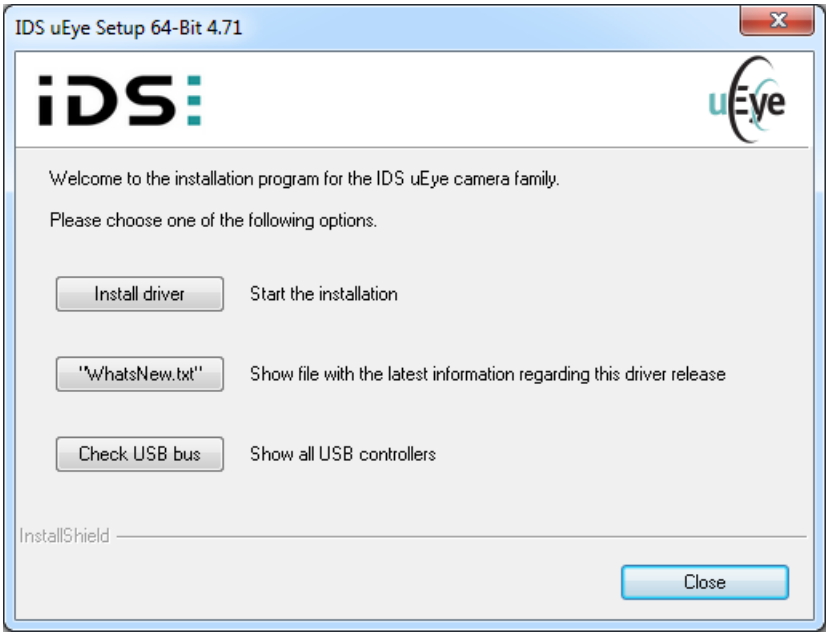


Figure 10: Installation Wizard

3. Start the following program:

   easyPickII-1.1.x-Installer.exe

   Follow the instructions always using the default selections.

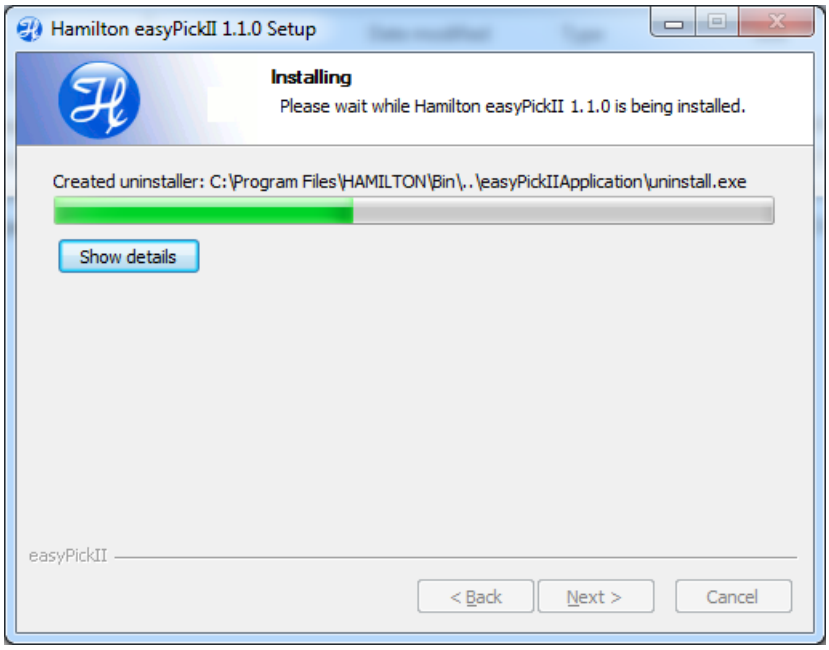


Figure 11: Installation Wizard

NOTE: *The last digit in the version can be different to the one shown in the picture*

4. Now connect the camera with the Controlling PC with the USB Cable. Use the free USB socket from the instrument.

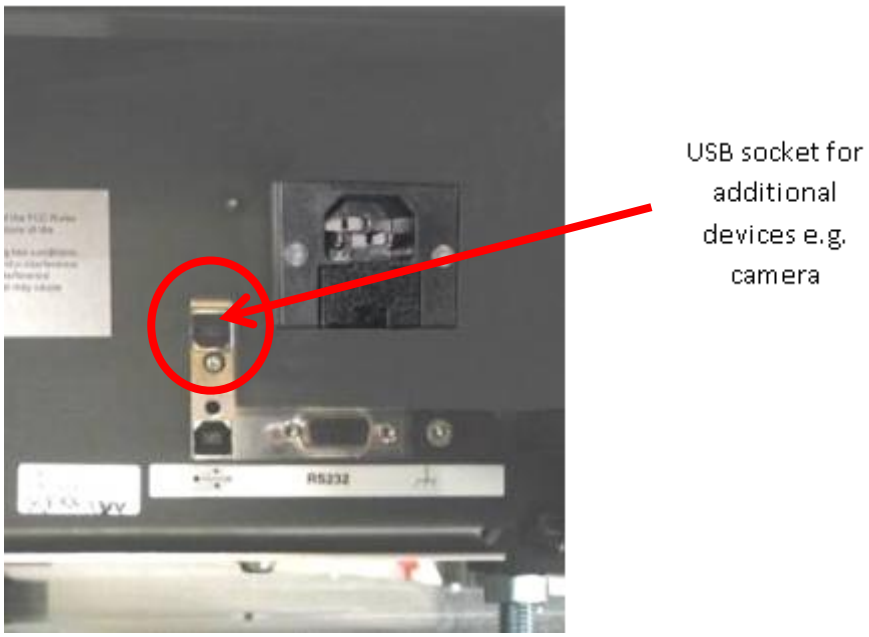


Figure 12: USB socket on the instrument

Windows 7: When connecting the camera into an USB port for the first time, a balloon tip "Installing device driver software" appears, that indicates installation of device driver. After a few seconds, the driver is installed and the device is ready.

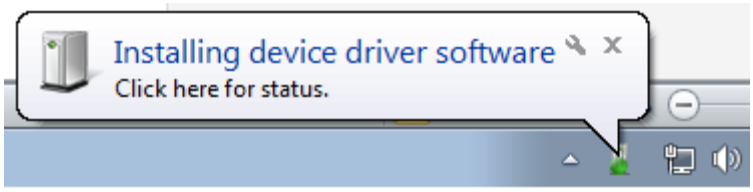


Figure 13: Balloon tip

5. During installation, the easyPickII Shortcut Icon will be created on the Desktop.

When the installation was successful, the green LED next to the USB connector on the camera case will light up.

ATTENTION: *Do not use a different or longer USB cable for the camera than the one provided! Do not use extensions for this cable.*

ATTENTION: *If the green camera LED does not light up try another USB port on the PC. Some PCs feature USB 1.x and USB 2.0 type ports.* ***The USB port must match USB 2.0 features.***

The USB camera might stop to work after reactivating your PC from Stand-by mode. Either disable all Stand-by and Sleep mode options on your PC or disable this option for your USB port specifically. To do this, open Control Panel, click System, and click Device Manager. Open the Universal Serial Bus controllers. In the Properties of the USB Root Hub disable "Allow the computer to turn off this device to save power".

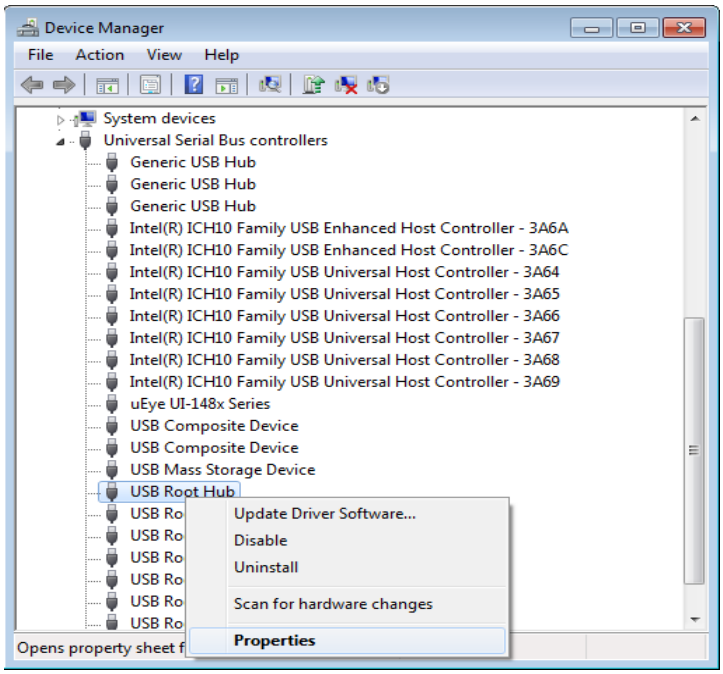


Figure 14: Device Manager

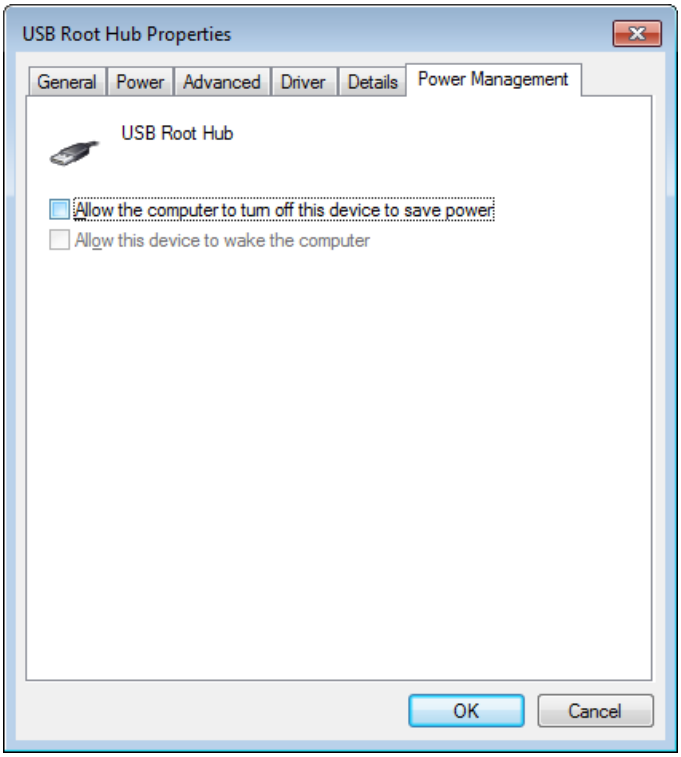


Figure 15: USB Properties

3.5 Software installation check

With the uEye Camera Manager you can check whether the camera is properly connected. Click on the uEye Camera Manager shortcut that has automatically been created on your desktop during installation.

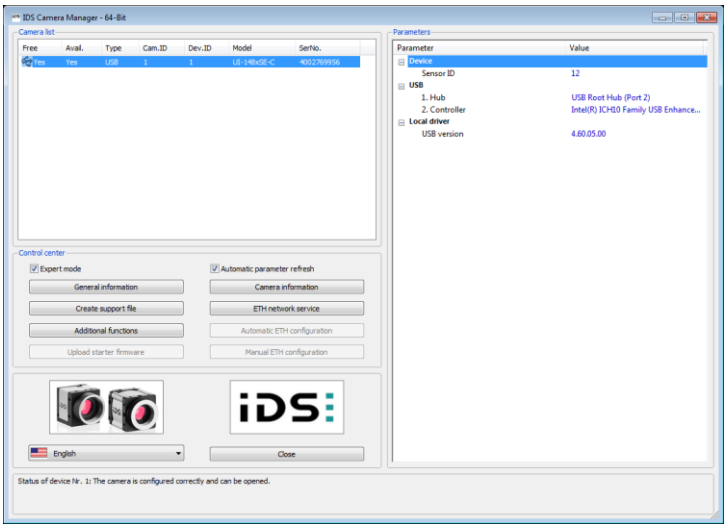


Figure 16: Camera Manager with correct installed camera

To check whether easyPickII has been installed correctly, click on the easyPickII shortcut that has also automatically been created on your desktop.

You should then see the easyPick startup screen as shown below.

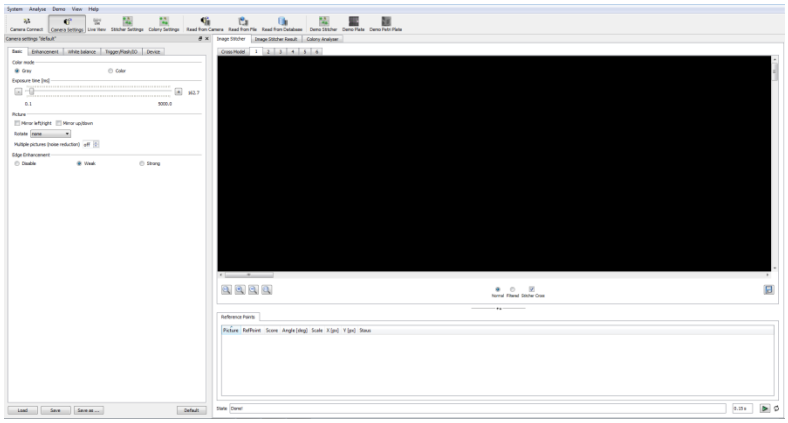


Figure 17: The easyPickII main window

Select the buttons "Camera connect" and "Live view" and you will see the live picture from the camera.

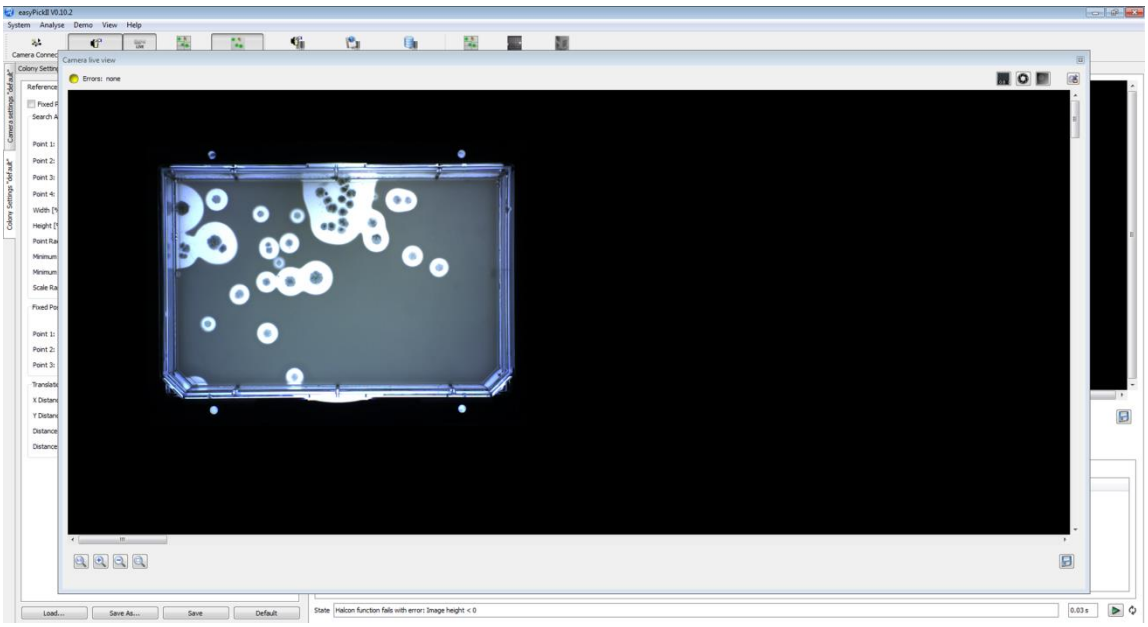


Figure 18: Live Image

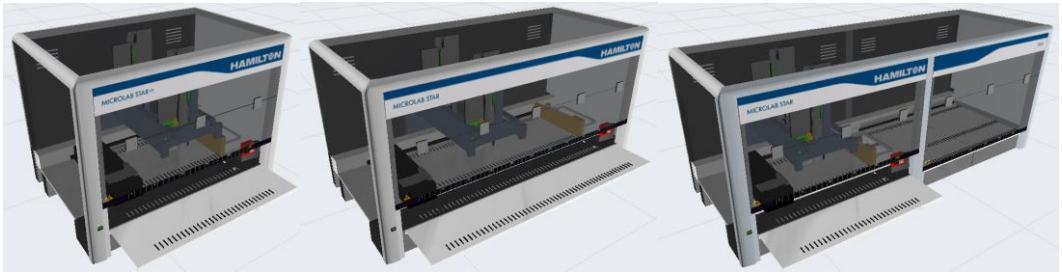
The easyPick software is running.

3.6 Adapt demo methods to Microlab STAR hardware

The demo methods are installed in the directory

C:\program Files\HAMILTON\Methods\easyPickIIMethods\

Adapt the Deck Layout to your Microlab® STARLet, STAR or STARPlus,



with the corresponding carrier:

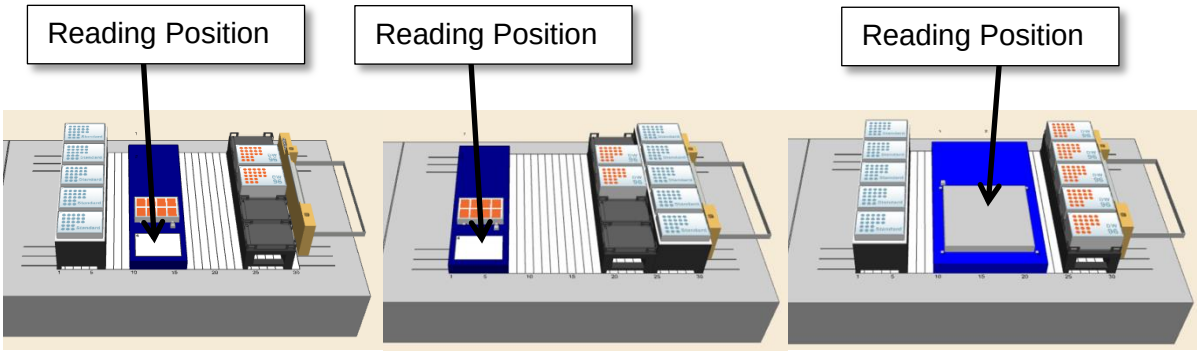


Figure 19: Demo deck layouts for easyPickII, easyPick Topeye and easyPickII XL

3.7 Adapt Connector for Light

In the method, select the DIV Connector (DIV2 or DIV3) of the STAR where the light table or light table XL is connected.

3.8 Demo methods

3.8.1 easyPick_AdjustReferencePosition.med

The method allows fine adjustment of the channels to optimize the picking position. This method modifies the Instrument.xml file in the sub folder "Settings".

3.8.2 easyPick_PlaceCameraToLightTable

The method allows fine adjustment of the camera towards the light table.

3.8.3 easyPick_TakePictureFile

The method takes a picture of the plate and saves it to a file.

3.8.4 easyPick_SearchColoniesDemo

The method takes a picture of the plate and analyzes it. It demonstrates all capabilities of the easyPick software

NOTE
All demo methods and libraries are in dedicated easyPickII sub-folders in the according main folders.

NOTE
All demo methods are also available for the XL light table and TopEye. See also description for check reference points

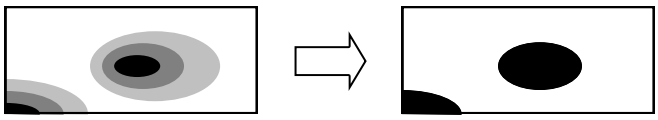
4 The Art of Colony Picking

4.1 Picking Principle

For bacterial colony picking based on image, processing a camera takes an image of the carrier object (e.g. a bacterial plate that can be divided into several wells). The software then searches for bacterial colonies within an area of interest defined by the user. The software is able to process several features to include colonies of interest in further processing and to exclude them otherwise. These criteria are for example size and color of colonies.
Packaged with the software comes a test image which allows the user to evaluate the effects of changing the parameters on the resulting selection of bacterial colonies by the application. It is also possible to try out new settings on any user-defined image stored in a file or even a database.

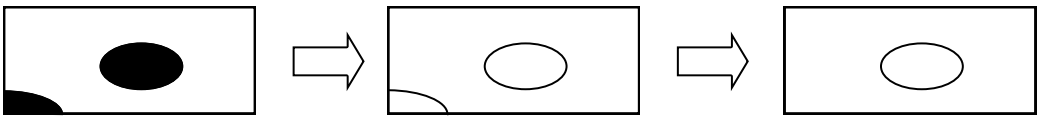
4.2 Threshold generation

There are two gray tone filters, a lower (dark) and an upper (bright) one. A gray level histogram of all pixels is evaluated such that a portion of the upper and the lower pixels is left out respectively. The end points of the threshold slider are set to correspond to these portions. By doing so, the application is able to exclude dark and/or bright noise up to an amount defined by the position of the threshold slider. Its value defines the upper border to pixel values included in the generation of an internal black and white (b/w) image.



4.3 Contour evaluation

The true b/w image allows for a contour evaluation. Only closed contours will be used for colony evaluation.



4.4 Colony properties

There are the following properties to be considered in the above described feature selection process:

- the area
- the circularity
- the distance to the nearest neighbor and
- the distance to the margin.

If color information has to be included, additional properties will be added for each color channel red, green and blue respectively.
The area property defines a minimal and a maximal area for the processing of closed contours. Smaller and larger colonies, even if they match all other properties, will be rejected. The other properties will cause a rejection of the object under consideration if they do not reach the minimum value. Increasing the normalized value leads not to be rejected but does not mean that the property is better than the specified value.
The circularity is the similarity of the contour with a circle. A circle is equal to 1, each other contour is less than 1:

c = 1 c = 0.5 c ~ 0.5 c ~ 0.16

The color properties allow to include/exclude color information. The coding is done in standard RGB or HSV. Within RGB color is represented by an additive combination of the three basic colors red, green and blue. Dark points or pixels have lower values in each color channel, bright pixels have higher ones, as shown in the following table:

name	**red**	**green**	**blue**	**color**
black	0	0	0	
maroon	128	0	0	
olive	128	128	0	
green	0	128	0	
teal	0	128	128	
navy	0	0	128	
purple	128	0	128	
gray	128	128	128	
red	255	0	0	
yellow	255	255	0	
lime	0	255	0	
aqua	0	255	255	
blue	0	0	255	
fuchsia	255	0	255	
white	255	255	255	

If the colonies have a teal color, the blue and green channels will have a relatively high parts but the red will be relatively low. So one can set the color properties to either satisfy a minimum value for both blue and green (for example 30/255 and check box reverse NOT marked) or a maximum of red (for example 0/50 and check box reverse marked).
An independent channel has to be set to be between 0 and 255 for minimum/normalize or normalize/maximum (meaning that in this case the check box reverse take no effect for color evaluation – but be careful using the weightage slider for the color properties: it's recommended to set them to zero). Within HSV the user can easily specify the color range which should be accepted or excluded. Furthermore the Hue, the saturation and intensity of the color can be specified.

4.5 Weightage of a colony

The weightage is used to define the sorting order of found colonies for further picking. Each property will be weighted with values ranging from 0 to 1 with respect to the minimum and maximum or the normalization value. Normalized values can only hold the maximum of 1 even if they are "better", e.g. a margin distance is greater than the specified value (see following sketch on the left).

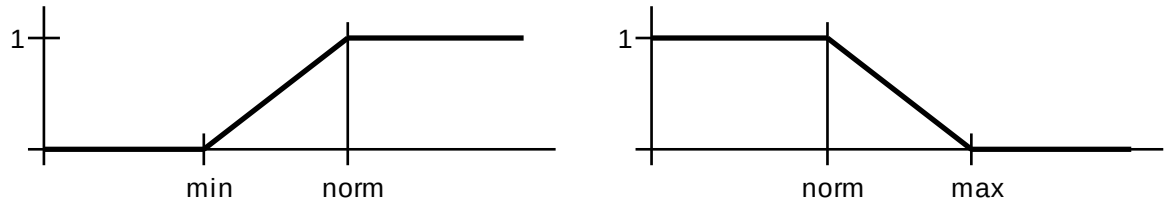


Figure 2: Property graphs: left min-norm., right reversed

The weightage of a colony describes how good a found colony meets the properties to satisfy a given definition of bacterial colonies. Each property will be multiplied with a weight to determine how important a property is. All weighted values then will be added and normalized. It's recommended to leave the color sliders on zero except the color is the important property to be picked for.

ATTENTION
At least one weightage slider has to be non-zero.

5 Five Steps to successful Colony Picking

NOTE
To adjust the settings of the picture acquisition do not forget to switch on the light of the light table by using the method ***easyPickII_PlaceCameraToLightTable.med***

5.1 Step 1: Take a picture

A good picture for colony analysis should provide a focused picture (pre set during camera installation) and also a good contrast of the colonies or objects to be picked. Therefore the brightness should be carefully adjusted.
Brightness of the picture can be set by:

- Aperture at the lens of the camera
- Brightness of the light table
- Exposure time of the camera

The latter two can be changed in the method, while the aperture of the lens cannot be changed during a run.
Bright light can help to obscure bubbles and dirt particles, so that they are not recognized as objects. This will reduce the time for the calculation of the position of all objects on the plate.
With low light conditions you can make small and pale colonies visible.

5.1.1 Camera settings

For a lot of applications the default settings should be suitable. However in some cases it might be necessary to change the camera settings.
The "Camera settings" window is activated per default and displayed on the lower left hand side of the main window.

There are 5 tabs in this window: "Basic", "Enhancement", "White balance", "Trigger/Flash/IO" and "Device".
With the buttons on the lower left hand side of this window the settings can be saved or existing settings can be loaded.

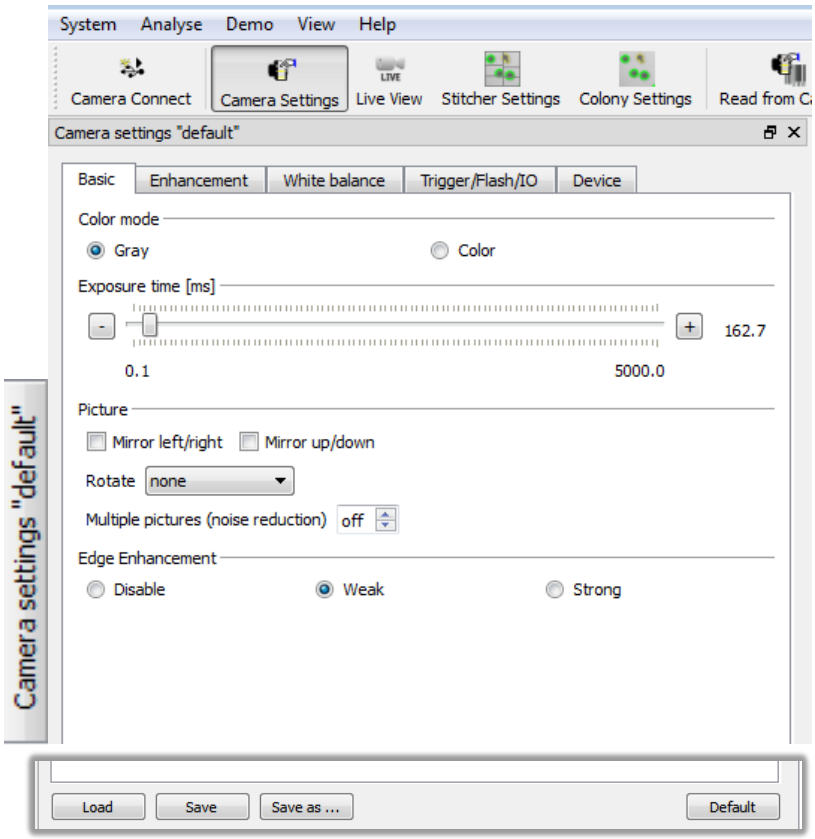
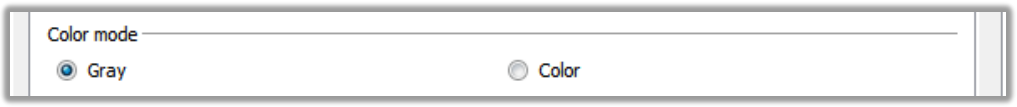


Figure 20: Window "Camera settings"

With the buttons on the lower left hand side of this Window, the Settings can be saved or existing settings can be loaded.

5.1.1.1 Tab "Basic"

With the radio buttons under color mode you can switch between "Gray" and "Color".



Choose "Gray" if you don't want to evaluate color information, otherwise choose "Color".

Underneath the "Exposure time" can be adjusted. e.g. this can be necessary for very dark plates. Increasing the time makes the picture brighter.

In the picture section the picture can be mirrored or even rotated.

Choose "Multiple pictures" to reduce the noise of the picture. Then a number of pictures will be added into one. The time for picture acquisition is then accordingly longer.

"Edge Enhancement" activates the image sharpness enhancement in the camera

5.1.1.2 Enhancement Tab

Use default values except when specifically recommended by experts.

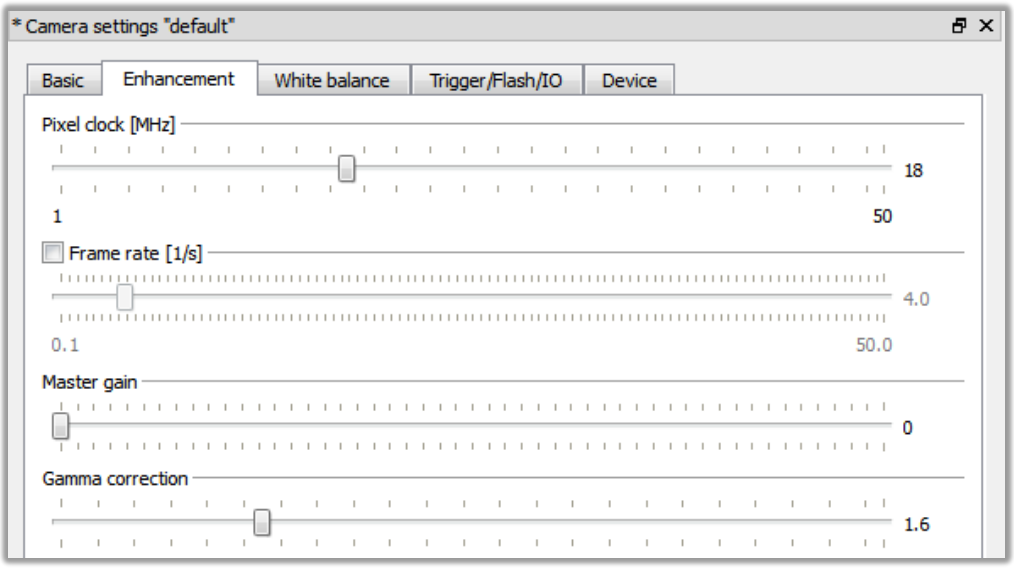


Figure 21: Camera Settings: Enhancement

The **Pixel Clock** is set on 18 MHz by default and should only be changed in case of USB connection problems. For detailed information see Section Problems with Camera Connection.

Frame Rate increases the rate of picture transfer. Do not change!

Master Gain activates the internal amplifier of the camera. It is useful for very poor lighting conditions, but will also enhance noise. Do not change!

Gamma Correction: Can be used to adapt color properties of the camera picture (do not change).

5.1.1.3 White Balance Tab

Mode disabled because it is either a black & white (gray) Camera or the color mode is defined as "gray".

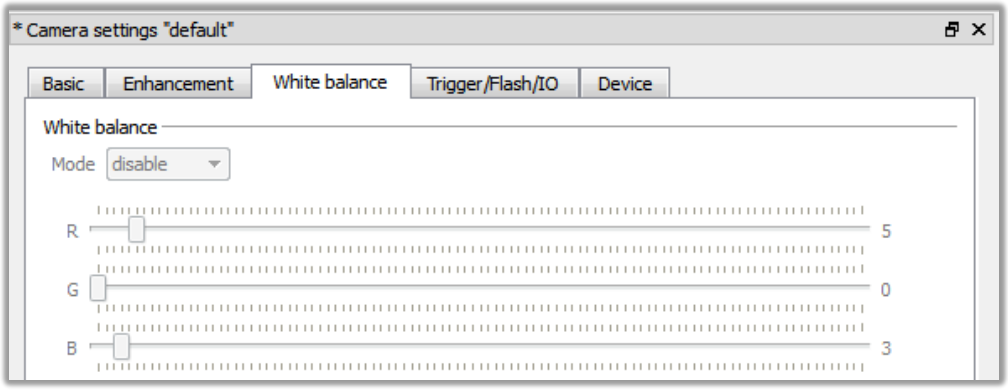


Figure 22: Camera Settings: White Balance

5.1.1.4 Trigger/Flash/IO Tab

”External trigger” and ”External flash“ should be switched OFF, because it is not relevant for the easyPick light table Carrier and the easyPick light table XL Installation.

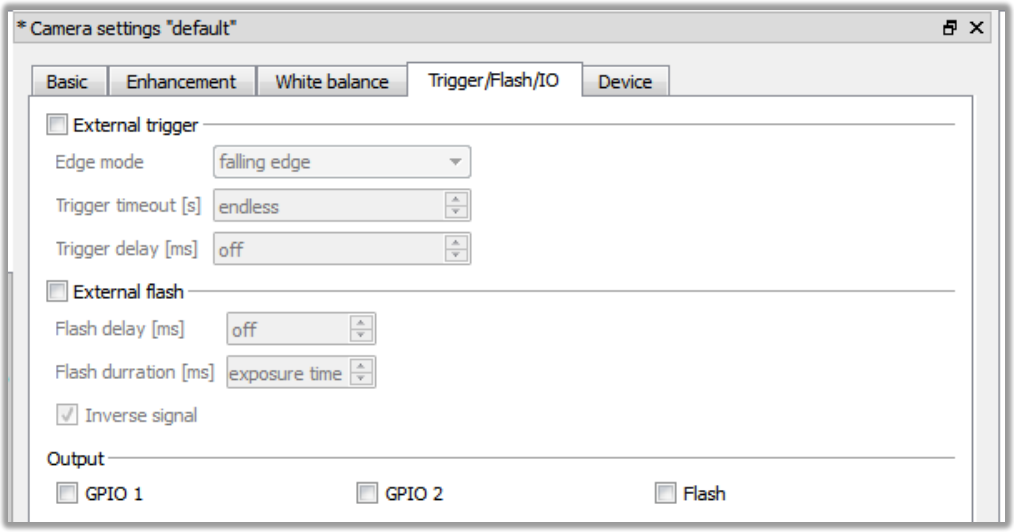


Figure 23: Camera Settings: Trigger/Flash/IO

The input and output ports of the Camera can be used to trigger picture acquisition with an external element or to control an external light.

5.1.1.5 Device Tab

In case only one Camera is connected to the USB, the **Default Camera** is automatically selected.

In a multiple Camera system, select the corresponding Camera, using preferable the connection mode “Camera serial number” (unique identification). Connection can be checked with the “Life view” Option, see also Section Problems with Camera Connection.

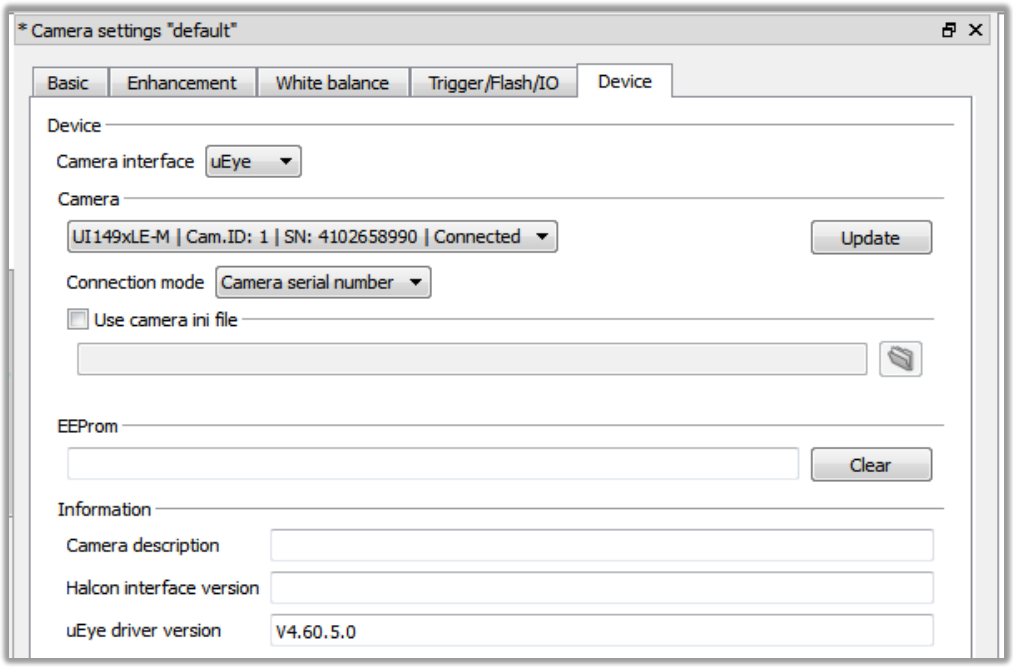


Figure 24: Camera Settings: Device

The **Device Tab** gives you the possibility to choose the correct device in case of more than one Camera is connected. The Camera can be identified by its Serial Number, its Camera ID or the type of Camera.

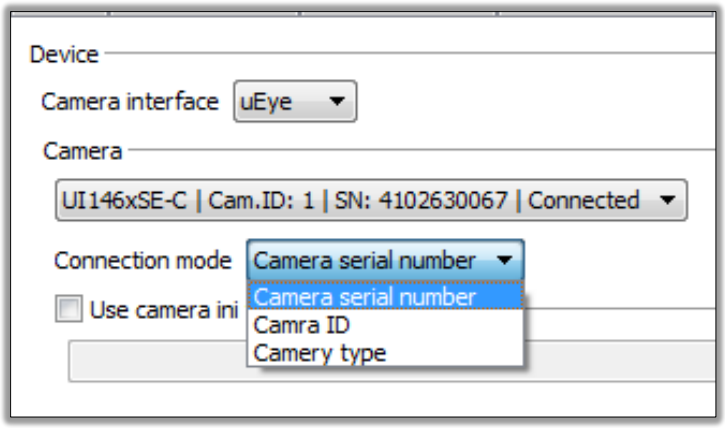
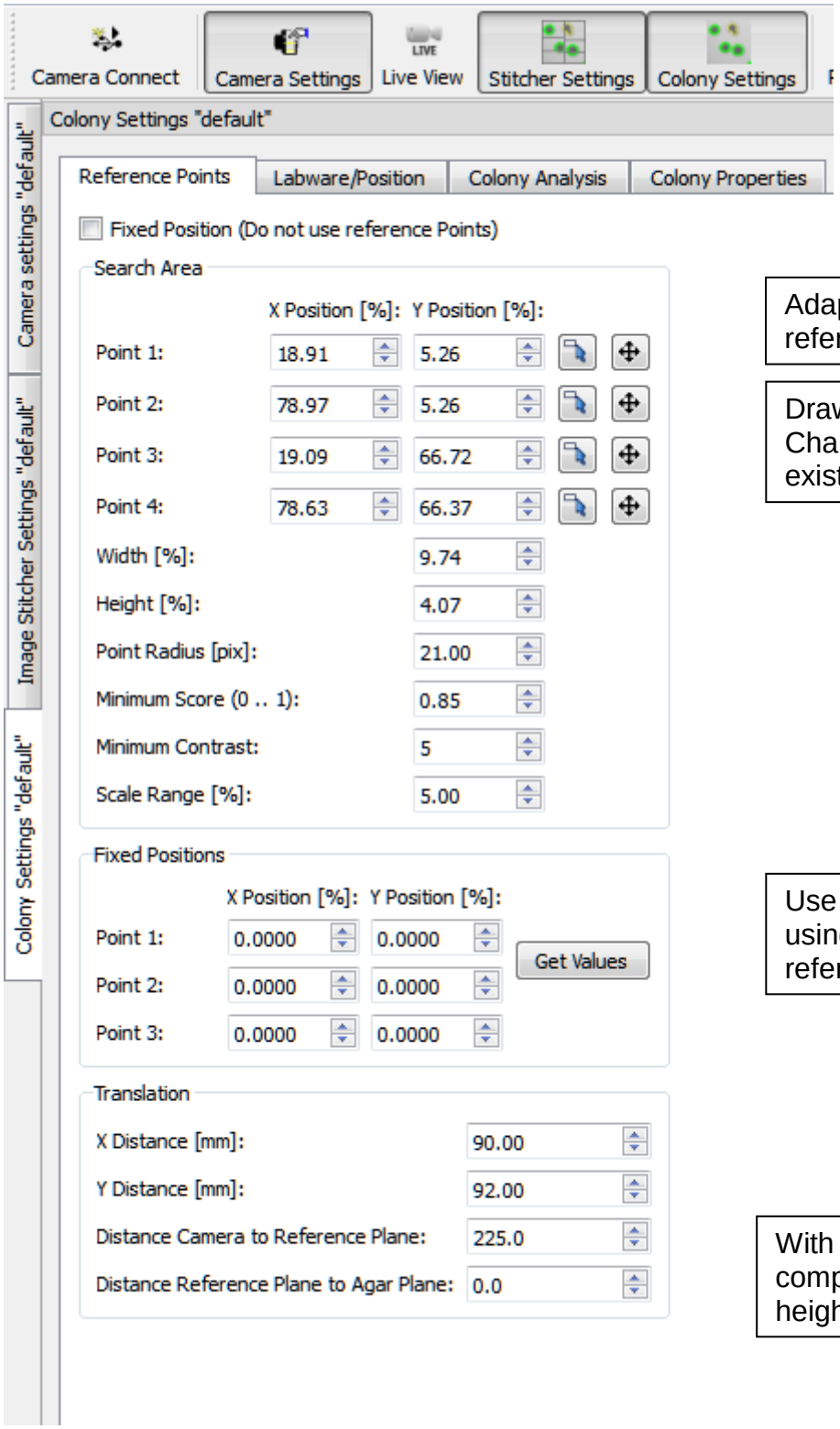


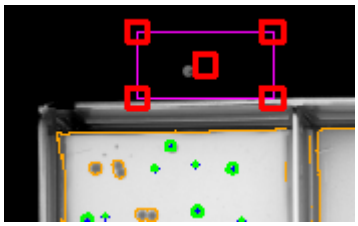
Figure 25: Camera Settings: Connection Mode

5.2 Step 2: Check the reference points

The reference points are crucial for the measurement of the exact position of the colonies. Make sure that they are visible in the picture and that the recognition zone of each point is correctly aligned. Change to the corresponding settings using the "Colony Settings" on the left side.

You can use fixed reference points or let easyPick look for these points in dedicated search areas.





Adapt the search areas for the reference points if necessary

Draw a new search area or Change the position of an existing area

Use these settings when you are using fixed positions for your reference points

With this setting you can compensate different agar heights

NOTE
For bigger Q – Tray plates on the XL-Light table the easyPickII_XL_AdjustReferencePosition.med will help to define the camera positioning and to match the reference points.

The camera will take 6 pictures of the Q-Tray plate with overlapping content so that all reference points on the neighborhood pictures as well according to the following scheme:

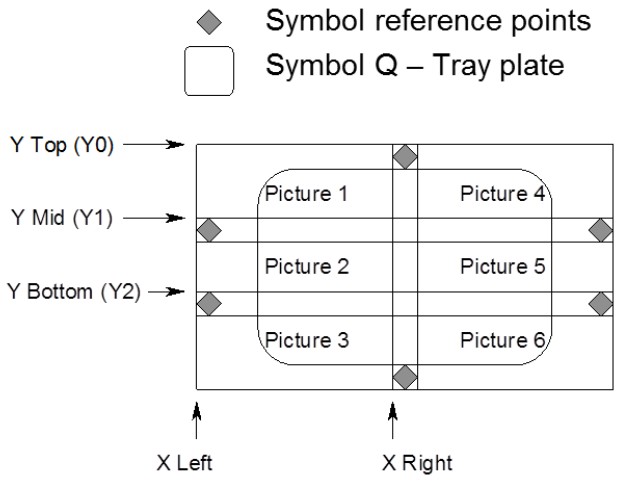
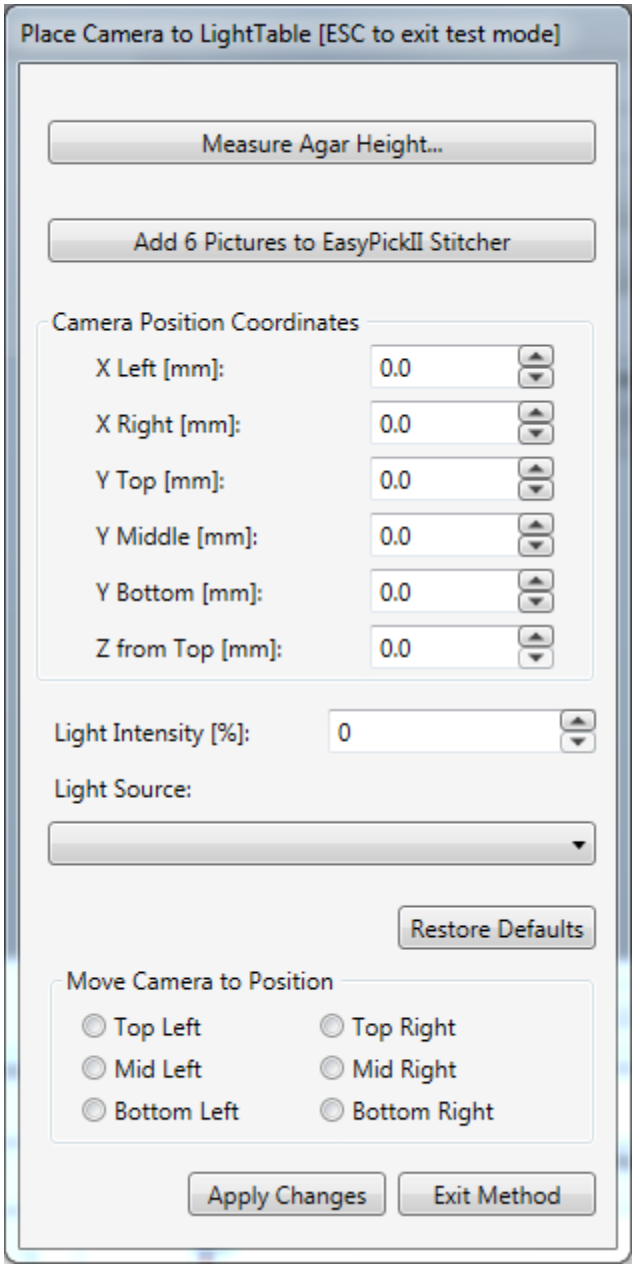


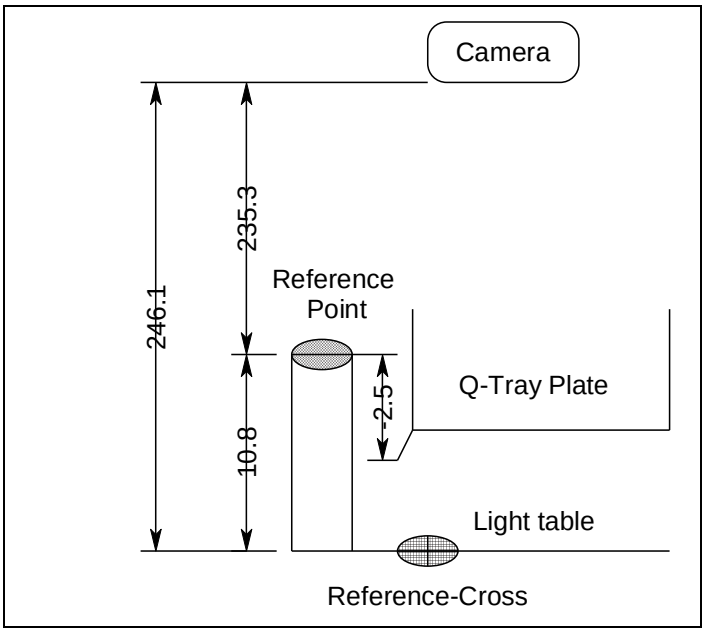
Figure 26: Camera coordinate system

The method easyPick_XL_PlaceCameraToLightTable shows the following dialogue which helps positioning the camera:



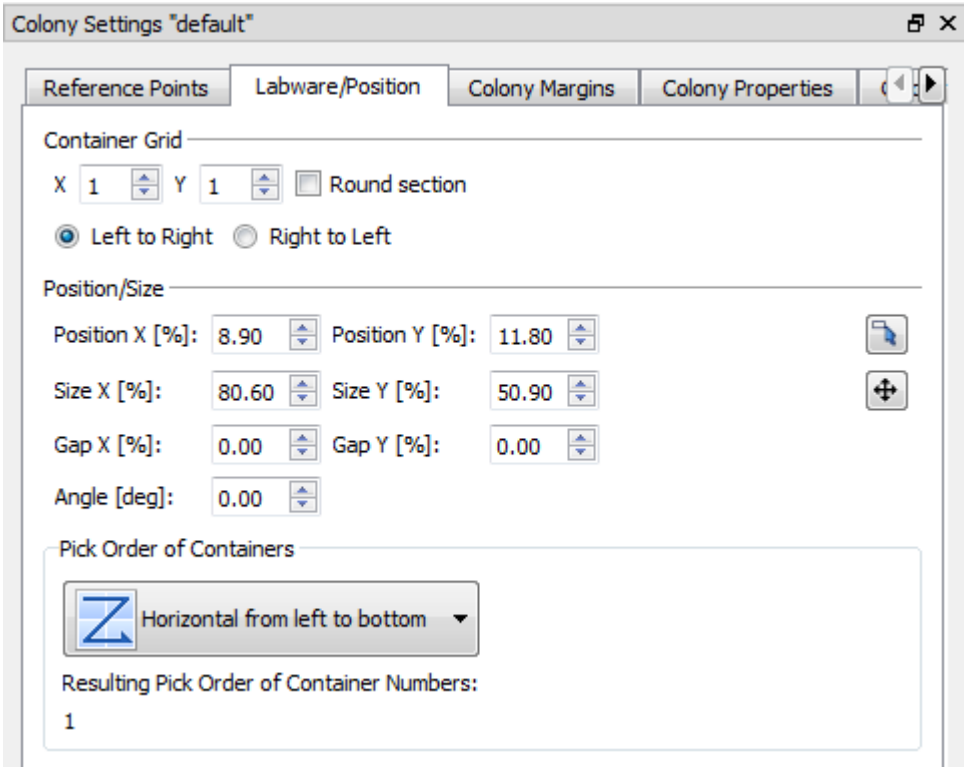
The positions 1 to 6 correspond with the positions in the drawing shown above. In order to work with this method the easyPickII application must be started first Position the camera in a way that you have at least 5 to 6 Pixel black pixel between plate and reference cross!
These coordinates will be transferred to the easyStep_ImageStitcher_TakePictures() sub-method which will take all required images and concenate the image to one big picture. Important here is that each image has overlapping reference points with the neighborhood images.
In order to correct the height differences from Q – Tray plate to light table you can adjust it in the Reference Points settings with Distance Camera to Reference Plane.
The agar height can be measured using the corresponding button.

The values belong to the following set up:



5.3 Step 3: Choose or adapt labware settings

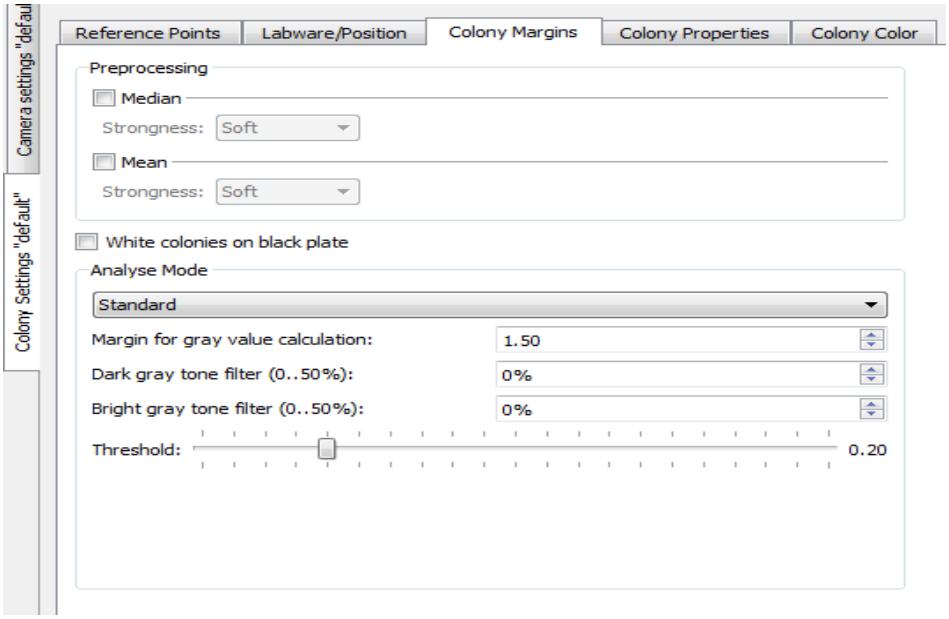
A labware (petri dish or plate) is defined by the number of its compartments (wells), its shape (rectangular or round) and its size



For a petri dish choose "round section", 1 section (for X and Y) and specify its diameter.
For an Omni Tray choose 1 section (for X and Y) and specify its size.

5.4 Step 4: Set the right values for colony analysis

easyPickII needs a certain threshold to detect objects that are darker than the background. A lower value is able to detect light and pale colonies. It is also possible to look for brighter objects on a dark background by selecting "White colonies on black plate". With the gray tone filter the range of the threshold can be specified. See also section 4The Art of Colony Picking



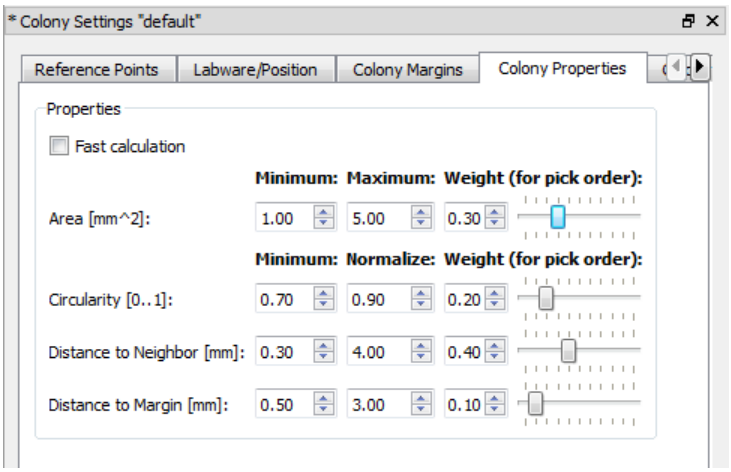
With the function Median and Mean you can glaze the picture in order to correct smaller picture errors which can disturb the analysis. Mean is working with an linear algorithm, is faster than Median but the correction is just concretive. Median is sorting the grey levels and correct them. Therefore this algorithm takes longer. For further information concerning the glazing algorithm please refer to the Halcon documentation from MVTec. If you do not have exact borderlines in your colony picture please try first the mean filter and afterwards the Median filter. Filtering can increase the recognition rate of colonies.

5.5 Step 5: Choose the right properties for colony picking

5.5.1 Colonies can be chosen by properties

- the area (size of the colony)
- the circularity (roundness)
- the distance to the nearest neighbor and
- the distance to the margin.

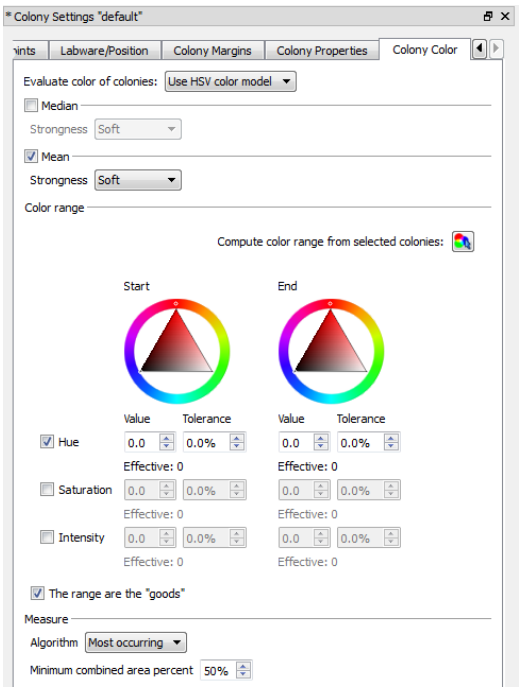
See also section 4 The art of colony picking for explanations of the settings.



At least one weight slider should be unequal to 0 to generate a hit order of interest.

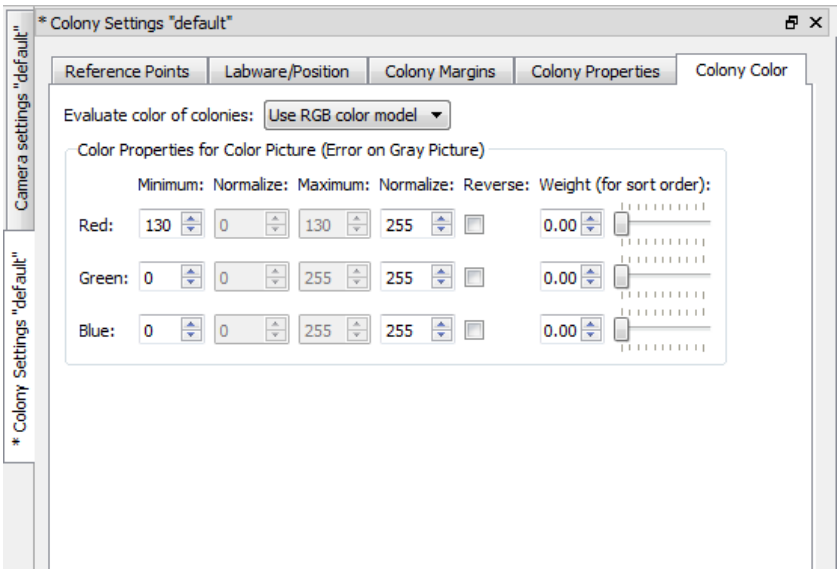
5.5.2 Colonies can be chosen by color

HSV Selection:



Define the start and end value of the color. It is also possible to sort additionally by saturation and intensity depending on the colony appearance.

RGB color selection:



If the RGB code of the to be picked is known the section can lasso done by using these settings. For proper selection please see also section 4 The art of colony picking for explanations of the settings.

6 Quick Guide to check the camera settings

1. Load the Camera Settings related to your colonies

   Default settings for the common colony detection are already prepared for your use.

   Specific parameters can be load in the window "Camera settings" with the "Load" Button".

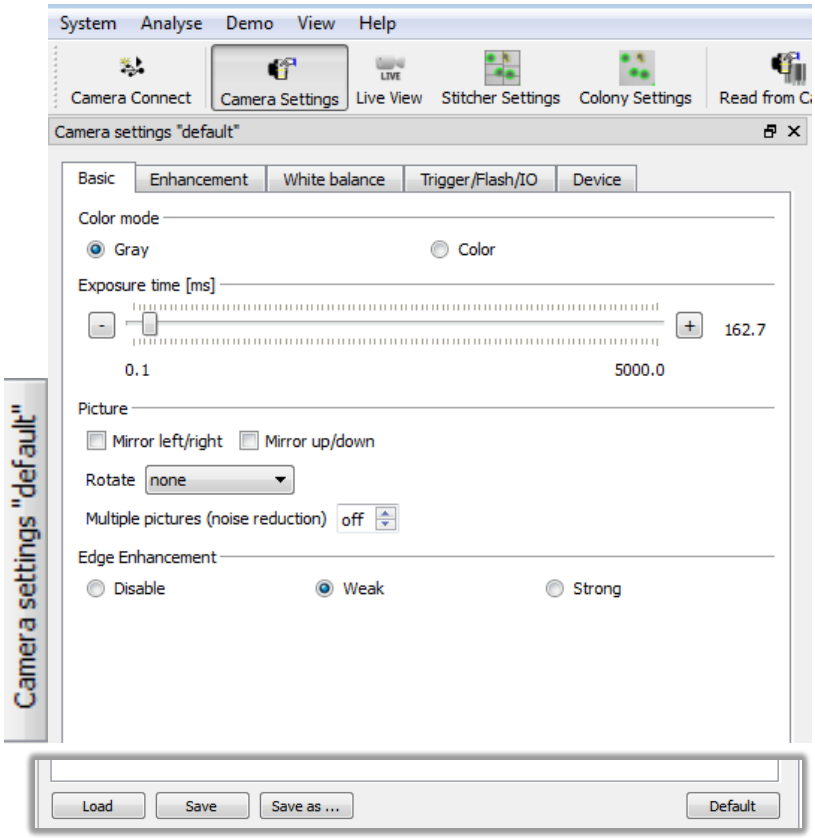
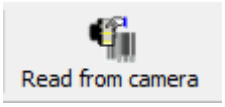


Figure 27: Load new camera Settings

2. Connect the Camera.

With this command, the easyPickII Software connects with the Camera. When the Camera has successfully been connected this button turns into the "Camera disconnect" Button.

3. Capture Picture from the Camera after moving the arm to the light table with a colony plate on it and the light table switched on.



4. Test your settings.

   Check picture quality regarding light, if not optimal, load other camera settings from the list or optimize picture quality by changing e.g. Exposure or manually the aperture at the lens.

7 easyPick Library for the Microlab® STAR VENUS Software

7.1 Structure

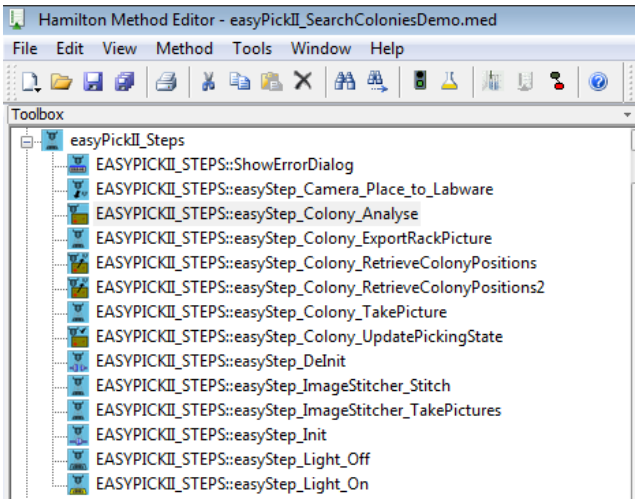
The complete library consists of three single libraries:

- EasySteps library (Helper library):
- SmartSteps library:
- General library:

All libraries were saved under the following path during installation:

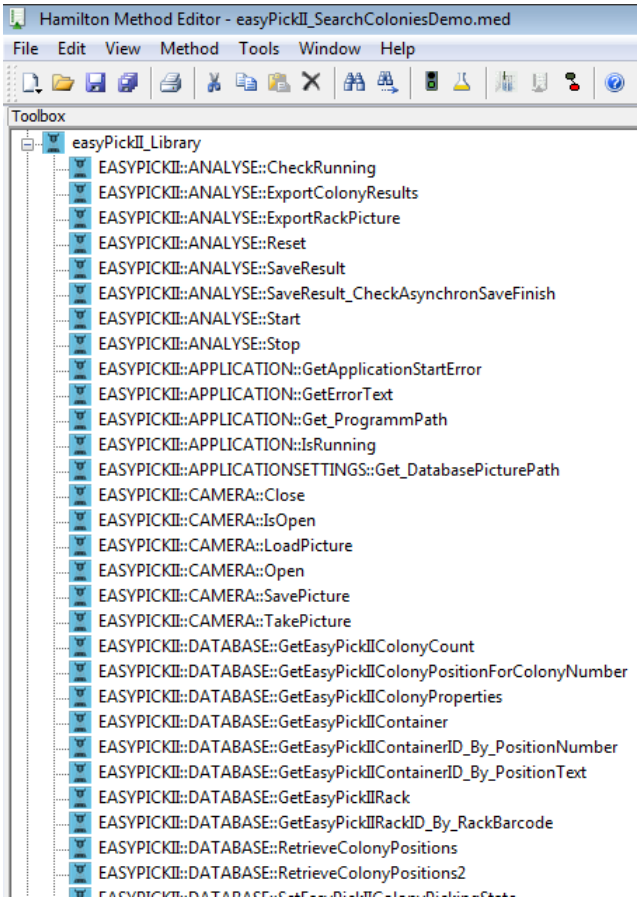
C:\Program Files\HAMILTON\Library\easyPickIILibrary

7.2 easyPickII Steps



For function description please refer to the help within the easyPickII Steps.

7.3 easyPickII_Library



For function description please refer to the help within the easyPickII Library.

7.4 Error Codes

The Error Codes are coded in namespace ERROR followed by a sub-namespace which groups the Error Codes, e.g. ERROR::GENERAL::_APPLICATION_ERROR. The only exception is the first group (example ERROR::_NONE):

7.5 Write a Colony Picking Method Based on the Demo Method

7.5.1 General

Start from the easyPickII_Demo Method as a reference. You may copy the whole method or parts of it into your method/workflow. If doing so make, sure to delete or disable the Microlab® STAR Init Step.

7.5.2 Init – DeInit – OnAbort

In the easyPickII sub method library "EasyPickII_Steps.smt" is built-in a sub method "EasyStep_Init". Insert easyStep_Init step after the ML_STAR-Initialize step.
In the easyPickII sub method library "EasyPickII_Steps.smt" is built-in a sub method "DeInit". It switches off the bar code light. Then it moves the camera channel to a save position and at least calls the library function "DeInit". The most advantage calling the sub method "DeInit" function is that the camera channel is moved to a save position what is not done with the "DeInit" library function.
Insert the easyStep_DeInit step at the end of the method and in the "OnAbort" sub-method.

The sub method "DeInit" should be called in each "OnAbort"-method to move the camera channel into a save position.

ATTENTION: *Be sure to include the easyStep_Init and easyStep_DeInit functions into your method and the DeInit also into the OnAbort-method to prevent crashes of the ML-Star pipetting channels and the camera channel!*

8 Troubleshooting

This section should be helpful when encountering problems with the EasyPickII Software.

8.1 General

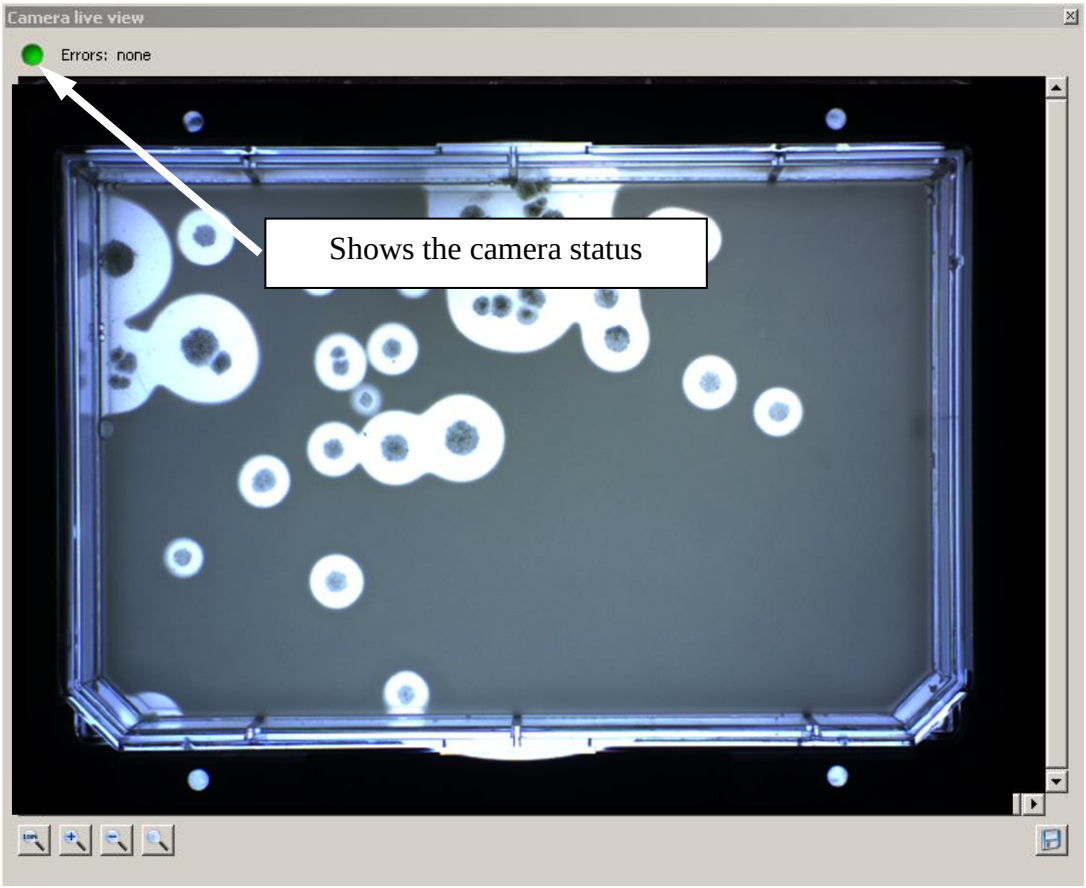
When contacting Hamilton's Technical Support refer to your version of your easyPickII software and make trace- and Data export files available for investigation. The respective information can be found in the Help menu under "About".

8.2 Camera not found

The camera is not connected or not initialized. Try another USB port on your PC – the USB port must match USB 2.0 features. Some PCs got both USB 1.x and USB 2.0 type ports.
Check with camera manager for the presence of the camera. Make sure that the camera is not occupied by a different task, possibly an unsuccessful closed "easy"application. In this case kill these tasks in the task manager.

8.3 Problems with camera connection

Press the button "Live view".
The window with the live camera image pops up.



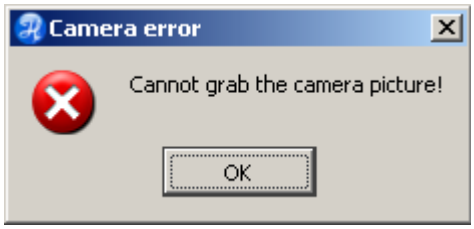
When the status light is green the camera is connected and capturing works properly. The flashing frequency indicates the capturing frequency which can be defined in the camera settings. Next to the status light it should say "Errors: none"

If the camera connection status is not green you can have the following problems:

Camera is not connected to software. Press button "Camera connect".

Physical connection has been interrupted or has transmission errors.
Check USB connection and wiring. The cable length must not be longer than 5m.
Furthermore the USB cable has to be connected directly to the PC and not via an additional hub. Next to the error status there is an error counter. It counts the numbers of connection interrupts. If the connection is working properly no number is visible

8.4 Camera cannot grab picture



The bandwidth of the USB connection does not fit to the frame rate of the camera. So from time to time the camera is not able to send the requested frame.

- Check the USB cable for proper connection, length and quality. The cable length must not be longer than 5m. Furthermore the USB cable has to be connected directly to the PC and not via an additional hub
- Check the USB ports of the computer and the bandwidth of the USB bus of the computer.

- Open easyPickII, connect to the camera and open "camera live view". With a pixel clock of 18 MHz (default) you should get no errors (red dot instead of green one). Good USB connections easily manage 30 MHz. Adjust the pixel clock until you do not get any errors anymore. Methods should still work with something like 7 MHz. Take care that the exposition time is still the same.

- Try to handle the error in your method by requesting a new picture if this error occurs

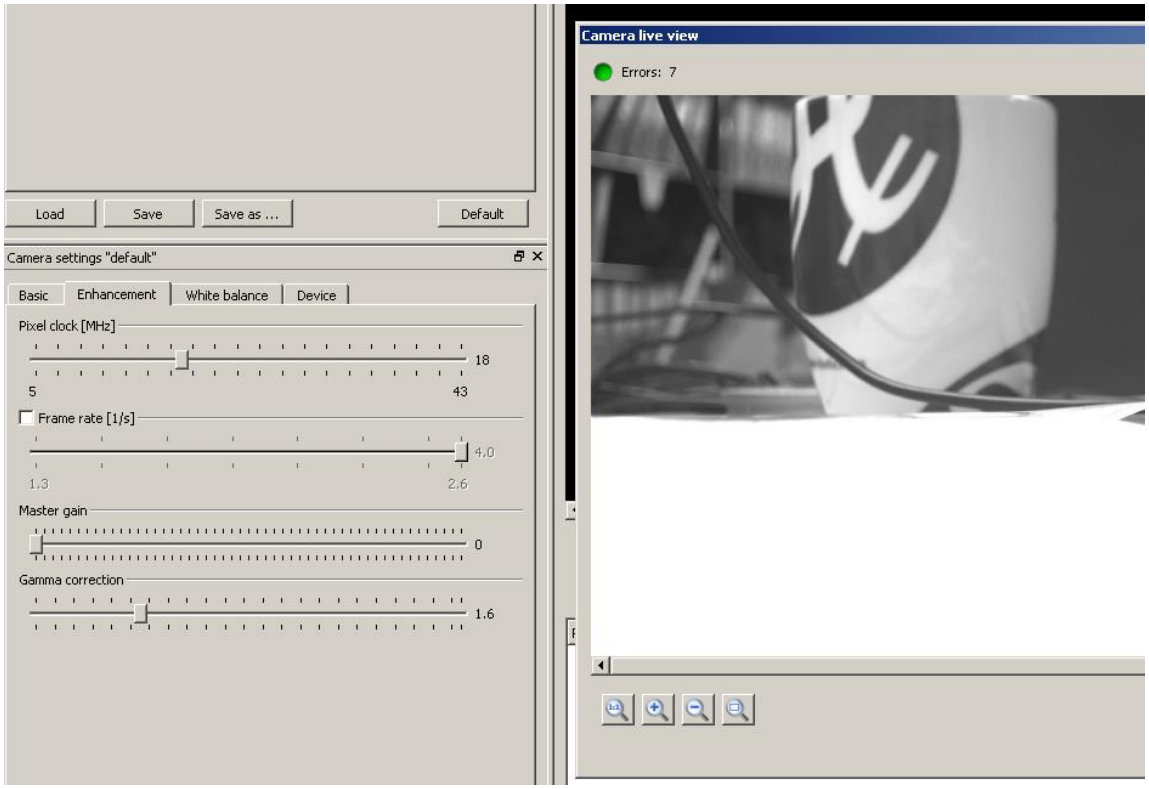
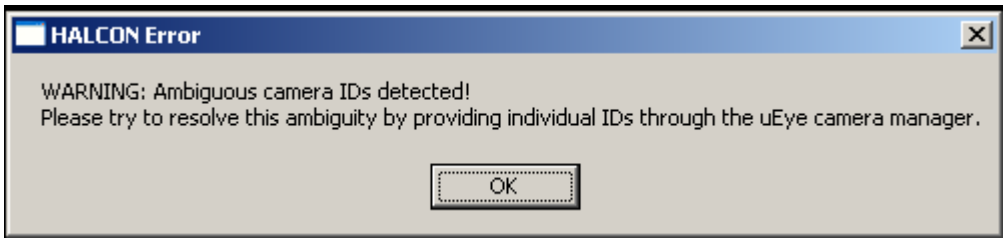


Figure 3: Changing the pixel clock

8.5 Ambiguous camera IDs detected!



If the device you are using has two cameras installed, be aware that, during driver installation, each camera is assigned a camera ID. Use the ID's camera manager software to assign a unique camera ID to each camera and use these ID`s in the easyPickII software.
The assigned ID can be found in the camera manager software under Start→All Programs→IDS→ uEye → uEye Camera Manager. Look in the camera list window under the tab Dev.ID and Cam.ID

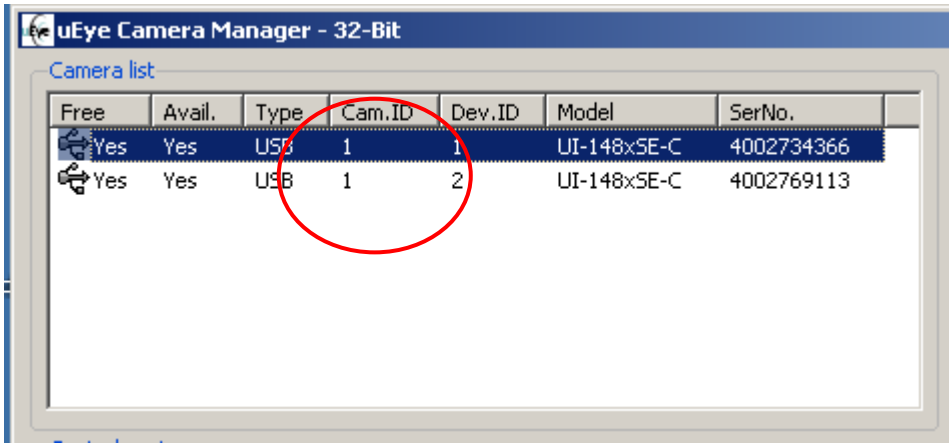


Figure 29: uEye Camera manager shows identical Cam.IDs

Choose "camera information" and set a new camera ID for the second camera

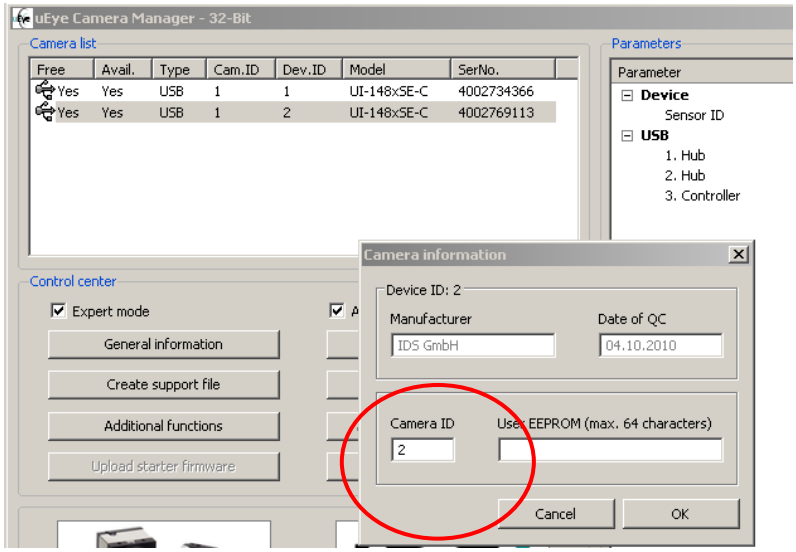


Figure 304: uEye Camera manager setting new Cam.IDs

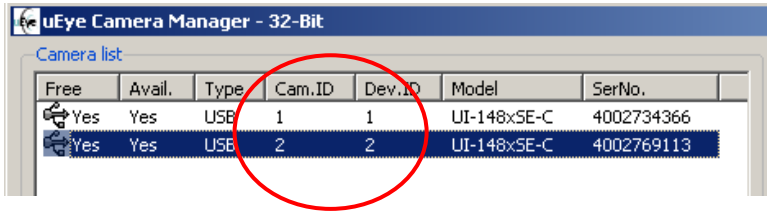
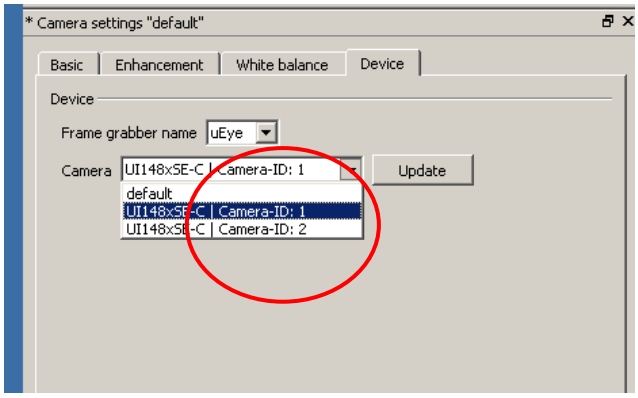
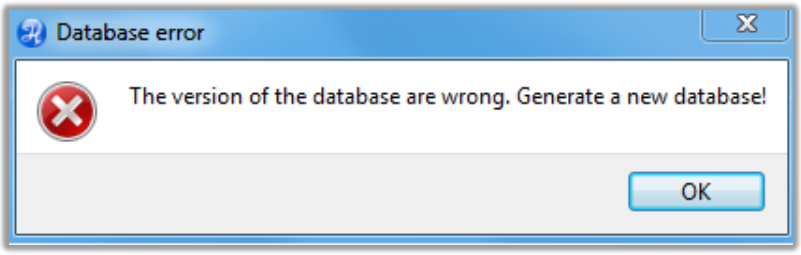


Figure 31: uEye Camera manager after change of Cam.IDs

After that you can choose in the camera settings of the easyPick software the device with the correct ID.



8.6 Problem with Database Installation

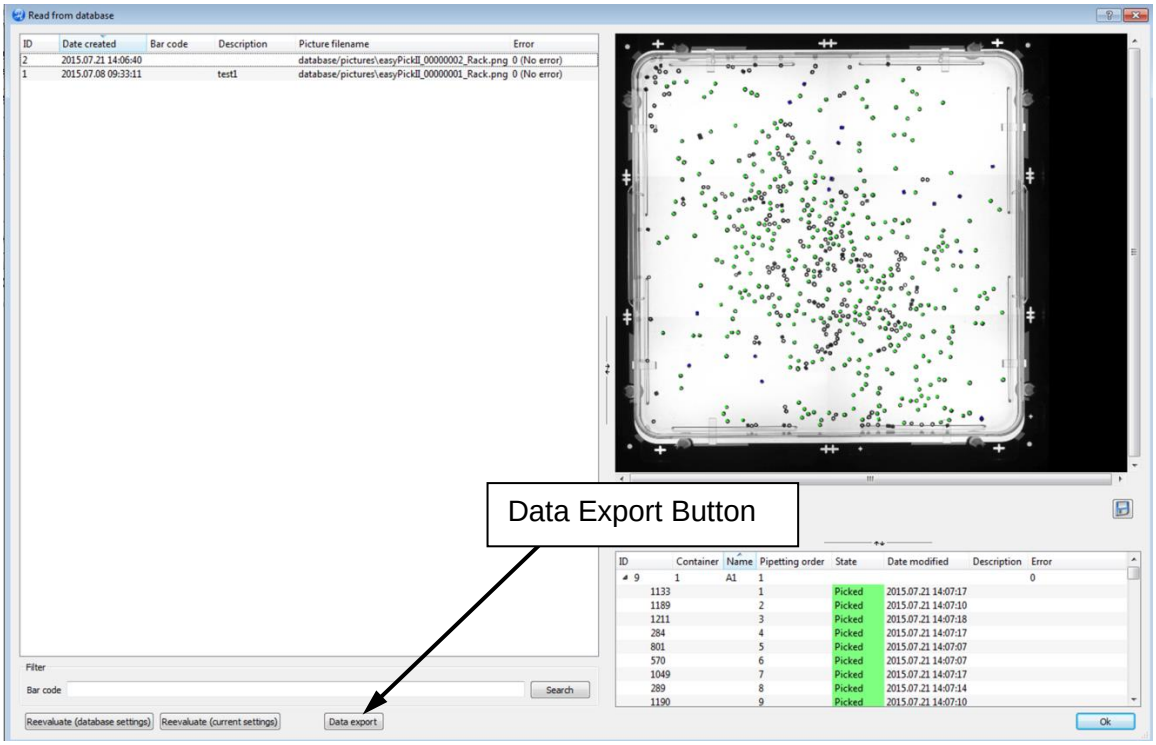


There is an error in the Database Installation. Go to the Application Settings, delete the database and create a new one (SQlite 3). After, restarting the application should work.

8.7 General Problems with the Evaluation

If you encounter problems during evaluation that cannot be solved by changing the settings proceed as follows:

Save the Image File including its settings to the Database. Press the "Read from database" Button, select the respective Evaluated Picture and press the "Data export" Button.



The Data Export Button creates a Zip File with the image and all configuration settings of the selected image. The Zip File can then be provided to Hamilton Technical Support.

9 Technical Specifications easyPick Light table

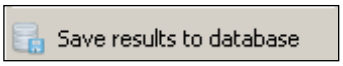
additional PC requirements	Interface	2x USB 2.0
Camera UI-1460SE-C	Resolution (pixel)	2480x 1536 RGB color
Light table	width (tracks)	7T
	Power 24V=/120mW	DIV2 or 3 (powered by MICROLAB® STAR)
	free space	for 2 MultiFlex modules *)
Light table XL	width (tracks)	13T
	Power 24V=/120mW	DIV2 or 3 (powered by MICROLAB® STAR)
	free space	for multiple MultiFlex modules *)
Camera channel	channel type	Imaging XL channel (odd numbering) mounted nearest to front

*) For MultiFlex Modules see MICROLAB® STAR Operator's Manual.

10 Appendices

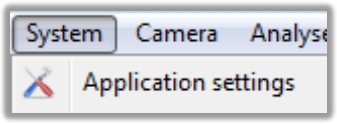
10.1 Database Settings

During installation, a database file is installed on your Controlling PC Hard Disk. Here you can store evaluated pictures (including their settings) that were captured by the Camera, loaded from a file from the Hard Disk or loaded from the Database. To store a picture, click the "Save results to database" Button in the Analyze Menu.



A Dialog Box pops up and asks for an Optional Description, e.g. "batch 1".

10.2 Application Settings



In the "System" Menu, click on the "Application Settings" Sub-Menu. This opens up a Dialog Box where the database settings can be changed.

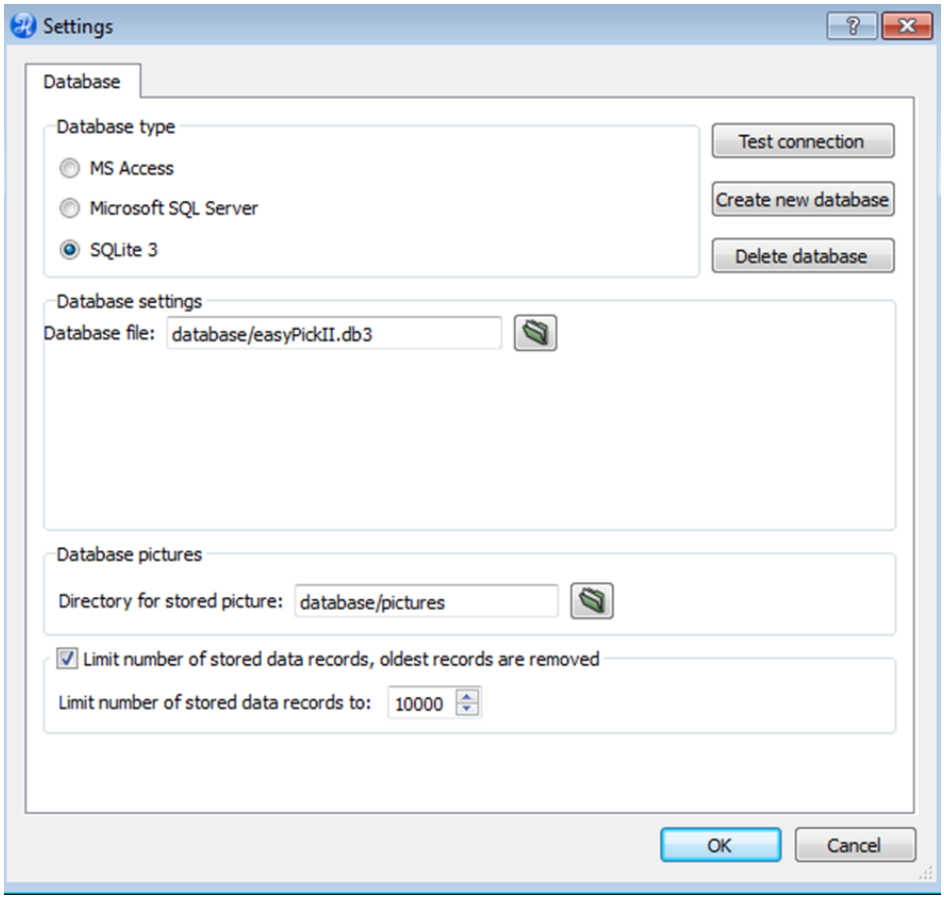


Figure 32: Database Tab of the Application Settings Dialog Box

The "Test connection" Button tests the connection to the database to check whether the settings are correct.

The "Create new database" Button generates a new database according to your settings.

The "Delete database" Button.

Database Type:

When using Microsoft Access, you have to define the following setting:

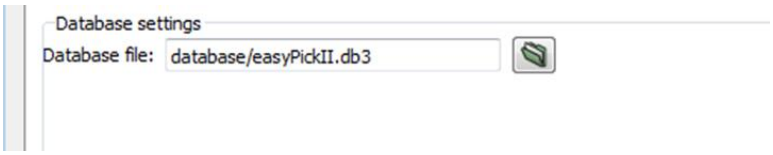


Figure 33: Database Settings for Microsoft Access

Database Pictures:

The directory for the stored pictures can be defined here. When "Auto delete stored data records" is checked, the maximum amount of stored data records can be defined here. When reaching this count the oldest database entry including its picture will be deleted.

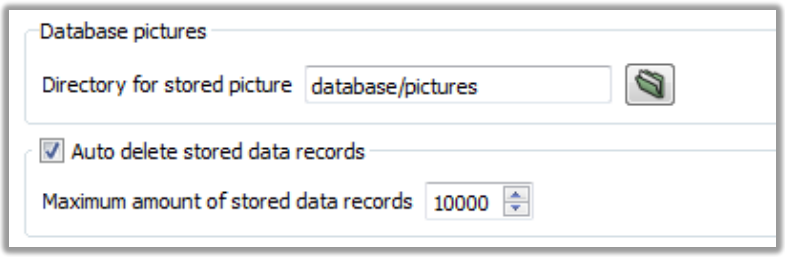
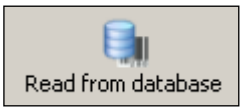


Figure 34: Database Pictures

10.3 Read From Database



With the "Read from database" Button in the Analyze Menu, you can load evaluated images with their settings from the database.

Figure 35: Read Bar Code from Database

In the window on the left, a list of all evaluated images is shown. In the "Filter" Window underneath, you can search for pictures with a specific content.

On the right side, a preview shows the database image which can be zoomed in, zoomed out, zoomed into a section and reset to the original size. With the "Disk" Button on the right, the image (without settings) can be saved separately.

On the lower right hand side the respective results are displayed.

In the window on the left select an evaluated image and choose one of the following functions to proceed:

Reevaluate (w/o new settings): When the image of interest is found press this button. The picture will be loaded and evaluated with the configuration settings stored in the database.

Data export: This button creates a Zip file with the image and all configuration settings of the selected image. When experiencing problems with evaluations, this information can be provided to Hamilton's Technical Support.

Ok: selected image is being loaded with appropriate evaluation.

10.4 Ordering Information

Microlab® STAR easyPick Light table		
easyPick Light table	Carrier for SBS formatted plates and Petri dishes, 7 Tracks Wide	**188092**

Microlab® STAR easyPick Light table XL		
easyPick Light table XL	Carrier for Q-trays, 13 Tracks Wide	**APE**

Microlab® STAR easyPickII		
easyPickII	Incl. camera channel, back light table, software and manual	**190980**
easyPickII Field upgrade	Incl. camera channel, back light table, software and manual	**188251_TS**

Microlab® STAR easyPickII Special Cloth		
Hamilton Cleaning Cloth	To clean the glass and mirror of the easyPick Light table	**692429**