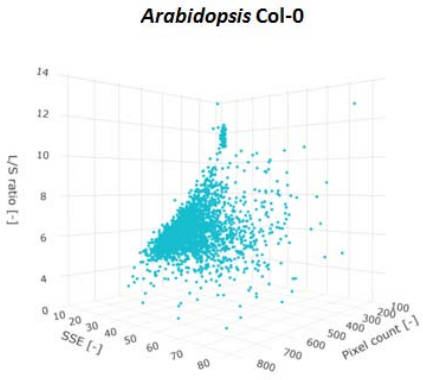
User Manual

Boxeed™ 2.1 System



Labdeers s.r.o.
Antonína Navrátila 1219/13,
680 01 Boskovice
+420 606 403 865
info@labdeers.com
CZECH REPUBLIC

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Labdeers reserves the right to change or make corrections in this manual at any time without prior notice. The current version of operation manual is available on our websites under www.labdeers.com/support.

The visualizations in this manual are illustrative.

This manual is an integral part of the product and its accessories and must be always easily accessible.

Read the operation manual thoroughly before using the product for the first time.

Table of content

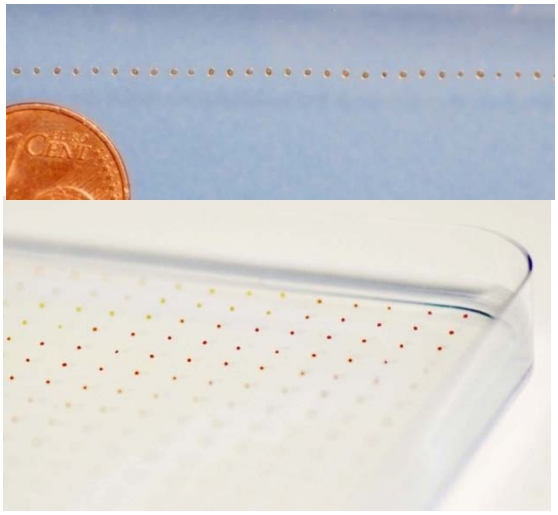
1. General information

BOXEED 2.1 IS A FULLY AUTOMATED LABORATORY DEVICE DEDICATED FOR STERILE AND NON-INVASIVE MANIPULATION WITH SEEDS AND PARTICLES IN BETWEEN THE STOCK AND WORKING AREA. IMAGING DATA ANALYSIS IS OPTIMIZED FOR SEEDS PHENOTYPICAL ANALYSIS.

Boxeed places individual samples on predefined lab plates formats directly from tubes, based on user-defined protocols. The use of parameterized sowing and robotically accurate placement is crucial for subsequent phenotypical analysis of plant growth, genotyping and seed-to-plant tracking analysis. Based on the image analysis, the seed phenotypical analysis is performed. Further the automatic placement on a plates or sorting into the test tubes is performed. Precise sowing and data archiving using the QR code labeling enables for further processing of experiments by other automated and analytical systems such as horizontal and vertical microscopes or plant-phenotyping robots

The Boxeed instrument is designed to work with dry samples. Its special configuration is optimal for sensitive and precise work with individual seeds and particles in laboratory. The process of sample manipulation is done by non-invasive way, regulating the air pressure and vacuum based on preset protocols.

All functions of Boxeed instrument are adequate to used nozzle, object weight shape and surface type.



1.1. Boxeed™ versions

The currently available versions of the Boxeed device are listed in the table. Other Boxeed versions for special purposes can be customized.

Type of instrument	Description
Boxeed 1.1	Manual inserting of samples and matrices
Boxeed 2.1	Manual inserting of samples and matrices

2. Safety

The safety instructions in this manual have the following danger levels:

DANGER	Will lead to severe injuries or deaths
WARNING	May lead to severe injuries or death
CAUTION	May lead to light to moderate injuries
NOTICE	May lead to product or material damage

2.1. Intended use

The Boxeed instrument is primarily dedicated for plant research and automated seeding to laboratory plates or for seed sorting based on phenotypical parameters. Boxeed automatically places individual seeds from various stocks to multiple plate formats. For precise robotic seeding and sorting is implementes image data analysis and partial 3D seed phenotyping.
Boxeed instrument is designed and constructed for solid dry samples manipulation. Instrument can gently and non-invasively work with dry objects starting on 80 µm of size. Boxeed instrument suits applications intended for sterile as well as for non-sterile laboratory work.

The Boxeed instrument might be operated only by trained lab stuff and users based on careful reading of the user manual. It is exclusively intended for use in research

Warnings for intended use

Please pay attention to the following general safety instruction before using the Boxeed device.

	**DANGER! – Danger of explosion** • Do not operate device in areas where work is completed with explosive substances • Do not use this device to process any explosive or highly reactive substances • Do not use this device to process any substances which could create an explosive atmosphere
	**WARNING! – Risk of electric shock** • Ensure that the housing is always closed and undamaged so that no parts inside the device can be contacted by accident. • Do not remove the housing of the device • Do not allow any liquids to penetrate the inside of housing • Only switch on the device if the device mains cable are undamaged • Use only device that has been properly installed or repaired by an authorized person. • In case of danger, disconnect the device from the mains supply
	**WARNING! – Risk of incorrect connection to the mains** • Connect device to a power source that compiles the specifications on the label • Use only supplied power cord and sockets with protective earth

	**WARNING! –Damage to health due to handling toxic or pathogenic particle** • When handling infectious, toxic or pathogenic materials it is needed to work with respect to national regulations and bio-safety rules • To protect health, wear personal protective equipment
	**WARNING! – Damage to health due to contamination of device** • Hazardous substances may result in personal injuries or product damage. Decontaminate the device or its accessories before transport or storage
	**WARNING! – Hazard when using flammable or explosive liquids** • Pay attention to your safety when handling ethanol • Disconnect device from the mains in case flammable liquid is spilled • Dry up spilled liquid immediately • Work in accordance with the decontamination instructions given in the safety data sheet
	**CAUTION! – Risk of injury** • Do not manipulate with Boxeed during operation • Do not reach into the Boxeed during operation • Turn on the protocol only in case the front protective cover is closed • In case of emergency push the button “emergency stop”
	**CAUTION! – Poor safety due to incorrect accessories and spare parts** • The use of accessories and spare parts other than those recommended by Labdeers may impair the safety, function and precision of the device. Labdeers cannot held liable or accept any liability for damage resulting from the use of incorrect or non-recommended accessories and spare parts or from the improper use of such equipment • Only use accessories and original spare parts recommended by Labdeers.
	**NOTICE – Contamination and incorrect sample handling** • Only use Boxeed with the fitted nozzles • Always use appropriate nozzle diameter – smaller in diameter than seed or particle to be handled • To avoid potential contamination of instrument or samples, keep attention to the purity of used nozzles. • Always use nozzles with filter to prevent damage and contamination of the laboratory instrument with microparticles • The Boxeed instrument may not come in direct contact with the liquid, this could lead to its damage. • Only dry object manipulation is permissible.
	**NOTICE – Impaired function due to vibration** • Do not place the Boxeed instrument on a surface that can vibrate. • Do not place Boxeed instrument on a surface with devices which generate vibration (e.g. vortex, centrifuge, thermomixer)
	**NOTICE –Damage of the instrument due to overheating** • Work within the specified operating temperature ranges

	**NOTICE – Impaired function due to high humidity** • The enhanced relative air humidity (RH), influence the dry-sample properties (especially the enhanced adhesion to surfaces), such a sample cannot be handled properly.

2.2. Product liability

In the following cases, the designated protection of the device may be compromised. Liability for any resulting property damage or personal injury is then transferred to the customer:

- The Boxeed instrument is not used in accordance with user manual.
- The Boxeed instrument is used outside of its intended use
- In case of special laboratory applications which are not listed in product intended use, customer needs the express written permission of Labdeers, to use the device outside of its intended use.
- The Boxeed instrument is used with accessories which are not recommended by Labdeers.
- The instrument is maintained or repaired by individuals or not authorized persons.
- The user makes unauthorized changes to the instrument

3. Product description

3.1. List of equipment

Carefully unpack the carton. You received following items:

Content	Quantity
BOXEED INSTRUMENT	1
CARROUSEL FOR 1,5ML TUBES	1
CARROUSEL FOR LARGE SEEDS	1
SERVICE AND MOUNTING KIT	1
TABLET	1
INDUSTRIAL USB FLASH DISC	64 GB
CLEANING KIT (VACUUM CLEANER, BRUSH)	1
CLEANING OPTICS KIT (MICROFIBRE CLOTH, CLEANING BALLON)	1
25MM OPTICS IN MAGNETIC HOLDER	1
16MM OPTICS IN MAGNETIC HOLDER	1
THERMO-LABEL ROLL (57MM)	1
AC POWER CORD	1
USB-C/MALE TO USB-C/MALE CONNECTOR	1
OPERATING MANUAL	1
NOZZLES WITH FILTER *(FOR ARABIDOPSIS)*	10+10
ANTISTATIC TUBES (1,5ML)	11
LARGE SEEDS CARROUSEL	1
LARGE SEEDS TUBES	11
LARGE SEEDS TUBE STAND	1
NOZZLE STAND	1
NOZZLE TRANSPORTING PIN	1
MOUNTING KIT (SCREWDRIVER, ALLEN SCREW, REPLACEMENT SCREWS)	1
NOZZLES FOR LARGE SEEDS (TESTING SET)	1

* Find detail list of currently available accessories at ***www.labdeers.com***

3.2. Technical product overview

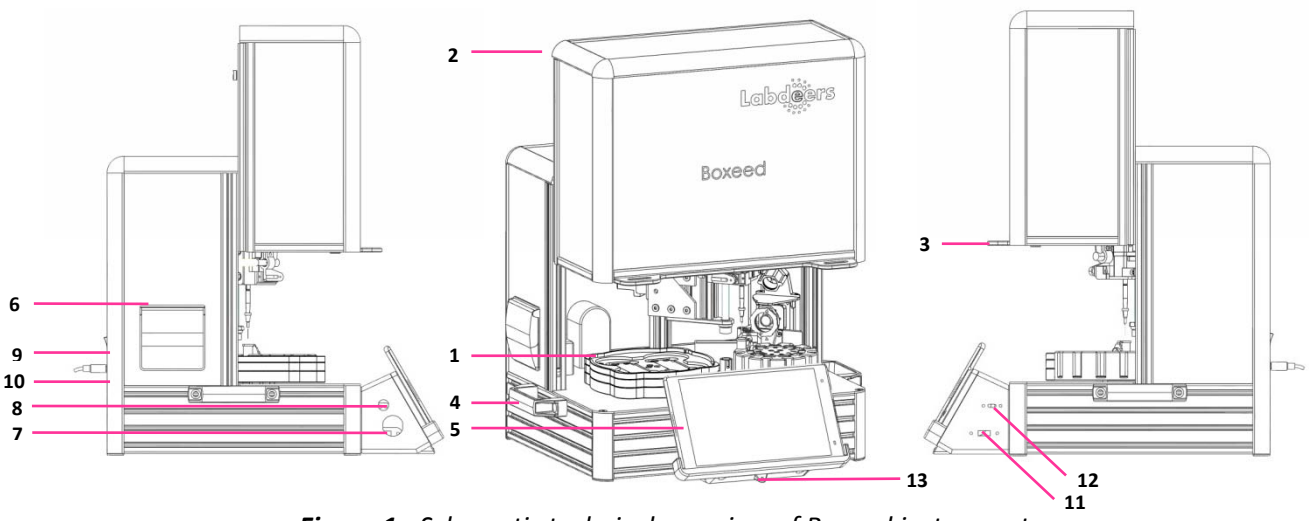


Figure 1 *- Schematic technical overview of Boxeed instrument*

1. Working area
2. Protective cover (lid)
3. Cover lid handles
4. Transporting handles
5. Tablet
6. Thermal printer
7. Ethernet socket
8. Emergency stop
9. AC power socket
10. Serial number
11. USB- socket (External memory, vacuum cleaner)
12. USB-C socket (tablet charging)
13. System ON/OFF - LED light indicator

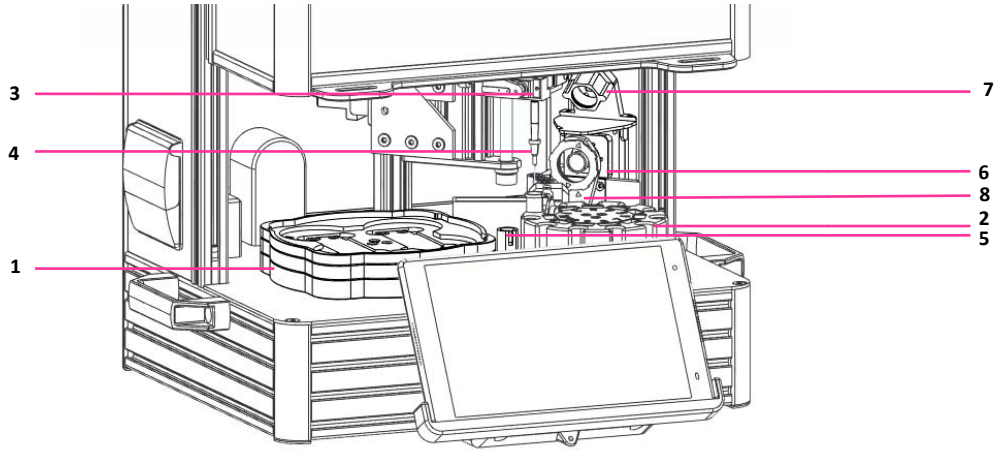
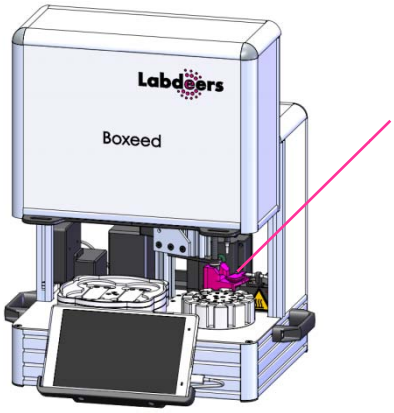


Figure 2 *- Schematic technical overview of Boxeed instrument - working area*

1. Multiple format plate holder
2. Tube holder (11 positions)
3. Transporting head
4. Nozzle holder
5. Nozzle cleaning station
6. Camera
7. Multispectral LED (W1) and W2 light source
8. Fluorescence module

4. Instalation

Installation of Boxeed instrument must always be carried out by Labdeers or a Labdeers service partner.



1. Make sure the transporting head is anchored when carrying the instrument, to prevent damage or de-calibration of Boxeed.
   Instructions for inserting the transport anchor into the instrument are shown in detail in Chapter 13.

2. Do not place Boxeed instrument on a surface with devices which generate vibration (e.g. vortex, centrifuge, thermomixer)

5. Operation

The sample picking, holding, sorting and precise placing is operated by using nozzles of various diameter and by changing the operating modes. Boxeed in a principle manipulate the objects by vacuum application followed by active air-pressure mediated sample layout. Impurities can get inside the device in case the nozzle with filter is not used.

Operation under sterile conditions: Boxeed can be used for seeding and sorting under sterile conditions in laminar hood. For sterilization temporarily subject the Boxeed instrument to the UV light at 254 nm (10-15min).

Operation under laboratory conditions: Do not place Boxeed instrument on a surface with devices which generate vibration (e.g. vortex, centrifuge, thermomixer). Do not place Boxeed instrument on a place with enhanced relative humidity.

5.1. First steps

CAUTION! – Before start remove the anchor from transporting head
CAUTION! – Before use ensure the appropriate nozzle is placed to the nozzle holder
CAUTION! – Never use nozzles without filter
CAUTION! – Take care the nozzle placed to a holder is straight to get optimal results
CAUTION! – Carefully insert plates with matrices and fasten them to holder tightly

1. Connect Boxeed to the mains
2. Switch on the Boxeed by the main switch. Light on the front panel (below the tablet) indicate the system is ON
   Wait until the Boxeed internal Wi-Fi turn-ON. IP addres for Boxeed WI-Fi: 192.168.6.1:8080

In case you are using the Ethernet connection wait until the Boxeed URL address gets active. The address is dynamic and is provided by your router.

3. Launch the Boxeed application/URL address.
4. After launching Boxeed system computer start in the menu **Manual**.
   - Activate the Initialization process by switching the button Execute.

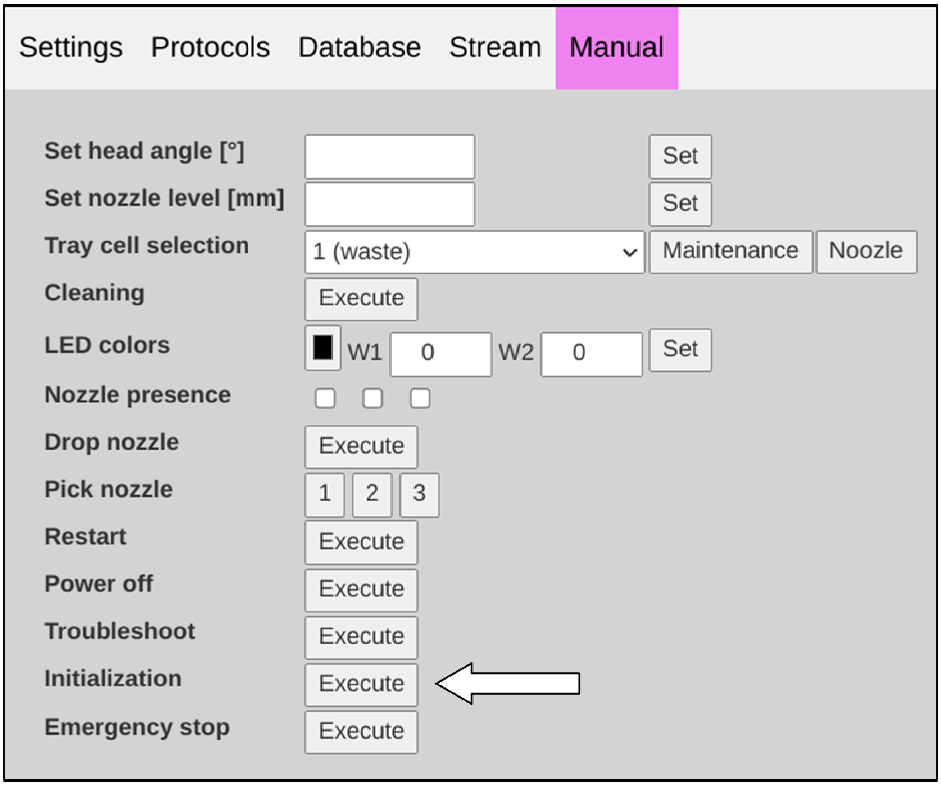


***Figure 3**: Manual mode*

5. Check Initialization process and correct position of:
   - plate holder – parallel position to the front of Boxeed instrument
   - tube holder – position 1 in bellow the nozzle
   - transporting head – nozzle in the middle of stream, above the carrousel

   System status is indicated by traffic light semaphore on a background of all SW menus:

   GREY – system out of order

   GREEN – system is ready to be used

   WHITE – system is operationg

   RED – system is in the error

6. Place tubes with sample and plates with matrices and initialize
7. Close front lid, set the nozzle and screen for seed analysis (Chapter 5.3 Stream)
8. Set protocol setting for seeding or sorting (Chapter 5.4 Working modes)

9. Close the front lid and start experiment

Each experiment can be terminated/interrupted by Emergency stop button. In the case at least 1 seed has been processed, this experiment can be restored. In the case the experiment was interrupted earlier it is needed to start new session.

5.2. General Settings

General setting to great extent influences the process of seeding or sorting. You can change the setting during process to make the seeding or sorting perfect.

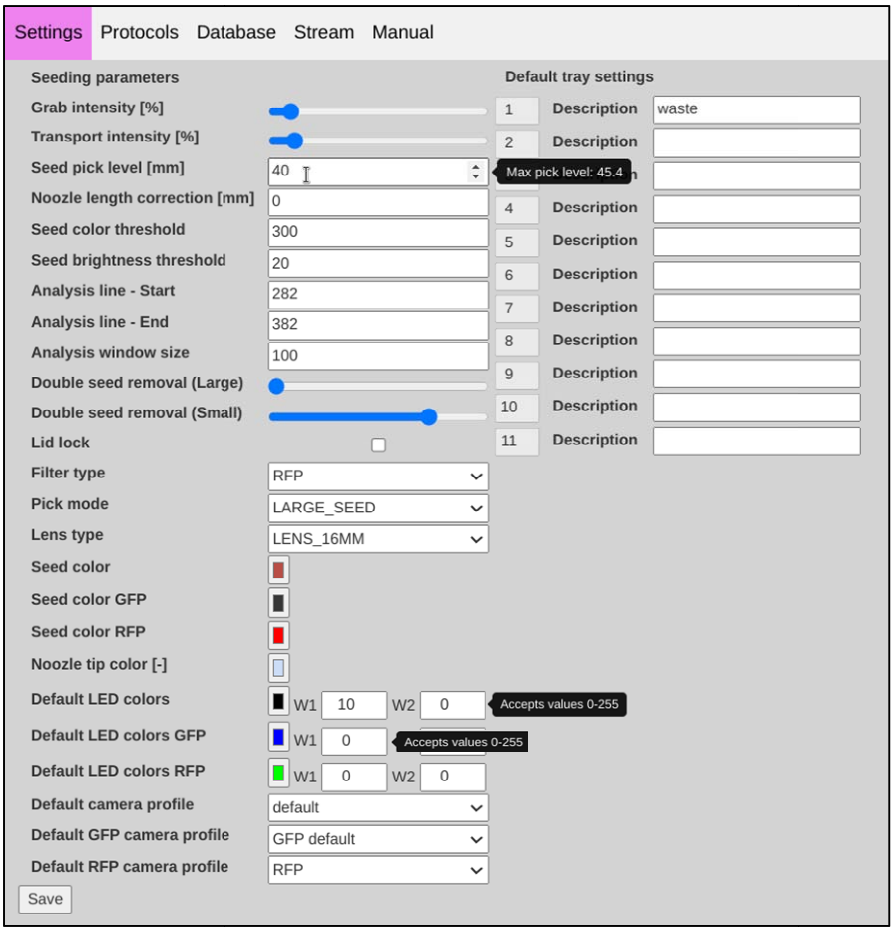


Figure 4: *Settings*

Right-click to see the values that can be entered (dark boxes).

- **Grab intensity (%):** Adjust intensity for seed picking, use the slider. The value of picking will be different for various nozzles based on the used gauge. All the time start from left to right. Lower grabbing intensities are important to prevent nozzle clogging. In the case the enhanced amount of multiple seeds is analyzed, decrease the grabbing intensity. In an opposite case there are not picked seeds from tube, or in low content, enhance the grabbing intensity.

- **Transporting intensity (%):** Fro transport use the same (similar) value or a bit lowered as is the grabbing intensity.
- **Seed pick level:** The maximal level you can reach is the bottom of tube. Check the maximal level by right click to this row. Use the lowest value only for picking the rest of seeds on bottom, otherwise you risk sample damage. When scooping large seeds, we recommend using values closer to the bottom and also depending on the size of the seed so as not to damage them. For Arabidopsis seeds, a level of approximately 3 mm from the bottom of the tube is appropriate.
- **Nozzle length correction (mm):** The nozzle correction setting is done on stream. Default value is 0. This value is important if you cannot see the tip of the nozzle you are using. Use the correction to ensure that even shorter nozzles can be used for seed manipulation.
- **Seed color threshold:** Set seed color pixels to be analyzed as seed/background. A higher number means less selective pressure on the selected color.
- **Seed Brightness threshold:** Set brightness threshold of selected pixels
- **Analysis line - start:** correspond to the top yellow bars in stream. Can be set in stream.
- **Analysis line - end:** correspond to the bottom yellow bars in stream. Can be set in stream.
- **Analysis window size:** Analytical window determine the area to be evaluated for the seed presence. Here is the width of window determined from the Analysis line –start to its end.
- **Double seeds removal (Large) -** Keep these value to a minimum unless it is necessary to increase them. By manipulating this value, the occurrence of multiple seeds being picked up at the same time is minimized. This function will only be used in the process of handling large seeds
- **Double seeds removal (Small) -** Keep these values to a minimum unless it is necessary to increase them. By manipulating this value, the occurrence of multiple seeds being picked up at the same time is minimized. This function will only be used in the process of handling small seeds
- **Lid lock:** To stop the running protocol when handling the lid, activate the lock
- **Filter type:** Select type of filter inserted
- **Pick mode:** Select type of seeds to be manipulated
- **Lens type:** Select optics to be used for the correct px/mm calculation
- **Seed color:** Color preset for seed recognition
- **Seed color GFP:** color preset for seed recognition using GFP fluorescence
- **Seed color RFP:** color preset for seed recognition using RFP fluorescence
- **Nozzle tip color:** color preset of nozzle tip, for the automated nozzle end detection
- **Default LED colors:** LED light preset for seed analysis (white light, green light analysis)
- **Default LED colors GFP:** LED excitation light
- **Default LED colors RFP:** LED excitation light
- **Default camera profile**
- **Default GFP camera profile**
- **Default RFP camera profile**
- **Default tray settings:** Setting of individual stocks' as default or for indexing using prefix.

5.3. Stream

The correct phenotypical analysis can be performed only based on careful setting of seeds to be analyzed. Be precise in the step of calibration of area to be analyzed and seed color and camera setting. It is influencing strongly seed phenotypical analysis and processes of seeding and sorting.

The stream allows continuous control of the ongoing process, setting the correct analysis, manual data analysis including image storage, possibility to monitor the seed from 0° and 90° position. These functions are available by right-clicking in the stream area. Real-time values are evaluated and displayed both graphically in the analysis window (right part of the image) and as calculated values below the stream. By culminating these values, the quality of the analysis performed can be inferred and the settings adjusted.

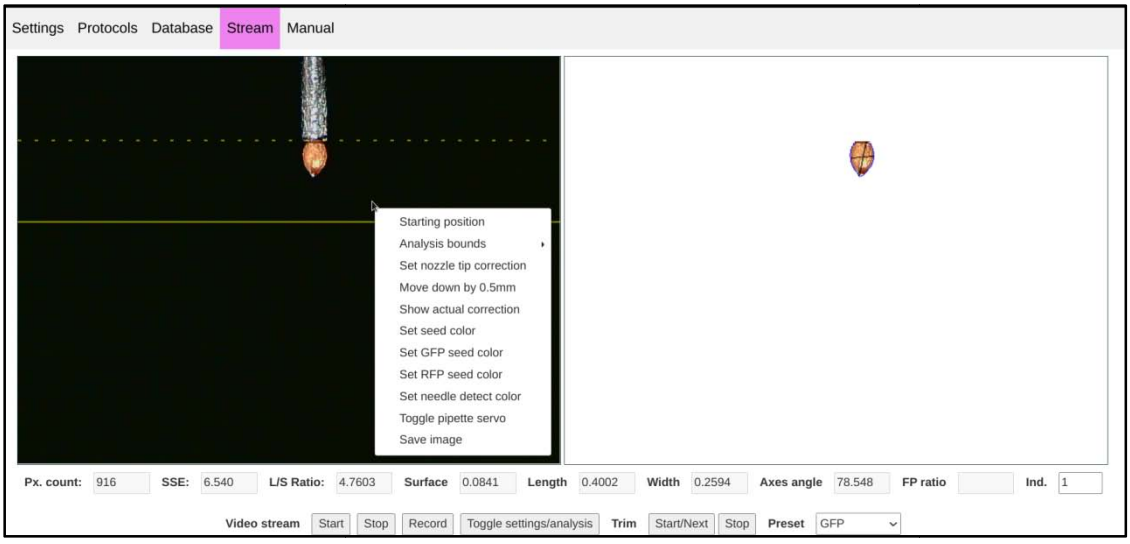


Figure 5 *– Stream (left), computed image (right). Quick assistive buttons accessible based on the right click to the stream/analytical window.*

5.3.1. System Calibration for seed analysis:

1. Turn on stream

2. Place the nozzle in front of the camera (standard situation based on initialization)

If not – Right -Click to any point in the stream view and select “starting position”

- Click the button “Start” of stream
- Close the lid and set camera

3. Calibration of camera: click button Toggle settings/analysis. Set camera setting, automatic (aperture priority) or saved profile. Do not forget to set the setting and in case you wish to save it use command save and save the profile as new one. During setting you can freely check the wiev of camera.

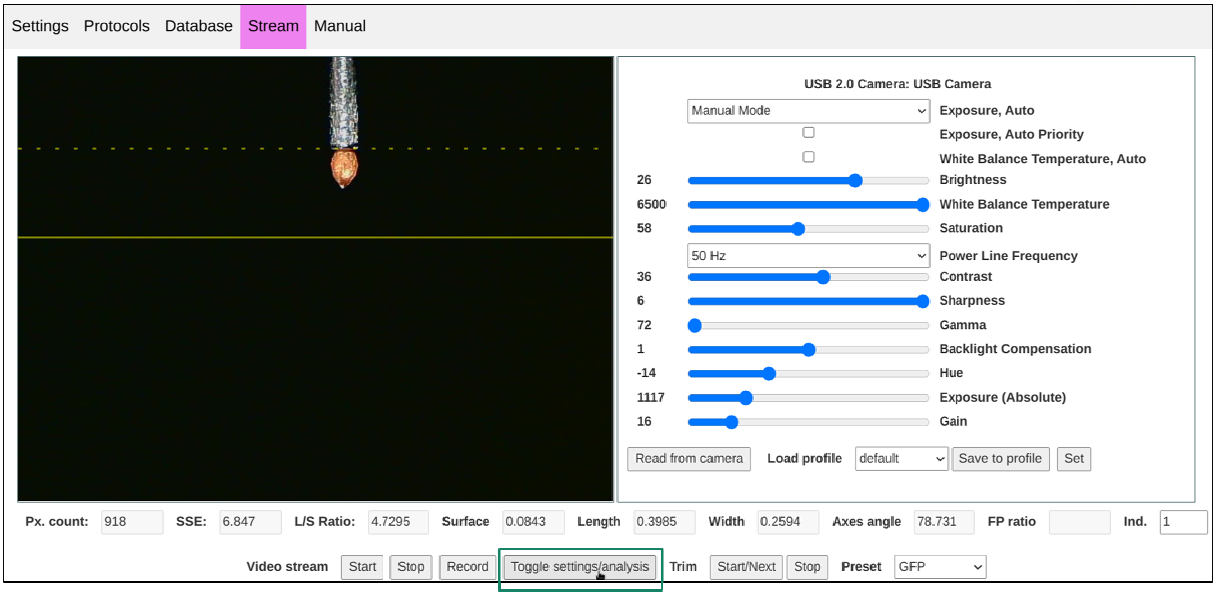


***Figure 6** – Camera setting*

4. Nozzle length calibration: In case the nozzle is not too long/short and it is close to the middle of screen it is not needed to calibrate the nozzle. In an opposite case it will be needed to carefully click the nozzle tip and click on the button nozzle tip correction. The nozzle will be automatically corrected and moved to position of upper yellow line. This line is analytical start. You can check once again the nozzle correction. Click the Starting point the nozzle will get to zero position, to reset this position go to Settings and manually delete the correction and preset 0.

In case the nozzle is not in a screen visible click the button move down by 0,5mm to set nozzle correction.

5. Analytical area setting: The correction of analytical start/end is defining area of seed phenotypical analysis. You can find its setting in Analysis bounds window. Click the nozzle tip and follow by Set analysis START. The yellow upper line will change its position to the nozzle tip, in case few pixels will be needed to calibrate do that ideally in General setting. Do the same for the analytical end, simply choose area which will be not exceeded by the seed. In case you need to manipulate the analytical lines in 1px line movements use 1px up/down button.

6. Seed color analysis: In the manual menu click on the Trim camera. Get back to the stream and use next to pick up another seed from stock. Click to the area of seed and select typical seed pixels. Select the Set seed color or Set GFP seed color in case you used filter for fluorescence for setting.

NOTE: Ensure the grabbing and transporting intensity is not to high (General setting). The setting could take several minutes in case of fluorescence or green light conditions. By setting lower values of grabbing and transporting intensity you protect the device. Carefully preset the Nozzle tip color as well to avoid its false positive analysis as seed.

Figure 7 *– the analytical window options allows for assisted thresholds selection*

7. Setting of seed phenotypical analysis: Check the original image (raw data) with the computed seed analysis, in case the shape borders or color are not in agreement with reality, you need to try different seed pixel color, or preset of camera. The setting of seed color threshold is important in calibration of pixels to be analyzed. The pixels in seed selected and the others are approximated to the selected color. By setting the threshold you are discriminating what is the seed color and what is background or potentially some impurity.

5.4. Working modes

5.4.1. Manual mode

This mode is active after start of the application. It is used for instrument initializing, restarting the system and for manual sample testing, presence of nozzles in the stack, prior using the automation.

- **Set head angle (°)** - The default head position is zero degrees. The maximal position pointing to the center of the plate is 118,284°. Do not exceed this value.
- **Set nozzle level (mm)** – Default position of nozzle is zero. There is Automatic protection which do not allow the system go deeper than the lowest position – the bottom of tube. However, be careful when setting this value, in positions where it is not possible to set the nozzle lower and a collision can occur.
- **Tray cell selection** – Turn the tube holder carrousel to the sample insertion position (Maintenance) or bellow the nozzle for seeds picking (Nozle).
- **Cleaning** – activates the process of nozzle surface cleaning
- **LED colors** – Temporarily set LED light spectrum by manipulating W1 (white LED1), W2 (white LED2) and RGB LED colors. (right click to identify maximal values).
- **Nozzle presence** – Fill the nozzle stock and mark the individual seeds in SW
- **Drop nozzle** – Nozzle attached to the transporting head will be removed to the waste container
- **Pick nozzle** – select nozzle to be picked from the nozzle stock

- **Restart** – The Boxeed system computer will be restated
- **Power off** – The Boxeed will be initialized and system computer turned off. Continue by turning OFF the machine by main switch.
- **Troubleshoot** - Connect Ethernet cable and contact the technical support via VPN.
- **Reload cache** – reload the user interface (only in application)
- **Initialization** – System will be initialized. During the first initialization LED light gets turned ON. Check Initialization process and correct position of:
  - plate holder – parallel position to the front of Boxeed instrument
  - tube holder – position 1 in front of camera, bellow the nozzle
  - transporting head – nozzle above the carrousel (No. 1)
- **Emergency stop -** Allows stopping all running processes.

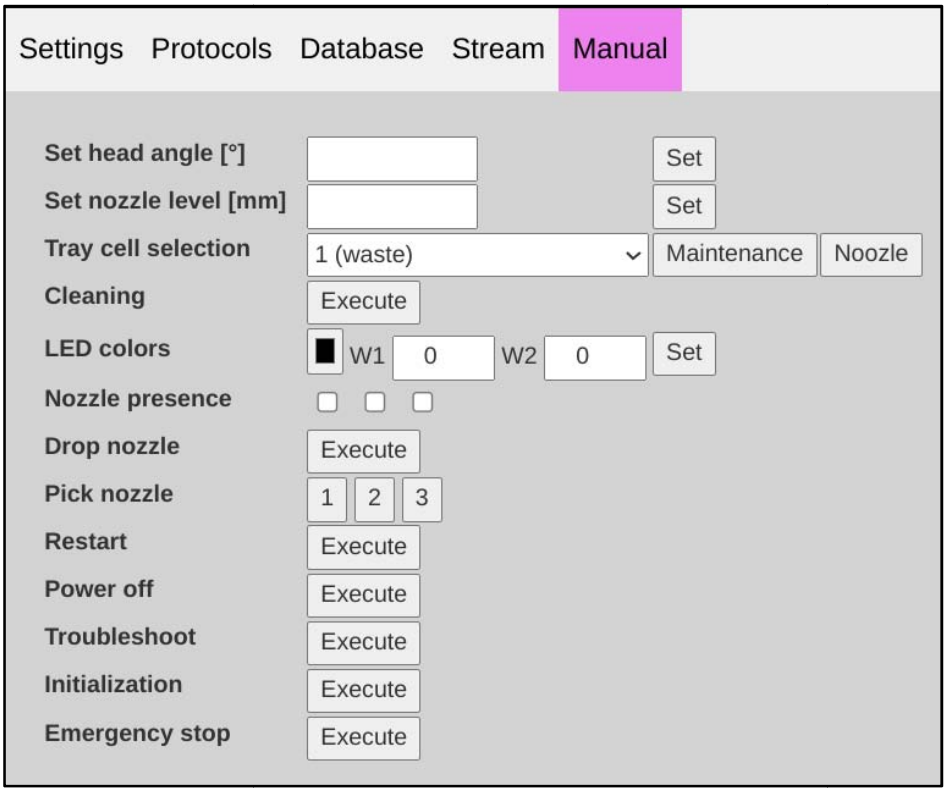


Figure 8 *- Manual mode*

5.4.2. Sorting

To preset sorting go to the protocols menu. Select an existing protocol from the list, or start a new one by pressing the new protocol button. Logs can be copied but also renamed and recompiled. Select also nozzle cleaning parameters for the type of illumination used camera profile and for standard measurement and analysis as well as for the use of Fluorescence profiles. In case you are setting up a new protocol, the basic settings are load from the menu settings. If you are copying a protocol, the settings are load from the already copied protocol.

Use the add button to add more categories for sorting. Unsuitable categories are deleted by using the remove button, chronologically from right to left. To consider setting up an experiment, it is necessary to keep in mind the hierarchy of the sorting process, which indicates that if it was not possible to insert a seed in the first category it is tested in the next category and so on. If the parameters for placement in any category were not met the seed is treated as a mismatch.

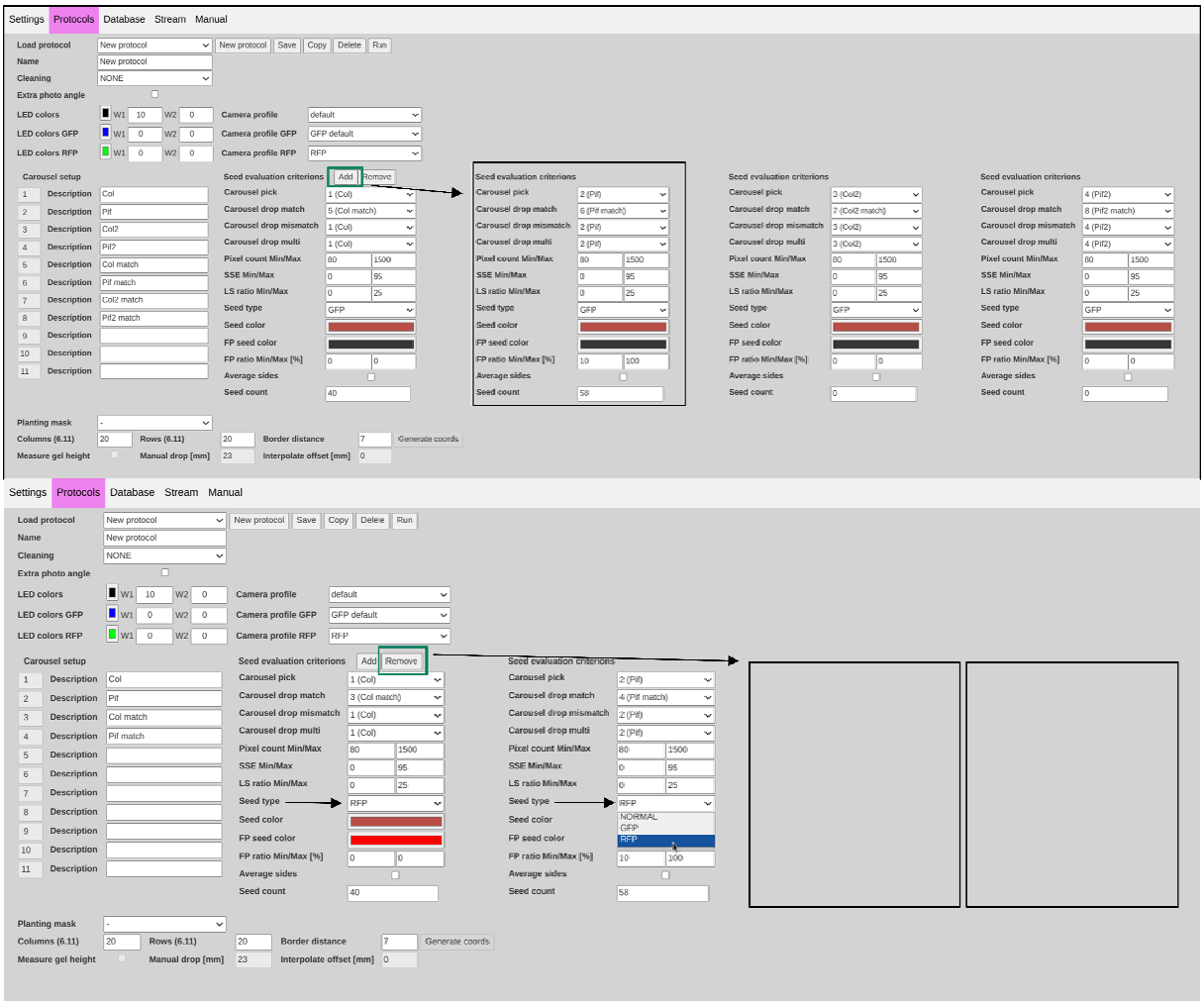


Figure 9 *– Sorting protocol setting*

Parameters:

- **Add/Remove** – adding of new window for another sorting thresholds
- **Carrousel pick** – Select tube No. - Tube used as source of seed material to be sorted
- **Carrousel drop match** – Select tube No. – Tube for seeds fulfilling the preset set criteria
- **Carrousel drop mismatch** – Select tube No. – Tube used for seeds which do not fit in thresholds. This is optimal to remove unwanted seeds from selection and to do not analyze them multiple times.
- **Carrousel drop multi** – Select tube No. – Tube for multiple seeds or doubled seeds in analysis. Based on sterilization process some of seeds can get “glued” each another.

- **Pixel count Min/Max** – Preset threshold for minimal seed size in px (*Arabidopsis* min. app. above 80px). Preset threshold for maximal seed size in px (*Arabidopsis* min. app. below 1500px)
- **SSE Min/Max** – Preset threshold for seed surface shape. Preset threshold for seed surface shape (Arabidopsis multiple seed app above 95)
- **L/S ratio Min/Max** – Preset threshold for seed shape
- **Seed type** – Standard analysis/fluorescence analysis. The morphometric analysis will be performed as well once FP analysis is selected.
- **Seed color** – Select seed color for the analysis
- **FP Seed color** – Select fluorescence seed color for the analysis
- **FP ratio Min/Max** – Select fluorescence intensity range for the analysis
- **Average values** – Preset method of sorting. In case Average parameters is checked, both measurements from photos in 0° and 90° (45°- extra angle button) orientation are used as one and counted in average. The average is then used to check preset criteria.
- **Seed count**– Preset amount of seeds to be selected in to the source tube.
- **Save the protocol** in case you want to use it in the future.
- **Proceed seeding**, turn the button RUN

5.4.3. Seeding

1. For seeding select again menu Protocols
2. Click to create protocol or edit existing protocol
3. Set the protocol properties in a menu including the setting of thresholds as described for the sorting protocols.

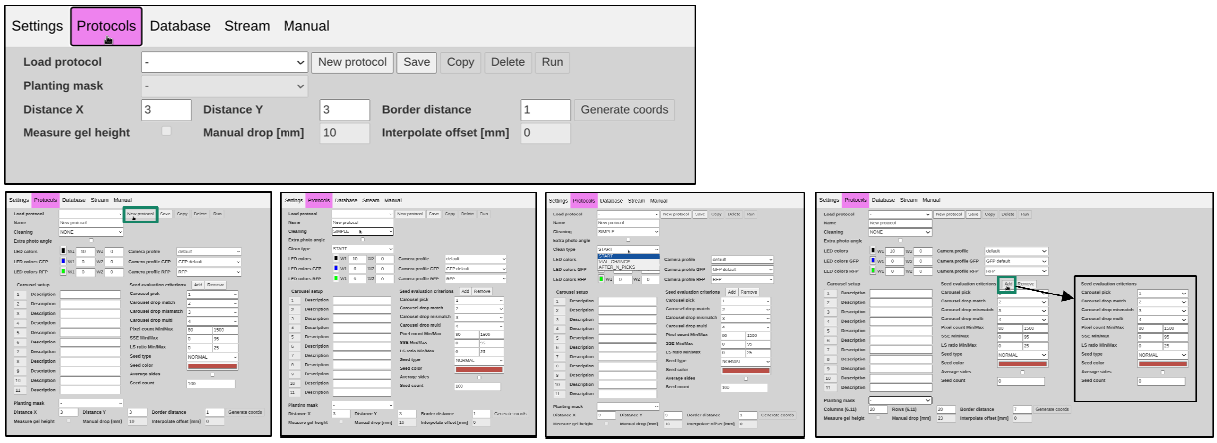


Figure 10 *– Setup of seeding protocol*

Protocol Name - will be visualized in archive of database, on QR code. In the case same experiment name will be used the date of seeding will be decisive. For the printed label only first 36 characters are used.

4. **Plate format** – select in pop-up menu the appropriate format of plate you use
    - **Petri dishes (5,5cm. 9cm, 13,85cm, square)**
    - **Multiwell plates (6, 12, 24, 48, 96, 384)**
    - **Slides**
    - **…**

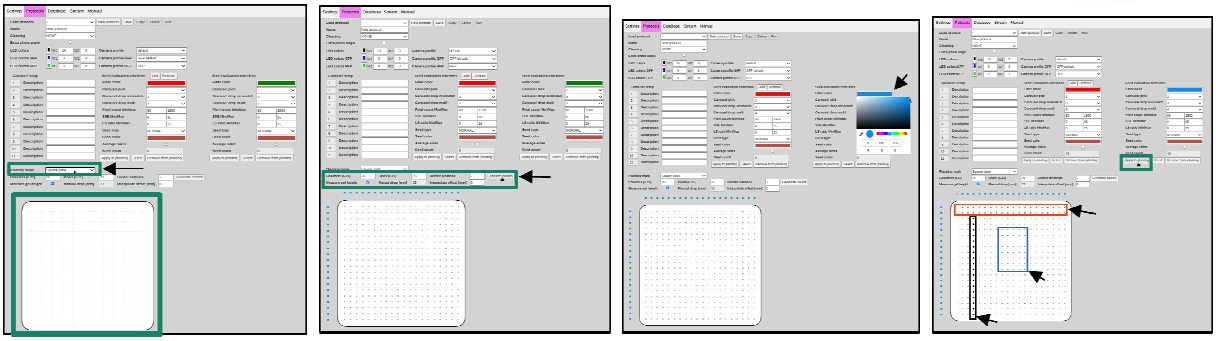


Figure 11 *– Seeding protocol setting of coordinates*

5. **Create the structure of coordinates**
   Column – select number of columns. Distance of columns (mm) is indicated
   Row – select number of columns. Distance of rows (mm) is indicated
   Border distance – select distance of samples from edges (mm) do not seed closer than listed values.
   Generate coordinates – Click this button to generate predefined coordinates
6. **Select coordinates** on plate and click: *apply to planting/remove from planting*, to the appropriate sorting parameters. The selected/unselected positions turn to the indicative seed color of individual sorting groups.
7. **Gel height measurement** - Check the button in case your preference is precise seeding. The gel surface will be analyzed by sensor 200µm above the gel surface. The 3D map of gel terrain will be used for further seeding in to coordinates. For manual dropout select value previously checked in Manual mode. The manual dropout value (mm) of plate surface is indicated, use always value closer to 0.
8. **Manual drop –** Select the height of Nozzle for the manual dropout. The gel height measurement is not applied.
9. **Interpolate offset** – This function allows the seed to be dropped onto the measured gel with the value reserve. The entire seed will be dropped on the gel from the height you define in this field. If no value is entered the seed is automatically placed exactly at the level of the gel height to be measured. This can be problematic for larger seeds. Therefore, for large seeds, always allow for an offset defined of at least the size of the seed to be sown. This offset can be used in hazardous media placement to minimize the risk of the nozzle contamination.
10. Save the protocol in case you want to use it in the future.
11. Proceed seeding, turn the button RUN

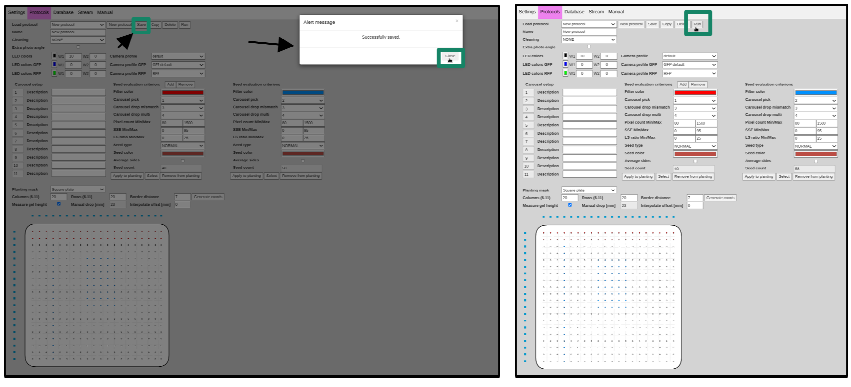


Figure 12 *– saving protocol and run experiment*

5.5. Database

All sown protocols are located in the database. The coordinates of individual seeds and raw data images are saved and can be exported. Closer information about stocks used and experiment details can be visualized in cloud (Protocol menu). In the database menu, the results can be viewed and analyzed graphically using histograms for individual measured parameters. Ongoing experiments can be paused, terminated or restarted later depending on the research needs. Results for individual tubes can be displayed during sorting. Print QR codes for archiving but also view individual seeds sown per plate or individual quintiles of seeds within the sort.

The results can also be deleted, If the results have been deleted in the instrument and have not yet been synchronized with the Cloud, they will not be saved to the Cloud. Otherwise, deleted results in the instrument are not automatically deleted in the Cloud system.

Results – Select performed experiment from a database
Quantile count – Select quintile size to adjust statistical graphs and resulted histograms
Average sides – average results from used projections is used
Filter slot – Filter the data based on individual sorted/seeded stocks categories.
Left/Total – Amount of seeds to be sorted for all and individual parameter from a total.

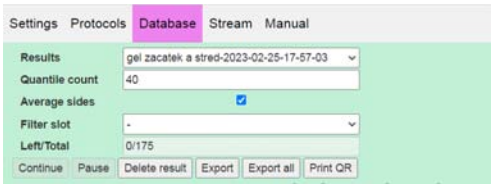


***Figure 13** – Database menu*

- **Continue** – Continue interrupted or incomplete sorting
- **Pause** – Interrupt sorting
- **Delete Result**– Delete selected experiment and all the entire data from database
- **Export** – export only data in csv.
- **Export all** – Download .zip file including raw data images and .csv file of metrics and measurements data export from experiment.
- **Print QR** –The QR code specific for selected experiment printed. QR code contain link to the database, name of experiment and date of sawing saved in the archive of tablet
- **Print QR cloud –** The QR code containing the Cloud database archive address.

***Figure 14** – QR label (QR code, date of seeding, Name of protocol – max 36 characters)*

- **Scan QR** – If connected device contain a reader or camera, the QR code from plates will be scanned. The used device has to be connected to a database to get full information about experiment. The provided tablet can also be used for scanning of QR codes, even in disconnected mode.

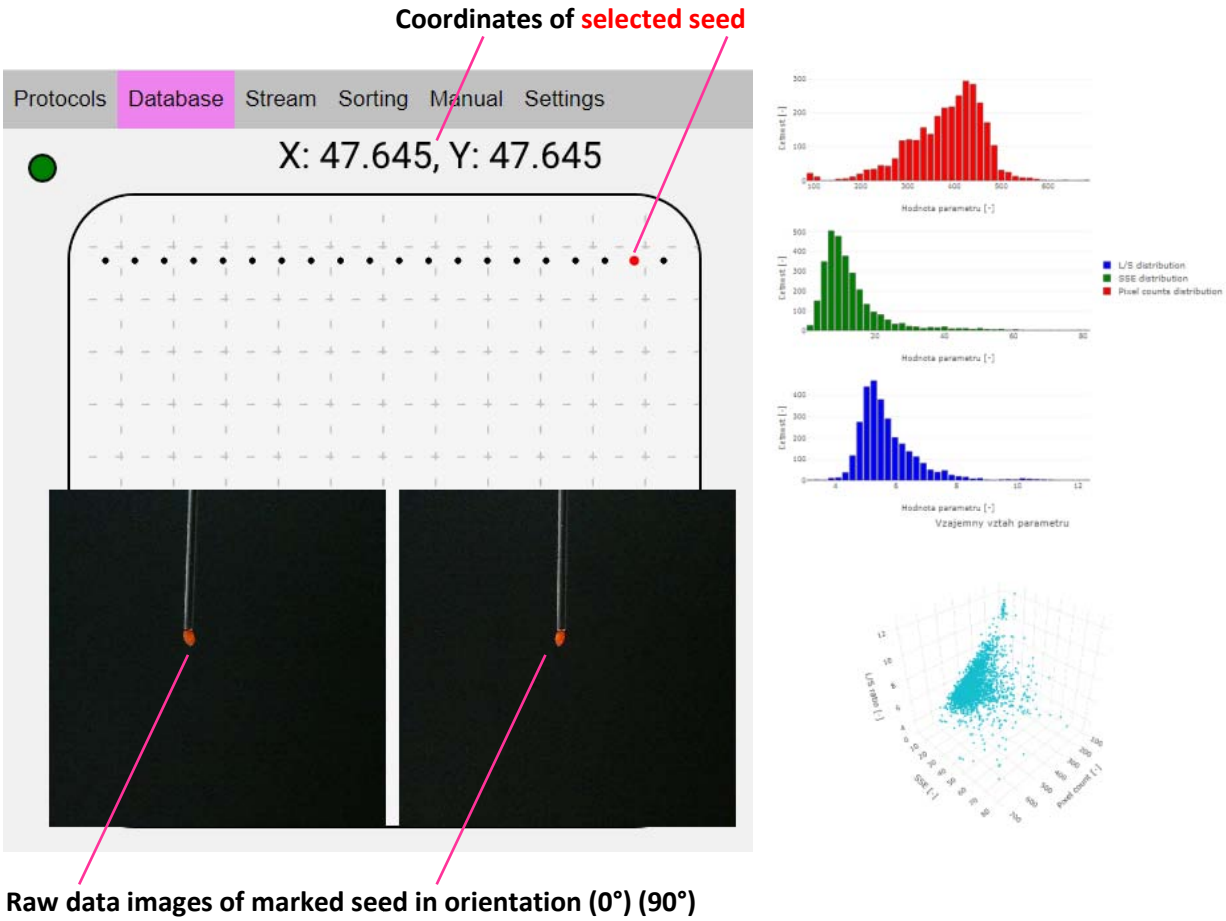


Figure 15 *- Database menu*

6. Sample

6.1. Sample preparation

For optimal operation of Boxeed, pay attention to the purity of sample. Ensure the large- and micro-sized impurities are removed from sample you are going to proceed. Large size impurities can reduce the speed of processes, by pulling down the sample from nozzle.

For *Arabidopsis thaliana* we do recommend cleaning using a sieve to discard large impurities. Protocol containing the rinse of seeds by 70% ethanol (30-60sec) followed by 96% ethanol (30-60sec) is recommended to discard micro-sized particles In case you cannot sterilize the sample and rinse the seeds use method of sanding seeds between papers to discard maximum of micro-sized particles.

Figure 16 *– Impurities in sample A) large-sized B) micro-sized C) cleaned sample*

6.2. Sample volume

The optimal seed volume in a 1,5 mL tube is 4-6mm.

- Do not exceed 10-12 mm of Arabidopsis seeds volume in a 1,5mL tube.
- In special regime can be sorted or sown almost all the seeds from tube (Arabidopsis). App. 2-3 seeds remain in a tube.
- For larger seeds do not use more than half of volume of the reservoirs. Prevent spilling the seeds into the machine by locking the lid and not exceeding the seed volume.

7. Plates and matrices, tubes

7.1. Plates and trays

WARNING! - Decreased safety and system functionality due to incorrect consumables use. No warranty will apply to any instrument that has been damaged or destroyed by using the incorrect consumables. Usage of consumables other than listed may impair the functionality, precision and safety of Boxeed device.

NOTE! - Thermal sterilization of consumables can change its dimensions and other parameters

Ensure you are using consumables specified for the standard plate holder ID: 20PL01 or for your customized plate holder. In the case of customized plate holders always use a plastic that is approved and agreed upon at the time of customization. In case the plate is not fitting well - is too tight or loose, do not use it. Make sure, that all 4 corners of the square plates and multiwell plates, are inserted well in the holder.

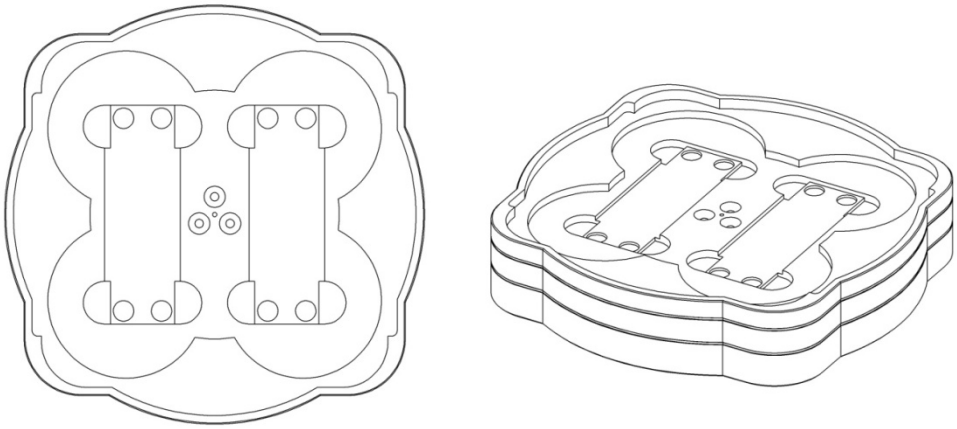


Figure 17 *- Plate holder 20PL01*

The standard compatibility is ensured for Boxeed 2.1 seeding and sorting system to the listed consumables by using the plate holder ID: 20PL01:

Consumables	**Manufacturer**	**Dimensions**	**Product ID**
Petri dish 60mm	Thermo Scientific	60mm x 10,2mm	Nunclon Sphera Dishes - 174944
Petri dish 90mm	Noex		BH903S25SQ
Petri dish 150mm	Medlab Products	150mm x 18mm	51-0150-0S 51-0150-0A
Square plate 120mm	Greiner bio-one	120mm x 120mm x 17mm	668 102
Multiwell plate 6-well	Greiner bio-one	127,8mm x 85,5mm x 19mm	CELLSTAR 657 160 657 165 657 185
Multiwell plate 12-well	Greiner bio-one	127,8mm x 85,5mm x 19mm	CELLSTAR 665 102 665 180 665 970
Multiwell plate 24-well	Greiner bio-one	127,8mm x 85,5mm x 19mm	CELLSTAR 662 102 662 160

Multiwell plate 48-well	Greiner bio-one	127,8mm x 85,5mm x 19mm	CELLSTAR 677 180 677 102
Multiwell plate 96-well	Greiner bio-one	127,8mm x 85,5mm x 14,2mm	CELLSTAR
Multiwell plate 384-well	4titude	127,9mm x 85,5mm x 10,6mm	4ti-0382
Microscopic slides	-	max. 76,4 x 26,2 x 1,2 mm	-

7.2. Matrices

WARNING! – Never use matrices which are of liquid state and can pour out!

CAUTION! – Never use matrices which are of bulk matrices and can spill out!

Ensure the matrices for seeding are wet. Boxeed is constructed for seeding of seeds to solid laboratory growth media (gel surfaces, wet paper). In case you are going to spread the sample across multi-well plate without matrices, be sure the seed dropout position is at least 3 mm below the edge of consumables you use (electrostatic charge on empty matrices can cause seed misplacement).

7.3. Tubes and seed holders

Seed reservoir type	**Manufacturer**
1,5 mL, antistatic tubes	Funakoshi, Japan
12mL, large-seeds inserts	Labdeers, Czech Republic

CAUTION! Usage of consumables other than listed may impair the functionality, precision and safety of Boxeed device.

8. External Devices

8.1. Thermal printer

The integrated thermal printer is operated by Boxeed software. The QR code specific for entire experiment including date of experiment is printed.

For better manipulations with stickers, the button on printer (1) should be hold on, to enlarge the ticket size. Status LED (2) shows the status of the printer during operation and - if it is equipped with a charging circuit

Paper replacing:

The thermal printer is specified for thermal paper rolls or labels with a width of 57.5 mm Ø 0,5mm and a winding diameter of 31 mm.

1. Unwind about 10 cm (4 ") of paper from the roll. Keep the layers wound tightly.

2. Open the printer cover by slightly pressing the LEVER in the cover upwards. The print roll is lifted from the mechanism together with the cover. The cover is now easy to open.

3. Insert the paper roll in the paper storage, so the thermosensitive part shows toward the printer mechanism. Only this side can be printed on.

NOTE: Standardly the side to print on is the outside. If you should have any doubts, just do the fingernail test: Quickly drive the edge of a fingernail with slight pressure over the paper. The thermosensitive side will turn black as a result of the frictional heat.

4. Close the cover by applying strong pressure. You can hear it snap shut. Now you can rip off paper at the tear bar (1) without the cover opening up or the paper sliding through the print head.

8.2. Tablet

Core 2.0GHz, RAM 3GB
Touch screen 8"
GPS, Wi-Fi 802.11ac, Bluetooth 4.2, USB-C,
4850mAh battery,
Google Android 7.0 Nougat
*NOTE technical specifications for the tablet (operating device) may change due to availability of optimal devices on the market

Tablet type and configuration may vary, follow the manufacturer's instructions for setup and maintenance.

Use the Boxeed app installed on provided tablet to easily operate the device.

8.3. USB storage disc

Always use the USB drive when the device is in use. We recommend a UTP connection for downloading data. If the instrument is in operating mode without external USB, the data will be stored in the internal memory of the instrument. This may cause the instrument to malfunction during prolonged use. You will be automatically notified if the data file limit on the disk is about to be exceeded.

9. Nozzles

The processed seed is sucked into the nozzle tip. The choice of the appropriate nozzle diameter is entirely the responsibility of the operator. Always use nozzles with an inner diameter smaller than the diameter of the object being handled.

9.1. Nozzles straightening process

One of the most important settings is nozzle *straightening*. If they are not *straightened* correctly the seed analyses will not be performed correctly. The data analysis may be biased and the performed results poor.

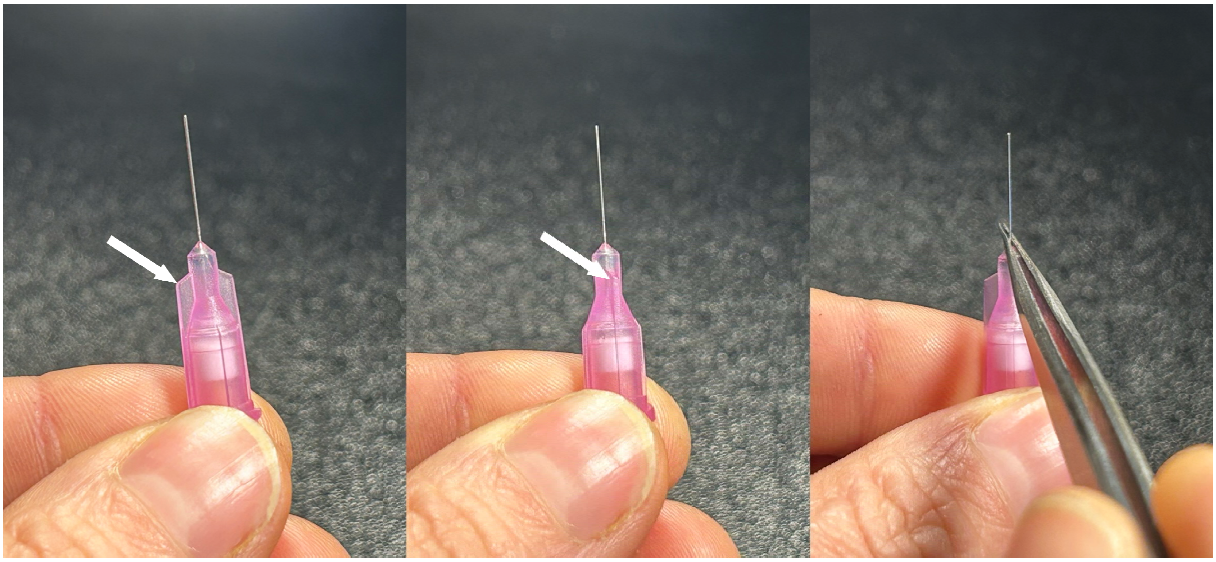


Figure 18 *- Nozzle straightening process. Use tweezers to rotate the nozzle to 0°/90°, applying slight sideways pressure to align the nozzle in both orientations*

9.2. Nozzle connection/release

9.2.1. Manual connection/release

1. To connect a nozzle, hold the nozzle hub and insert the nozzle directly into the nozzle connector.
2. To connect a sterile nozzle manually, the nozzle must be handled in such a way as to avoid contaminating the nozzle.
3 . To release the nozzle, repeat the procedure in reverse order.

9.2.2. Automatic connection/release

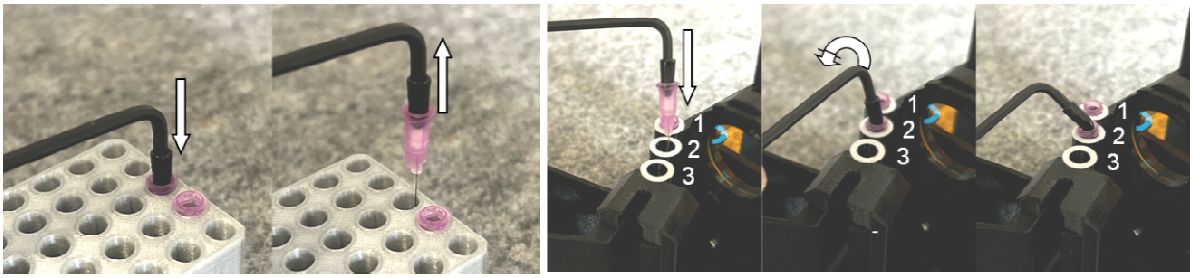


Figure 19 *- Slide the nozzle transport tool onto the nozzle. Carefully insert the nozzle into the nozzle holder inside the Boxeed. Apply slight pressure to release the nozzle from the pin. Push the*

transporting tool sideways until the nozzle is released from the pin. The nozzle positions are marked and correspond to the SW. White arrows indicate the direction of movement of the nozzle transporting tool.

1. Insert the nozzle into the nozzle holder, making sure it is straight and taking care not to bend the nozzle when handling it.

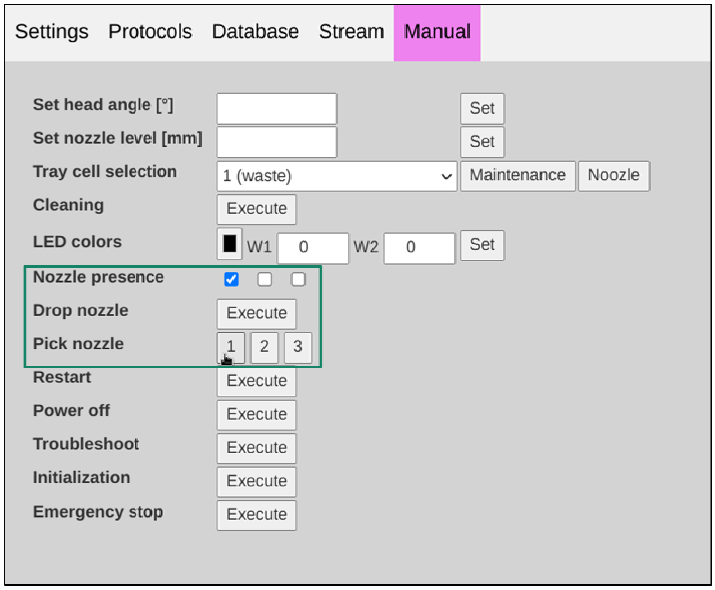


Figure 20 *– Manual menu nozzle stock and operation buttons*

2. In Manual Mode, mark the position where the nozzle is inserted and click on the pick nozzle command to select the nozzle position number.
3. To remove the nozzle, go to the manual menu and use the drop nozzle command. Make sure the waste container is placed in the instrument.

NOTICE! - Avoid producing strong power to fix the nozzle in to the locking system.
The strength of locking does not influence the proper function of Boxeed instrument
NOTICE! – Always use nozzle gauge smaller than seeds to be manipulated
NOTICE! – Nozzles can be reused repeatedly with careful cleaning and maintenance (Chapter 10).

10. Care and maintenance

10.1. Cleaning of Boxeed

All parts of Boxeed can be cleaned by following procedure.

1. Moisten a cloth with cleaning agent
2. Clean the device form external contamination
3. Moisten the cloth with clear water and wipe down the housing to remove rests of cleaning agents

WARNING! – Health risk and damage due to penetration of liquids. The device itself is not waterproof and may not come into contact with the liquid, always use wet cloth for cleaning. Switch of the device before wet cleaning.

NOTICE! – Damage to device by unsuitable cleaning agents or by sharp objects. Do not clean the device by corrosive cleaning agents, strong solvents or abrasive polishes. Never use the organic solvents or acetone. Do not use sharp or pointed objects for cleaning. Check the compatibility and chemical resistance of materials before the use of cleaning agents.

10.2. Disinfection of Boxeed

For disinfection of Boxeed wipe the outer surfaces with decontamination agent or 70% isopropyl.

Sterilizing with UV light – Boxeed can be temporarily subjected to the UV light at 254 nm.

NOTICE! – Damage due to incorrect handling. Do not use any additional disinfectants, decontamination agents during UV sterilization.

NOTICE! - Color change due to UV light exposure. Exposure to UV radiation may cause changes in the color of some parts, but the functionality of the instrument is not affected. A color change is not considered a defect.

10.3. Maintenance of consumables

Filters – cleaning station maintenance

Filters can be sterilized temporarily with UV light at 254 nm.
Dirty filters or filters with burrs replace with new ones.

Nozzles maintenance

Nozzles can be used repeatedly based on the careful cleaning and maintenance.
Clean the nozzles immediately after use.

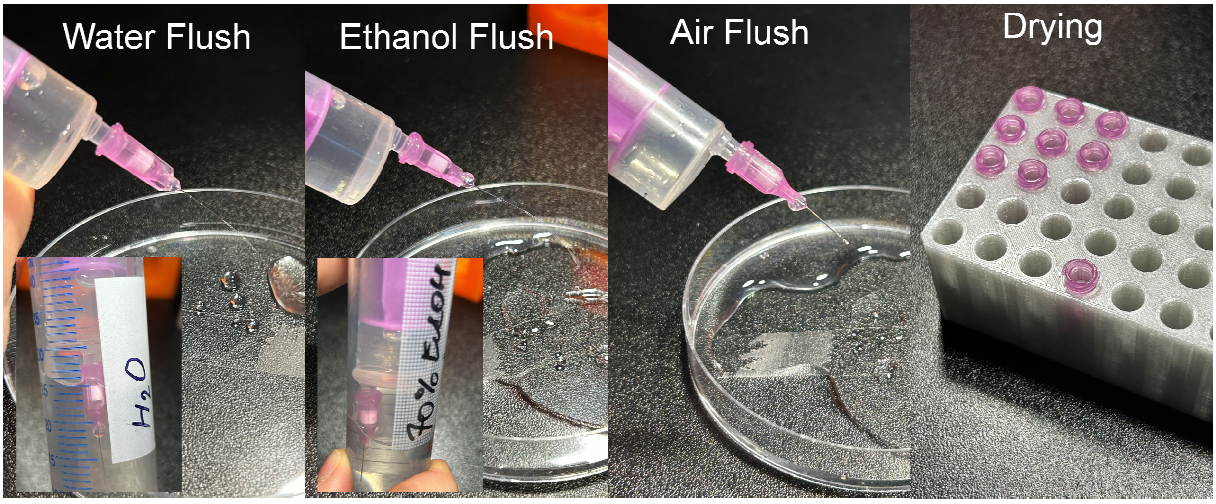


Figure 21 *– Nozzles cleaning process*

1. Use cleaning agent or decontamination agent or 70% isopropyl/ETOH for cleaning of nozzles surface
2. Clean the inner parts by cleaning agent or decontamination agent or 70% isopropyl/ETOH using the syringe.
3. Remove the rests of cleaning agents by clear water.
4. Use syringe mediated air flush to remove rests of water or disinfectants from inner parts of nozzles.

Nozzles can be sterilized temporarily with UV light at 254 nm.

11. Technical data

The proper device functions were verified by employee of Labdeers. Each Boxeed device was adjusted and tested in all the operation modes.

11.1. Technical Processes

11.1.1. Door Opening/Closure mechanism

Both the closing and opening of the lid are done in the upward direction. Never use a downward closing motion, do not place objects on the open door or lean against it. In a SW a lid lock can be activated to protect the experiment if the door is accidentally opened. Lid lock stops the protocol when the door is opened. The ongoing machine movement is completed and the instrument stops. When the lid is closed, the protocol can be started to continue, the process will continue.

NOTICE! - Always use both hands to close and open the door mechanism to avoid damage

NOTICE! - Never use a downward closing motion

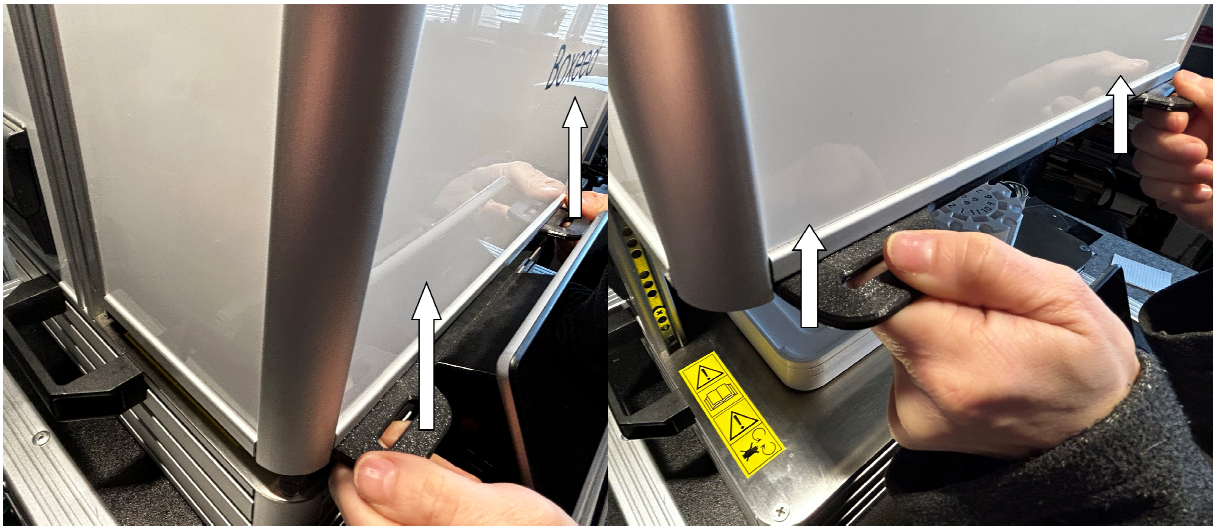


Figure 22 *– Lid opening (left) and lid closing (right). Always force in the upward direction.*

11.1.2. Locking and transporting head mounting

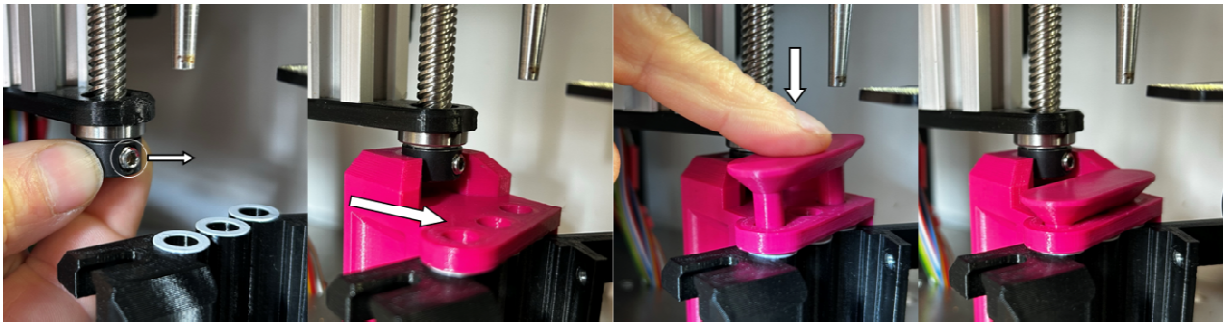


Figure 23 *- Turn the shaft of a head with two fingers on the underside so that the locking screw points towards the camera. Insert the anchor and fasten the head with the enclosed pin. The head is now secured for transport. (The white arrows indicate the direction of movements.)*

11.1.3. Replacement of the carousel

The Boxeed system comes with two carousels. A carousel for 1.5 mL tubes and a carousel for 12 mL inserts for large seeds. The carousel for large seeds is supplied with a lid, the carousel should always be secured with this lid after inserting the tubes. Follow these instructions to attach the carousel to the Boxeed.

1. Insert the key into the screw hole in the carousel, at the same time push the key through the socket on the motor.
2. Push the carousel evenly against the motor.
3. Move the key from left to right with gentle pressure, to ensure that the carousel is placed correctly on the motor.
4. Insert the 2 screws, then slide the key out and insert the remaining screw.
5. Never tighten the bolts individually, making sure to tighten the bolts one at a time, all the same height.

6. To remove the carousel, unscrew all screws and gently remove the carousel from the motor carrier with a rocking motion.

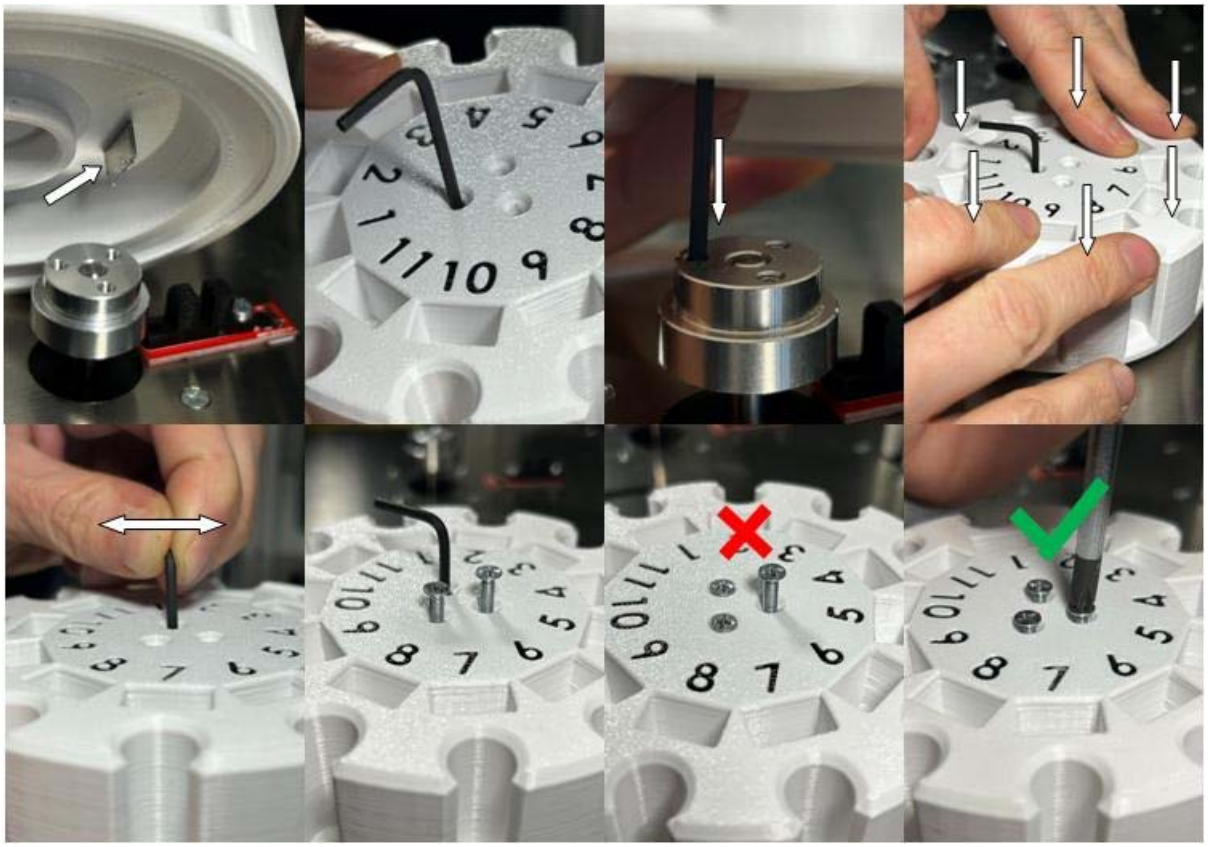


Figure 24 *– carousel deployment process (White arrows indicate the direction of movement)*

NOTICE! - Take care not to damage the end cap on the bottom of the carousels (1st photo)

11.1.4. Stocks placing

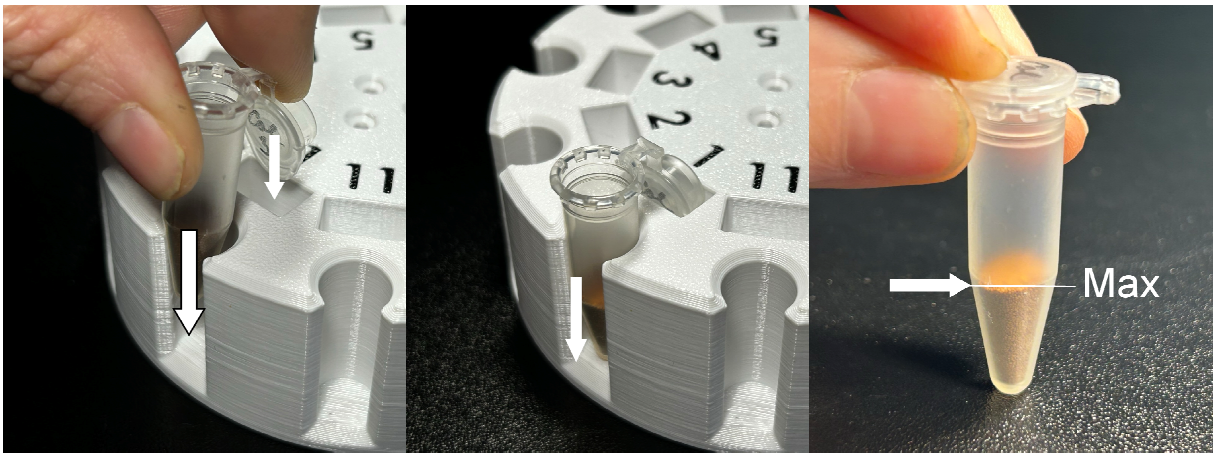


Figure 25 – 1,5mL tubes insertion into the carrousel

Insert the tube vertically into the carousel, making sure that the tube cap is seated in the hole in the carousel. Push the tube to the bottom of the carousel. (The white arrows show the direction of seating and the maximum seed fill.)

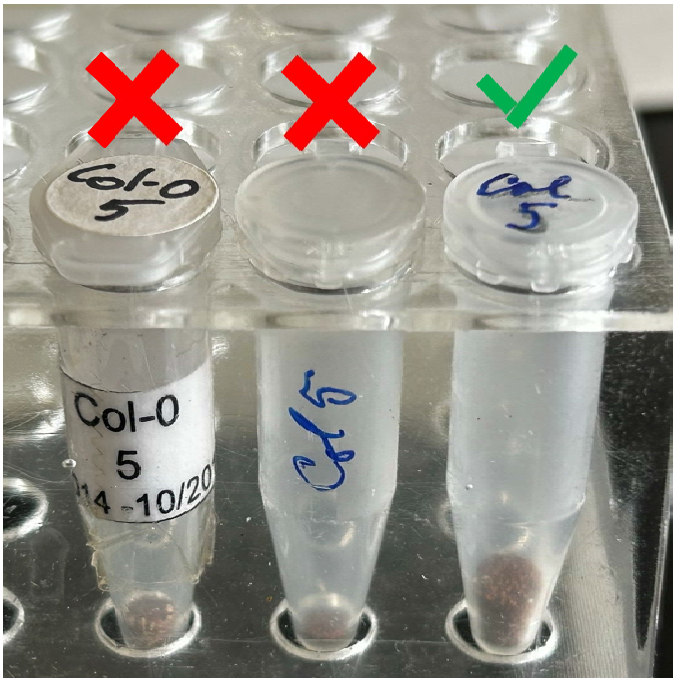


Figure 26 *– correct sample description*
Insert stocks into the Boxeed labeled on the top of the tube

NOTICE! – All the samples volume should not exceed more than half volume of used tubes or reservoirs. With larger volumes of seeds there is a risk of spilling samples into the instrument.

11.1.5. Installation of waste container

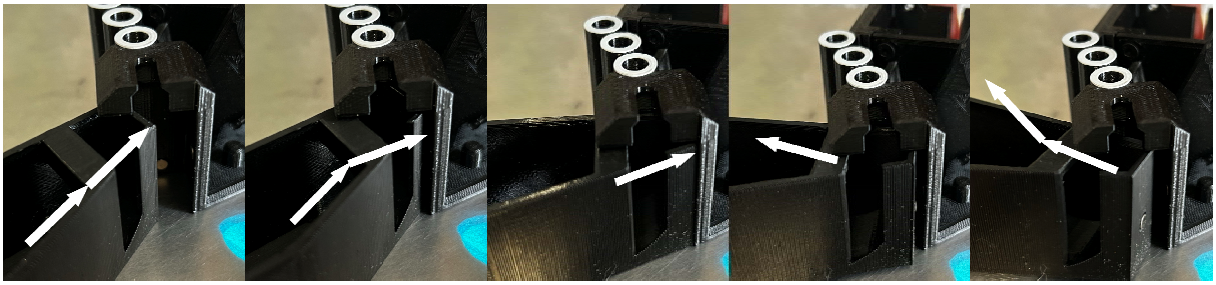


Figure 27 *– Waste container installation*

1. Insert the nozzle waste container into the holder at a slight angle from the front position of the machine. Make sure the edge of the nozzle container fits tightly against the edge of the holder.
2. To eject the waste container, proceed in the reverse order of the insertion process.

The white arrows indicate the direction of container positioning during the container insertion and removal process.

11.1.6. Cleaning station

Insert the sterile filter into the holder using tweezers. Follow the photo instructions. Change the filter regularly to prevent contamination of the working environment or seed samples.

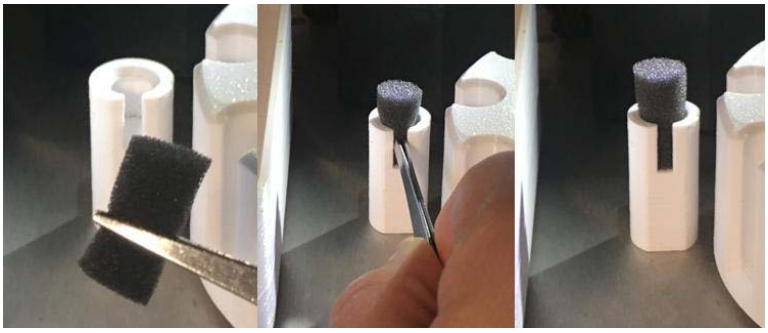
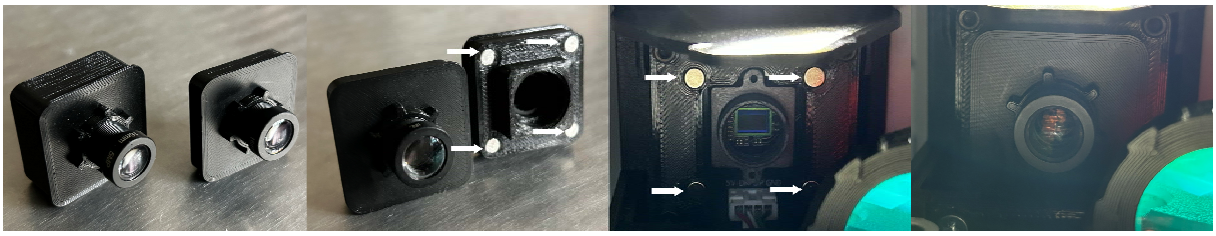


Figure 28 *– Cleaning filter placement to the clearing station*

11.1.7. Replacement of optics



7. Attach the optics on side of the magnets to the camera. The white arrows show the positions of magnets.
8. The optics is calibrated; avoid its screwing out to the magnetic holder.
9. Place the optics by the descriptive sign down.
10. Never leave the camera chip exposed and without optics. If it becomes dusty, use an air blower to remove impurities from the chip.

11.1.8. Installation of optical filters

Insert the 2 pins of the filter glass holder into the hole on the filter holder. Rotate the filter glass clockwise to lock into the filter holder. (The white arrows show the pin and lock positions.)

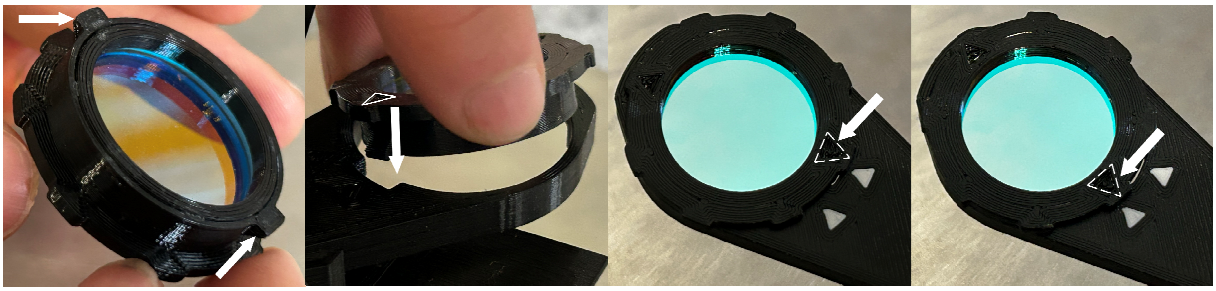


Figure 29 *– Installation of optical filters into the Fluorescence module*

11.2. Technical Specifications

Description and technical data	
Manufacturer	Labdeers s.r.o.
Mechanical parameters	
Height	355mm
Width	425mm
Depth	390mm
Weight	~15kg
Electrical parameters	
Connected load	100-240V; 50/60Hz; 2.2A
Max. power consumption	90W
Operating conditions	
Temperature	15°C-30°C
Relative humidity	10% - 50% RH
Atmospheric pressure	795 hPa – 1060hPa
Interface	
Connection	RJ-45 (LAN), Wi-Fi
Data storage input	USB 2.0
User interface	
Internet browser	HTML5 enabled web service/browser (chrome, firefox, IE)
Cloud service	seed.labdeers.com (optimized for chrome)
Tablet	Boxeed application
Data archive management	
Sticky label printer	Standard paper width 58mm, QR code archive
Cloud service	Secure free service*
External memory	64GB
Optical parameters	
Light source	R/G/B/W multichannel LED
Camera	2MP/ sensor ½", COMS, low light sensitivity
Seeding and sorting parameters	
Seed type	Dry seed samples Optimized for Arabidopsis, suited for other small seed species, optimal shape - rounded/oval.
Seed size	80µm up to 3mm
Sorting and Seeding capacity	11 stocks
Sorting and Seeding capacity	Tens to Thousands of seeds (Arabidopsis) * residual seeds count ~5pcs (Arabidopsis)
Seeding accuracy	~ 100µm (Arabidopsis)
Seeding/Sorting speed	~ 6-9sec per seed, tested on Arabidopsis *NOTE seeds purity/quantity/size influence the speed
Seeding/Sorting setting	Single and multiple parameterized Non-parameterized
Phenotypical analysis projections	0°/45°/90° orientation
Calculated phenotypical parameters	Seed area (px/mm2) Seed length (mm) Seed width (mm) Seed shape - L/S ratio (length/area ratio) Seed shape – SSE (sum of square errors parameter) Seed Fluorescence intensity (%)
Double seeds removal	Implemented
Gel height detection	Automatic mode (for all plate types up to 24 well format) Manual dropout-height setting mode
Nozzle cleaning station	Single-step, mechanical cleaning of contaminants
Nozzle exchange station	Reservoir for 3 nozzles, waste container Function of automated nozzle replacement

Sample holder (1,5ml tubes)	11 positions
Sample holder (5ml holders)	11 positions
Standard plate holder	20PL01

System accessories	
External memory	64 GB
Tablet	Core 2.0GHz, RAM 3GB Touch screen 8" GPS, Wi-Fi 802.11ac, Bluetooth 4.2, USB-C, 4850mAh battery, Google Android 7.0 Nougat *NOTE technical specifications for the tablet (operating device) may change due to availability of optimal devices on the market
Power supply	included

System accessories available on a request	
Fluorescence measurement	
Fluorescence Module	not included in the standard system version Ex:455-465nm LED; Ex: 520- 530nm LED One filter position
Filter for RFP detection / mounted ring Ø25 mm	565 - 800nm (550nm 50%TR)
Filter for GFP detection / mounted ring Ø25 mm	505 - 800nm (490nm 50%TR)
Other accessories	
Custom plate holder	Customized plate holder including SW customization for the provided plate samples.
Custom sample holder	Customized sample holder including SW customization

Consumables	
Cleaning kit	Brush, antistatic cloth, vacuum cleaner
Thermo label ROLL (57mm)	Thermal printer sticky labels, 5pcs/PCG Width 57mm, diameter 31 mm
Antistatic tubes (1,5ml)	Funakoshi - antistatic tubes, 500pcs/PCG
Stainless steel nozzles, 1/2" incl. filter	Gauge 14, 15, 16, 18, 20, 21, 22, 23, 25, 26, 27, 30, 32 *NOTE – the object manipulation depends on used caliber of nozzle, object shape, surface and weight

Working set	**Quantity**
Nozzles with filter (for Arabidopsis G30, G32)	20+20
Nozzles for large seeds	10
Antistatic tubes (1,5ml)	11
Large seeds tubes	11
Filters for cleaning station	5
Large seeds carrousel	1
Large seeds tube stand	1
Nozzle stand	1
Nozzle transporting pin	1
Thermo-label roll (57mm)	1
Cleaning kit	1
Mounting kit (screwdriver, allen screw, replacement screws)	1

NOTE: Consumables can be ordered separately from Labdeers s.r.o.

The consumables provided as starting working set are not the subject of the purchase.

11.3. Materials used

The components of Boxeed device, accessible to the user are made of the following material. Please check the chemical resistance before using organic solvents or aggressive chemicals which are in contact with Boxeed. For cleaning please follow the cleaning instructions in part of maintenance.

NOTICE! – Aggressive chemicals may damage the Boxeed or supplied accessories

Assembly	Material
Boxeed chassis	Polyethylene (PET-G) Dibond Aluminum Stainless steel
Boxeed parts	Polyethylene (PET-G)
Nozzle connector	Stainless steel
Filters	Polypropylene (PP)
Nozzles	polypropylene (PP) hub stainless steel canula polypropylene (PP) canula

11.4. Accessories – overview

Type	Description
Cleaning kit	Brush, antistatic cloth, vacuum cleaner
Thermo label ROLL (57mm)	Thermal printer sticky labels (5pcs/PCG
Antistatic tubes(1,5ml)	Special antistatic tubes 500pcs/PCG
Stainless steel nozzles, 1/2" (tab.2)	Gauge 14, 15, 16, 18, 20, 21, 22, 23, 25, 26, 27, 30, 32

1/2" STRAIGHT STAINLESS STEEL NOZZLES

Gauge	ID mm	Hub Color
12	2.27	Navy Blue
13	1.78	Magenta
14	1.60	Olive
15	1.37	Amber
16	1.20	Black
17	1.4	White
18	0.84	Green
19	0.70	Brown
20	0.60	Pink
21	0.51	Purple
22	0.41	Blue
23	0.33	Orange
24	0.31	Sky Blue
25	0.25	Red
26	0.23	Beige
27	0.20	Clear
30	0.15	Lavender
32	0.8	Yellow

12. Statement of warranty

This Limited warranty applies for 6 months for the battery of tablet (do not include capacity loss), valid from the date of the product purchase.

Contact directly Labdeers Ltd. in case of warranty claims. The warranty is return-to-base only.

No warranty is given in case of:

- normal wears and tears of the instrument
- misuse, abuse, negligence, or accident howsoever caused
- connected, installed, adjusted, or used product contrary than in accordance with the user manual
- instrument was modified, altered, repaired or opened by unauthorized persons
- defects or damage due to spillage of liquids or particles, corrosion, rust, or the use of wrong voltage

Excluded from the warranty are:

- reduced battery capacity
- instruments with defected or removed serial numbers
- instruments without any prove of purchase

If at any time within the warranty period the instrument does not function as warranted, the product will be repaired or replaced at no charge. The customer is responsible for shipping and for covering of insurance charges (for the full product value) to Labdeers company. Labdeers is responsible for shipping and insurance on return of the instrument to the customer. Returned instruments must be packaged sufficiently so as not to assume any transit damage. If damage is caused due to insufficient packaging, the instrument will be treated as an out-of-warranty repair and charged as such.

The Labdeers Ltd. offers out-of-warranty repairs. These are usually returned to the customer on a cash-on delivery basis. Labdeers repairs or replaces faulty instruments as quickly as possible. All replaced parts or equipment shall become to property of Labdeers.

13. Transport

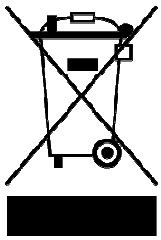
NOTICE! - Always use an anchor to transport and move the instrument. (Chapter 11.1. Technical processes)

CAUTION - Clean and decontaminate the instrument prior shipment. Perform cleaning of device before shipping as described in chapter 4. Maintenance. Hazardous substances may result in personal injuries or product damage.

14. Storage and disposal

Store the device in secure storage location, where the device cannot be exposed to aggressive gases for an extended period. Do not store device with attached nozzles. It is recommended to charge the batteries fully in the period of two months, to sustain the optimal battery capacity.

Storage conditions



Ambient temperature: -5°C / + 45°C

Relative humidity: 10% - 5%

Atmospheric pressure: 700hPa – 1060hPa

Disposal

Follow the relevant locally applicable legal regulations in case the product is to be disposed.
Product disposal in EU is regulated by EU Directive 2002/96/EC pertaining to waste electrical and electronic equipment. According to these regulations electrical and electronic equipment supplied in business-to business sphere, may no longer be disposed of in municipal or domestic waste.