InVitroSys

MyoDish 1

Laboratory Tissue Culture System

Operation Manual

This guide serves as an operating manual for the Laboratory Tissue Culture System “MyoDish 1” (name plate: MD-01-01), in the following also referred to as “device”.

An addendum with additional information for the MyoDish MD-1.2 device is attached to this operating manual.

Igelgarten 10
82166 Gräfelfing
Germany

info@invitrosys.com

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0 Addendum

0.1 Changes introduced with the new version of the MyoDish system (MD-1.2)

The MyoDish system MD-1.2 (v1.1) has been improved in several ways over the first version:

- free positioning of culture chambers
- full direct current suppression

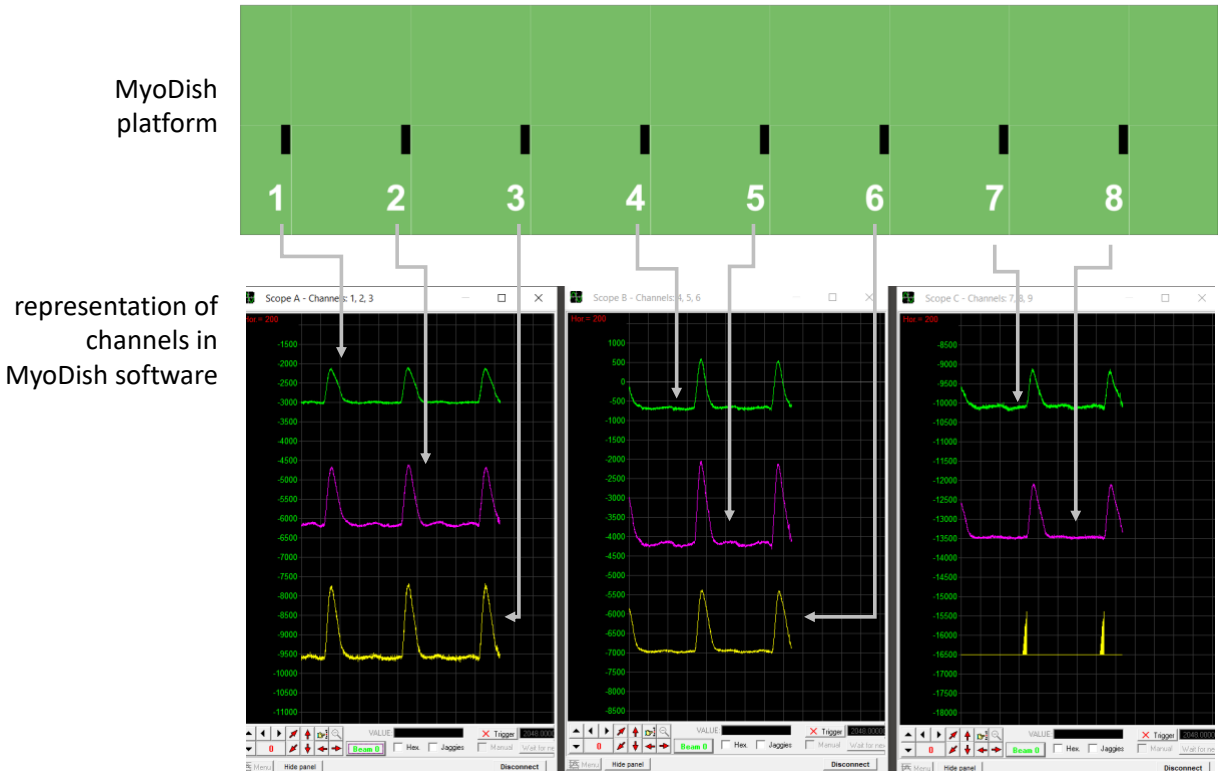
The model MD-1.2 comes with two major enhancements which are described here.

0.2 MD-1.2 handles chambers and sensor boards in any combination and position

The Manual describes for the previous version (MD-1) that each magnetic sensor of the MyoDish chambers is identified by one of 4 different communication addresses (marked as "A", "B", "C", or "D") which may not be placed in duplicate within the first 4 channels and the second 4 channels of the MyoDish platform. This restriction no longer applies to model MD-1.2. Although the sensor boards delivered with your system are still labelled with the respective address letter, this just serves downward compatibility purposes, and has no relevance for the operation of your system.

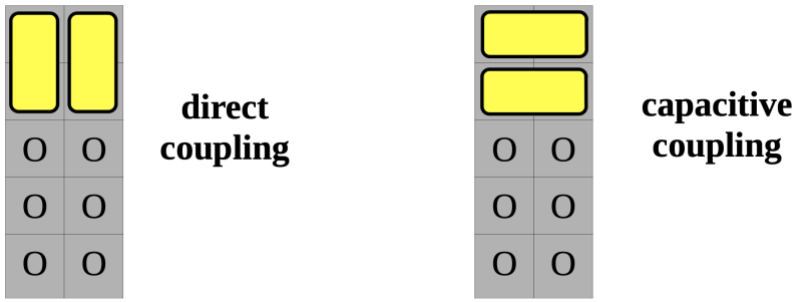
The chamber positions of the MD-1.2 platform are labelled with numbers 1-8, which represent the "channel" number of any chamber placed at a specific position. The "channel" identifies the target of force recording and stimulation as well as its display position in the MyoDish software.

Thus, operating the MD-1.2 system, you may ignore the A, B, C, D labels on the sensor boards. However, you may still use them as an arrangement system. To maintain flexibility and avoid confusion in positioning, we have equipped the sensor boards with interchangeable number labels. We suggest attaching a yellow plastic ring to each sensor board according to the channel of the respective chamber. In this way, the correct position of each chamber is easily determined and can be changed if the chambers are rearranged.

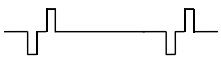
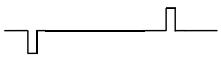
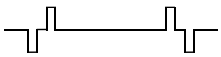
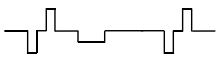
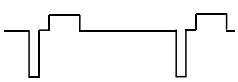
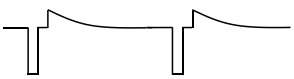


0.3 MD-1.2 can fully suppress direct electrode current, even with asymmetric pulses

The previous MyoDish model's integrated pulse generator emitted current-controlled, biphasic pulses with equal positive and negative charges when using identical charge and discharge durations. The MD-1.2 system can still operate in this mode, but the new option to add a capacitor in series with the stimulation electrodes improves the suppression of direct electrode current. This feature can be activated for each chamber by rotating the two yellow plastic handles, which also act as placement limiters, in the sensor board connector sockets.



Applying stimulation impulses via direct coupling (mode 1, shown on the left) is favored due to many years of positive experience. However, theoretical models suggest that to minimize electrochemical reactions at the electrode surface, direct current should be eliminated, achievable with capacitive coupling. This setup maintains charge balance regardless of differing impulse charges (durations x currents) for the pulse's positive and negative phases. Capacitive coupling adjusts the dominant pulse's charge to match the reverse pulse's charge. This is particularly useful for creating asymmetric pulses, where a strong initial pulse for stimulation is offset by a longer, possibly sub-threshold intensity discharge current (modes 5 and 6). A table comparing direct and capacitive electrode coupling applications follows.

mode	specification	direct	capacitive
1	bipolar impulses with identical currents and durations of charge (negative) and decharge (positive) pulses (recommended default mode) 	√	√
2	monopolar impulses with constant currents and durations applied at alternating polarities 	√	√
3	bipolar impulses with alternating order of polarities 	√	∅
4	non-excitatory monopolar impulses for contractility modulation 	√	∅
5	bipolar asymmetric impulses with poor balance of charging and decharging pulses (duration × current) 	∅	√
6	maximum efficacy bipolar impulses with excessive decharge current 	∅	√

1 Application

1.1 How to Apply this Manual

- Please read and understand the complete manual before using the device for the first time. If relevant, please also refer to the accessories instructions for use.
- This manual is part of the product and should therefore be kept near by the product itself.
- Please also include this operating manual if distributing the product to third parties.
- You will find the up-to-date version of this operating manual in all languages available under https://www.invitrosys.com/downloads.

1.2 Warning Signs and Accident Levels

The safety symbols contained in this manual refer to the following warning signs and accident levels:

1.2.1 Warning signs

	Potential danger		Biohazard
	Risk of electric shock		Risk of explosion
	Property damage		

1.2.2 Accident levels

DANGER	Will lead to severe injury or death
WARNING	May lead to severe injury
CAUTION	May lead to slight to moderately severe injury
ATTENTION	May lead to property damage

1.3 Flags

	Please note		Tip

1.4 Abbreviations

rpm	revolutions per minute
O_2	molecular oxygen
CO_2	carbon dioxide
BDM	2,3-butanedione monoxime
PFA	paraformaldehyde
PBS	phosphate-buffered saline
bpm	Beats per minute

2 General Safety Instructions

2.1 Intended Use

The MyoDish 1 Laboratory Tissue Culture System serves for in-vitro cultivation of muscle tissue, in particular for mammal heart muscle tissue (including human tissue).
The MyoDish 1 Laboratory Tissue Culture System shall exclusively be intended for indoor use or for use in laboratory cell-culture incubators. The specific country and institutional safety requirements for operation of electric devices in laboratory environments and for handling of biomaterial must be adhered to.
The MyoDish 1 Laboratory Tissue Culture System is **not** intended for use in private households.

2.2 User Requirements

The device as well as all device accessories shall be operated only by trained personnel with the knowledge required for handling electric devices in laboratory environments.
Users must observe the specific country, institutional and laboratory safety instructions for handling of biomaterial and must wear adequate personal protection equipment (gloves, lab coat, safety goggles, and face mask, if applicable).
Please carefully read this operating manual and the instructions for use of accessories before use and make yourself familiar with the operation of the device.

2.3 Limits of Use

DANGER **Risk of explosion**

Do not operate the device in environments in which explosives are used.
Do not use this device with explosives or highly-reactive substances.
Do not use this device with substances or material that may produce an explosive atmosphere.

Due to its structural design, the MyoDish 1 Laboratory Tissue Culture System is not suitable for use in a potentially explosive atmosphere.
The device shall only be used in a safe environment, for example in the open-plan environment of a vented laboratory or inside an incubator with a mixture of air/CO_2 or air/O_2. The content of oxygen in the ambient air must not be higher than 50%. Do not use substances that lead to or contribute to a potentially explosive atmosphere. The user shall be responsible for any risk arising from the use of such substances.

DANGER **Risk of electric shock**

The device shall only be connected to power supplies delivering 15V DC.

The MyoDish 1 Laboratory Tissue Culture System must only be operated with 15V DC. Power supplies exceeding 15VDC may lead to device damage and to dangerous electric shock, i.ºe. personal injury.

2.4 Warranty

The culture chambers, sensor circuit boards and electrodes are accessories and wear parts with limited warranty. Warranty for these parts shall be limited to their condition before first use.
Defects arising from wear and tear (performance, aging) of such parts shall not be deemed to be a defect of the product.
The warranty for the entire product shall also be forfeited if:

- the device is not used as described in this operating manual.
- the device is not used according to its intended use.
- the device is used in combination with consumables not recommended by InVitroSys GmbH.
- changes are made to the device that have not been authorized.

2.5 Product Liability

Liability for any personal injury or property damage shall be transferred to the operator (user) if:

- the device is not used as described in this operating manual.
- the device is not used according to its intended use.
- the device is used in combination with consumables not recommended by InVitroSys GmbH.
- changes are made to the device that have not been authorized.

2.6 Hazards arising from Intended Use

2.6.1 Personal Injury or Device Damage

WARNING Electric shock of the device or the power unit or cord

- Only switch on the device if the device, the power unit and the power cord show no damage.
- Only operate devices that have been installed or maintained in a technically correct manner.
- In case of danger, unplug the device. Pull the power plug either from the device or from the power outlet. Use adequate disconnection equipment (for example, emergency stop switch in the laboratory).

Hazards arising from inadequate voltage

- Exclusively connect the device to voltages fulfilling the electrical requirements as per the name plate of the power unit.
- Exclusively use CE-certified power units delivering 15V DC and at least 250mA.

WARNING **Health damage due to infectious liquids, infectious biomaterial and pathogenic germs**

- When handling infectious liquids, pathogenic germs or other potentially infectious biomaterial observe the national regulations, the biosafety level of the laboratory, institution-specific regulations as well as the safety data sheets and instructions for use of the manufacturers.
- Please note that the lids of the culture chambers are not absolutely tight. Therefore, pathogenic germs or infectious aerosols may escape or culture medium may be spilled or leak during operation.
- Exclusively operate the device with culture chambers that have been closed with a matching lid.
- Before inserting or removing culture chambers switch off the stepper motor in order to reduce the risk of spilling the medium.
- When working with pathogenic high-risk germs, take protective measures and use personal protective equipment to protect yourself from aerosols.
- Wear personal protective equipment.
- For comprehensive regulations on how to handle germs or biomaterial belonging to risk group II or higher, refer to the *Laboratory Biosafety Manual* (World Health Organization, Laboratory Biosafety Manual, in the relevant valid version).

CAUTION **Safety hazards due to damaged or wrong accessories or replacement parts**

The use of damaged accessories or replacement parts that have not been recommended by InVitroSys may impair the safety, function and/or precision of the device. Any warranty or liability by InVitroSys for damage arising from accessory or replacement parts that have not been recommended or from improper use shall be ruled out.

- Exclusively use accessory parts recommended by InVitroSys and original manufacturer replacement parts by InVitroSys.
- Protect all accessories from mechanical or chemical damage.
- Always inspect all accessories for damage before use. Exchange all damaged accessory parts.
- Replace damaged accessories or parts.
- Do not use any accessories or parts if their maximum service life has been exceeded.

ATTENTION **Device damage due to spilled liquids**

Make sure that the stepper motor is switched off when inserting or removing the culture chambers. The motion of the main circuit board will otherwise increase the risk of the culture medium or other liquids being spilled.
How to proceed in case of spilled liquids:

1. Switch off the stepper motor.
2. Unplug the device (remove the USB plug and the power plug).
3. Carefully clean the device and the accessories according to the instructions for cleaning and sanitizing the device included in this operating manual.
4. If intending to use a cleaning and sanitizing method other than the one described in this manual, contact InVitroSys to ensure that such method will not damage the device and/or accessories.

Device damage due to condensation water

Condensation water inside the device or on the outside of the device may occur after transfer of the device from a cool to a warm environment (e. g. from a cold lab room into an incubator). During operation, the controller and stepper motor will warm up, preventing condensation.

- Put the device into the incubator only after it has reached at least room temperature.
- Devices within the incubator currently not used for an experiment shall be remained connected to the power supply and the stepper motor kept on with low cadence (20-30 rpm). The USB connector at the PC, however, may be unplugged.
- Operate the device at a maximum of 85% of relative humidity.

2.6.2 Wrong Handling of the Culture System

Damage or functional impairment due to mechanical shock or motion of the device in operation

The sensors of the device and the culture chambers are sensitive to motion. Only grab the baseplate in order to lift or move the device. Irreversible damage may result from mechanical stress imposed on all other components.

- Do not shift the device during operation.
- Exclusively grab the device by the baseplate in order to shift the position of the device.

Device damage or functional impairment due to external magnetic fields

The sensors of the devices are sensitive to external magnetic fields.

- Do not bring any permanent magnets or electromagnets (e. g. electric coils) into the near environment of the device.

Device damage due to corrosive substances or strong solvents

- Any contact of the device with corrosive substances (e. g. strong alkaline solutions or acids) as well as with strong solvents (e. g. acetone) should be avoided.
- Do not use any corrosive substances or strong solvents to clean the device or accessory parts of the device.

Device damage due to overheating

- Do not expose the device to temperatures exceeding 40°C.
- The device must not be autoclaved.

2.6.3 Wrong Handling of Accessories

Damage to the culture chambers due to dislocation of the magnet

Dislocation of the magnet fixed at the spring wire may lead to malfunction or failure of force sensing.

- Do not touch or apply any sort of mechanical stress (e. g. by tweezers or pipette tips) to the magnet.

Damage to the culture chambers due to overexpansion of the spring and adjusting wires

The wires integrated into the culture chambers must not be overexpanded when inserting and removing biomaterial or culture medium.

- Always use the adjusting bolt to adjust the position of the adjusting wire as to avoid overexpansion of wires in order to insert or remove biomaterial.
- Avoid contacting wires with pipette tips when inserting or removing the culture medium.

Damage to culture chambers due to excessive mechanical stress of the adjusting wire assembly

The adjusting wire of the culture chambers protrudes from the outside into the inside of the chamber, transversing a seal. In case of excessive mechanical stress, the seal and the nut to which the adjusting wire is fixed may be damaged.

- Always turn the adjusting bolt slowly.
- Use a hex ball driver of the right size (2.0mm).

Damage to the culture chambers due to high temperature

Heating the culture chambers or sensor boards to more than 50°C will lead to permanent damage.

- Always store the culture chambers and sensor boards at room temperature.
- Do **not** autoclave the culture chambers or sensor boards.

Damage to the culture chambers or sensor boards due to corrosive substances or strong solvents

- Avoid contacting the culture chambers, sensor boards or electrodes with corrosive substances (e. g. strong alkaline solutions or acids) and strong solvents (e. g. acetone).
- Do not use any corrosive substances or strong solvents to clean the culture chambers, sensor boards or electrodes.

Damage to sensor circuit boards due to spilled liquids

During operation, the sensor circuit boards, in particular the insertion points for the electrodes, must not come into contact with liquids.

- When adding or removing culture medium as well as when handling culture chambers, make sure that no liquids are spilled onto the sensor circuit boards.
- Ensure that sensor boards are completely dry before any use.

How to proceed in case of spilled liquids:

1. Switch off the stepper motor.
2. Immediately remove the relevant sensor circuit board together with the culture chamber.
3. Check if liquids have been spilled also onto other parts of the device. If so, unplug the power and USB cords and remove liquids immediately.
4. Immediately and thoroughly clean the sensor circuit board according to the instructions for cleaning and sanitizing the device included in this operating manual.

If intending to use a cleaning and sanitizing method other than the one described in this manual, contact InVitroSys to ensure that such method will not damage the device and/or its accessories.

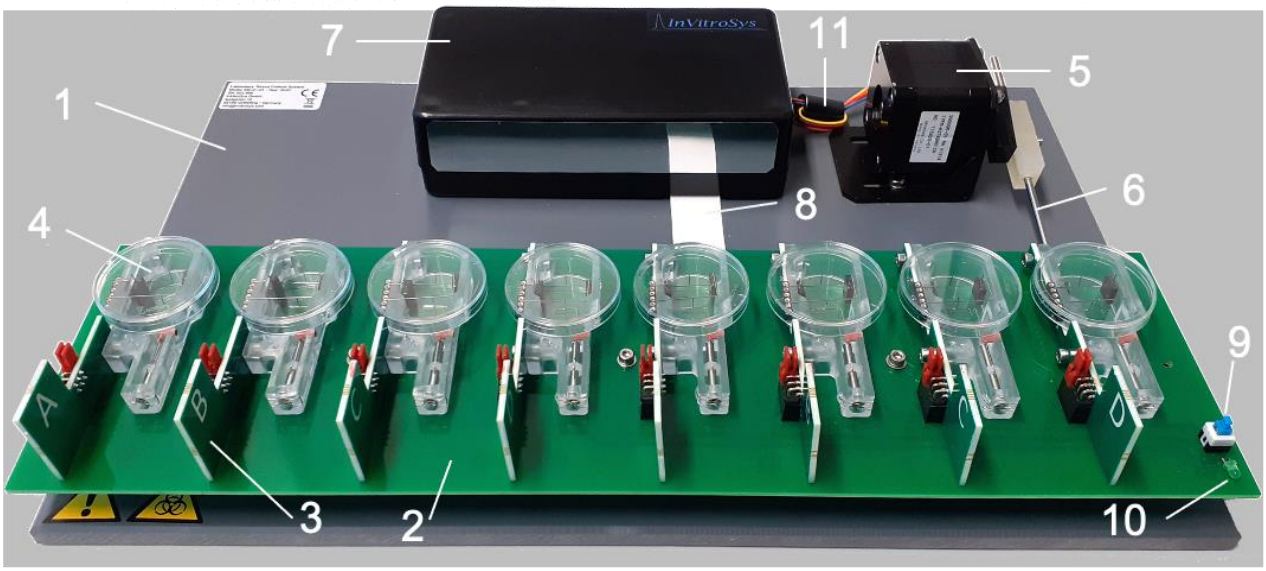
2.7 Safety Symbols on the Device and Accessories

Symbol	Meaning
	**Attention** Observe the safety instructions included in the operating manual
	**Warning** Warning of biological risks when handling infectious liquids, biomaterial or pathogenic germs
	Please refer to the operating manual

3 Description of the Product

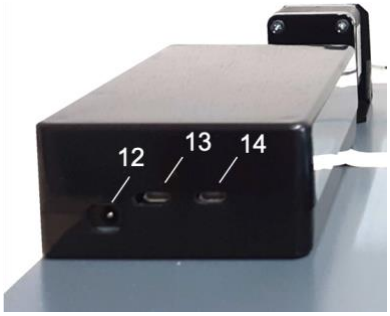
3.1 Product Overview

3.1.1 Device with all Components and the Chamber Set



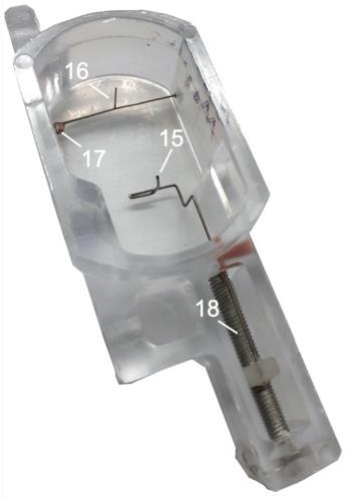
1	**Baseplate**	is the mounting basis of the other components
2	**Main circuit board**	contains the slots for the culture chambers with sensor circuit boards, is mounted on a movable bearing and is moved by the stepper motor
3	**Sensor circuit boards (8x)**	are bolted to the culture chambers, communicate the sensor information to the controller via the main circuit board, contain insertion connectors for the electrodes
4	**Culture chambers (8x)**	serve for insertion of the tissue and for containing the culture medium
5	**Stepper motor**	moves the main circuit board
6	**Piston rod with friction bearing**	transfers the movement of the stepper motor onto the main circuit board
7	**Controller unit**	for voltage supply and control of the system, serves as an interface to the computer, contains two USB connectors and the power unit connector (power unit requirements: 15V DC, ≥250mA)
8	**Ribbon cable**	electrical connection between the controller and the main circuit board
9	**Push-button switch**	for starting and stopping the stepper motor
10	**Light-emitting diode (LED)**	signals the electrical stimulation
11	**Connection cable**	electrical connection between the controller unit and the stepper motor

3.1.2 Controller Unit

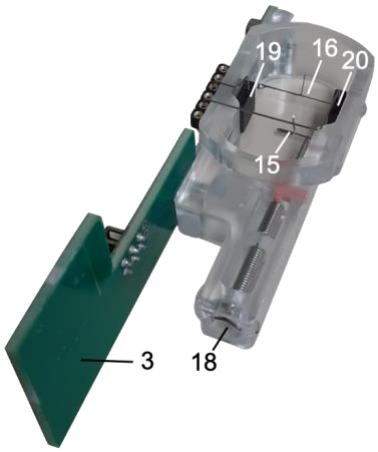


12	**Power unit connection socket**	required power unit: 15VDC, ≥250mA
13	**USB connection socket 1**	reserved for future versions
14	**USB connection socket 2**	connection to Windows computer

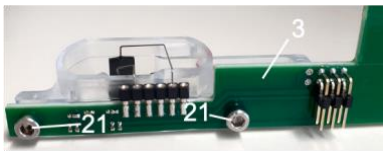
3.1.3 Culture Chamber



15	**Adjusting wire**	fastening of the tissue slice
16	**Spring wire**	fastening of the tissue slice, force measurement
17	**Magnet**	force measurement
18	**Adjusting bolt**	adjusting pretension

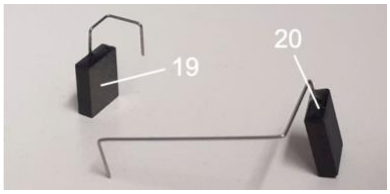


3	**Sensor circuit board**	
15	**Adjusting wire**	fastening of the tissue slice
16	**Spring wire**	fastening of the tissue slice, force measurement
18	**Adjusting bolt (head)**	adjusting pretension (preload)
19	**Electrode with short wire**	electrical stimulation
20	**Electrode with long wire**	electrical stimulation



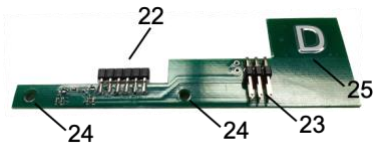
3	**Sensor circuit board**	
21	**Mounting bolt**	fastening of the sensor circuit board to the culture chamber

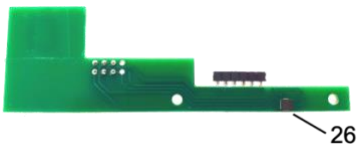
3.1.4 Electrodes



19	**Electrode with short wire**	electrical stimulation
20	**Electrode with long wire**	electrical stimulation

3.1.5 Sensor Circuit Board





22	**Connectors**	insertion of the electrodes
23	**Connection plug**	connection of the sensor circuit board with the main circuit board
24	**Drill holes**	fastening of the sensor circuit board with the culture chamber (by means of **21**)
25	**Communication address**	

3.2 Scope of Delivery

3.2.1 MyoDish 1 Laboratory Tissue Culture System

Number	Component or device
1	MyoDish 1 Laboratory Tissue Culture System
1	USB cable
1	Power unit

- Check the delivery for completeness.
- Inspect all parts for transport damage.
- Keep the delivery box and the packaging material in order to safely transport and store the device.

3.2.2 Accessories: MyoDish Chamber Set

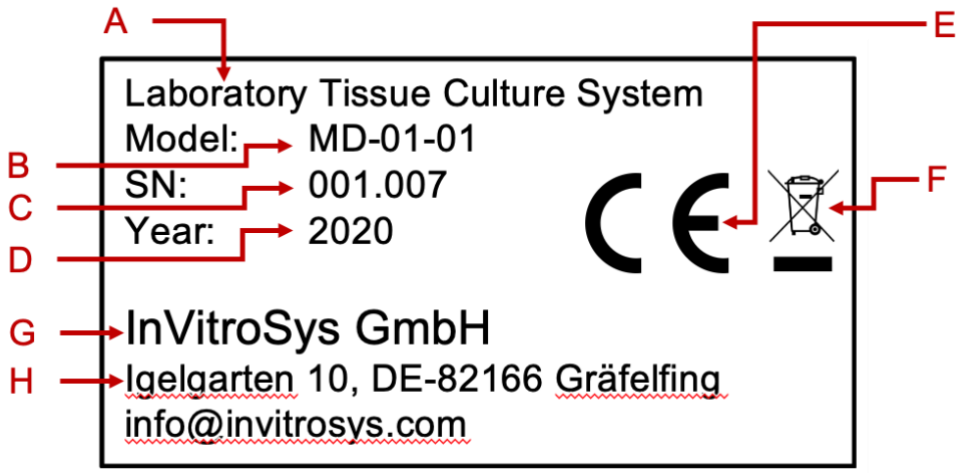
Number	Component or device
8	Culture chamber
8	Electrode pairs (each with long and short wire)
8	Sensor circuit boards
16	clamp bolts

- Check the delivery for completeness.
- Inspect all parts for transport damage.
- Keep the delivery box and the packaging material in order to safely transport and store the device.

3.2.3 Accessories: MyoDish Starter Set

Number	Component or device
1	Forceps set
1	2mm hex ball driver
1	2.5mm hex ball driver
2	Foils with laser-cut triangles

3.3 Name plate



A	Product category
B	Product name
C	Serial number
D	Year of manufacture
E	CE marking
F	WEEE marking (waste electrical and electronic equipment)
G	manufacturer
H	Contact address of manufacturer

3.4 Scope of Operation and Product Characteristics

The MyoDish 1 Laboratory Tissue Culture System is suitable for permanent operation within the usual limits of a cell culture incubator at temperatures ranging from 20 to 37°C and at a maximum relative humidity of 85% and an atmosphere with 5% of CO_2.
Tissue slices from the heart muscle can be kept in culture and electrically stimulated with the system, with maximum voltage pulses of +/- 12V.

The tissue contraction force is measured using patented technology (EP3176252A1) by means of the culture chambers and the sensor circuit boards and is continually communicated to the controller unit. The Laboratory Tissue Culture System can be controlled and experimental data be recorded by means of the MyoDish software through a personal computer connected by USB. For details on installation and operation of the MyoDish software, please refer to the relevant software manual.

3.4.1 Operation of the Controller

- The controller serves as an interface between the USB cable-connected computer and the culture system.
- The controller autonomously controls the speed of the stepper motor (leading to a "rocking main circuit board" or "rocker") as well as the stimulation pulses and frequency for the electrodes in the culture chambers. Each culture chamber is identified and controlled with the help of the address of its sensor circuit board. For this reason, the order and names of the sensor circuit boards must be observed when installing the culture chambers on the device (see chapter 5.3.5). The stimulation pulses are transferred in series.
- The controller is provided with information from the sensors of the sensor circuit boards about the contraction of the tissue, i. e. about the position of the spring wire. The sampling rate is 400Hz. The controller communicated the data to the MyoDish software via the USB cable.
- The controller takes commands from the MyoDish software via the USB cable. The commands accepted by the controller refer to settings of the rocker board, the stimulation pulses and the chronological order of the stimulation pulses (stimulation frequency). Furthermore, commands for noise reduction and for an extended measurement range can be communicated.
- The controller settings will be kept even if the USB connection is lost but not after restart of the controller or after device restart after disconnection from power.

3.4.2 Generation of Electrical Stimulation Pulses

Stimulation pulses are generated by the controller by briefly connecting an adjustable current source via a multiplexer with the electrodes of the culture chambers. Bipolar pulses are used, which are defined by the duration of positive and negative phases and the intermittent pause. Impulse strength can be controlled by the generated current. Impulse parameters can be adjusted individually for each culture chamber and then apply to all stimulation pulses of that chamber.
Time points of single stimulations are defined within a stimulation sequence, which will be repeated after a pre-defined stimulation period. Within the stimulation period, stimulation time points of each chamber can be defined by the user. Simultaneous stimulation of more than one chamber is not possible. Between the end of one stimulation pulse and beginning of another stimulation pulse, at minimum pause of 3 ms is mandatory.
Definition of stimulation pulse parameters and of the stimulation sequence is possible through commands submitted by the MyoDish software to the controller. Here you will find the most useful commands that can be communicated to the controller using the MyoDish software. For more details and more commands, please refer to the software manual.

Command	Meaning
`chargeDuration`	duration of the first (negative) stimulation pulse in microseconds (µs)
`dechargeDuration`	duration of the second (positive) stimulation pulse in microseconds (µs)
`pauseDuration`	duration of the stimulation-free interval between the first and the second stimulation pulse in microseconds (µs)
`stimCurrent`	amperage of the stimulation pulse current in milliampere (mA)

Command	Meaning
`stimPeriod`	duration of the repetitive sequence of stimulation pulses in milliseconds (ms)
`stimTime`	times at which the channels (culture chambers) are electrically stimulated within the stimPeriod, in millisecond (ms)
`stimFrequency`	Stimulation frequency (bpm)

The commands chargeDuration, dechargeDuration, pauseDuration, stimCurrent and stimFrequency can be set differently for each channel (culture chamber) within certain restrains. The command stimPeriod will always address all channels. After execution of stimPeriod, the stimulation sequence as per the settings made for stimTime will begin again (see examples). The settings will remain active as long as they are not replaced by submission of a new command.

Example A	`stimPeriod = 1000ms`	`stimTime channel 1: 0ms`
		`stimTime channel 2: 10ms`
		`stimTime channel 3: 20ms`
		`stimTime channel 4: 30ms`
		`stimTime channel 5: 40ms`
		`stimTime channel 6: 50ms`
		`stimTime channel 7: 60ms`
		`stimTime channel 8: 70ms`

Example B	`stimPeriod = 500ms`	`See above`

Example C	`stimPeriod = 1000ms`	`stimTime channel 1: 0ms and 500ms`
		`stimTime channel 2: 10ms and 510ms`
		`stimTime channel 3: 20ms and 520ms`
		`stimTime channel 4: 30ms and 530ms`
		`stimTime channel 5: 40ms`
		`stimTime channel 6: 50ms`
		`stimTime channel 7: 60ms`
		`stimTime channel 8: 70ms`

In example A, all channels are stimulated with a frequency of 1Hz as they are stimulated exactly one time within 1000ms.
In example B, all channels are stimulated with a frequency of 2Hz as they are stimulated exactly one time within 500ms.
In example C, channels 1 to 4 are stimulated with a frequency of 2Hz as they are stimulated exactly two times, each time after 500ms, within 1000ms. Channels 5 to 8 are stimulated with a frequency of 1Hz.
Any point in time can be chosen for stimulation (up to 100 per stimPeriod). However, the settings selected for the stimulation pulse per channel (stimCurrent, chargeDuration, dechargeDuration, pauseDuration) will always apply to all stimulation times of the respective channel.

3.4.3 Operation of the stepper motor

The stepper motor (5) generates the rocking motion of the main circuit board (2) by means of a piston rod (6). The rocking motion leads to circulation of the culture medium, improving oxygenation of the medium and transport of oxygen to the cultivated tissue. Moreover, this motion generates a convection

leading to a facilitated discharge of metabolites from the tissue into the medium. This rocking motion is absolutely necessary for successful cultivation of heart tissue.
The initial speed of the motor when starting the controller is approximately 30 rpm. The rocking speed can be changed through a command submitted by the MyoDish software:

Command	Meaning
`rockerSpeed`	Rocking speed of the stepping motor (rpm)

After setting the speed, it is kept until changed by a command to the controller or until power supply is interrupted. The maximum speed of the motor is 100rpm. The motor can be stopped and restarted by means of the push-button switch (9) on the main circuit board.

3.4.4 Operating Mode of the Culture Chambers

The culture chambers consist of polystyrene and contain the adjusting wire (15), the spring wire (16) with magnet (17) as well as an adjusting bolt (18). The culture chambers may accommodate tissue slices with dimensions from 10mmx 10mm x 0.3mm (WxHxD) as well as the glued-on plastic triangles. The volume and the shape of the chambers serve to accomodate up to 2.5ml of culture medium, ensuring that no medium is spilled due to the rocking motion of the step motor. The chambers are designed as to be closed by means of a common Petri dish lid with a diameter of 35mm (e. g. Falcon #353001). The lid will not close the chamber tightly so as not to hinder gas exchange.
The adjusting wire is movable and can be pushed forth and back by means of the adjusting bolt (18). The head of the adjusting bolt is a 2.0mm hexagon socket. One full turnaround of the adjusting bolt will shift the adjusting wire by 0.5mm. By means of this function, the distance between the adjusting wire and the spring wire can be adjusted to the size of the tissue slice and pretension can be applied.
The spring constant of the spring wires is 50-75mN/mm (spring stiffness #3). Chambers with harder or softer wires are available upon request (see also chapter 6.3).

3.4.5 Role of the Plastic Triangles

The plastic triangles consist of polyethylene terephthalate (PET) and serve as the connecting pieces between biomaterial (tissue) and the wires of the culture chambers. They are glued to the tissue by means of cyanoacrylate glue (Histoacryl® is recommended). The plastic triangles have a hole which allows them to be pushed onto the spring wire.

3.4.6 Operating Mode of the Sensor Circuit Boards

The sensor circuit boards (3) are screwed to the culture chambers from the outside, which means that they are not in direct contact with the culture medium or the tissue. They consist of a printed circuit board, a magnetic field sensor, connectors (22) for the electrodes as well as the connection plugs (23), by means of which they can be plugged onto the main circuit board.
Each sensor circuit board has one of 4 addresses for communication with the controller, identified by A, B, C and D (25). The main circuit board is designed as to control and read out 2x4 addresses by means of the controller via two serial busses (one bus for each set of sensor boards A-B). Within one bus, no duplicate addresses are allowed. This results in 2x4 = 8 sensor circuit boards per device (A, B, C, D, A, B, C, D).
The sensor circuit boards register the position of the magnet that is mounted to the spring wire by means of the sensor. The positional information is communicated to the controller, which processes the data and communicates them to the connected computer via USB.

The below figure shows the relationship between the amplitude of the spring wire (x axis) and the sensor value communicated to the computer (y axis):

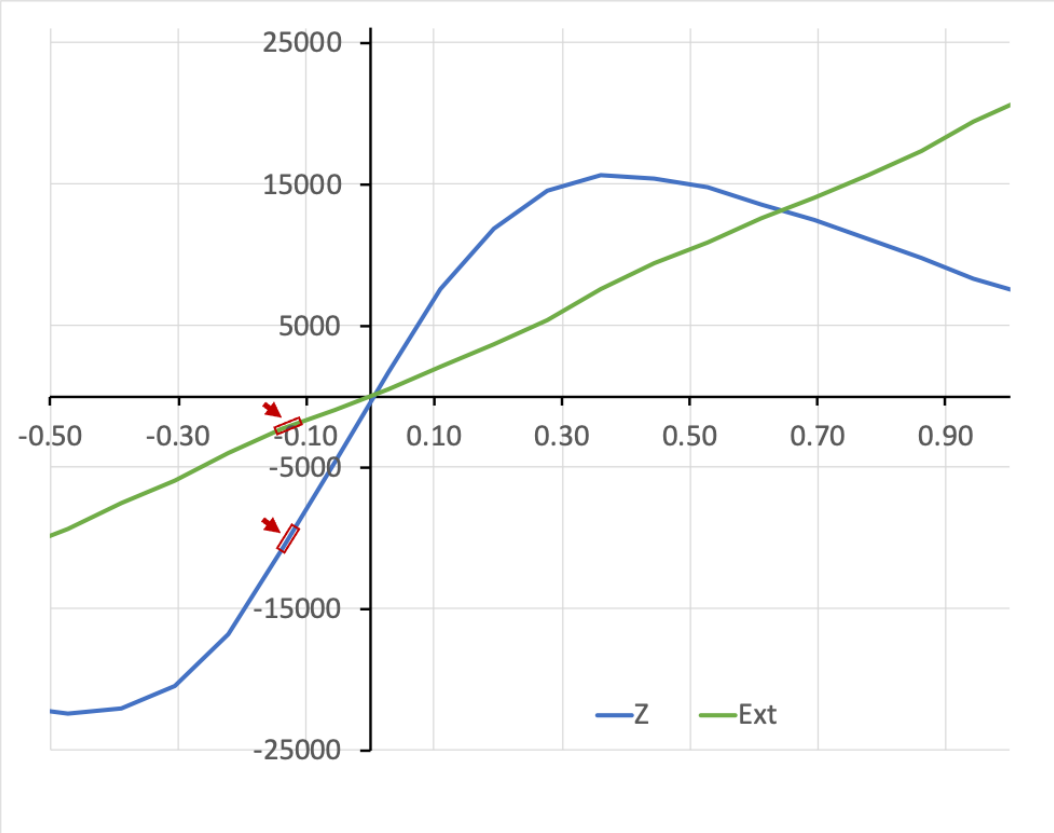


Figure: sensor value (y axis) vs amplitude of the spring wire in mm (x axis)

As can be seen, there are two sensor modes, the regular Z mode (blue line) and the extended mode (green line). They differ in their range of linear value relationship and in their sensitivity. While the regular mode is more sensitive (steeper rise), it has a smaller range of linear value relationship. The linear measurement range is from approx. -0.15mm (sensor value -12000) to +0.15mm (sensor value +12000), i.ºe. approx. 0.3mm in total. This mode is preset. If you wish to measure tissue contractions that are greater than 0.3mm, you can switch to the extended mode by means of the MyoDish software. The linear measurement range is then from -0.5mm to +1.0mm, i.ºe. more than approx. 1.5mm. The sensitivity of the extended mode is, however, lower (less steep rise). The regions highlighted in red show the starting position of the adjusting wires (baseline). It lies around approx. -0.13±0.2mm and translates into sensor values between -8000 and -12000 in the regular mode and/or -3000 to -4000 in the extended mode. The effectively usable linear range is thus a bit smaller than the linear range available in total and in the regular mode is approx. 0.28mm, and approx. 1.13mm in the extended mode).
The culture chambers are calibrated before delivery so you will know a calibration constant of the spring wire of each chamber and thus the contraction force can be calculated. In a chamber with spring stiffness #3 (spring constant of 75mN/mm), a force of 7.5 mN will deflect the wire by approx. 0.1 mm, resulting in an increase of the sensor value by 7500 units. Thus, sensitivity in normal Z mode is approximately 1000 units per mN. The extended sensor mode offers a reduced sensitivity by factor 3, which required an independent calibration. To display contraction force in µN, the calibration values included with the chamber on delivery can be entered in the MyoDish software program.

3.4.7 Operating Mode of the Electrodes

The electrodes consist of a connecting wire made from stainless steel and a graphite board. The graphite boards are immersed in the medium and, following a stimulation pulse, will generate an electrical field propagating in the medium and the tissue between the electrodes (field stimulation). The maximum voltage that can be generated is approx. ±12V, the maximum current is approx. 80-100mA.
To avoid electrolysis, positive and negative stimulation pulses should have the same power and duration. Therefore, *chargeDuration* and *dechargeDuration* shall have identical values. When using M199 as a culture medium, electrolysis can be recognized by small gas bubbles close to the electrodes and acidification (decolorization or yellow discoloration) of the medium.

4 Setup and First Steps

4.1 Choosing the Right Position

WARNING Hazard due to inadequate voltage

- Observe the power unit specifications.
- Exclusively connect the device to power supplies that fulfill the electrical requirements of the power unit.

The device is fit to be operated inside and outside of an incubator.
During operation, always ensure that a safety distance of the device of at least 10cm is kept from all material or items and remove those, if applicable.
Choose a place for setting up the device that fulfills the following criteria:

- connection to power according to name plate of the power unit is possible
- horizontal minimum distance of 10cm to other devices and wall structures is ensured
- upward vertical minimum distance of 20cm is ensured
- stable horizontal table plate (if operated outside incubator) or horizontal, stable plane within incubator is ensured
- make sure you will be able to easily reach the slots for the culture chambers and the push-button switches for the stepper motor (9)

4.2 Preparing the Setup

Device Damage due to Wrong Handling during Transport

Only the baseplate (1) is able to carry the weight of the device. All other components (e. g. main circuit board) are sensitive to mechanical stress and are not strong enough to carry the weight of the entire system.

- When unpacking, packing or transporting the device always only grab it by the baseplate.

Take the following steps in the order described below:

- Open the box, i. e. remove the packaging.
- Remove the accessories and the tissue culture system and check for completeness.
- If applicable, remove transport safety devices.
- Install the device on a suitable lab tabletop or on a clear stage within the incubator.
- Lay out the USB cable and the power unit (with power cord).
- Let the device warm to ambient temperature since condensation water may have been produced due to the changing temperatures during transport.

Device damage due to condensation water

After transport of the device from a cool to a warm environment (e. g. from a cold lab room into the incubator) condensation may have been built up in or on the device. During operation, the controller and stepper motor will warm up, preventing condensation.

- Put the device into the incubator only after it has reached at least room temperature.
- Devices within the incubator currently not used for an experiment shall be remained connected to the power supply and the stepper motor kept on with low cadence (20-30 rpm). The USB connector at the PC, however, may be unplugged.
- The device should be operated at a relative humidity of max. 85%.

4.3 Setting up the Device

Device damage due to wrong plugging and unplugging of the USB cable

- Ensure that the USB plug is always (un)plugged in a straight manner, i. e. horizontally, on the USB connection. Unplugging the plug with an upward motion may damage the USB connection.

Device damage due to electrostatic discharge

When setting up the device, the power circuits of the PC and the MyoDish system will be connected. In rare cases, this may cause electrostatic discharges that can damage the MyoDish controller. To prevent this damage, always unplug the power supply mains connecter of the device before plugging in or unplugging the USB or power cords at the MyoDish system.

- Always disconnect the power mains connection before performing any connector changes at the device.
- Connect the power mains connection at the very end of the installation process, i. e. after installing all the other cords and connectors (power cord, USB cord, ground cable, if applicable).
- If the controller is damaged, it may overheat. Never operate a device of which the top of the controller unit is more than lukewarm.

4.3.1 Setting up the Device: Outside an Incubator

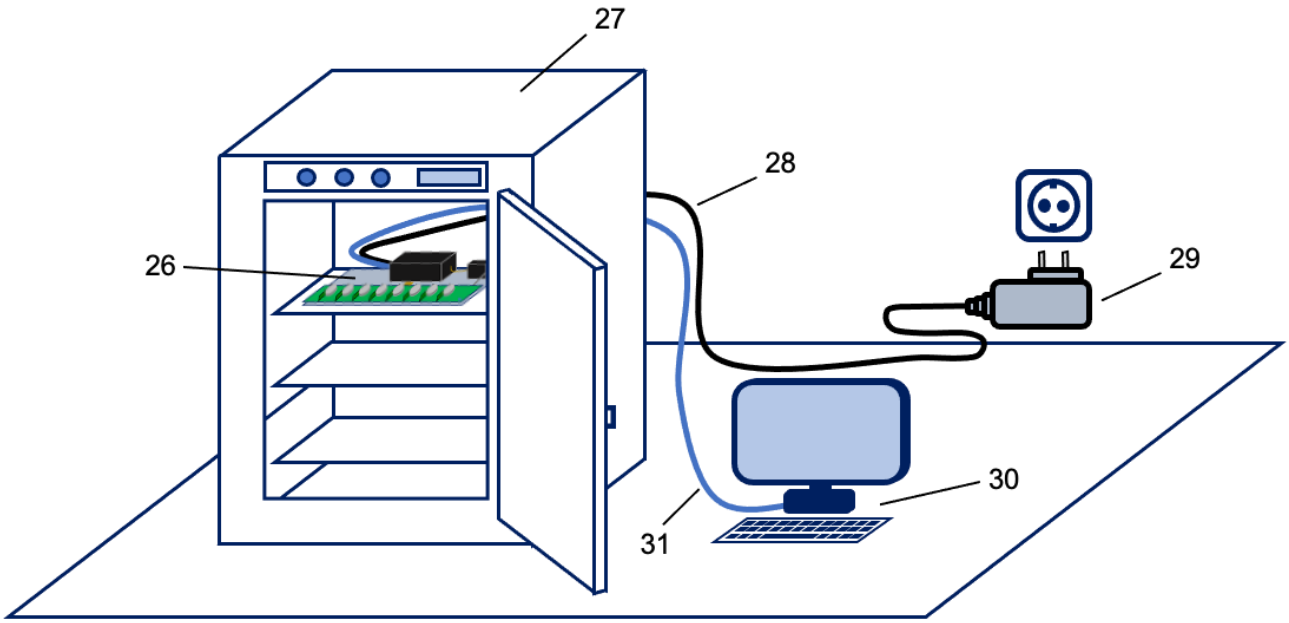
Take the following steps in the order described below to install the device outside an incubator:

- Plug the barrel connector of the power unit into the connection socket (12) on the device.
- Connect the device to a suitable computer using the USB cable: To do so, plug the USB-type A connector into the dedicated computer connector slot and the smaller USB-type micro B connector into the USB connection 2 on the right side of the device’s controller unit (14).
- Observe the line voltage and frequency and make sure that it fits to the requirements of the power supply.
- Plug the power plug of the power unit into the socket-outlet.

4.3.2 Setting up the Device: Inside an Incubator

For cultivating myocardial tissue, CO2 incubators with subdivided inner doors are recommended. It is also recommended to use incubators with an air-tight feedthrough for power cords and other cables at the back. Contact InVitroSys for a list of proven and tested incubator models.
To set up a MyoDish system inside an incubator, follow the steps described above (4.3.1) while paying attention to the following particularities:

- Insert the power cord (side of the barrel connector) and the USB cable (side of the micro B connector) from the outside into the inside of the incubator through the air-tight feed-through of the incubator, which in most incubator models is located on the rear of the incubator.
- Should the incubator have no such feed-through on the rear, both cables can also be fed through at the top of the incubator door.
- Ensure incubator tightness after having fed through the cables.
- Observe the instructions described in the incubator operating manual.
- Up to three MyoDish devices can be installed within one incubator and can be connected with one power supply via Y-shaped distribution cables. Contact InVitroSys for help. It is also possible to connect up to three devices to one USB hub. This limites the number of cables that need to be fed through into the incubator.
- The computer should be placed in close proximity of the incubator, i. e. next to or on the incubator if acceptable.



26 MyoDish 1 Culture System

Place the device on a horizontal level within the incubator. Ensure adequate upward clearance (at least 20cm) in order to manually access the connection cables, the push-button switch (9) for the stepper motor and the slots for the culture chambers. Ensure stability of the device.

Commercial incubators are able to accommodate up to three culture systems at the same time.

27	**Incubator**	We recommend incubators with an air-tight rear-side feed-through for cables. Insert the power cord (28) and the USB cable (31) through said feed-through into the incubator. First plug the USB cable into USB connection 2 (14), then plug the power cord into the power unit connection socket (12).
28	**Power unit cable**	For connecting the power unit (29) to the device
29	**Power unit**	The power unit should have an output voltage of 15V DC and ≥250mA.
30	**Computer system**	For controlling the culture system and recording data by means of the MyoDish Control Software
31	**USB cable**	For connecting the computer system to the culture system

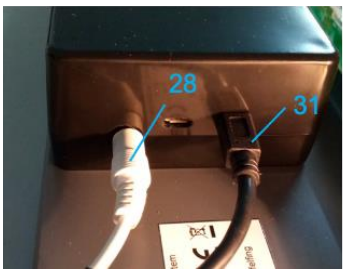


Figure: Controller unit (7) with barrel connector of the power cord (28) and micro-B connector of the USB cable (31) connected.

4.3.3 Grounding the Power Supply

It is not necessary to ground the power supply for proper operation of the device. However, if the operator is electrically charged, it is possible that an electrostatic discharge between the operator and the device causes a rest of the controller. The risk for discharge can be reduced by briefly grounding the operator before touching the device. Additionally, using a Y-shaped distribution cable together with a clamp, the power supply can be grounded. This should be done by connecting a clamp, which must exclusively be connected with the negative pole of the power supply cord, to a grounded plate of the incubator.

Risk of Electric Shock

Make sure that the polarity of the clamp is correct (negative pole) and that the connection inside the incubator is not current-carrying, but grounded. Let the wiring check by an expert and adhere to the operating manual of the incubator as well as to the safety regulations of your institution.

4.3.4 Setting up Several Devices Inside an Incubator

If the power supply unit of the MyoDish device provides 1 A or more current (15V DC), it is possible to connect several devices to one power supply. The delivered power supply unit (15V DC, 2A) is suitable. To connect several devices, connect the barrel connector of the power supply cord with a Y-shaped distribution cable. One connecter of the Y cable can be connected to a device, the other

connector to a second device or to another Y cable. By this means, up to three devices can be connected.

4.4 Starting the Device

After having connected the device to power, the stepper motor will automatically start to run at a frequency of approx. 30rpm. Push the push-button switch (9) to stop and/or restart the stepper motor. The device can be operated permanently inside an incubator or in a laboratory environment if ambient conditions are adequate.
After restarting the device the culture chambers will not be electrically stimulated. To start electrical stimulation and to alter the stepper motor speed, install the MyoDish software included in the scope of delivery on the computer connected to the device. The software serves for controlling the device and starting electrical stimulation.

4.5 Stopping the Device

Take the following steps in the order described below to stop device operation:

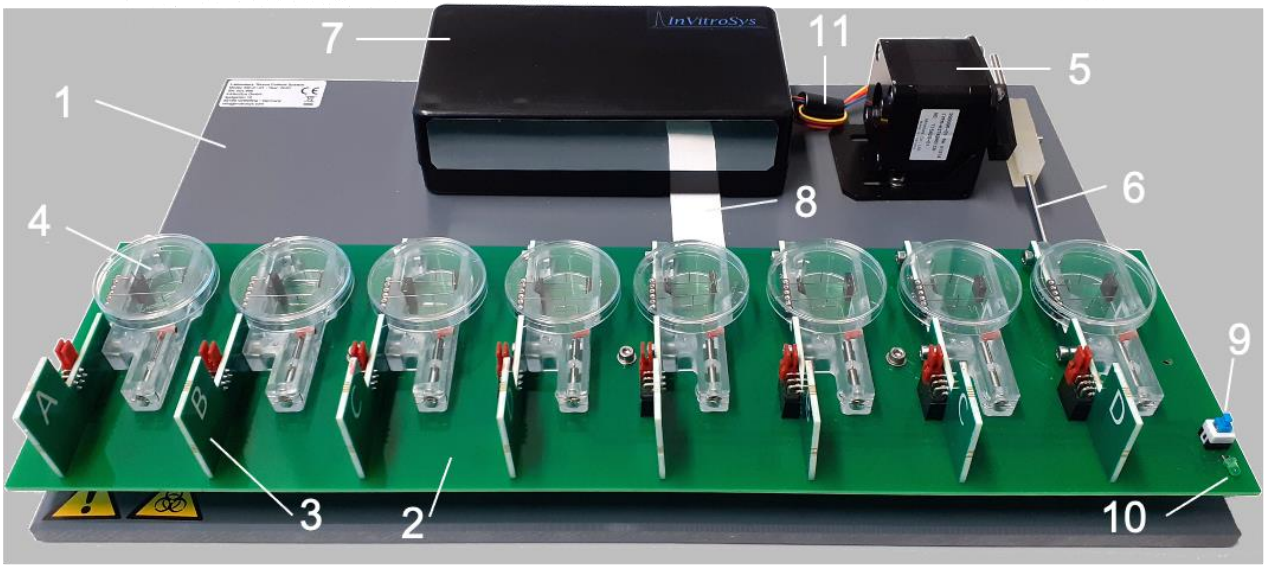
- Stop the stepper motor by pushing the push-button switch (9).
- Disconnect the USB cable from the computer.
- Unplug the power unit plug from the socket-outlet.

During brief periods of time (few days) between two experiments, the device may be kept inside the incubator. Let the device connected to power and the stepper motor run at low speed (20-30 rpm) to avoid water condensation.

5 Operation

5.1 Operating Elements

Before first using the MyoDish 1 Laboratory Tissue Culture System, make yourself familiar with the components and operating elements of the device and with the MyoDish software for controlling the device and recording data.



1	**Baseplate**	is the mounting basis of the other components
2	**Main circuit board**	contains the slots for the culture chambers with sensor circuit boards, is mounted on a movable bearing and is moved by the stepper motor
3	**Sensor circuit boards (8x)**	are bolted to the culture chambers, communicate the sensor information to the controller via the main circuit board, contain insertion connectors for the electrodes
4	**Culture chambers (8x)**	serve for insertion of tissue and culture medium
5	**Stepper motor**	moves the main circuit board
6	**Piston rod with friction bearing**	transfers the movement of the stepper motor onto the main circuit board
7	**Controller unit**	serves for voltage supply and control of the system, serves as an interface to the computer, contains two USB connectors and the power unit connector (power unit requirements: 15V DC, ≥ 250mA)
8	**Ribbon cable**	electrical connection between the controller and the main circuit board
9	**Push-button switch**	for starting and stopping the stepper motor
10	**Light-emitting diode**	signals the electrical stimulation
11	**Connection cable**	electrical connection between the controller unit and the stepper motor

5.2 Software for Controlling the Device and Recording Data

Below you will find an overview of the main features of the MyoDish software for controlling the device. For a more detailed and complete description, please refer to the software manual.

5.2.1 System Requirements

The software should be installed on a computer fulfilling the below system requirements:

- Operating system: Windows 10 or higher
- RAM: at least 4GB
- .NET Framework 4.72 or higher
- Hard-disk drive available: 10 MB for the software, at least 10 GB are recommended for data logging. Depending on the storage rate, the amount of data stored may be up to **622 MB** per 24hrs. Since up to three MyoDish 1 Laboratory Tissue Culture Systems can be connected to a computer at the same time, this sums up to 1.8 GB per 24 h. A typical experiment runs up to 1-2 weeks and can generate up to 10 GB of data for each device.

5.2.2 Installing the Device Driver and the MyoDish Software

Typically, a device connected to a PC will be visible as a USB drive named "Daplink" and a COM port interface. The identification of the drive with a drive letter and the COM port number are somewhat randomly chosen by the computer system. Thus, they may change when disconnecting and reconnecting the device.

- Should a device be connected and visible as a USB device, but not assigned to a COM port, please install the device driver *mbedWinSerial_16466.exe* included in the scope of delivery. Leave the device connected during installation of the driver. You will need administrator rights to do so.

To install the MyoDish Software on your computer, follow these steps:

- Copy the folder "MyoDishSoftware" to the hard disk, e. g. into the folder C:\Users\Shared\MyoDish\Software\.
- For convenience, create a link to the program file "MyoDishSoftware.exe" by dragging the file to the desktop while pressing ALT or by dragging the file to the taskbar.

After connecting and starting the device as well as after installing the device driver and the MyoDish software, start the MyoDish software by double-clicking on *MyoDishSoftware.exe*. This will open a main window and three scope windows (see figure).

5.2.3 Entering Calibration Constants and Sensor Offsets in the MyoDish Software

The calibration window can be opened by clicking on Settings → Calibration… Here, the spring compliance (unit: AU/mN) of the used chambers can be entered as well as the sensor offsets.

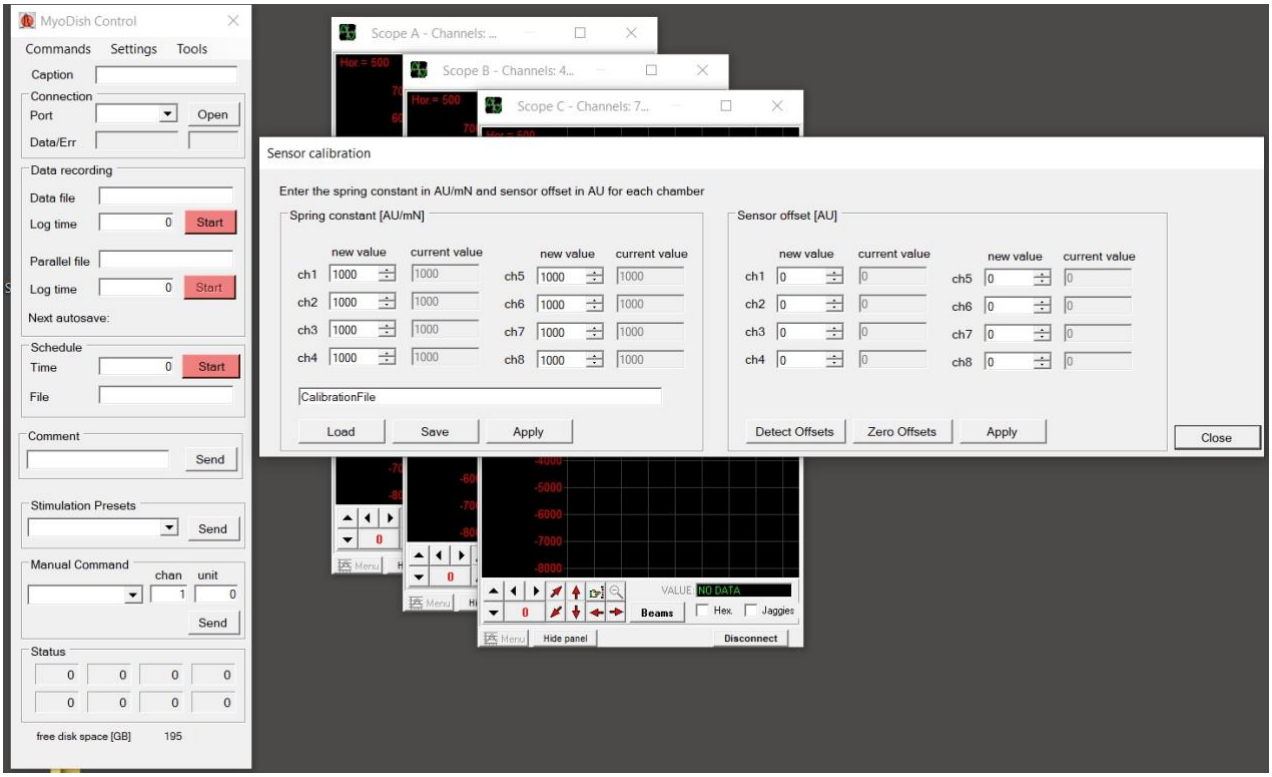


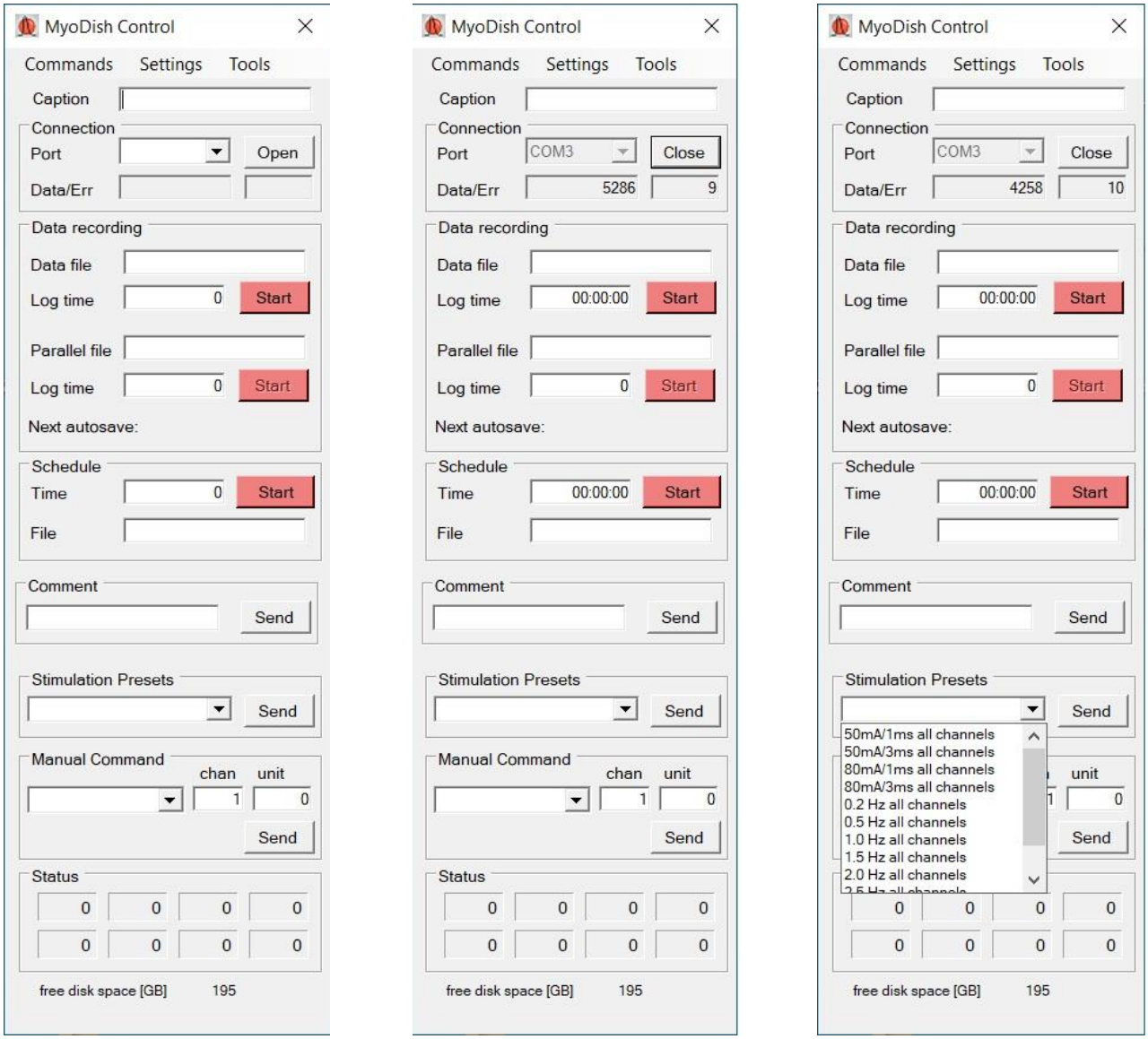
Figure: Start screen of the MyoDish software with main window (left), three scope windows (scopes A, B, C) and the sensor calibration window (sensor calibration).

Sensor offset values can be automatically detected when connecting empty chambers, i.e. chambers that do not contain any tissue. We recommend doing this while the culture medium in the chambers is warmed or kept warm before inserting the slices into the chambers.

The calibration constant C of each chamber (unit: AU/mN) can be entered later or during the data conversion process after the experiment. Thus, if you do not have the calibration constant at hand, you can enter these values later. Calibration constants are delivered together with the chambers by InVitroSys.

Please note that the calibration constants of chambers (closely related to the spring constant and spring compliance of the spring wire) may change over time, especially when chambers are used multiple times. InVitroSys provides a special device, the *MyoDish Tester*, that can be used to obtain the current values before each experiment. See also chapter 6.3.

5.2.4 Connecting the Device and Starting Stimulation



Left: In the main window, you will find an overview of the main features.

Center: To connect to the device, click on the drop-down menu arrow next to *Port* in the *Connection* panel to select the COM port via which the device has been initialized. Then, click on *Open*. The data package counter *Data/Err* will start counting.

Right: In the *Stimulation Presets* panel, select the stimulation strength (current/duration), e. g. *50mA/ms all channels*. Then, click on *Send*. Now select the stimulation frequency, e. g. *0.5 Hz all channels*. Then, click on *Send* again.

5.2.5 Setting the Rocking Board Speed

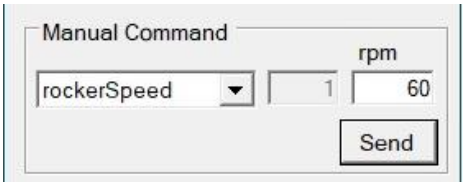


Figure: Setting the rocker speed.

To change the rocking speed, select the command *rockerSpeed* in the panel *Manual Command* of the main window and enter the target frequency into the field *rpm*. We recommend to set it between 30 and 60 rpm. Then, click on *Send*.

5.2.6 Checking the Stimulation Current

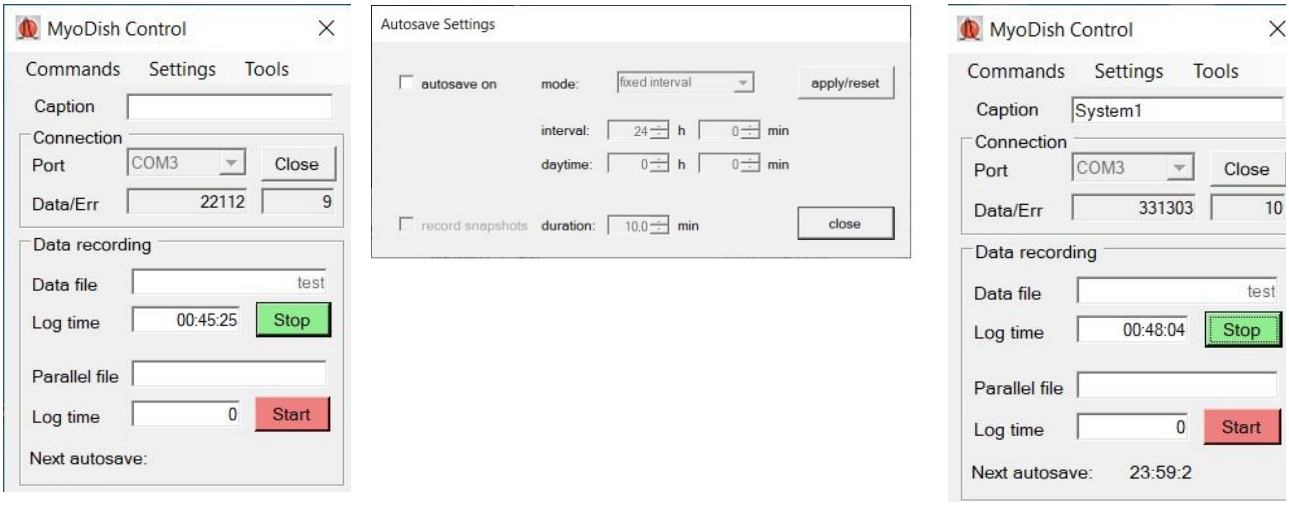
Figure: Fields highlighted in red indicate culture chambers where the set current amperage (here: 50mA) cannot be reached.

At the bottom of the *Status* panel in the main window, you will find the current settings of the command selected under *Manual Command*. The order of the channels displayed from left to right is channel 1, 2, 3, 4 in the upper row and channel 5, 6, 7, 8 in the lower row. A channel field highlighted in red (see figure: channels 2 and 4) indicated that the set stimulation current (in this example = 50mA) is not reached.
This may be the case for individual channels with stimulation current of more than 70mA without the problem being caused by the electrodes. Check the electrodes in the relevant culture chambers if the target current is not reached for lower current settings.

Do not set current values permanently that cannot be reached as this may damage the tissue or the culture medium due to electrolysis caused by an imbalance between positive and negative pulsing.

We recommend checking whether stimulation currents are reached before inserting tissue slices into the chambers in order to avoid tissue damage should electrodes have to be adapted, if applicable. We recommend checking it in the culture chambers while equilibrating the culture medium.

5.2.7 Starting Data Recording



Left: In the *Data recording* panel, double-click on the text field next to *Data file* to select the storage location and enter the file name. Then, click on the *Start/Stop* button right below the *Data file* text field. This will start logging.
Center: In the menu field *Settings* of the main window, click on *Autosave…* . This will bring up the *Autosave Settings* window. You will now be able to enable the *autosave* function and set the time period after which or a daytime at which a new data file is to be generated. This function serves to avoid the generation of excessively large files. Logging 24hrs will generate a file of approx. 700 MB.
Right: The time remaining before reaching the next increment of the file counter (autosave) is shown in the Data recording panel ("Next autosave: …").

5.3 Starting the Tissue Culture

If possible, always use sterile or germ-free tools, material and gloves in order to avoid infections or contamination of the cultivated tissue slices and culture medium. Wear appropriate protection equipment (lab coat, face mask), if applicable, and adhere to the instructions of your laboratory. If possible, carry out all working steps in a sterile or germ-free environment (e. g. filtered air, laminar-flow bench).

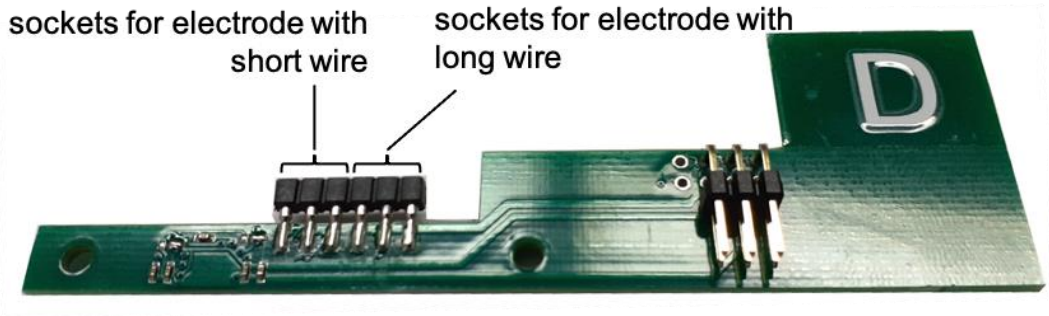
5.3.1 Preparing the Culture System

- Ensure that the incubator and the culture system are dry and clean. Refer to the instructions for cleaning and maintenance.
- Start up the device.
- Start the MyoDish software and open the COM port connection to the device.
- Set the target stepper motor frequency (recommendation: 60rpm).
- Set the target stimulation strength and frequency (e. g. 50mA/1ms and 0.5Hz).

5.3.2 Preparing the Culture Chambers

- Sanitize the chambers by immersing them briefly (≥ 3 min) in 100% isopropyl alcohol and subsequently leaving the chambers to dry in a sterile or low-germ environment (e. g. laminar-flow bench).

- Always use autoclaved electrodes or electrodes that have been sanitized with 100% isopropyl alcohol. Make sure to leave the electrodes to dry completely before using them. Leaving the graphite boards to dry may take a while (approx. 1hr).
- Fasten the sensor circuit boards (3) to the chambers using the mounting bolts (21).
- Observe the identification letters (25) of the sensor circuit boards (A, B, C, D): Each letter may be used twice. This means that for one MyoDish culture set with 8 chambers sensor circuit boards 2xA, 2xB, 2xC and 2xD can be used.
- Connect one electrode with a long wire and one electrode with a short wire to the sensor circuit board connectors (22) of each chamber such that the graphite boards of the two electrodes are positioned opposite to each other.
- When connecting the electrodes, you will notice that there are 3 different connectors for each electrode (see figure):
    - The electrode with a long wire (20) can be connected to one of the three connectors that are located on the side facing the adjusting wire.
    - The electrode with a short wire (19) can be connected to one of the three connectors facing away from the adjusting wire.



- Ensure that the graphite board of the short-wire electrode does not hinder movement of the spring wire (16). A distance of 2-3mm should be ensured between the spring wire and the graphite board.
- Fill the culture chambers with 2.5ml of culture medium each.
- Ensure that no medium or other liquid is spilled onto the sensor circuit boards.
- Cover the culture chambers using an appropriate lid (recommendation: lid of a sterile plastic Petri dish with a diameter of 35mm, e. g. Falcon #353001)
- Insert the culture chambers into the dedicated lots of the culture system.
- Ensure the correct order of the sensor circuit boards:
  A – B – C – D – A – B – C – D
- Let the chambers filled with medium incubate for at least 30min in the incubator (for most applications: 37ºC and 5% CO_2)
- Check electrode function. With stimulation enabled, the chambers for which the target stimulation current is not reached, are highlighted in red in the MyoDish software main window status panel.

Device damage due to spilled liquids

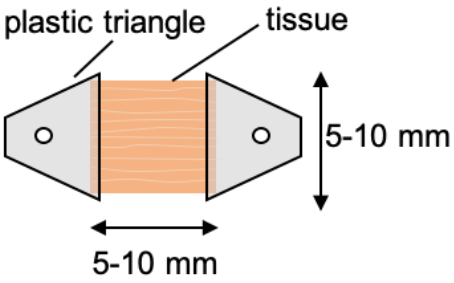
- Fill the culture chambers with a maximum of 2.5ml of medium. Spilling will occur if higher amounts of medium are used.

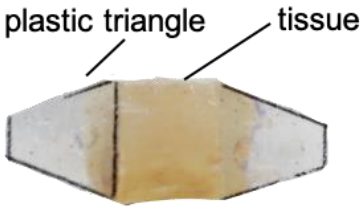
5.3.3 Preparing the Tissue

For cultivating heart muscle tissue, tissue slices of a thickness of approx. 300µm and a length by width of 5x5mm to 10x10mm are recommended.

The best method to produce the tissue slices is using a vibratome. Feeding speeds of 0.05 to 0.1mm per second, amplitudes of 1mm to 1.5mm and vibration frequencies of approx. 80Hz have proven to be particularly useful.

- If applicable, residues of agarose should be removed from the tissue slices.
- In direction of the fibers, glue two plastic triangles to both ends of each tissue slice, e. g. using Histoacryl® glue.
- Store the prepared tissue slices at approx. 4°C in Tyrode's solution containing BDM.





5.3.4 Mounting Tissue into the Chambers

- Take one culture chamber with warm and equilibrated medium out of the incubator.
- Push one of the plastic triangles onto the adjusting wire (15) of the culture chamber.
- If necessary, adjust the distance between the adjusting wire and the spring wire by means of the adjusting bolt (18) to make the second triangle reach the spring wire (16) without having to stretch or pull the tissue or the spring wire.
- Push the second triangle onto the spring wire (16).
- Now carefully use the forceps to push onto the two triangles from above to completely submerge them in the liquid.
- Adjust the distance of the two wires by means of the adjusting bolt (18) so as to make sure that the tissue is neither wavy or bent nor expanded.
- Cover the chamber with a Petri dish lid (e. g. Falcon #353001)

Work on the system without delays, making sure that the culture medium does not cool or becomes too alkaline due to CO_2 loss.

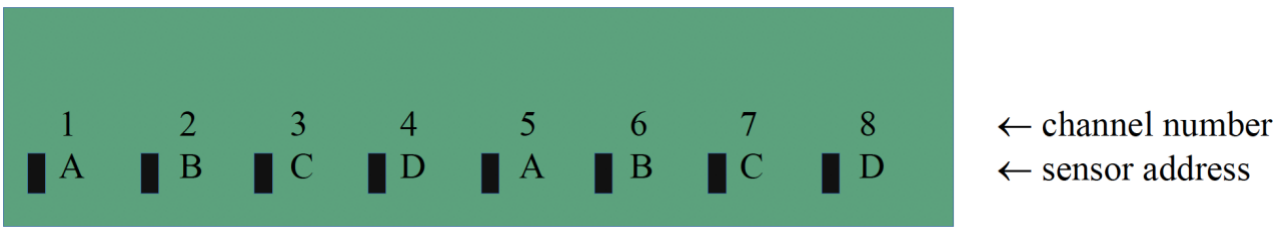
Make sure not to overexpand the tissue and/or the spring wire.

Using forceps with bent tips is recommended for putting the plastic triangles onto the wires (see below photograph).

5.3.5 Installing the Chambers with Tissue in the Culture System

- After mounting the tissue, push the chamber into the slot on the main circuit board (2) of the culture system.
- Refer to the scope window of the MyoDish software that displays the relevant channel of the culture chamber.
- Adjust the targeted pretension of the tissue by means of the adjusting bolt (18). Turn the bolt clockwise to increase tension until the displayed force increases visibly in the MyoDish software scope window.
- Reduce tension again (turn the bolt counter-clockwise) until the force stops decreasing. This value corresponds to the sensor base value (sensor offset).
- Now increase tension again until the intended diastolic preload has been reached.

When inserting the chambers, mind the labels of the sensor circuit board (25), i. e. the proper order to ensure correct assignment of the chambers to the channels displayed in the MyoDish software (from left to right: A B C D A B C D, see figure below).



Note that in the Myodish version MD-1.2 and later the position of sensor boards can be chosen freely (see Addendum).

Device damage due to wrong sensor board placement

- Incorrect positioning of a sensor circuit board into the socket typically disturbs the communication of all coupled addresses, but does not damage the device.
- However, do not remove the guiding pin of the sensor board socket. If the sensor board gets into contact with one of the connectors protected by the pin, the magnetic field sensor will be damaged permanently.

When plugging in sensor circuit boards (culture chambers), electrostatic discharges are possible. They typically cause a reset and restart of the device controller. Stimulation settings and rocker speed then must be resent by the MyoDish software. By grounding the power supply and/or the operator, this can be prevented (see also chapter 4.3.3).

For human heart tissue, preload values ranging from 1000 to 2000 µN are recommended. Pretension should be checked and, if applicable, be adjusted 24hrs after mounting the tissue.

5.3.6 Calculating the Stretch-Force Relationship of the Tissue

One full turnaround (360°) of the adjusting bolt will shift the adjusting wire by 0.5mm. Directly after installing a new tissue slice into a chamber, the slice should be at slack length (loose, without any tension, spring wire in its start position). You can check if this is the case by turning the adjusting bult counterclockwise. If no change of the sensor value can be observed on the screen, the tissue is unstretched. Otherwise, keep turning counterclockwise until the sensor value remains stable.
Now start turning the adjusting bolt slowly in clockwise direction until you observe a very slight increase in the sensor value. Note this value. From now on each turn of the bolt increases the preload force and, at the same time, stretches the tissue. Turn the bolt in steps of 60°, which can be achieved easily by using the hexagon Allen key provided. After each step, note the sensor value. Continue this process until the desired stretch force has been reached.
Afterwards you can calculate the strain-stress (stretch-force) relationship as follows:

- Movement of the adjusting wire, l_a: 0.083 mm per 60°
- Movement of the spring wire: l_s: 0.013 mm per 1000 AU
- Elongation of the tissue $\Delta l = l_a - l_s$
- Force F: approx. 0.5-1 µN/AU (depending on the calibration constant of the chamber, see 6.3)

Using these values, you can calculate the relationship between tissue elongation and force. If you measure the slack length l of the tissue (distance between triangles), for example using a caliper, you can calculate the strain, $e = \Delta l / l$, or the stretch ratio, $\lambda = (\Delta l + l) / l$, and set it in relation to the applied force.

5.4 Maintaining the Tissue Culture

5.4.1 Adaptation Period

In the first 24 to 48 hours after starting the tissue culture adaptation processes take place that make the tissue particularly sensitive to stress, e. g. by CO_2 and temperature fluctuations or high stimulation frequencies.

We recommend to adjust pretension once or twice and to stimulate the tissue with no more than 0.5Hz during this period.
To re-adjust the preload, use the driving bolt of the adjusting wire. Turning the bolt counter-clockwise until the base value (offset) of the corresponding sensor is reached, i. e. the value stops decreasing. Increase tension again by turning the bolt clockwise until the preload has reached the desired value (for example base value +1500 units).
The medium should preferably be changed for the first time 48hrs after starting the tissue culture.

5.4.2 Exchanging the Medium

The cultivated tissue slices consume substances within the medium, e. g. glucose, and produce final metabolites, such as lactate and signal molecules, e. g. cytokines. The medium should therefore be renewed on a regular basis, however, not all medium should be exchanged at once. When renewing the medium, it has proven favorable to leave part of the "old" medium in the chamber (preconditioned medium).

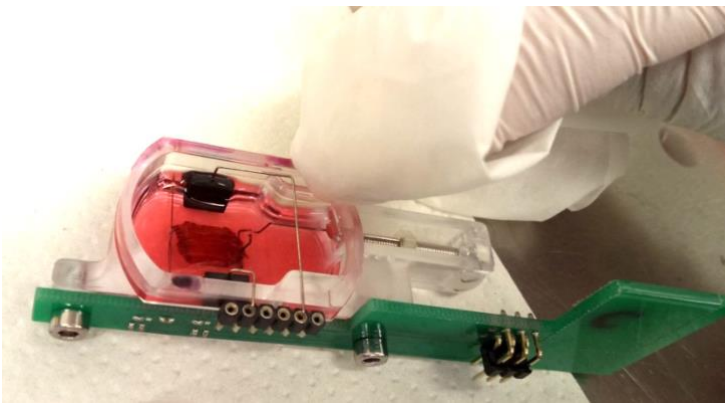
- Heat fresh, aseptically filtrated culture medium to 37-38°C.
- Remove a culture chamber from the culture system and exchange the medium.
- Change the medium in all culture chambers, one after another.
- Ensure that the atmosphere in the incubator is not cooling down too much and that not too much CO_2 is lost. This is critical for successful cultivation.

The temperatures of the medium and the tissue slices must under no circumstances drop below 35°C. Avoid delays during your work steps and do not place the culture chambers on a cold surface. If possible, use a medium-heating device with heat control and a surface preheated to 37°C.

All work steps should be carried out under aseptic conditions by using sterile pipette tips and working in a low-germ environment, e. g. on a laminar-flow bench.

Change the medium every 24 hours (0.8ml) or every 48 hours (1.6ml). Since part of the medium will evaporate, add 0.9ml or 1.8ml of fresh medium, respectively.

If during handling of a culture chamber medium is spilled and gets between the chamber and its lid, remove the medium immediately. We recommend using a cleansing tissue prepared with 100% isopropyl alcohol to clean the surface of the chamber and the inside of the lid (see figure below). When the chamber and lid are dry, the lid can be replaced and the chamber plugged back into the device.



5.5 Ending the Tissue Culture

If you wish to stop cultivation of tissue slices in all chambers, you can stop data recording in the MyoDish software and stop the stimulation. Or you can remove only individual chambers and continue cultivating the remaining tissue slices.

5.5.1 Removing the Tissue

- Remove the relevant culture chamber and place it on a hard and stable surface (e. g. laminar-flow bench).
- First, remove the lid and remove the two electrodes (19 and 20) using forceps.
- Place the electrodes into a container containing deionized water (see cleaning).
- Remove the sensor circuit board (3) by loosening the mounting bolts (21). Rinse off remnants of medium and salts with deionized water. Spray the sensor circuit board with 100% isopropyl alcohol to clean it and leave it to dry on a clean, antistatic surface (see cleaning).
- Reduce the pretension by turning the adjusting bolt (18) counter-clockwise until tissue tension is visibly reduced.
- Carefully remove the plastic triangles from the adjusting wire (15) and the spring wire (16) and remove the tissue slice.
- Take the desired additional working steps for the tissue (additional experiments, fixation, freezing, etc.).

If you wish to fixate the tissue in PFA/Formalin, it is recommended to do so under pretension directly in the culture chamber. In this case, first remove the electrodes. Then remove and dispose the medium. Before loosening the adjusting wire, add approx. 2ml of the fixing agent (e. g. 2% PFA in PBS) into the chamber and leave it to take effect for the desired time. Then proceed with the steps described above.

Device damage due to corrosive substance, strong solvents or fixing agents

- Avoid any contact of the device and accessories with corrosive substances (e. g. strong alkaline solutions or acids) and strong solvents (e. g. acetone).
- Do not use any substances other than Formalin or PFA to fix tissue slices in the chambers. If you do use such substances, remove the tissue from the chamber before using the substances.
- The electrodes must not come into contact with PFA or other fixing agents. Always remove the electrodes before using such substances on the device.

5.5.2 Disposing of the Tissue and Culture Medium

When terminating an experiment or when changing the medium, the medium has to be disposed of according to the applicable safety regulations.

Health hazards due to infectious liquids, infectious biomaterial and pathogenic germs

- Keep in mind that the cultivated tissue slice and the medium are potentially infectious.
- Adhere to the safety regulations of your institutions.
- Wear your personal protection equipment.
- For detailed instructions on how to handle germs or biomaterial of risk level II or higher, refer to the *Laboratory Biosafety Manual* (World Health Organization, Laboratory Biosafety Manual, in the relevant valid version).

5.5.3 Terminating an Experiment and Stopping the Culture System

- After removing all culture chambers, stop the MyoDish software.
- Stop device operation by taking the steps described under 2.5.

Clean the device and accessories immediately after terminating the experiment.

6 Maintenance, Cleaning and Storage

6.1 Cleaning and Storing the Device

For optimum function and a long life cycle of the device, any contamination (e. g. dried medium residue) has to be removed after every experiment.
In case of contamination, take the following steps in the order described below:

- Disconnect the device power cord and unplug the USB plug.
- Remove the device from the incubator.
- Place the device on a firm, clean surface, if possible in a low-germ environment (e. g. laminar-flow bench).
- Moisten a soft, antistatic cloth (if possible, sterile) with deionized water to wipe and clean the device of dirt.
- Apply 100% of isopropyl alcohol to the device and clean it with a soft and antistatic cloth.
- Leave the device to dry.

For short periods of time (few days) between experiments the device can also remain in the incubator. Leave it connected to the power supply and let the stepper motor run at low speed (20-30 rpm). For long-time storage, store the device in a dry and clean place. You may also use the delivery box for storage.

6.2 Cleaning and Storing the Accessories

6.2.1 Cleaning and Storing the Electrodes

To reuse the electrodes, they have to be thoroughly cleaned after each use.
Carry out the following steps in the order described below after each experiment:

- Remove the electrodes from the chamber using forceps (see also chapter 5.5).
- Rinse the electrodes under running water.
- Place the electrodes into a container with at least 500ml of deionized water plus 10% isopropyl alcohol. More than one pair of electrodes can be placed in a container together.
- Place the container onto a shaker to slightly agitate the water.
- Leave the electrodes to soak in the water for at least 12 hours.
- Discard the water and rinse the electrodes with fresh, deionized water.
- Leave the electrodes to dry in a clean, if possible low-germ, place (e. g. laminar-flow bench).
- Autoclave the electrodes.
  OR
  Place the electrodes into a container with at least 50ml of pure isopropyl alcohol and leave them to soak in it for 2 to 3 hours. After that, leave them to dry again.
- Store the electrodes in a sterile container or repeat the previous step immediately before re-using them.

6.2.2 Cleaning and Storing the Culture Chambers

To reuse the chamber cultures, they have to be thoroughly cleaned after each use.
Carry out the following steps in the order described below after every experiment:

- Remove the electrodes and the sensor circuit board.
- Remove any remaining medium or tissue.
- Rinse the chamber under running water.
- Place the chamber into a container with at least 500ml of deionized water plus 10% of isopropyl alcohol. Several chambers and electrodes can be placed into a container together (see figure).
- Leave the electrodes to soak in the water for at least 12 hours.
- Discard the water and rinse the electrodes with fresh, deionized water.
- If applicable, remove stubborn dirt deposits with a cotton swab and rinse the chamber again.
- Immerse the chambers into 100% isopropyl alcohol and leave them soak for at least 5 min.
- Remove the chambers and leave it to dry in a clean, if possible low-germ, place (e. g. laminar-flow bench).
- Store the chamber in a clean, dry, low-germ and closed container, e. g. in a plastic box for pipette tips.

Damage to culture chambers due to high temperature

Heating the culture chambers to more than 50°C will lead to irreversible damage.

- The culture chambers must not be autoclaved.

Additional cleaning steps can be performed with anionic detergents, enzyme cleaners and/or application ultrasound. If these procedures are suitable should be tested by the user. Always rinse the chambers thoroughly after each cleaning step.

6.2.3 Cleaning and Storing the Sensor Circuit Boards

To reuse the sensor circuit boards, they have to be thoroughly cleaned after each use.
Carry out the following steps in the order described below after each experiment:

- Disconnect the sensor circuit board from the chamber by loosening the connecting bolts.
- Inspect the sensor circuit board for dirt/contamination (e. g. dried medium residues) and liquids.
- Use a cotton swab moistened with deionized water to carefully remove any dirt deposits.
- Apply 100% isopropyl alcohol onto the sensor circuit board and leave it to dry on a clean, if possible low-germ, place (e. g. laminar-flow bench).
- Store the sensor circuit boards in a clean, dry, low-germ and closed container.

Damage to sensor circuit boards due to liquids

- Various metals of the sensor circuit boards will corrode when getting into contact with water. Except for brief rinsing or wiping with deionized water, sensor plates must always be kept at a dry place and must be dry when being connected to the device.
- If, during operation, any medium or other fluid gets into the gap between sensor board and chamber, remove the chamber immediately, remove the sensor board and clean both parts.

6.3 Calibration of the Culture Chambers

The culture chambers are calibrated before delivery. The position of the spring wire is adjusted and the spring rate is determined (see also chapter 3.4.6). For conversion of the sensor values into contraction force, you will have to know the values from calibration. When starting the MyoDish Software program, you can open window prompting you to enter the values (see chapter 5.2.3). Depending on how and for how long the culture chambers are used, these values may change over time.

To calibrate MyoDish chambers we highly recommend to use the *MyoDish Tester*, a device designed specifically for this purpose (see picture below).

The following steps describe how to calibrate chambers with a MyoDish Cultivation System. Take special care to not damage any parts!

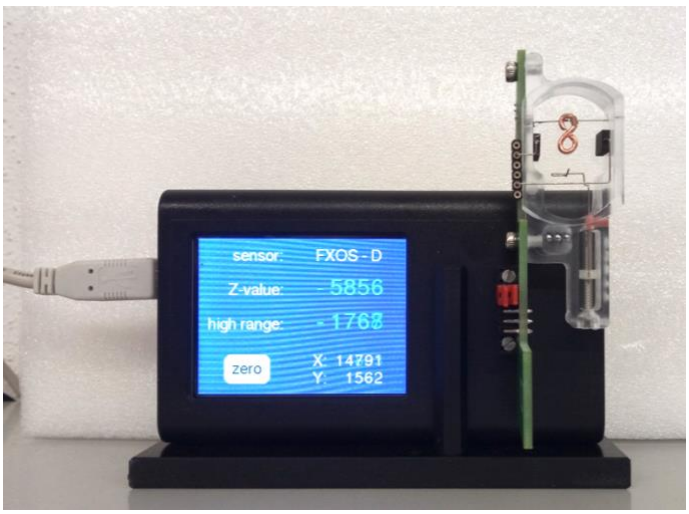


Figure: The MyoDish tester with a chamber containing a test weight.

For manual calibration, take the steps below in the order described:

- Take a non-magnetic wire (for example, a paperclip made of copper) and measure its weight using a precision balance.
- Shorten the paperclip or wire to make it weigh approx. 0.3 g. The weight of 0.3 g translates into 3mN.
- Bend the clip or wire so as to form a small eye, which you will use later on to suspend the test weight on the spring wire of the chamber.
- Switch off the stepper motor of the MyoDish device by pressing the push button.
- Disconnect the power unit and USB cable and take the device out of the incubator.
- Place the device on a stable tabletop.
- Reconnect the USB cable and power unit.
- For the following step, you will need the **help of another person:**
- Bring the device into a vertical position with the controller housing pointing upwards. **Make sure to grab the device by the baseplate only and don't let it tip over to avoid damage device**.
- Take a note of the sensor values (S1) of the chambers to be calibrated.
- Suspend the test weight on each spring wire of the chambers to be calibrated and again take a note of the sensor values with the weight suspended (S2)
- The spring rate K in µN per sensor value in arbitrary units (µN/AU) for each calibrated chamber will then result from the following equation

$$K = g \bullet m/(S2-S1) \bullet 10^6,$$

wherein g is the gravitational acceleration (9.81N/kg) and wherein m is the mass of the test weight in kg. The multiplication by 10^6 is required to convert the resulting unit from N to µN.

Please note that the **calibration constant C** that is entered into the MyoDish software and the file converter to display sensor values in units of force (µN) is not equivalent to the spring rate K, but corresponds to the compliance in AU/mN:

$$C = 1/K \bullet 1000$$

MyoDish culture chambers are available in four different categories of spring wire stiffness, indicated by the number of points on the chambers. The more points, the stiffer is the spring wire, i.e., the lower is the calibration constant C. The following table provides average values of the expected calibration

constants for the different chamber types. Please note that for very precise measurements, the calibration constant for each chambers needs to be determined individually as described above.

Chamber type	Calibration constant C Unit*: AU/mN	Spring rate K Unit*: µN/AU	Spring constant k Unit: mN/mm	Comment
1-point (•)	6000-7500 AU/mN	0.133 – 0.167	11-13	Recommended for stem-cell derived cardiac tissues
2-point (••)	3000-4000 AU/mN	0.25 – 0.33	20-30	Recommended for small slices, e. g. atrial tissue or mouse ventricular tissue
3-point (•••)	1100-1600 AU/mN	0.625 – 0.91	50-80	Recommended for most applications, especially for ventricular tissue
4-point (••••)	200-300 AU/mN	3.3 – 5.0	266-400	for special applications; results in nearly isometric contraction

* AU refers to sensor values in arbitrary units

6.3.1 Conversion from Sensor Values to Deflection (Contraction)

75 000 AU correspond to a wire deflection of 1 mm in the normal sensor mode (default).
25 000 AU correspond to a wire deflection of 1mm in the extended sensor mode (see chapter 3.4.6).

6.4 Adjustment of the Spring Wire Position

If the initial value of the sensors with empty chambers strongly differs from its target baseline, you may try to adjust the position of the spring wire. The target value is approx. -8000 to -12000 in regular sensor mode and approx. -3000 to -4000 in the extended sensor mode. Deviations of up to approx. 4000 sensor units in a regular mode or approx. 1000 sensor units in an extended mode can be tolerated. The initial value is determined by the position of the magnet (17) at the spring wire (16) in relation to the magnetic field sensor (26) at the sensor circuit board.

During all works on the spring wire, make sure not to touch the magnet. Magnet dislocation in relation to the spring wire will forfeit functionality of the chamber!

In the starting position of the spring wire the Magnet is located approx. 1mm above the bottom of the chamber and approx. 0.2mm behind the center of the magnetic field sensor (see figure). If the actual position strongly deviates from the starting position, you may try to adjust the position by carefully bending the spring wire using a forceps.

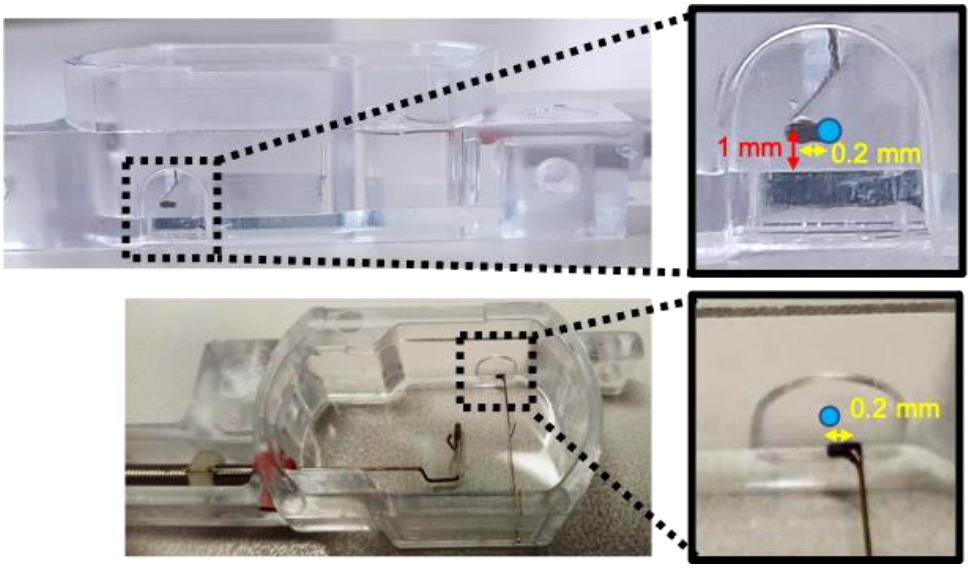


Figure: The blue circle in the enlarged pictures shows the point where the magnetic field sensor is located when the sensor circuit board is screwed on. The ideal position of the magnet on the spring wire is approx. 0.2mm behind the center of the sensor and approx. 1mm above the bottom of the chamber.

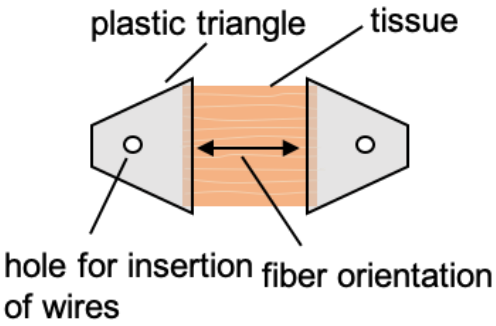
7 Troubleshooting

The recommended steps for troubleshooting (chapters 7.1 – 7.3) refer to preparations from human myocardium with at least 5x5mm^2 surface area and 0.3mm thickness and culture conditions as described in the literature (Fischer et al., 2019, Nat. Commun.).

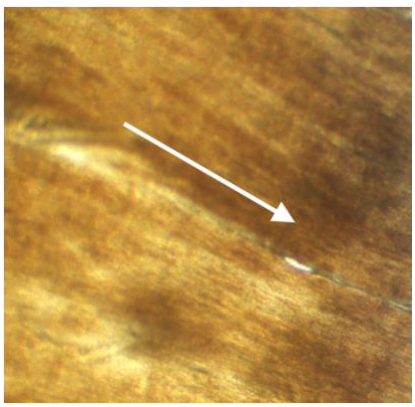
7.1 Problems with Preparing and Mounting Tissue Slices

7.1.1 Problems with Recognizing Fiber Orientation

Muscle fiber orientation should allow connection of the two opposite triangles (see figure). Fiber orientation can be recognized easily by looking at the tissue texture, when the tissue slice is placed on a non-transparent surface (e. g. Petri dish) and held up against the light.



In addition, fiber orientation can be identified under an optical microscope with a 10x or 20x lens. To do so, place a tissue slice in cool cutting solution into a Petri dish and focus on the tissue. You will then be able to see the muscle cell orientation (see photograph).



Left figure: Fiber orientation shown by arrow

7.1.2 Problems with Placing the Plastic Triangles onto the Wires

- Check if the hole for inserting the wire is big enough. If not, use a cannula with a larger diameter (e. g. 22G/0.7mm or 20G/0.9mm) to punch the hole.
- Ensure that any plastic remains and shreds, which may block the hole, have been removed completely after punching. We recommend to carefully burr the edge with a scalpel.
- Do not apply too much glue so as not to block the holes.
- Do not overexpand the tissue or the spring wire even if it is difficult to insert the wire.

7.2 Problems with Starting the Tissue Culture

7.2.1 No Tissue Contraction

- After mounting the tissue in the culture chambers and starting the stimulation, it may take several seconds to minutes before you will see a contraction.
- Ensure that no electromechanical decoupling agent, e. g. BDM, remains in the culture medium.
- Check the MyoDish software for whether the stimulation rate is set appropriately. Resend the commands for stimulation pulses (e. g. *50mA/1ms all channels*) and frequencies (e. g. *0.5Hz all channels*). The LED on the platform should be blinking with each stimulation pulse.
- Check whether the target stimulation current is reached (if not, the relevant channel will be highlighted in red in the status display at the bottom of the main window). If applicable, check the electrodes.
- Ensure that the rocking board speed is at least 30rpm. At lower frequencies, the tissue is not sufficiently supplied with oxygen.
- Ensure that fiber orientation has been observed when gluing the plastic triangles.
- Ensure that the triangles are still properly attached to the tissue slice.
- Ensure that the spring wire is not blocked by an electrode.

7.2.2 Hyper-contraction of Tissue

- If tonic contracture occurs after installation of the tissue (slow increase of diastolic force with or without phasic contractions), this indicates hypercontracture of cardiac muscle cells, which is a result of tissue damage.
- Presumably, the stronger the hyper-contraction is, the more serious is the tissue damage.
- Hyper-contractions ranging from 1000 to 4000µN may be reversible within the first hour of cultivation and do not prevent successful cultivation.
- If this range is exceeded, the tissue is seriously damaged.
- Try to find out the cause of the damage (quality of the tissue sample, delivery conditions, preparation of the tissue slice, composition of Tyrode's solutions, temperature fluctuations, ischemia-reperfusion injury, etc.).

7.3 Problems with Maintaining the Culture

7.3.1 Infection of the Culture

- If the medium turns cloudy, starts to smell foul or if you can see mycelium growth, this indicates an infection of the culture.
- The content of infected culture chambers should be discarded. The described cleaning and decontamination steps (Chapter 6) have been proven effective. However, although fungal infestation can be removed by these steps, additional cleaning may be necessary, e.g. surgical instrument cleaner.
- The electrodes, which are made of graphite, can be autoclaved. Nevertheless, graphite is a porous material, such that it is not possible to ensure sufficient cleaning or disinfection of its surface. To prevent the release of heat-stable toxins (e. g. lipopolysaccharides, mycotoxins), we recommend to discard contaminated electrodes and replace them with new ones.
- To prevent infections, use only sterile medium (if applicable, use sterile filtration) and provide for low-germ and, if possible, sterile conditions in all steps of preparation and medium change.

7.3.2 Weak or no Tissue Contraction

- In the adaptation period, i. e. within the 24 to 48 hours after starting the tissue culture it is normal for the contraction strength to decrease. After that, it should, however, increase again.
- A sudden decrease in contraction strength at a later point in time indicates tissue damage.
- Common causes for tissue damage are:
    - infection
    - electrolysis due to deficient stimulation (shown by decolorization of the medium)
    - temperature fluctuations
    - CO_2 and pH fluctuations
    - wrong pretension (preload)
    - wrong composition of the culture medium (electrolytes, vitamins, growth factors etc.)
    - mechanical tissue damage
    - excessively high stimulation frequency
    - too low frequency of the rocking board (recommended: 60 rpm)
    - addition of toxic pharmacological substances or hormones
    - previously existing damage of the tissue sample
    - deficient medium change or medium change that is not appropriate to the tissue (recommended: remove 0.8ml/1.6ml every 24hrs/48hrs and add 0.9ml/1.8ml of fresh medium; this regimen should be adapted depending on the culture conditions and the origin of the tissue (e. g. the species))
- Optimal culture conditions may depend on the species and age of the tissues.

It is extraordinarily important to avoid fluctuations in CO_2 or temperature. We recommend incubators with subdivided inner doors to reduce gas exchange when opening the door. Additionally, try to minimize the frequency and duration and of opening the incubator doors as well as the time of culture chambers outside the incubator (e. g. during medium exchange or when adding chemicals or substances).

7.4 Technical Problems

7.4.1 Problems with the Stimulation

- The maximum voltage of stimulation is approximately 12.5 V. With the included graphite electrodes and commonly used culture media, this allows for currents of up to 80 mA when using 1 ms pulses, or 60 mA when using 3 ms pulses. If the set current is not reached, this will be indicated in the status panel of the respective culture chamber in the MyoDish software main window. Only if the maximum current is significantly lower than the described values, the electrode is defect.
- If typical stimulation currents (e. g. 50mA) are not reached, remove the culture chamber and check whether the electrodes are inserted correctly into the culture medium if whether the connectors are plugged in correctly. If not, insert them correctly.
- Keep in mind that there are three connectors for electrodes with a long wire and three connectors for electrodes with a short wire. Try alternative connectors.
- If the stimulation current is still not reached, replace the electrodes by a new pair of electrodes.
- Keep in mind that the culture medium serves as an electrical conductor. If the fill level of the medium is too low, this may prevent the set target current from not being reached. If the level is too low, fill the chamber with medium (but not more than 2.5ml in total).
- Maximum stimulation intensity can be reached by prolonging the stimulation pulses to 3-5 ms. If the current limit indicator shines red only intermittently, this indicates that the maximum stimulation intensity is reached.

- Operation with the current limit indicator shining red permanently (with each stimulation) is possible, but includes the risk that positive and negative pulses are not balanced. This may result in electrolysis, acidification of the medium as well as damage of the tissue and electrodes.

7.4.2 Discoloration of the culture medium

- Constant currents of same polarity cause electrolysis and generate HCl within the medium. This leads to acidification and yellow discoloration of the pH indicator phenol red. Additional signs of electrolysis include gas bubbles and disintegration of the electrodes. The most common cause of electrolysis is a conductive connection between the electrode wires and a power source, for example if medium gets into the gap between the sensor circuit board and the culture chamber.
- Discoloration of the culture medium may also occur through chemical decomposition of the phenol red dye. Such a reaction is possible if the medium is constantly connected to the electrode wire. This may happen preferably with electrodes where the surface is not properly insulated or where the graphite block is damaged. Damaged electrodes must be replaced.

7.4.3 Problems with the USB Connection

During operation, it may be possible from time to time that the USB connection to the computer is interrupted. This does not affect the stimulation or the settings of the rocker because these functions are controlled autonomically by the controller of the MyoDish system. However, data recording is stopped.
Perform the following steps to restore the connection:

- Close and open the relevant COM port again.
- If the COM port is not shown in the drop-down menu of the MyoDish software, unplug the USB plug from the computer for a few seconds and plug it back in again.
- The computer should now detect the USB device and the COM port should show in the menu. Open the COM port and start data recording.
- If the USB device is still not detected, check whether the USB cable has been inserted correctly into the device controller unit.
- If the problem persists, switch off the device completely:
    - Disconnect the power unit plug from the socket-outlet.
    - Disconnect the USB cable from the computer.
    - Wait a few seconds.
    - Reconnect the USB cable to the computer.
    - Reconnect the power unit plug to the socket-outlet.
    - Restart the MyoDish software.

7.4.4 Restart after Power Outage or Electrostatic Discharge

After a power outage or electrostatic discharge, the device will restart automatically. Electrostatic discharge may occur when a culture chamber is inserted if a person has not discharged himself before.

- After restart, the controller settings are reset:
    - discontinuation of electrical stimulation
    - rocker speed of approx. 30rpm
- The connection (COM port) has to be reopened and data recording has to be restarted.
- The target settings (stimulation pulse settings and frequency as well as the rocking board speed) have to be submitted again by means of the MyoDish software.

- Incidents due to electrostatic discharge can be prevented by grounding the power supply and/or the operator.

7.4.5 Troubleshooting Sensor Problems

The following errors may occur if the correct order of the sensor circuit boards is not adhered to:

- The order of the culture chambers does not match the order of the channels shown in the MyoDish software
- Stimulation pulses are wrongly delivered to the chambers
- Registrations within a sensor groups (channels 1-4 or 5-8) interfere with each other or fail completely (flat line)

To avoid these errors, make sure to use the correct order of the sensor circuit boards:

- from left to right: A, B, C, D, A, B, C, D.

The initial value of the sensors may have shifted. If so, the sensor value after inserting the empty chamber (without tissue) will strongly deviate from the target initial value. In the regular mode, it should range between -8000 and -12000, in the extended sensor mode between -3000 and -4000 (see chapter 3.4.6).

- Slight deviations (up to +/- 4000 sensor units) are tolerated
- The position of the magnetic field sensor may have been displaced due to wrong mounting of the sensor circuit board to the chamber
- The position of the magnet may be displaced due to a loose or overbent spring wire. Small displacements can be corrected manually by re-adjusting the spring wire position (see chapter 0).

8 Transportation, Storage and Disposal

8.1 Transportation

Device damage due to wrong handling

Only the baseplate is able to carry the weight of the device. All other components (e. g. main circuit board) are not strong enough to carry the weight of the entire system.

- Only grab the device by the baseplate when transporting and packing the device.
- Use the original packaging for transport.

8.1.1 Conditions for Transport

Air temperature	-25°C to 50°C
Relative air humidity	10% to 85%
Air pressure	30kPa to 110kPa

8.2 Storage

- If possible, store the device in a dry place at room temperature, protect it from dust, liquids and other external influences.

8.2.1 Conditions of Storage

Air temperature	-5°C to 40°C
Relative air humidity	10% to 85%
Air pressure	30kPa to 110kPa

8.3 Disposal

Observe the common legal instructions for disposal of the product. **Regulations for disposal of electrical and electronic devices in the European Community**: Within the European Community, the disposal of electrical devices by national instructions based on the EU Guideline 2012/19/EU on Waste Electrical and Electronic Equipment (WEEE). According to this guideline, this device must not be disposed in the municipal waste or in the household waste but has to be disposed of according to the Waste Electrical and Electronic Equipment or has to be taken back by the manufacturer. The following icon has been attached to the device to reflect this:

Please note that the waste disposal instructions within the EU may vary between the countries.

9 Technical Data

9.1 Power Supply

Power supply	15 V DC
Current consumption	250 mA max.
Power consumption	max. 4 W
EMC interference	EN 61326-1/EN 55011 – Category B
EMC immunity	EN 61326-1

9.1.1 Power Unit Specifications

Input	100...240V, 50Hz–60Hz
Output	15 V DC, 1-2 A
barrel connector	5.5/2.1mm, positive centre

9.2 Environmental Conditions during Operation

Environment	For indoor use only
Environmental temperature	10°C to 40°C
Relative air humidity	10% to 85%
Air pressure	60kPa to 110kPa For use in elevations of 4000m max. above NHN

9.3 Weight and Dimensions

Weight	2.5kg
Dimensions (WxDxH)	38.5x25x7cm

9.4 Noise Level

Noise level	<50dB (A)

9.5 Service Life of Accessories and Wear Parts

9.5.1 Service Life of Wear Parts

The chamber units are deemed to be wear parts, each consisting of a culture chamber, a pair of electrodes and a sensor circuit board. Basically, these accessory parts can be reused multiple times provided that they are used and cleaned properly. However, InVitroSys cannot guarantee a minimum service life of the wear parts because the wear and tear heavily depends on the type and scope of use. Failure arising from the wear and tear (service life, age) of the wear parts are not deemed to be a defect in the product. Thus, the guarantee exclusively covers the state of these parts prior to first use.

The moving parts of the rocker mechanics are designed for continuous operation, but underlie wear. Bearings and axles can be replaced by the customer and will be provided for free by InVitroSys within the warranty period

Declaration of Conformity

The product mentioned below fulfills the requirements of the cited guidelines and standards. Unauthorized modifications or changes in the product or unintended use of the product will render this declaration null and void.
This declaration of conformity is issued under the sole responsibility of the manufacturer.

Product name: MyoDish 1, incl. components
Product type: Laboratory device for tissue cultivation
Relevant guidelines/standards:

2006/42/EC:	EN ISO 12100
2014/35/EU:	EN 61010-1, EN 61010-2-020, IEC 61010-1, IEC 61010-2-020
2014/30/EU:	EN 61326-1, EN 55011
2011/65/EU:	EN 50581

Gräfelfing, 25. November 2020

Dr. Andreas Dendorfer
Managing Director

Dr. Thomas Seidel
Managing Director

InVitroSys GmbH • Igelgarten 10 • 82166 Gräfelfing • Germany • info@invitrosys.com