multichannel systems

QT-Screen*

User Manual

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1 Introduction

1.1 About this Manual

This manual comprises all important information about the first installation of the hardware and software, and about the daily work with the instrument. It is assumed that you have already a basic understanding of technical and software terms. No special skills are required to read this manual.

If you are using the device for the first time, please read the **important safety advice** before **installing the hardware** and **software**, where you will find important information about the installation and first steps.

The **printed manual** and **Help** are basically the same, so it is up to you which one you will use. The Help offers you the advantage of scrolling through the text in a non-linear fashion, picking up all information you need, especially if you use the **Index**, the **Search** function, and the **Browse Sequences**. If you are going to read larger text passages, however, you may prefer the printed manual.

The device and the software are part of an ongoing developmental process. Please understand that the provided documentation is not always up to date. The **latest information** can be found in the **Help**. Check also the MCS Web site (www.multichannelsystems.com) for downloading up-to-date manuals and Help files.

2 Important Information and Instructions

2.1 Operator's Obligations

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely.

Personnel still undergoing training may only work on the device under the supervision of an experienced person.

2.2 Guaranty and Liability

The *General conditions of sale and delivery* of Multi Channel Systems MCS GmbH always apply. The operator will receive these no later than on conclusion of the contract.

Multi Channel Systems MCS GmbH makes no guaranty as to the accuracy of any and all tests and data generated by the use of the device or the software. It is up to the user to use good laboratory practice to establish the validity of his findings.

Guaranty and liability claims in the event of injury or material damage are excluded when they are the result of one of the following.

- Improper use of the device
- Improper installation, commissioning, operation or maintenance of the device
- Operating the device when the safety and protective devices are defective and/or inoperable
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device
- Unauthorized structural alterations to the device
- Unauthorized modifications to the system settings
- Inadequate monitoring of device components subject to wear
- Improperly executed and unauthorized repairs
- Unauthorized opening of the device or its components
- Catastrophic events due to the effect of foreign bodies or acts of God

2.3 Important Safety Advice

Warning: Make sure to read the following advice prior to install or to use the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.

Warning: Obey always the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks.

The product has been built to the state of the art and in accordance with recognized safety engineering rules. The device may only

- be used for its **intended purpose**;
- be used when in a **perfect condition**.
- Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Warning: The device and the software are **not** intended for medical uses and **must not** be used on humans.

Malfunctions which could impair safety should be rectified immediately.

High Voltage

Electrical cords must be properly laid and installed. The length and quality of the cords must be in accordance with local provisions.

Only qualified technicians may work on the electrical system. It is essential that the accident prevention regulations and those of the employers' liability associations are observed.

- Each time before starting up, make sure that the **mains supply** agrees with the specifications of the product.
- Check the **power cord** for damage each time the site is changed. Damaged power cords should be replaced immediately and may never be reused.
- Check the **leads** for damage. Damaged leads should be replaced immediately and may never be reused.
- Do not try to insert anything sharp or metallic into the vents or the case.
- Liquids may cause short circuits or other damage. Keep the device and the power cords always **dry**. Do **not** handle it with wet hands.

Requirements for the installation

- Make sure that the device is not exposed to direct sunlight. Do not place anything on top of the device, and do not place it on top of another heat producing device. Never cover the vents, not even partially, so that the air can circulate freely. Otherwise, the device may overheat.

Requirements during operation

For more information on the operation of the Tecan MiniPrep 60 liquid handler, please consult the MiniPrep Operator's Guide from Tecan.

- Do not enter the QT-Screen robot with your hands during operation. The moving needles can lead to severe injuries.
- The system liquid has to be free of any particles. Only use clean reservoirs and particle-free solutions.
- The system liquid has to be distilled or deionized water to prevent contaminations of the tubing.
- The system should be flushed before and after each run with the manual controls to avoid contaminations of the tubing with sticky compounds that can lead to wrong results, algae growth, or other microbiological contaminations leading to a decreased lifetime of the tubing.
- The system liquid should have ambient temperature for best pipetting accuracy.
- Basic QT-Screen functions such as the pipetting accuracy should be verified in regular intervals. The system should only be used in perfect condition.

2.4 Terms of Use for the program

You are free to use the program for its intended purpose. You agree that you will not decompile, reverse engineer, or otherwise attempt to discover the source code of the software.

2.5 Limitation of Liability

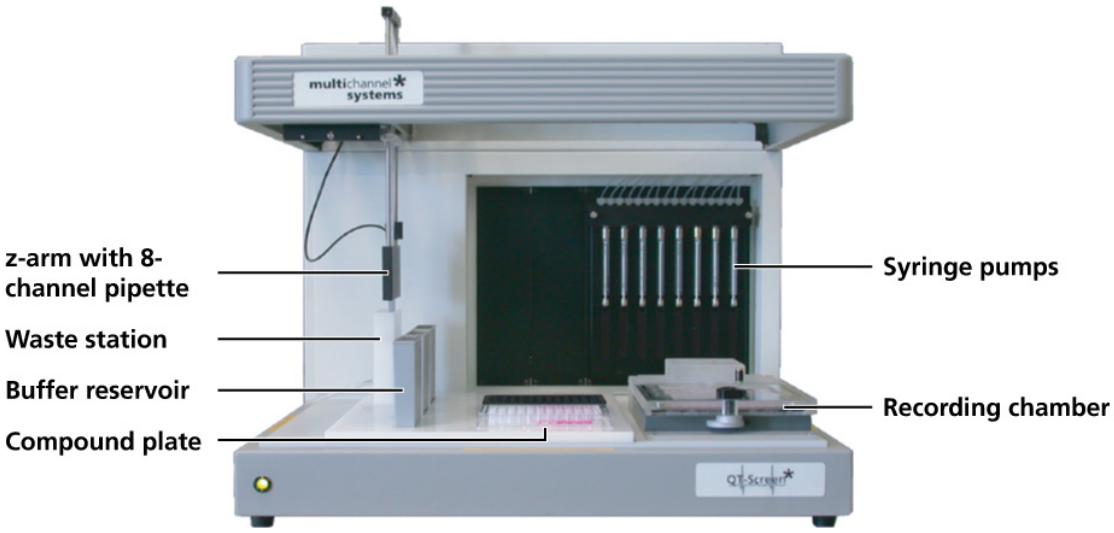
Multi Channel Systems MCS GmbH makes no guaranty as to the accuracy of any and all tests and data generated by the use the software. It is up to the user to use good laboratory practice to establish the validity of his findings.

To the maximum extent permitted by applicable law, in no event shall Multi Channel Systems MCS GmbH or its suppliers be liable for any special, incidental, indirect, or consequential damages whatsoever (including, without limitation, injuries, damages for data loss, loss of business profits, business interruption, loss of business information, or any other pecuniary loss) arising out of the use of or inability to use the program or the provision of or failure to provide Support Services, even if Multi Channel Systems MCS GmbH has been advised of the possibility of such damages.

3 First Use

3.1 Welcome to the QT-Screen

QT-Screen robot

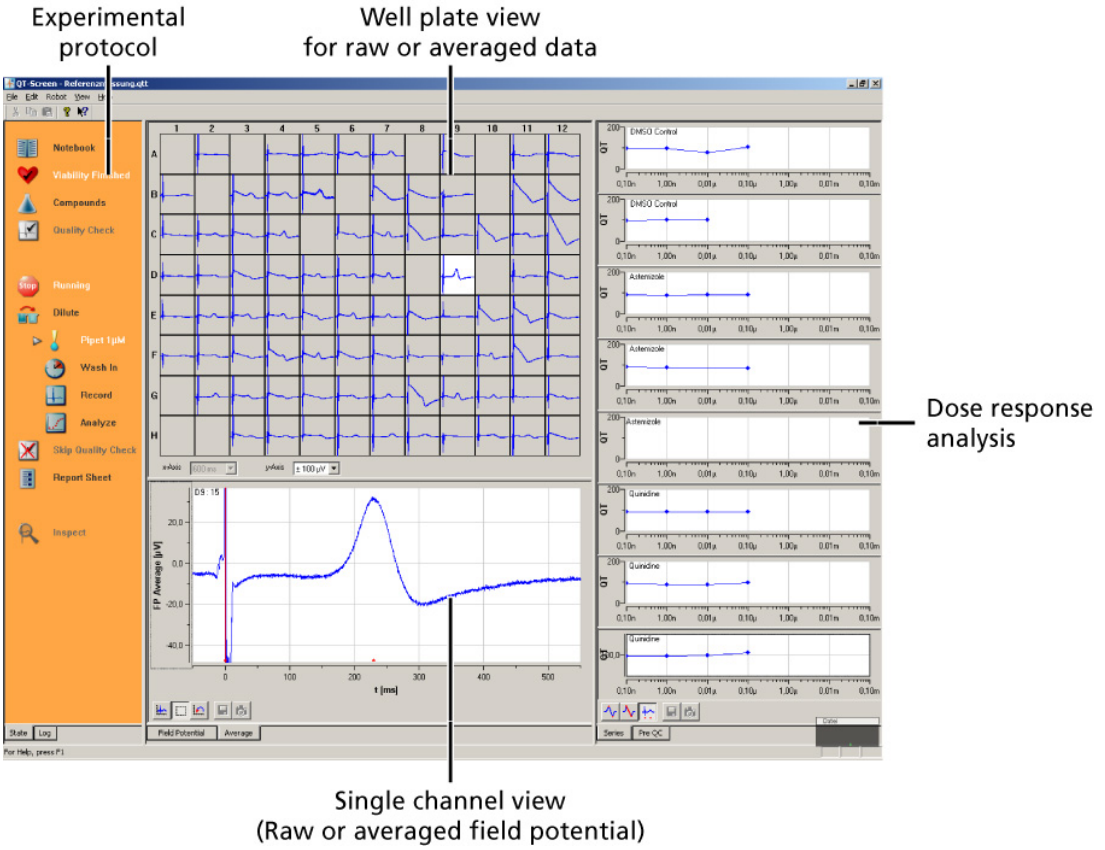


The QT-Screen is an automated system for safety-pharmacological screenings of a drug-induced QT prolongation in conformity with the regulations of the Food and Drug Administration (FDA) in the United States of America. The QT-Well plate carrying cardiomyocyte cultures in 96 recording chambers is placed onto the QT-Screen, and test compounds are applied to the cell cultures in 12 or duplicates by an 8-channel liquid handler integrated in the QT-Screen. This results in 8 or 16 compounds that can be screened using a single QT-Well plate.

Several concentrations of the compounds are tested consecutively on the same well, starting from the lowest concentration to the highest. The QT-Screen prepares the final test solutions in the user-specified concentrations from stock solutions plated into standard 96-well plates.

3.2 About the QT-Screen Program

QT-Screen program overview



The electrical activity, that is, the field potentials, is recorded by the data acquisition computer. The QT-Screen program controls all hardware functions and the ambient parameters of the cell cultures, and analyzes the QT interval and other user-specified parameters like the sodium peak amplitude, or the slope.

The experimental protocol is visualized by icons on the left orange pane of the QT-Screen main window. The icons can be clicked for editing the protocol. Recorded data traces are displayed in the well plate view. You can zoom to any channel by clicking on the corresponding well in the well plate view. The zoomed channel is displayed in the larger single channel view pane. In the single channel view, you can switch between the raw data and the averaged data traces.

The user specified analysis parameters are extracted from the automatically detected waveforms. The detection points are marked with red dots. The parameters are brought into relationship with the compound concentration, resulting in dose-response curves for each test compound.

Compound saving strategies, viability checks, and negative and positive controls are also included in the QT-Screen program. Backups of raw data are saved for later reference. Results are documented in a report sheet and filed into a database.

3.3 Setting Up and Connecting the QT-Screen

1. Connect the QT-Screen directly to the provided **computer** by the provided Ethernet cable. The appropriate connector on the rear panel of the computer is tagged with a "QT-Screen" label.

2. Plug the power cord into the **AC power line input**.

3. Place the free ends of the **system liquid tubing** into a bottle filled with **distilled water**. Make sure the bottle contains enough water so that all lines can be filled. Make sure the distilled water is replaced in regular intervals to avoid contaminations. It is recommended to replace the system liquid daily.

4. Connect the **waste tubing** to the **waste receptacle**. Place the free end of the waste tubing into a **waste bottle**. Make sure the bottle is emptied in regular intervals to avoid spillage. It is recommended to empty the waste bottle before each experiment or at least daily.

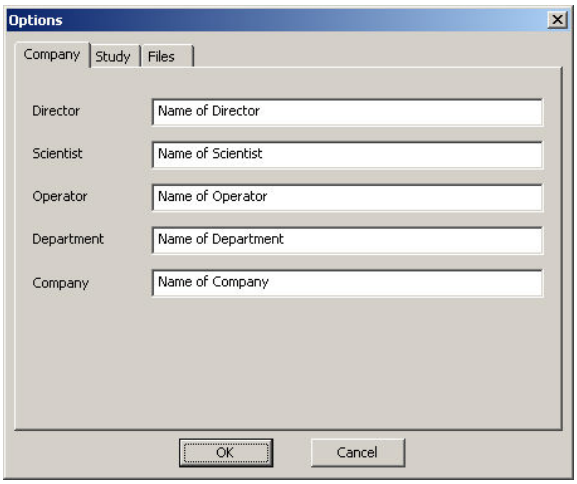
3.4 User Defined Settings

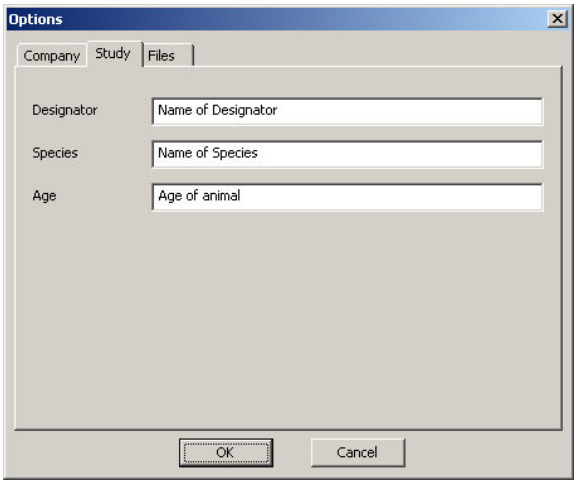
You can enter **default settings** that are specific for your company and department, and for the study and biological material. Each time you set up an experiment, the default settings are loaded into the graphical user interface, and saved together with the data for documentation. (You can still modify them for a particular experiment.)

Also, you need to define the **file paths** for the recorded data.

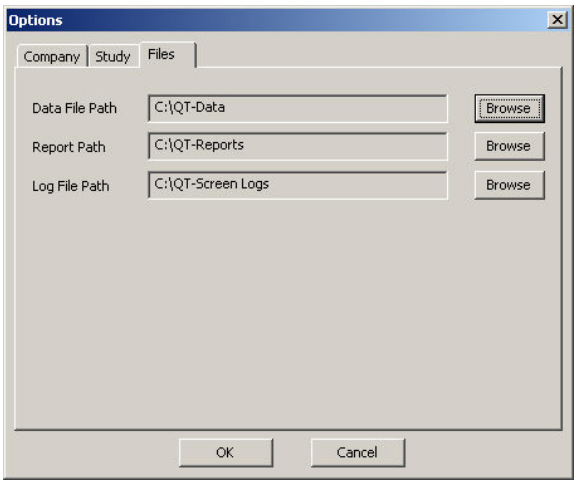
- Click **Options** on the **Edit** menu to open the dialog.

User specific settings





File paths



Data File Path The recorded data is saved to the specified folder.

Log File Path The log files are saved to the specified folder.

Report Path The automatically generated reports (PDF files) are saved to the specified folder.

4 Theoretical Background

4.1 Application

4.1.1 Safety Pharmacology

National and international boards constitute guidance for safety-pharmacology studies. In the year 2002, casualties caused by drug-induced arrhythmias of the heart led to the guideline S7B announced by the FDA. This guidance has been acknowledged by the EMEA and other organizations. The guideline "Safety Pharmacology Studies for Assessing the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals" states that all new pharmaceuticals, even those, that do not target the heart directly, have to tested for an abnormal prolongation of the QT interval.

4.2 Method

4.2.1 Extracellular Recording and Field Potential

Over the last 30 years, **non-invasive extracellular recording** has developed into a widely used standard method.

The semi-permeable lipid bilayer cell membrane separates different ion concentrations (charges) on the inner and outer side of the membrane. Conventional methods measure the membrane potential that results from the electrochemical gradient directly with an **intracellular** electrode. When ion channels are opened due to chemical or electrical stimulation, the corresponding ions are moving along their electrochemical gradient. In other words, the resistance of the membrane is lowered, resulting in an inward or outward flow of ions, measured as a **transmembrane current**.

The extracellular space is conductive as well, and though the resistance is very low, it is not zero. According to Ohm's law (U=R*I), the extracellular current results in a small voltage that can be measured with extracellular electrodes. Extracellular signals are smaller than transmembrane potentials, depending on the distance of the signal source to the electrode. Extracellular signal amplitudes decrease with increasing distance of the signal source to the electrode. Therefore, a close interface between electrode and cell membrane is very important for a high signal-to-noise ratio.

The transmembrane current and the **extracellular field potential** follow the same time course. The field potential is roughly equal to the first derivative of the transmembrane potential, thus revealing an information that is comparable to conventional methods. This has been shown for different types of signals derived from neuronal preparations as well as cardiac preparations.

This convenient method is used by the QT-Screen. Simultaneous recordings of action potentials (with intracellular electrodes) and field potentials (with extracellular electrodes) have shown that there is a linear relationship between the **rise time** of the cardiac action potential (AP) and field potential (FP) as well as between AP and FP **duration**. The contribution of different ionic transmembrane currents can be identified in the shape of the FP waveform as well, as shown in the following picture. The correlation between the waveform components and the ion channel activities was shown by using ion channel blockers or depleting the medium of the respective ions (see reference below).

You can clearly see the rapid component of the **depolarizing sodium current** and the slow calcium current.

The **slow rectifying K^+ current** (IKr) is represented either by a positive or negative peak. The polarity of the peak depends on several parameters, for example, the proximity of the cell layer to the measuring electrode, and cannot be predicted, which does not matter for this assay.

The **field potential duration** corresponds to the action potential duration, which can be correlated to a **QT interval** in an electrocardiogram. It is measured from minimum of the Na^+ peak to the maximum/minimum of the IKr current peak.

Field potential from cardiac myocytes recorded with the QT-Screen

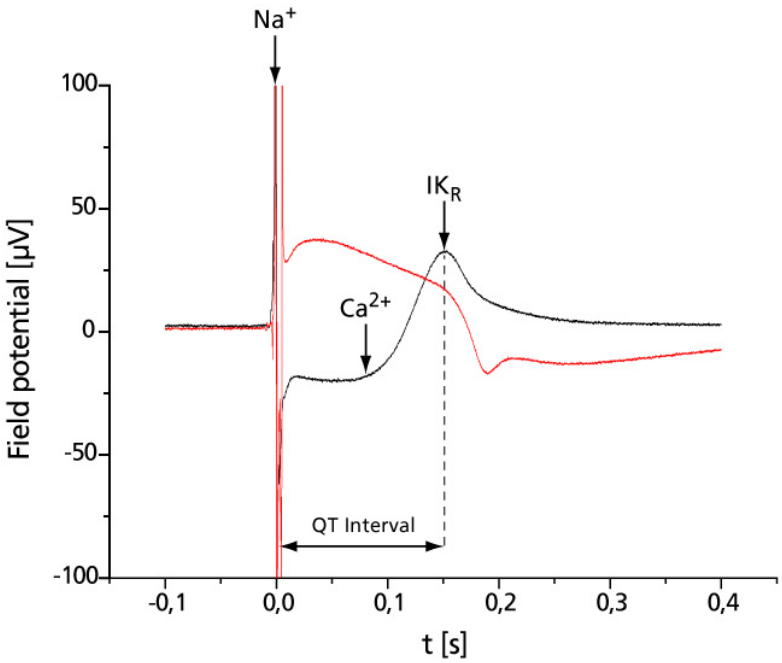


Fig. 1: Identification of waveform components in a cardiac field potential.
The illustration shows two typical raw data traces recorded from chicken cardiomyocytes with the QT-Screen. Waveform components of the black trace are labeled. The red trace shows an example of a field potential recorded from a separate well. The waveform is slightly different; the IKr peak shows the opposite polarity, but all components can be clearly identified as well.

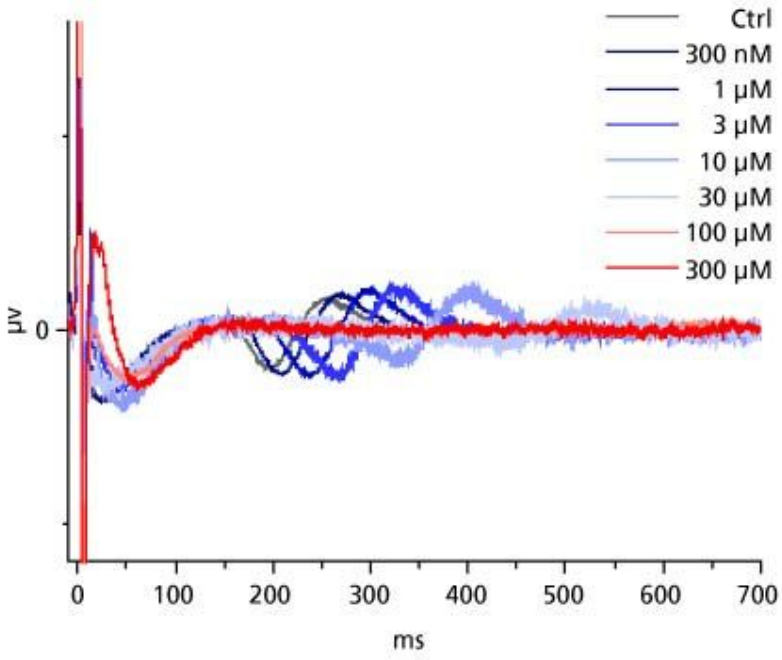
(Reference: "Estimation of Action Potential Changes from Field Potential Recordings in Multicellular Mouse Cardiac Myocyte Cultures", Marcel D. Halbach et al., Cell Physiol Biochem 2003;13:271–284)

4.2.2 QT Prolongation

QT prolongation effects are measured as the prolongation of the field potential duration. The following overlay plot shows a prolongation of the QT interval on chicken myocytes induced by different concentrations of quinidine. Quinidine is used as an antiarrhythmic agent, which predominant electrophysiological effect is the block of the fast inward sodium channels. The drug blocks the rapid rectifier potassium channels (IKr), in an inverse rate-dependent manner and slowly inactivates steady-state plateau inward sodium and calcium currents.

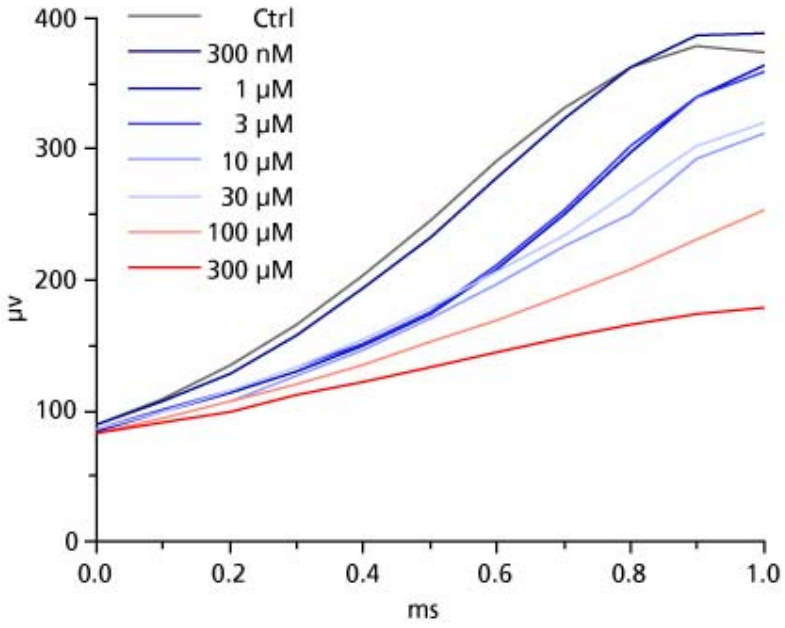
The plot shows that the field potential duration **increases** with increasing concentration of quinidine and that the Ikr is almost completely **blocked** at a concentration of 100 µM.

Quinidine induced QT-prolongation



The next plot shows the same signals but on a much lower time scale. You see that quinidine blocks the fast sodium currents in a concentration dependent manner.

Effects of quinidine on the rapid depolarization



4.2.3 Pharmacological Validation

The major goal of the pharmacological validation of the system was to challenge cultivated cardiac myocytes with a selection of well described drugs and compare the results with data obtained by other, established assay systems. The validations studies have been performed with cardiomyocytes from embryonic chicken ventricles removed after 12–13 days. The cells have been cultivated and recorded on standard QT-Well plates.

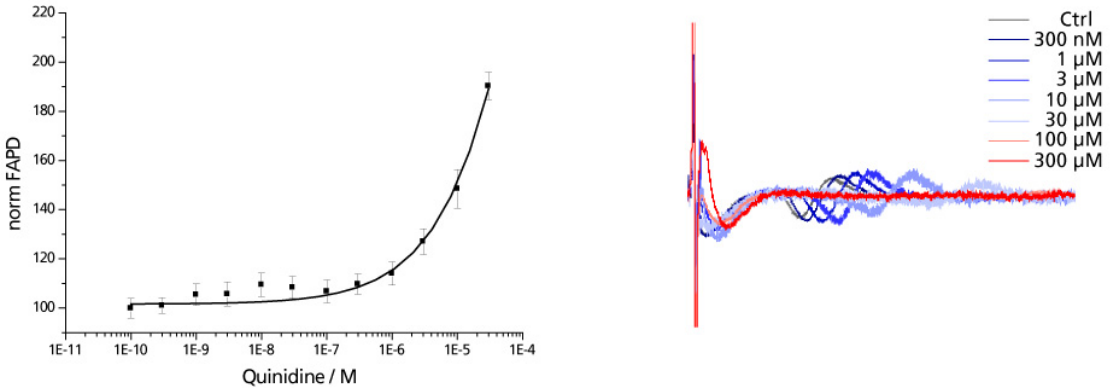
The pharmacological tools used to validate the system have been selected in order to cover various therapeutical classes (for example, antiarrhythmics and antihistaminics).

Drugs that are known to give false positive results in other assays (for example, Verapamil) are specific inhibitors of ionic channel classes (E4031) or are approved drugs that have been taken from the market because of later revealed QT prolonging effects (for example, cisapride). A selection of drugs with a known effect on the ventricular action potential have been tested. In general, the results are in line with the literature and data obtained in established systems. Drugs tested in the validation studies include the following. The illustrations show dose response curves (for n > 8) and typical raw data traces demonstrating the drug effects on the field potential shape.

Antiarrhythmics

- Class IA antiarrhythmic quinidine — fAPD prolongation of more than 200 %. Quinidine also blocks sodium channels and reduces the slope and amplitude of the rapid initial peak.

Quinidine induced QT prolongation

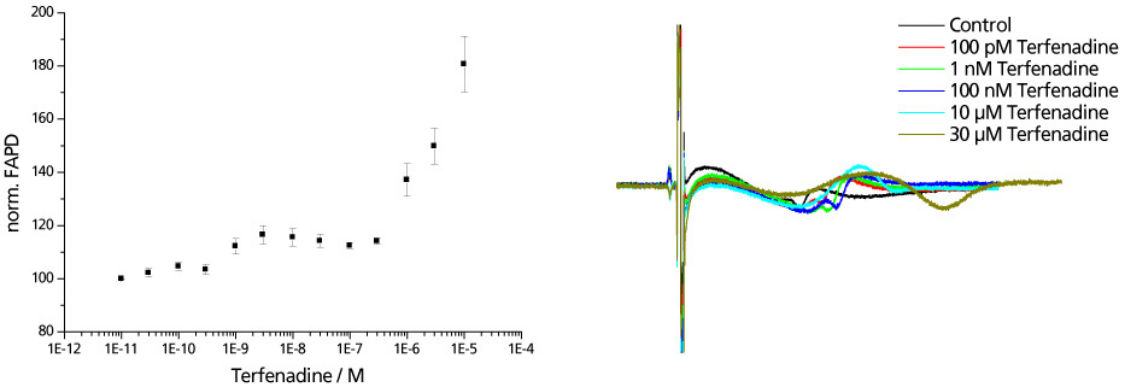


- Sotalole — significant fAPD prolongation (100%)

Antihistaminics

- Terfenadine — fAPD prolongation at nanomolar concentrations

Influence of terfenadine on cardiac field potential

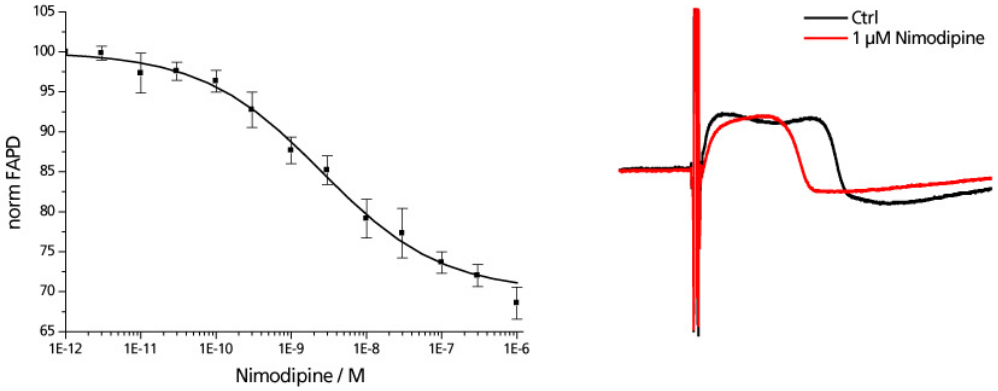


- Astemizol — fAPD prolongation with nanomolar concentrations
- Fexofenadine — no effect

Calcium channel blockers

- Nimodipine used to treat symptoms resulting from a ruptured blood vessel in the brain (hemorrhage) — causes a clear dose dependent shortening of the field potential.

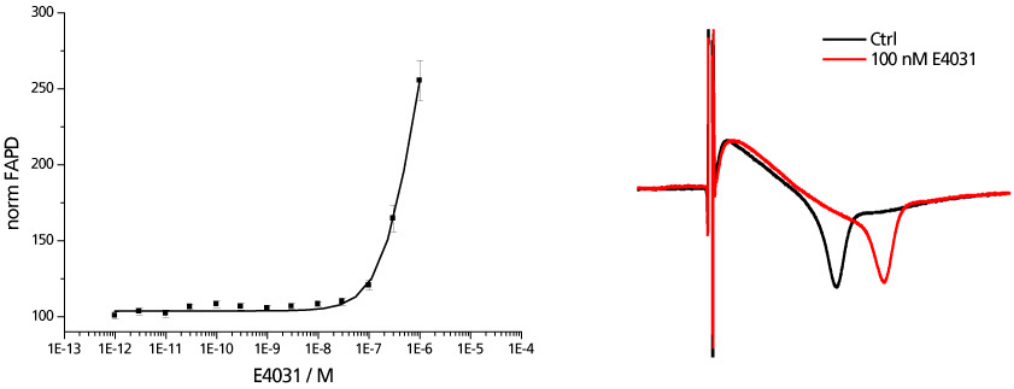
Influence of nimodipine on cardiac field potential



Other

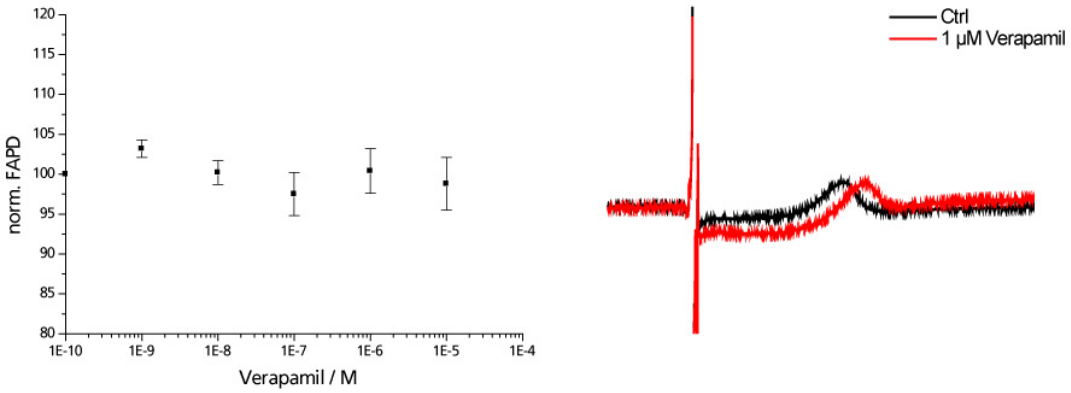
- E4031 as a hERG blocker — nanomolar concentrations cause fAPD prolongation in submicromolar concentrations and arrhythmias.

Influence of E4031 on cardiac field potential



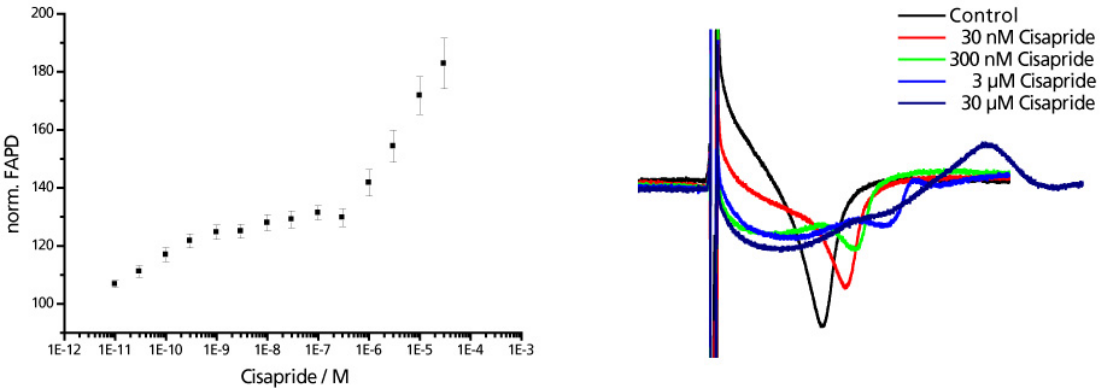
- Verapamil as a false positive in a conventional hERG assay — shows no significant effect with the QT-Screen system.

Influence of verapamil on cardiac field potential



- Cisapride as a drug removed from the market due to QT prolongation — subnanomolar concentrations cause effective fAPD prolongation

Influence of cisapride on cardiac field potential



4.3 Technology

4.3.1 QT-Well Plate

Cardiomyocytes are grown in culture in the QT-Well plates, which are plates with 96 small wells holding a volume of about 250 µl. In the bottom of each well, a measuring and a reference electrode are integrated. The outer golden ring is the reference electrode. A tiny golden measuring electrode with a 100 µm diameter is positioned in the center of each well.

After about 4–5 days in cell culture under standardized conditions like temperature and pH, cells form a heart tissue, which shows a spontaneous electrical activity and rhythmical contractions (like a heart beat), and which is comparable to a native heart tissue in many ways.

The QT-Well plate carrying the cardiomyocyte culture is placed onto the QT-Screen and test compounds are applied to the cell cultures in 12/6 duplicates by an 8-channel liquid handler integrated in the QT-Screen.

4.3.2 Temperature Control

Heating elements are integrated into the QT-Well plate carrier and the lid. The heating is active, but the cooling is passive. Therefore, the minimum temperature is limited by the room temperature.

For cardiomyocytes, you generally need a temperature in the range of 37–39 °C. The heating of the recording chamber is set to 37 °C.

Evaporation is minimized by the mechanics in the lid that open the perfusion holes only during compound delivery and aspiration.

You can adjust the **Wash In** step in the experimental protocol for minimizing temperature effects on the signals.

4.3.3 Data Acquisition

The QT-Screen has an integrated A/D (Analog to Digital conversion) board that converts analog signals in real time into digital data streams at sampling rates of up to 10 kHz for all 96 channels. The sampling rate is high enough for measuring the peak-to-peak amplitude of the fast component.

The digital data streams are led to the data acquisition computer via a protocol. The QT-Screen program controls the recording and the analysis of the recorded data.

4.3.4 Data Analysis

The QT-Screen program detects the fast sodium peak automatically and uses it as a reference point for the subsequent data analysis. You can define a threshold value for the detection of the peak, that is, all peaks that cross the threshold are detected. The other option is to detect the peak in relation to the noise level. In this case, the standard deviation of each data trace is used to compute the detection threshold. A time interval of 1 s is used to calculate the standard deviation. You define the factor (usually between –5 and –20), by which the standard deviation is multiplied to set the threshold.

Detected cardiac field potentials are then aligned and averaged to increase the signal-to-noise ratio and to make the subsequent QT detection more reliable.

The minimum or maximum following the sodium peak is used for detecting the IKr component of the waveform. The QT interval is detected as described in the chapter "Extracellular Recording and Field Potential" and plotted against the dose of the test compound.

The rise time of the falling edge of the sodium peak is analyzed as well.

4.3.5 Liquid Handling

The liquid handling of the QT-Screen is derived from an approved technology from Tecan. It is a volume-based 8-channel liquid handler.

5 Setting up an Experiment

5.1 Preparations

1. In the **Manual Robot Control** dialog box, use the **Fill System** command at least once or twice for filling the system with the system liquid (distilled water).
2. Optically control the tubing system. It should be filled completely without visible air bubbles. Use the **Fill System** command again if any air bubbles are visible.
3. You can use the manual controls for making pre-dilutions of the compound stock solutions, if necessary. The final dilutions will be made during the experimental protocol.
4. Fill the buffer reservoir with recording buffer.
5. If using the quality check feature, fill a column of a well plate (not the compound plate) with the working solution of the standard reference compound. Place the well plate on one of the free well plate slots. Hint: You can use the manual robot controls for preparing the working solution in the wells.
6. If using an aeration: Make sure the CO_2 valve of the QT-Screen is closed and adjust the pressure of the CO_2 bottle. The pressure should be set just slightly above atmospheric pressure. You should hear a minimal outflow when opening the valve.
7. Place the QT-well plate onto the amplifier and close the lid.
8. If using an aeration: Use the manual controls to open the CO_2 valve. There should be practically no flow while the recording chamber is closed.

5.2 Running an Experiment

The graphical user interface of the QT-Screen is easy to use: Just enter the settings for the experimental protocol from top to bottom on the left pane of the main window (**State** pane). You can then save the customized protocol as a template for future experiments.

The following provides an overview on the experimental protocol. For more information, please follow the links.

Warning: Do not enter the QT-Screen robot with your hands during operation. The moving needles can lead to severe injuries.

1. Click **Open Template** on the **File** menu to open a preset experimental protocol.
2. Click **Settings** and enter any information on the experiment into the lab book.
3. Perform the **Viability Test** and select all wells containing viable cardiomyocyte cultures. The data from the viability test will not be saved to hard disk.
4. Fill in the compound information, and pipet the specified volume of stock solutions into the **first column** of the compound plate, that is, A1, B1, C1, and so on. The final dilutions will be made during the experimental protocol.
5. Enter all experimental settings, like buffer reservoir / dilution settings, wash in time, recording settings, analyzer settings, and report generator settings.
6. (Optional) Run a quality check and deselect all wells that do not meet your quality criteria. The data from the quality check will be saved together with the data. Therefore, it is not possible to change any experimental protocol settings after a quality check was performed.

7. You can review the averaged data directly after the experiment with the **Inspect** feature. You can also review the averaged and the raw data traces later with the QT-Analyzer program.

5.3 After the Recording

The following is recommended for preventing microbial growth and for extending the lifetime of the tubing.

Important: Make sure that the ends of the system liquid tubing are placed into a waste bottle before using the **Empty System** feature. The system liquid will **not** be emptied into the standard waste bottle (through the pipet tips), but in the reverse direction.

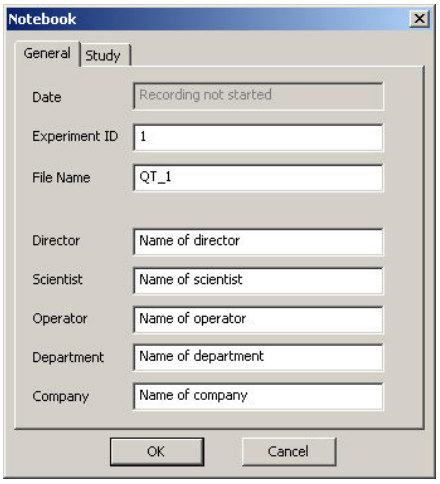
1. Remove the compound plates and the QT-Well plate from the QT-Screen. Wipe all surfaces with 70 % alcohol for preventing contaminations.
2. In the **Manual Robot Control** dialog box, use the **Fill System** command at least once or twice for flushing the system with the system liquid (distilled water).
3. Empty the waste bottle.
4. After the last experiment run on a working day, click **Empty System** once or twice until the system liquid is removed.
5. Replace the system liquid bottle with 100 % alcohol, and use the **Fill System** command at least once or twice to fill the tubing with alcohol.
6. Incubate for 10 min.
7. Click **Empty System** once or twice until the system liquid is removed.

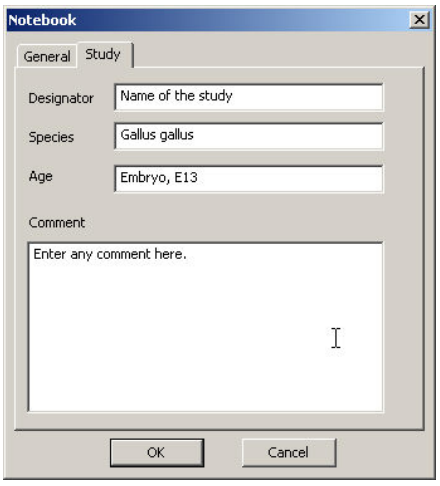
5.4 Experimental Protocol Settings

5.4.1 Lab Book

Please enter all information relevant for the experiment, like the date, experiment ID, and information on the biological test model into the virtual lab book. The information will be saved together with the data, and will be used for generating reports.

- Click the **Settings** icon to open the **Lab Book**.
  Company-specific default information will be loaded from the Options dialog box.



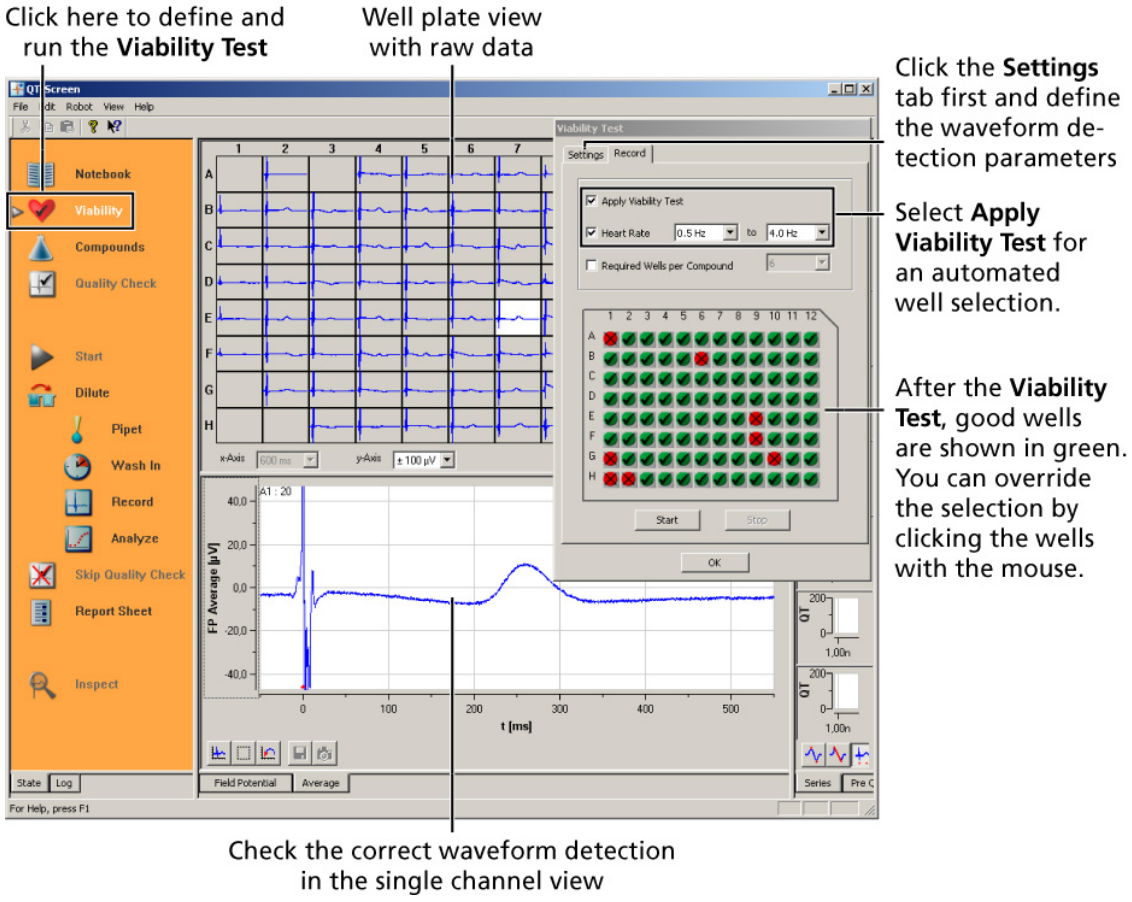


5.4.2 Viability Test and Waveform Detection

First, a viability test guarantees that the cell cultures are fine and compounds are not wasted on non-viable cells. You start and stop the viability test manually. During the test, the beating frequency and the amplitude of the R wave are monitored and evaluated automatically by the system, but the user has also the option to control the system before starting an experiment. You can control whether the general signal quality is sufficient for detecting a QT interval, and whether the signal activity is stable for a sufficient number of replicates.

Based on the results, the user can define which QT-Wells should be used and which not. Based on customizable parameters, the QT-Screen program suggests which QT-Wells should be used and which not. You can change the selection manually if you disagree with some results of the test.

Viability Test



Recording and analysis parameters

You can adjust the parameters that are used for discriminating the desired field potential waveform from the raw data stream. The sodium current is detected by the rapid change in the field potential. You can either define a **fixed value** for the minimum field potential change (ΔV), for example, –60 µV, or use the **standard deviation** to detect a significant change in the field potential. The standard deviation is used to estimate the individual minimum change for each channel separately. A time interval of 1 s is used to calculate the standard deviation. You set the factor, by which the standard deviation is multiplied. The rise time is used to detect only signals that show the expected kinetics of the waveform component.

To compensate individual differences in the noise level of channels, it is recommended to use the standard deviation as detection parameter. For example, if the noise level of a well is 10 µV peak-peak, that is, a standard deviation of about 5 µV, and you expect a minimum sodium amplitude of –100 µV, you can use a standard deviation of –20. The data is scanned for a situation, where the minimum amplitude (either fixed or relative to the standard deviation) is met and the rise time is in the defined range as well. Limit the rise time range to exclude artifact signals that are in the range of the defined amplitude. As the sodium peak is generally very fast, small rise times such as 100–400 µs can usually be used. The higher you define the FP change parameter, the higher you need to set the rise time as well, as a higher FP change needs longer than a smaller one.

The raw data signals are averaged to increase the signal to noise ratio. The averages are triggered on the maximum of the sodium peak. See also "Analyzing Data". The length of the signal waveforms that is used for the averaging is specified by the **Average Interval**. The **Average Interval** should be shorter than the interspike interval, but longer than the longest expected field potential.

Field potential detection

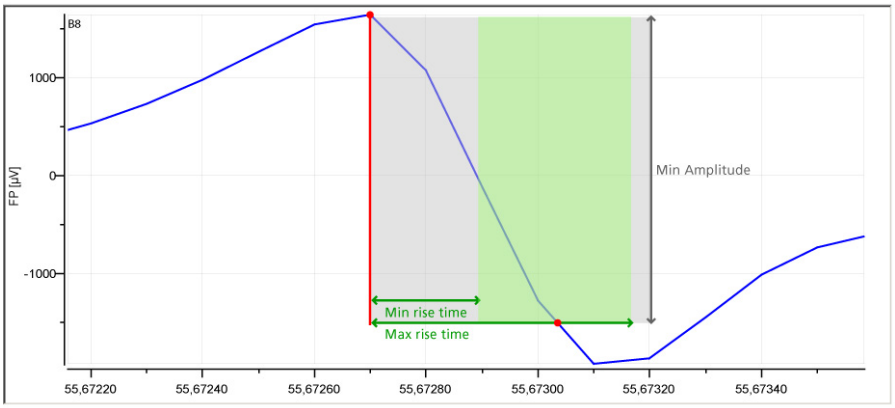


Fig. 2: Automated waveform discrimination.
*This illustration shows how the QT-Screen routine detects the fast sodium peak by the three parameters **field potential change**, **minimum** and **maximum rise time**. The software scans the recorded data points, and it checks whether following data points meet the required criteria. For example, when the waveform detection routine arrives at the data point marked with the red dot in the figure, the following data points meet the **amplitude** criterion (shaded in gray). The signal reaches this field potential change in the preset range of the **rise time** (shaded in green). The data point, at which both the FP change and the rise time criteria are met is marked with a second red dot in this figure. Would the signal be smaller than the minimum FP change or faster or slower, it would not be detected. In other words, the signal needs to cross the bottom border of the gray shading and the green shading at the same time.*

Any signal component that matches the criteria will be detected. The FP change parameter is the main parameter that should discriminate biological signals from noise. The rise time parameter ensures that biological or artifact signals that meet the FP change criterion, but have a shorter or longer rise time than the sodium peak, are not detected.

Frequency (Hz) The range of the signal frequency that is acceptable. Standard values are between 0.5 Hz and 4 Hz.

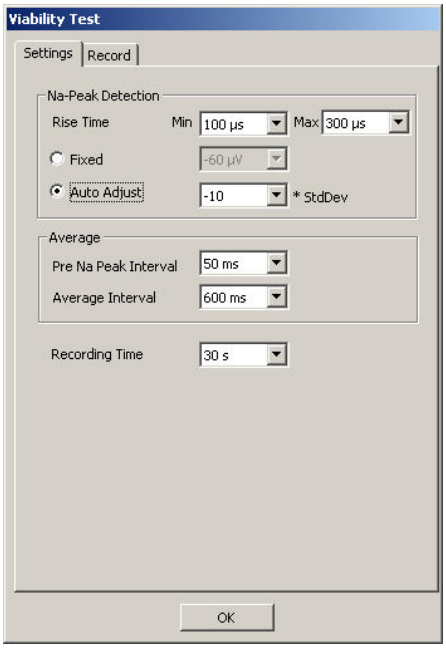
Running the viability test

Important: Please check the **signal detection parameters** and the length of the **averaging interval** very carefully before starting an experiment. Inappropriate parameters will lead to a wrong signal detection and to wrong results. Please take into account that the signal waveform may change during the experiment.
Too rigid settings will lead to **signal loss**, that is, the cardiac signal is not detected at all in some or all wells.
Too loose settings will lead to a **wrong signal detection**, and thus to wrong results, especially of the sodium amplitude and slope analysis, and to a mismatch in the overlay plots.

Note: Please note that deselecting **single** wells will significantly **increase** the time needed for compound application / pipetting. Deselect only **whole** columns on the QT-Well plate to **minimize** the pipetting time.

1. Click the **Viability** icon to open the **Viability Test** dialog box.

2. Select a test time and the Na^+ peak detection parameters on the **Settings** page. If you are doing your first experiments with the system, you should rather select not too strict parameters (for example, a **threshold** of **–60 µV** or a **StdDev** of **–5**, **min rise time** of **100 µs** and **max rise time** of **700 µs**) in order to get as much signals as possible. Once you have established the system in your lab, you can stepwise increase the strictness of the detection in order to optimize your results. Make sure to select an averaging interval according to the expected interspike interval. Please take into account that the signal length will possibly increase due to QT prolongation during the experiment. For example, for a standard culture with 1 Hz signal rate, an averaging interval of **800 ms** is recommended.



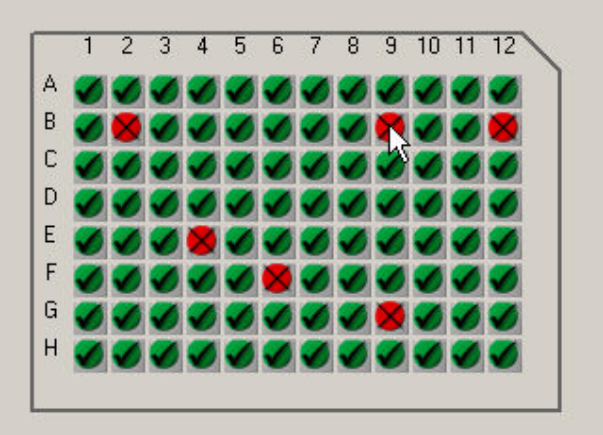
3. Deselect all empty wells (if applicable) by clicking on the well icon or the column or row number for **deselecting complete columns** or **rows**.

4. Click **Start** to run the test.

5. Monitor the traces on the screen. Use the display functions to zoom traces and check the signal and waveform detection quality. Especially, check for a correct **detection** of the sodium peak and an appropriate length of the **averaging interval**. If the averaging interval is **too short**, you will not be able to detect the **end** of the field potential, especially, if the test compounds will prolong the QT interval. If the averaging interval is **too long**, you will get detect **multiple** signal as a single event (and thus miss the additional events in the averaging interval).
   If the **Apply Viability Test** option was selected, the QT-Screen program evaluates the data and suggests a selection of viable cultures.

6. Simply click on a well icon to change the state of the well from accepted to not-accepted, if necessary. If you are unsure about the results, you can select a longer test time and click **Start** to repeat the test.
   Wells that are marked as not-accepted will not be used for the experiment. If there are too few accepted wells in a row, the complete row will not be used for the experiment. You can specify the number of **required** replicates for each compound.

7. If you want to **reselect** a well, click the icon again to toggle the state of the well. For reselecting **complete columns** or **rows**, hold down the **SHIFT key**, and click the column or row number.



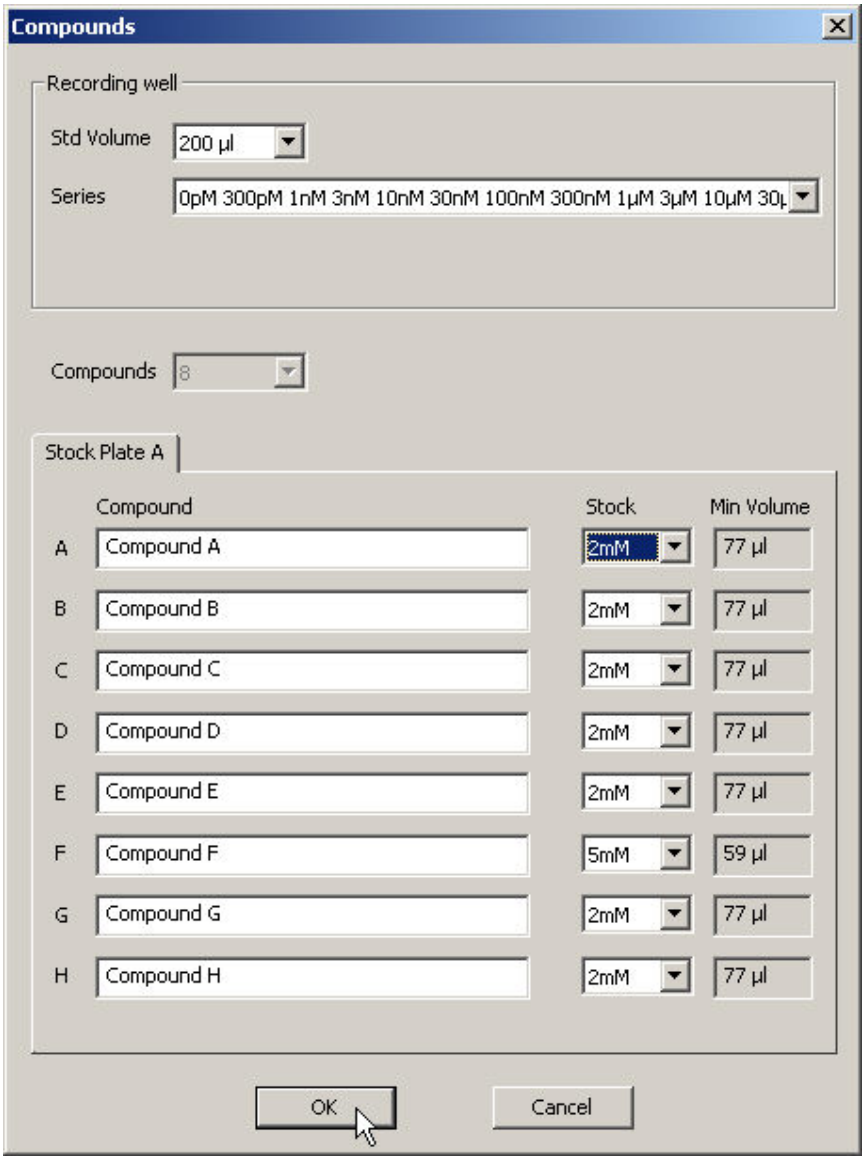
5.4.3 Compound Settings and Stock Solutions

8 or 16 different test compounds can be applied to a single QT-Well plate. You can choose between three positions for the active compound plate. The compounds have to be filled into the **first column** of compound plates, that is, A1, B1, C1, and so on. The dilutions are then prepared using the empty columns of the well plate.

The final test solutions are then applied to the cell cultures starting from the lowest concentration to the highest, each compound into a single row.The same concentration is applied to all replicates in a row. The dose response series is applied consecutively to the same wells, starting with the lowest concentration.

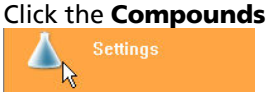
In the **Compound** dialog box, you specify the compound names and stock concentrations. The compound names and test concentrations are automatically filed into the database. The volume of stock solution that has to be filled into the first well of the compound plate is calculated for your convenience.

In the current version of the QT-Screen program, only **8 compounds** and a **log 10** and a **log 3 concentration series** are supported. Please select the concentration series first, as the required stock concentrations depend on the final concentrations.



Definition of compounds and stock concentrations

1. Click the **Compounds** icon to open the **Compounds** dialog box.



2. Select the **Std volume**, that is, the total volume in each well during the experiment. (Standard is 200 µl.)

3. Choose a scheme for the **concentration series** from the **Series** list. When using a **Custom** series, enter the doses into the text field and click the **Set** button to confirm the settings..

4. Enter a **compound name** for each accepted (see Viability Test and Waveform Detection) row from A to H.

5. Specify the **stock concentrations**.

6. Pipet at least the **minimum compound volume** as specified in the dialog box into the appropriate wells in **column H** of the compound well plate.

Defining a custom dose response series

You can use the **Custom** series feature to define longer or shorter dose response series, or enter custom doses. The following requirements need to be fulfilled. Otherwise, the system does not support the pipetting step, and an error message will show up. See also chapter "Error Messages" for an explanation of possible error messages.

Minimum dilution factor between two following concentration steps	1 : 2
Maximum dilution factor between two following concentration steps	1 : 50
Minimum number of doses	2
Maximum number of doses	11
Supported **measuring units**	pM, nM, µM

The series must start with the **lowest** concentration and the concentrations must be entered in **increasing** order.

Doses must be separated by a **space** character.

The number and the measuring unit must **not** be separated by a space character.

Click the **Set** button to confirm the settings. Otherwise, your changes will **not** be applied.

Compound saving strategies

It is recommended that you use about six QT-Wells per compound to obtain a statistical relevant result. You can define the minimum number of viable cell cultures that are needed for testing a compound by selecting a number from the **Required wells/compound** list. If the number of QT-Wells that can be used in a row, that is, accepted in the **Viability Test** and the **Quality Check**, is lower than the selected number, the compound assigned to that row is not applied. You need to test this compound on another QT-Well plate.

The program suggests the volume of the stock solution that should be filled into the compound plate individually for each compound. It is calculated based on the stock and test concentrations, and on the outcome of the viability test and quality check. Thus, you need only the volume that is actually used with essentially no compound wastage. For example, in the preceding screen shot, the program suggests a minimum volume of 59 µl for compound F (in comparison to 77 µl for the other compounds), because the stock concentration of compound F is higher. Wells that were not accepted during the viability test also decrease the required volume.

If the compound is not a limiting factor, you may prefer to fill a standard volume of for example 200 µl into each well for saving time and work, regardless of the cell viabilities and the actually required volume.

5.4.4 Quality Check

You can apply a substance with a known effect as a positive or negative control before and/or after running the experiment. This way, you can exclude any external influences like temperature effects that may affect your results. It is recommended to use a compound and a dose that will not permanently damage the cultures. A typical positive control would be, for example, 10 µM Quinidine or 3 µM Astemizole. You can then accept only wells that show the expected behavior, for example, a compound effect greater than 20 %.

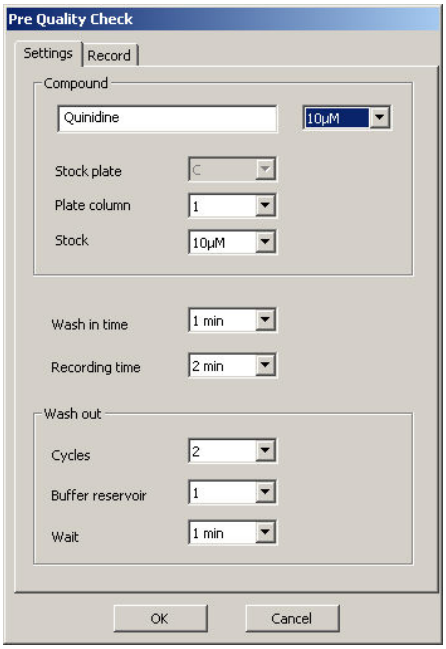
This step is optional. You can deselect the **Quality Check** by deselecting the option **Enable Quality Check**.

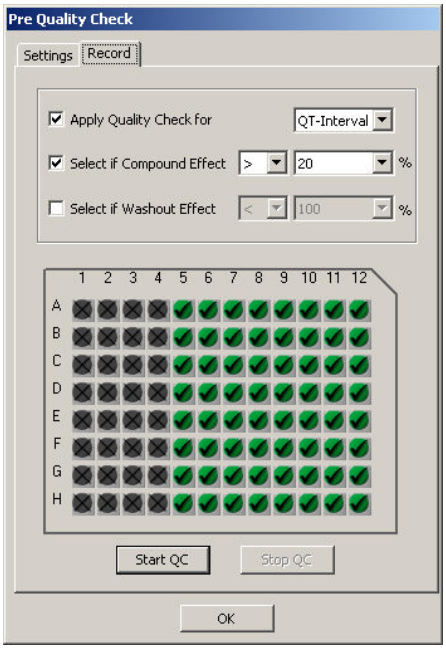
A second quality check after the compound testing will be supported by future software versions.

Note: The data recorded during the quality check will be saved in the data file. Therefore, you cannot change any critical parameters like the sampling rate anymore after starting the **Quality Check**. Check all parameters before starting the **Quality Check**.

1. Under **Compound**, enter the compound name and the test concentration.
2. Specify the **Plate** position (generally a different plate is used than for the test compounds), the **Well Plate** column, and the **Stock** concentration.
3. Enter the wait time between application and recording (**Wash In**).
4. Enter the **Recording** time.
5. Define the **Wash Out**: Specify the number of cycles of removing and adding buffer, the **Buffer Reservoir**, and the **Wait** time between each cycle.
6. If you want to automatically accept only wells that show the expected behavior, select the option **Apply Quality Check** on the **Record** tabbed page. Select the parameter of interest.
7. Select a threshold for the **compound effect** and/or for the **washout efficiency**.

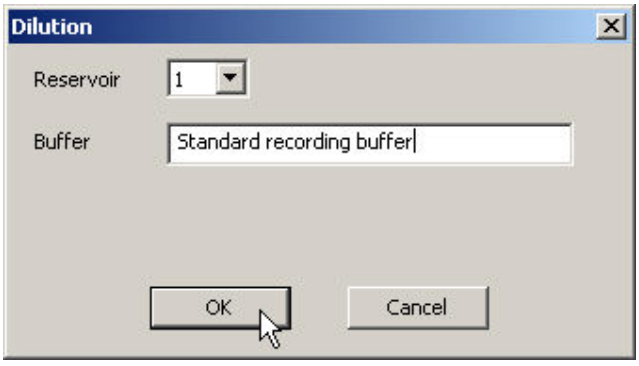
8. Click **Start QC** to start the **Quality Check**.
The compound will be washed in and washed out according to the settings. If the option **Apply Quality Check** is selected, all wells that do not match the requirements will be automatically excluded from the recording protocol. Otherwise, you may make your own choice of accepted wells.





5.4.5 Preparing Dilutions

The QT-Screen prepares the concentration series fully automatically according to the specified stock and test concentrations.



1. Specify the number of the **Buffer Reservoir**. Buffer from the specified reservoir is used for preparing the dilutions. Check that the reservoir is filled with buffer.
2. Specify a **name** for the buffer used for the dilutions (for documentation).

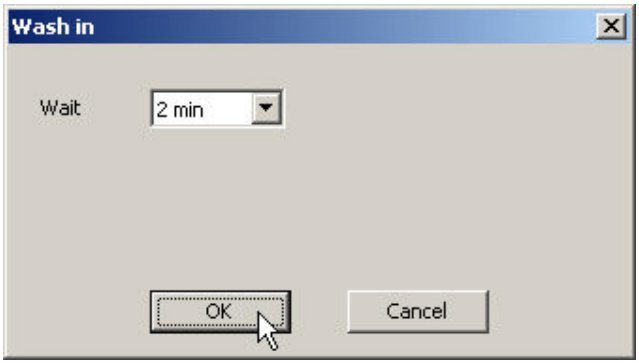
5.4.6 Wash In

After the compound application, the cell cultures are incubated for a user-specified time mainly to warm up the bath solution after compound application and to allow the cells to recover from the temperature and mechanical shock inflicted by the compound application. It may also take some time until the test compounds show some effect.

It is recommended that the required **Wash In** time is estimated empirically once by the user by performing an experiment with the buffer alone, without any test compounds. Generally, a **Wash In** time of two minutes should be sufficient.

Note: Please note that the compound application time can vary, according to the concentrations and the selected wells. Compound application on a full plate can take up to 8 min. Therefore, wash in times vary from the wells where the compound was applied first to the last wells. An appropriate **Wait** time should compensate for the delay.

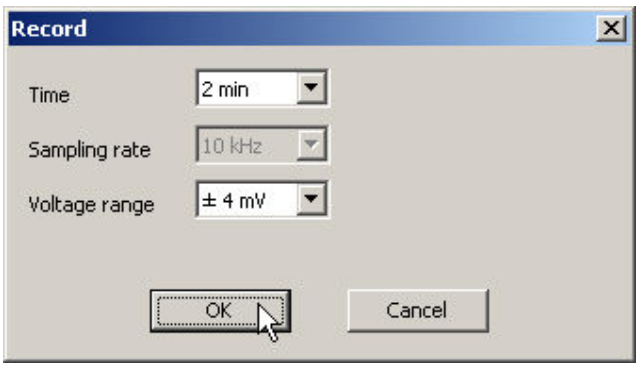
Tip: If timing is critical and you want to spare compound application time, select the **same** wells in **each** row, that is, deselect **whole** columns on the QT-Well plate. Otherwise, the pipetting algorithm gets more complex and time consuming.



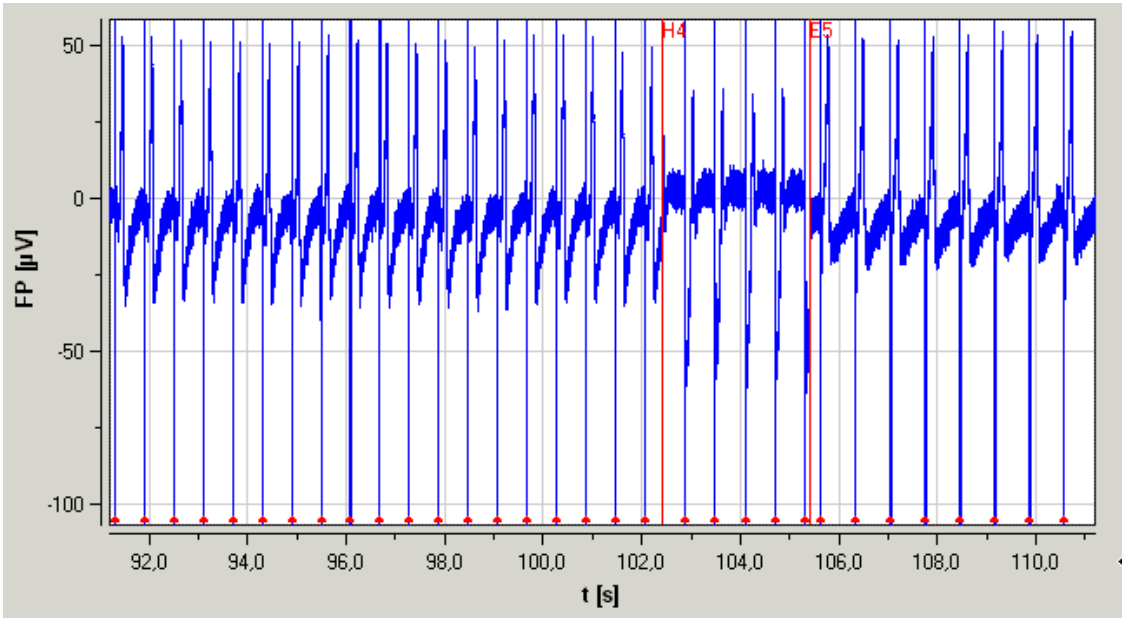
- Specify the **Wait** time in minutes from the drop-down list.

5.4.7 Recording

Data from all QT-Wells that have passed the viability test is recorded and displayed on the screen. Raw data is stored on the hard disk. You can specify the **recording time**, and the **sampling rate** and **voltage range** of the data acquisition card.



5.4.8 Real-Time Monitoring



Well plate view

The virtual well plate view serves as a virtual oscilloscope. You can monitor the ongoing recording in real-time on all recording wells. Wells that failed the Viability Test or the Quality Check will not be recorded, and thus also not displayed on the screen (see gray empty slots).

You can adjust the overall x- and y-axis ranges in the well plate view.

Single channel view

You can zoom to a single recording channel by clicking on a well. The selected well is highlighted. The zoomed in channel is displayed in the single channel view below the well plate view. You can toggle between the **Field Potential** and the **Average** display by clicking the appropriate tab.

Single channel view toolbar

	**Zoom to fit**	Adjusts the ranges of the axes to fit all data of the current recording.
	**Zoom box**	Zooms in a user selected region of the display.
		
	**ASCII export**	Exports display contents in ASCII file format. Only available after recording protocol was completed.
	**Graphics export**	Exports display contents in a graphics file format. Only available after recording protocol was completed.

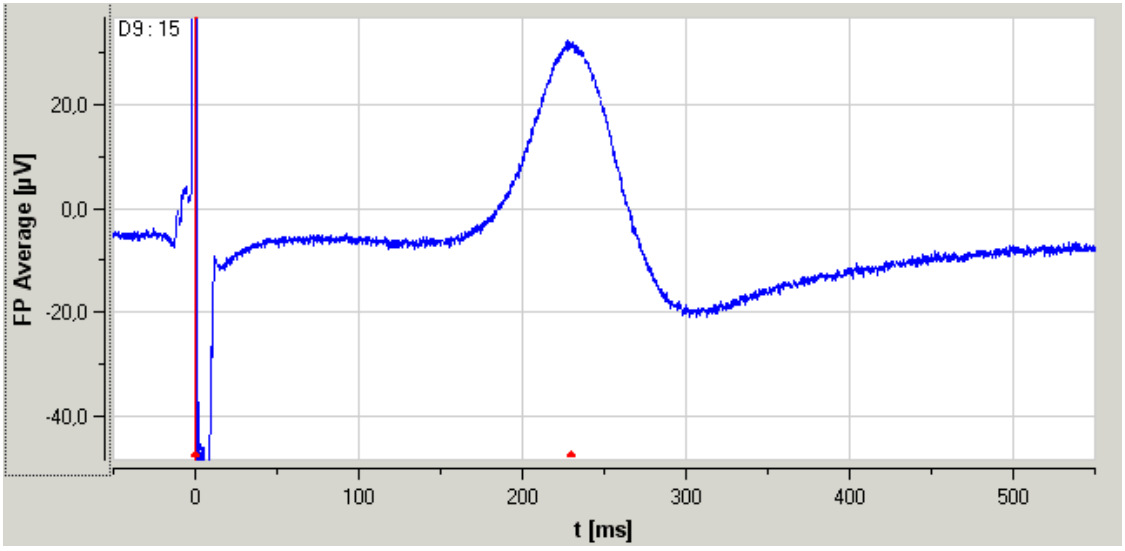
Field Potential display

This display shows the ongoing activity in the selected well in real time. Each time you switch to another well, a marker in the Field Potential display indicates the well number. The detected sodium peak is marked by a red dot.

Averages display

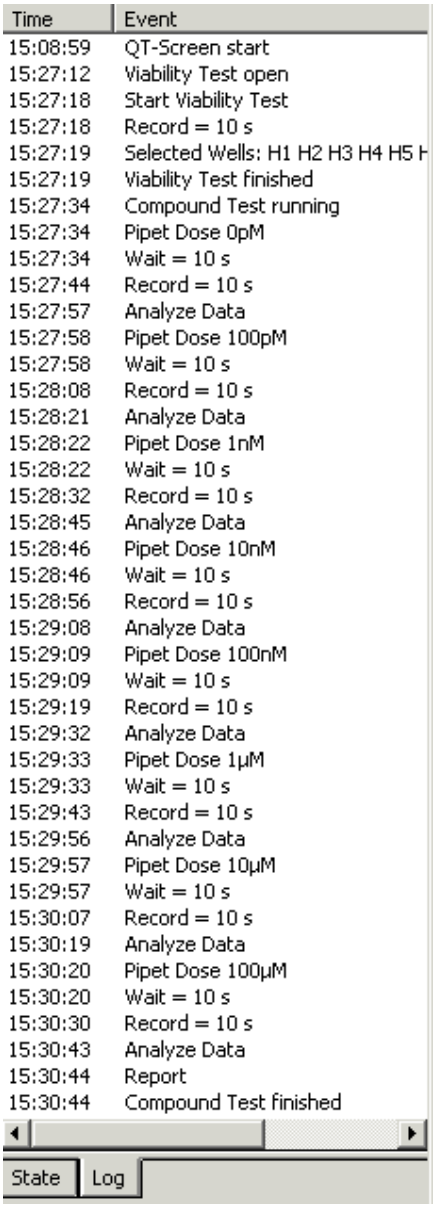
After the recording was completed, the average of all detected waveforms are averaged for each well. You can toggle between the **Field Potential** and the **Average** display by clicking the appropriate tab.

Please note that the Average display of a well is empty if no signal was detected, for example, after a cell culture died due to a higher concentration of an effective test compound.



Log

All QT-Screen actions are documented in the **Log**. Use the **Log** view to inform yourself on the recording process, and use the information for troubleshooting, for example, if you need to contact the technical support.



Time	Event
15:08:59	QT-Screen start
15:27:12	Viability Test open
15:27:18	Start Viability Test
15:27:18	Record = 10 s
15:27:19	Selected Wells: H1 H2 H3 H4 H5 H
15:27:19	Viability Test finished
15:27:34	Compound Test running
15:27:34	Pipet Dose 0pM
15:27:34	Wait = 10 s
15:27:44	Record = 10 s
15:27:57	Analyze Data
15:27:58	Pipet Dose 100pM
15:27:58	Wait = 10 s
15:28:08	Record = 10 s
15:28:21	Analyze Data
15:28:22	Pipet Dose 1nM
15:28:22	Wait = 10 s
15:28:32	Record = 10 s
15:28:45	Analyze Data
15:28:46	Pipet Dose 10nM
15:28:46	Wait = 10 s
15:28:56	Record = 10 s
15:29:08	Analyze Data
15:29:09	Pipet Dose 100nM
15:29:09	Wait = 10 s
15:29:19	Record = 10 s
15:29:32	Analyze Data
15:29:33	Pipet Dose 1µM
15:29:33	Wait = 10 s
15:29:43	Record = 10 s
15:29:56	Analyze Data
15:29:57	Pipet Dose 10µM
15:29:57	Wait = 10 s
15:30:07	Record = 10 s
15:30:19	Analyze Data
15:30:20	Pipet Dose 100µM
15:30:20	Wait = 10 s
15:30:30	Record = 10 s
15:30:43	Analyze Data
15:30:44	Report
15:30:44	Compound Test finished

State | Log

5.4.9 Analyzing Data

Field potential waveforms are detected automatically by the QT-Screen program and averaged, that is, the waveforms are **aligned** by the maximum of the fast sodium peak and added up successively. Averaging signals reduces the noise level and enhances the signals, because noise usually stems from arbitrary signals, where "real" signals will generally follow a regular pattern. Therefore, the added up noise signals cancel each other out and the biological signals will improve. The **QT interval**, the **amplitude** and the **slope** of the **fast sodium peak** are extracted automatically from the averaged traces. Please see chapter Viability Test for more information on signal detection.

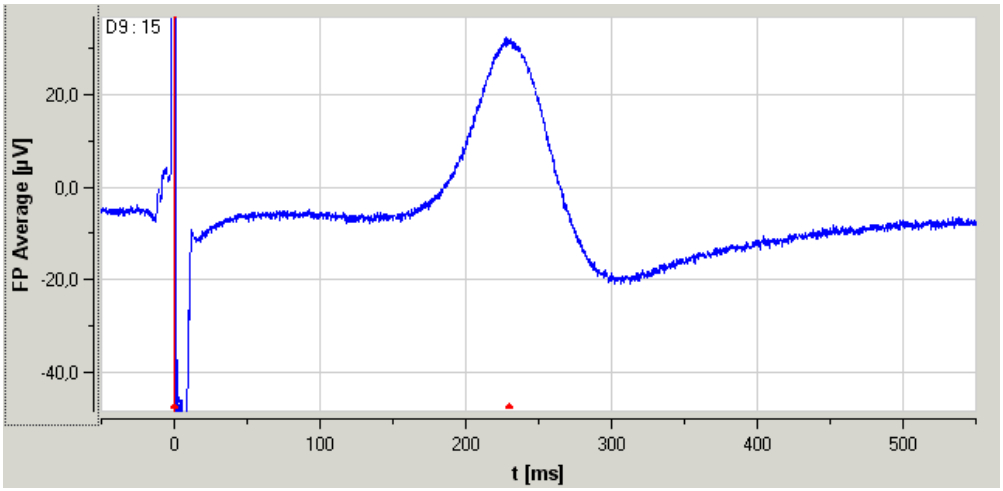


Fig. 3: ***Averaged waveform.***
The numbers in the upper left corner are the well number (D9 in this case) and the number of waveforms used for the averaging (15). The higher the number of waveforms used, the higher is the signal to noise ratio. The start and end point of the detected QT interval is marked with a red dot on the time axis after the averaging has been completed (that is, at the end of the recording).

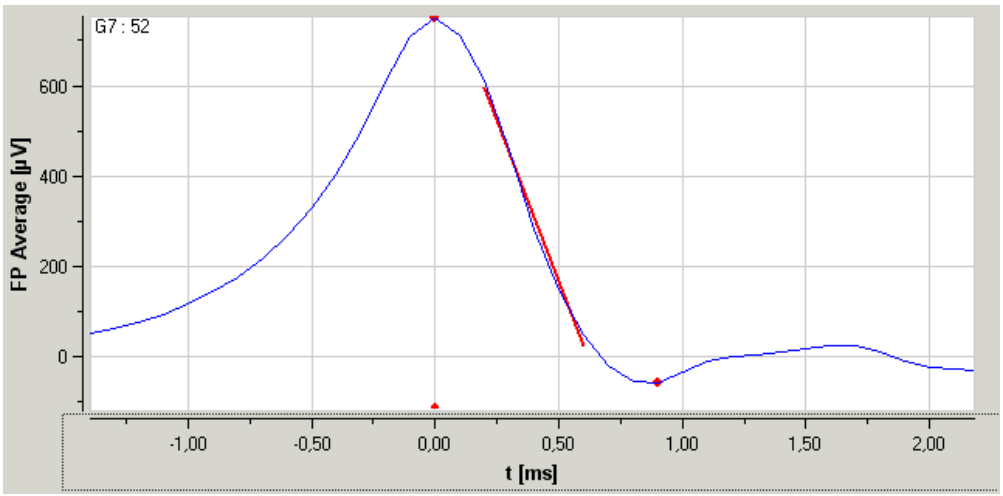
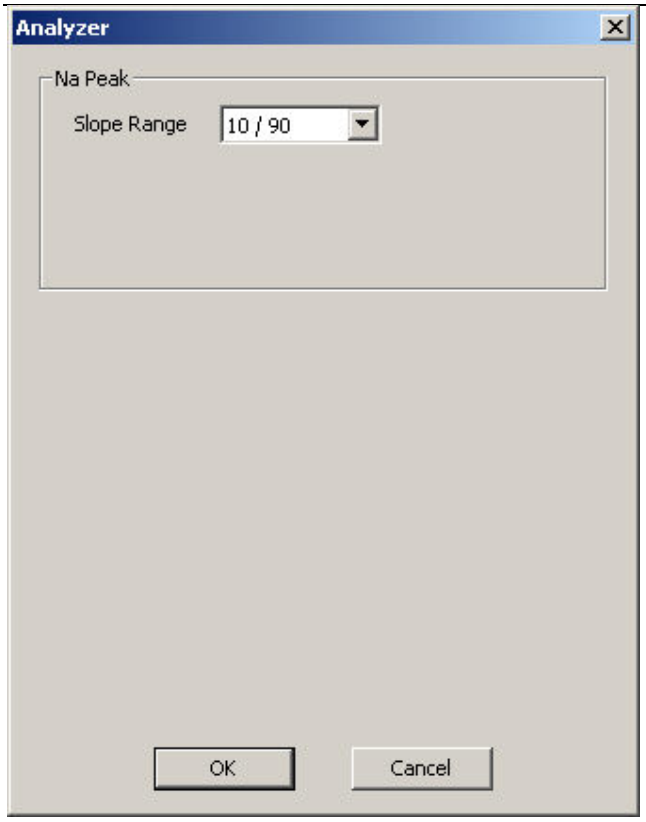


Fig. 4: ***Zoomed in sodium peak.***
The minimum and maximum of the sodium peak are marked with a red dot. The fast signal component is fitted by linear regression for analyzing the slope. You can extract the slope from the minimum and maximum of the sodium peak (0/100) , from 10–90 % as shown in the screen shot, or from 30–70 % between max and min points by choosing the appropriate parameter of the ***Analyzer****.*



Dose response analysis

The parameters extracted from the averaged data are plotted automatically as dose response curves in real time during the experiment. The standard deviation is plotted as **error bars**. See chapter "Display Settings" for more information on the zoom and display features.

You can switch between three different parameters of interest:

 Amplitude (peak to peak) of the sodium peak

 Slope of the sodium peak according to the **Analyzer** settings

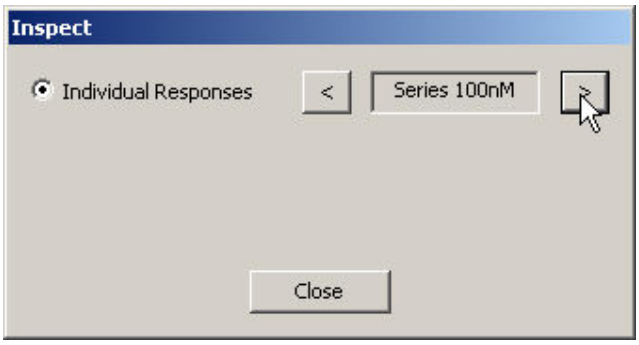
 Field potential duration = QT interval

5.4.10 Reviewing Data After the Experiment

With the **Inspect** feature, you can review the averages from an experiment immediately after the experiment, before starting a new one. It is recommended to roughly check all results after an experiment, to exclude that were any general problems with the recording or the waveform detection.

1. Click the **Inspect** icon to open the Inspect dialog box.

2. Click the arrow buttons to move through the viability test data, or the compound applications sorted by concentrations. Please note that **only** the **averages** are updated accordingly. Use the **QT-Analyzer** for reviewing the raw data, if necessary.



3. Click any well to select the individual recordings and use the scroll and zoom features of the displays for reviewing the averaged traces.

4. You can use the ASCII or graphics export features for extracting the displayed graphics.

6 General Software Features

6.1 Manual Control of the Liquid Handling

The manual control of the liquid handling allows standard procedures for filling, testing, and cleaning the perfusion system such as priming the lines, rinsing the tubing, and so on.

For more information on the operation of the Tecan MiniPrep 60 liquid handler, please consult the MiniPrep Operator's Guide from Tecan.

Warning: Do not enter the QT-Screen robot with your hands during operation. The moving needles can lead to severe injuries.

- Under **Robot** on the menu, click **Manual Control** to open the **Manual Robot Control** dialog box.

In the following list, you will find a description of the single commands.

Arm to component	*Moves vertical arm to components of the QT-Screen.*
Reset	Vertical arm performs reset movement.
Rinser	Vertical arm moves to pipette rinsing station.
Waste	Vertical arm moves to waste receptacle.
Reservoir	Vertical arm moves to selected reservoir 1, 2, or 3.
Plate	Vertical arm moves to the selected column 1 to 12 of the selected compound plate A, B, C or of the recording plate R.
Arm to height	*Moves vertical arm to different heights relative to the well plate.*
Travel	Vertical arm moves to the default travelling height, that is, a height that is higher than the microplate.
Dispense	Vertical arm moves to the default dispensing height.
Liquid	Vertical arm moves to the detected liquid level plus the offset entered into the text box in µm.
Max	Vertical arm moves to the maximum depth into the well.
Dilutor	*Controls actions of the syringe pumps.*
Reset	Resets dilutor.
Wash	Moves vertical arm to rinsing station and rinses the pipette tips.
Fill System	Primes the lines by running 7 cycles of the syringe pumps, resulting in filling each tube with 3.5 ml system liquid (usually distilled water). Used for removing air bubbles from the system.
Empty System	Empties the system liquid into the waste receptacle.
Aspirate	Aspirates the specified volume.
Dispense	Dispenses the specified volume.
Arm to Position	*Moves vertical arm to specified position.*
Shutter	*Controls the lid of the recording plate.*
Open	Opens the lid of the recording plate.
Close	Closes the lid of the recording plate.
CO_2	*Controls the CO_2 delivery to the recording plate.*

Valve

Open	Opens the valve. CO_2 delivery is switched on.
Close	Closes the valve. CO_2 delivery is switched off.

6.2 Display Settings

In this topic, general features of the displays are described.

Display toolbar

Several tools for zooming and scrolling the data as well as exporting the graph are provided on the toolbar of the offline displays. Using these tools will not change the general settings in the **Display Settings** dialog box.

If you manually zoomed the axes, recorded data will not show up outside the selected range. Please right-click on the axis and select the option **Tracking Enabled** to enable the display to follow the incoming data traces.

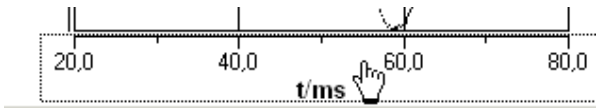
	**Reset axes**	Resets the axes to default ranges.
	**Zoom box**	Zoom in a particular region of the display.
	**Zoom to fit**	Adjusts the ranges of the axes to fit all displayed data.
	**Save as ASCII**	Exports the graph as an ASCII file.
	**Snapshot**	Exports the graph as an image file.

Scrolling and zooming with the mouse wheel

1. Click an axis to select it.
   This is indicated by a tiny dotted line around the selected axis.
2. Use the **mouse wheel** for **scrolling** the axis. Press the **CTRL key** at the same time for **zooming** the axis.

Scrolling with the mouse or keyboard

1. Click the axis you like to scroll.
   The mouse pointer becomes a hand.



2. While holding down the mouse button, move the mouse pointer to the left and right, or up and down.
   — OR —
   You can use the keyboard: Press LEFT ARROW or DOWN ARROW to move the selected axis to the left (down), RIGHT ARROW or UP ARROW to move the selected axis to the right (up).
   — OR —
   Use the PAGE UP and PAGE DOWN keys for a faster scrolling (larger steps): PAGE DOWN to move to the left (down), PAGE UP to move to the right (up).

Zooming with the zoom box

The zoom box allows the user to outline a region in the display for zooming into. The box can start at any point, but must be dragged to the lower right to actually cause a zoom. The zoom action will affect all axes.

1. Click  to enable the zoom box.
2. Move the mouse pointer over the display.
   The mouse pointer becomes an arrow with a little box.
3. Click any point in the display and move the mouse pointer to the lower right.
   The zooming box appears around the outlined region.
4. Release the mouse button for zooming into the selected region.

7 Offline Analysis with the QT-Analyzer

7.1 Offline Analysis with the QT-Analyzer

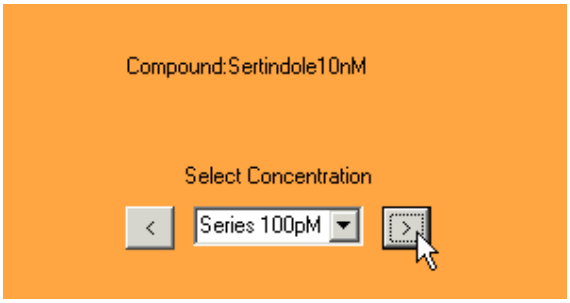
The QT-Screen program controls the QT-Screen robot and runs the experiments. The QT-Analyzer program is intended for reviewing and graphical representation of recorded experiments. In future version, offline analysis features will be added.

Loading a data file

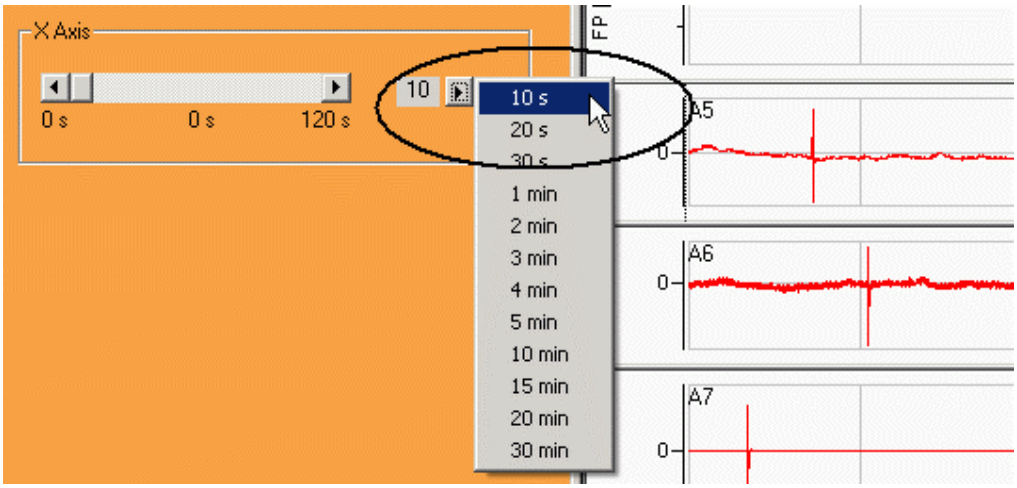
- Click Open on the File menu and select a data file of your choice. A single data file contains one tested compound, that is, a single row of a QT-Well plate.

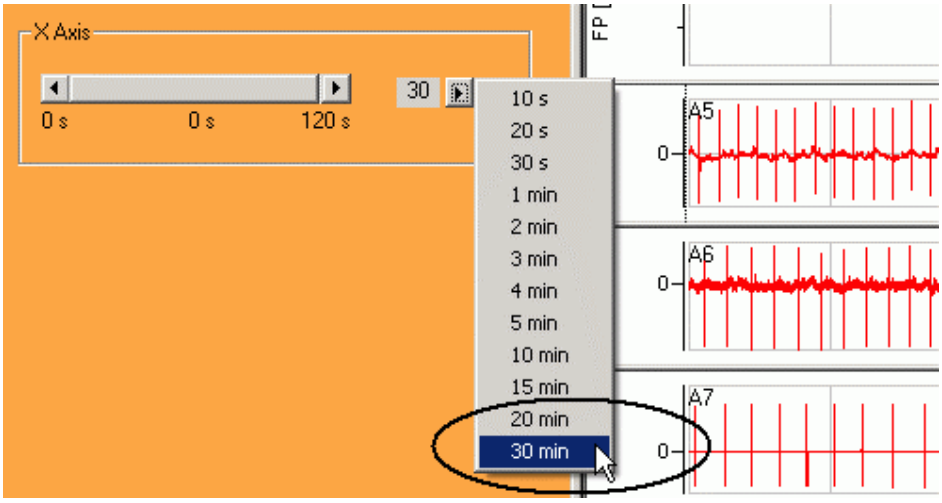
Reviewing raw data

1. Click the Raw Data tabbed page for reviewing the raw data of all recorded wells of the loaded row.
2. Move from a tested concentration to another with the arrow buttons or by choosing from the drop-down list. It will take some time to load the new information, depending on computer performance.

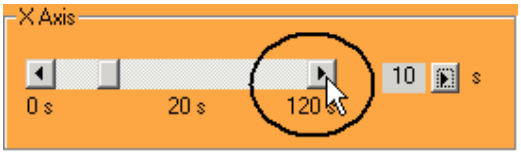


3. Choose the appropriate time interval that you want to display on the panel from the x-axis drop-down list.





4. Browse through the data file by clicking the **arrow buttons** next to the slider. The step size is the selected x-axis interval. You can also move the **slider** for jumping to a specific position.



5. Double-click a channel window to zoom into the data recorded from the specific well. There are several tools for zooming and graphics export (ASCII, graphics format, and clipboard) available in single channel mode.

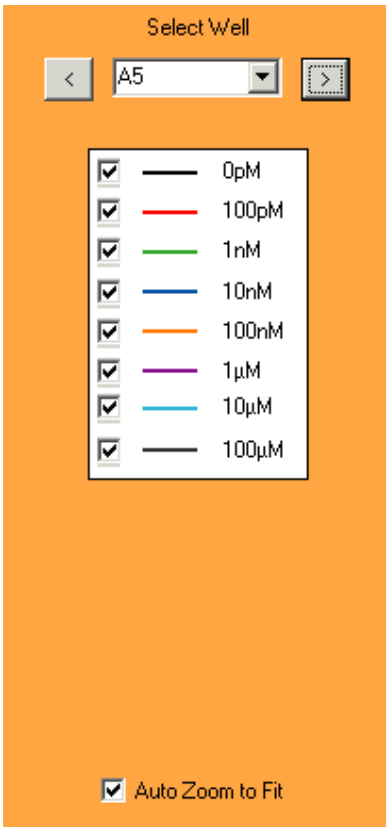
6. Double-click again to go back to multi-channel view mode.

Dose-response curves and overlay plots

1. Click the **Results** tabbed page for reviewing the result output of the QT-Screen program. The dose response curves for all normalized parameters are shown, and can be exported as ASCII, in graphics format, or to the clipboard. The averaged data recorded for the different compound concentrations are overlaid for each well separately. The display on the bottom left shows the complete waveform, the display in the middle focuses on the sodium peak, and the bottom right display shows the inter-spike interval (ISI).

2. Move from a well to another with the arrow buttons or by choosing from the drop-down list. There are several tools for zooming and graphics export (ASCII, graphics format, and clipboard) available in single channel mode.
   The signals are zoomed to fit each time you move to a new well. You can switch off the zoom to fit if you want to keep your zoom settings by deselecting the **Auto Zoom to Fit** option.

3. You can hide traces in the overlay plot by deselecting the check boxes next to the legend.



8 Appendix

8.1 Contact Information

Local retailer

Please see the list of official MCS distributors on the MCS web site.

User forum

The **Multi Channel Systems User Forum** provides the opportunity for you to exchange your experience or thoughts with other users worldwide.

Mailing list

If you have subscribed to the QT-Screen Mailing List, you will be automatically informed about new software releases, upcoming events, and other news on the product line. You can subscribe to the list on the MCS web site.

www.multichannelsystems.com

QT-Screen

multichannel systems

QT-Screen robot

Operating temperature	15 °C to 37 °C
Storage temperature	0 °C to 50 °C
Relative humidity	30 % to 85 % RH, non-condensing
Dimensions (W x D x H)	559 mm (22'') x 615 mm (24.2'') x 515 mm (20.3'')
Weight	38 kg
Maximum power consumption	350 W
Supply voltage	110/230 VAC
Positioning accuracy	0.2 mm in x/y dimensions and 0.4 mm in z dimension
CO_2 Connector	For 4 mm OD hoses

Amplifier

Recording channels	96
Input voltage	-4 mV to +4 mV
Gain	1000
Bandwidth	0.6-2.2 kHz
Filter slope	60 db/decade (low pass), 40 db/decade (high pass)
Input impedance	10^{11} Ω parallel to 10 pF
Input noise	< 800 nV_{RMS}
Noise density	$e_n = 15\ nV/\sqrt{Hz}$

Heating element (separate base plate and lid heating)

Heating temperature	37 °C
Accuracy	0.1 °C
Recovery time	0.5 min to 2 min
Typical time constant	50 s
Temperature sensor	PT 100
Resistance	22 ± 2 Ω (base plate), 30 ± 2 W (lid)
Thermal resistance	6 °C / Watt
Calibration constant	$T = c * (R-R_0)/R_0$ for c = 259.7 °C

Data acquisition

Sampling rate	10 kHz
Data resolution	14 bit
Crosstalk (channel to channel)	max 0.1 %, typically 0.01 %
DC Offset	max ± 2 mV (1.8 µV for unamplified signal)

Perfusion system

8-channel liquid handler (MiniPrep 60) from Tecan

Pump type	500 µl syringe pumps
Tubing	ID 1.5 mm (0.059''), OD 2.0 mm (0.079'') and ID 1.0 mm (0.039''), OD 2.4 mm (0.094'') FEP tubing
Compound plate format	96 well plate (SBS standard)
Travel speed incl. Liquid level sensor	894 mm/s in x, 569 mm/s in y, 393 mm/s in z

Software package	QT-Screen, QT-Analyzer
Operating system	Windows 2000, Windows XP, English and German versions supported
Data export	ASCII file format
Connection to the QT-Screen via Ethernet	

Model test probe

Resistor	220 kΩ
Capacitor	1 nF

QT-Well plate

Temperature compatibility	0 °C to 80 °C
Dimensions	96 well plate (SBS standard)
Contact pad and track material	Gold
Electrode diameter	100 µm
Electrode type	Planar gold
Electrode impedance	approx. 30 kΩ
Number of electrodes	96 (one per well)

multichannel systems

MEA Application Note:
Primary Culture Cardiac Myocytes from Chicken Embryo

Printed: 2006-11-21

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Table of Contents

1 Material

1.1 Biological Materials

- About 20 or more chicken eggs (*Gallus gallus*) day E13 are required per QT well plate. (Incubate more for replacement in case that eggs are not fertilized.)

1.2 Technical Equipment

- MEA System or QT-Screen (with amplifier and data acquisition, see Suggested MEA System)
- 1 sterile QT-Well plate
- Sterile workbench
- Egg incubator
- Incubator set to 37 °C, 5 % CO_2
- Ice
- Centrifuge (for1mL and 50 mL tubes)
- Cell counter, for example, CASY Model DT from Schärfe System GmbH, Reutlingen, Germany
- Sharp forceps
- Large forceps
- Curved forceps
- Dumont forceps
- Small scissors
- Pipettes (5 mL,1-1000µL)
- 8- or 12-channel pipette (100-300 µL)
- Automatic 10 µl dispenser
- Petri dishes (2 large dishes, 1 small dish)
- 50 mL Falcon tubes
- 15 mL Falcon tubes
- 100 µm nylon mesh cell strainer

1.3 Chemicals

- Sterile distilled water
- 70 % Ethanol for sterilizing the workbench and instruments
- **Ham`s F-12** without L-Glutamine, ~500 mL (PAA Laboratories GmbH, E15-016)
- Fetal Calve Serum (**FCS**) Gold, ~120 mL (PAA Laboratories GmbH, A15-151)
- Penicillin/Streptomycin (**Pen/Strep**) 100 x Concentrate (~20 ml) (PAA Laboratories GmbH, P11-010)
- Dulbecco's Modified Eagle Medium (**DMEM**) high Glucose, without L-Glutamine (Cambrex, 12-614F)
- **HEPES** (~1g) (Sigma-Aldrich, H 4034)
- Trypsin (Sigma-Aldrich, T 7409)
- Phosphate buffered saline (**PBS–**), Ca^{2+}/Mg^{2+}-free (ATCC, SCRR-2201)
- Cellulose nitrate or PEI coating solution (see MEA User Manual)

1.4 Media

20% FCS Medium

- 158 ml Ham`s F12
- 40 ml FCS
- 2 ml Pen/Strep 100 x

3% FCS Medium

- 168 ml DMEM
- 30 ml 20% FCS Medium
- 2 ml Pen/Strep 100 x

0.05% Trypsin Solution

- 25 mg Trypsin
- 50 ml PBS–

Recording Buffer

- 200 ml DMEM
- 10 mM HEPES
- Adjust the pH to 7.3 with NaOH/HCl

2 Methods

2.1 Preparations

1. Place about 20 or more fertilized eggs into an egg incubator for 13 days.
2. Prepare the cellulose nitrate or PEI coating solution (see MEA User Manual).
3. Treat the QT-Well plate with a plasma cleaner for 2 min shortly before use (maximum of two days).
4. Prepare two large Petri dishes with about 20 mL, and one small Petri dish with about 2 mL DMEM medium.

2.2 QT-Well Plate Coating

You can coat the recording area either with cellulose nitrate or PEI solution (see MEA User Manual). In case either method fails, that is, cells do not attach or signal quality is not ok, try the other one.

Coating with cellulose nitrate

As coating with cellulose nitrate is a very fast procedure, it can be done immediately before the preparation or even later during the incubation steps.

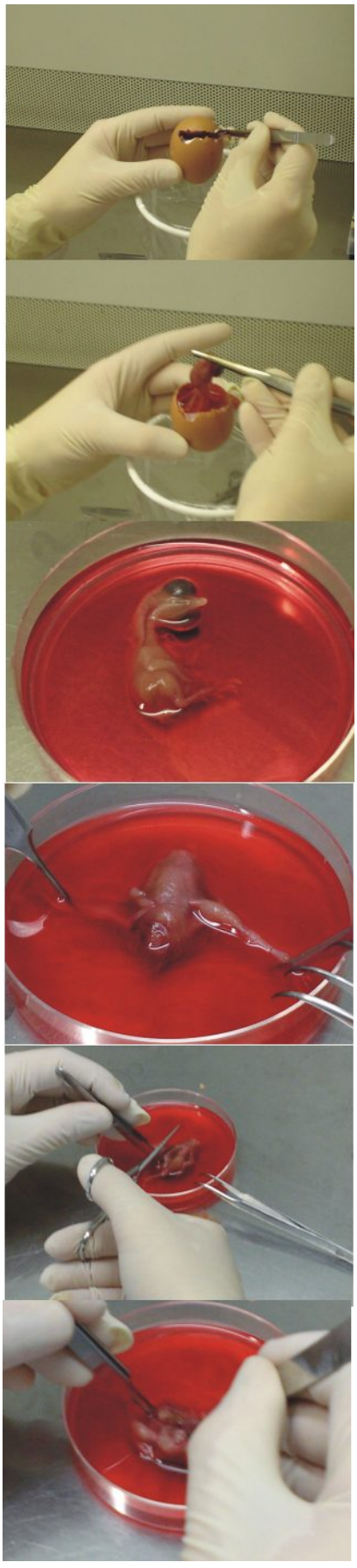
1. Pipette 5 µl cellulose nitrate coating solution into each well.
2. Remove the coating solution and let the wells air-dry.

Coating with PEI

1. Pipette 50 µl PEI coating solution into each well.
2. Incubate 1–2 h under sterile conditions and UV light.
3. Remove the coating solution and wash the wells three times with sterile distilled water.
4. Let the wells air-dry under sterile conditions and UV light.

2.3 Harvesting Cardiac Tissue

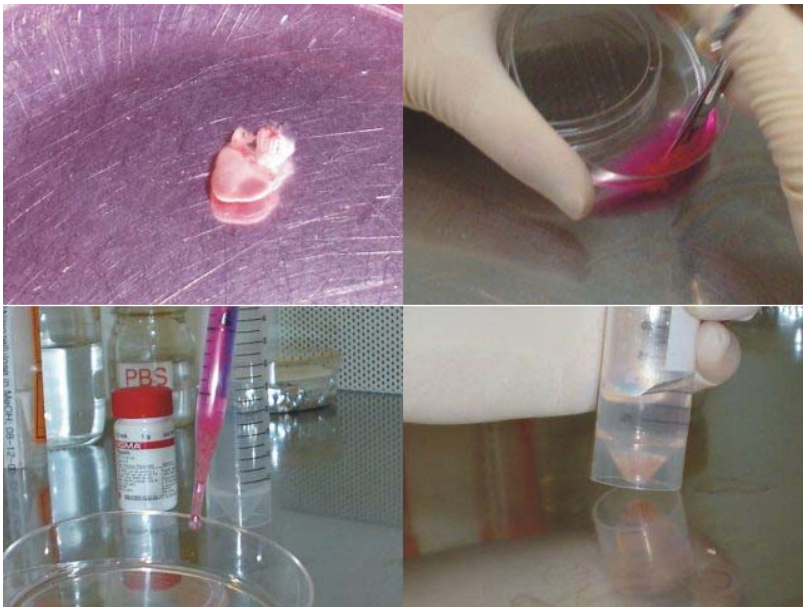
1. Remove the embryonic chickens from the eggs and decapitate the chickens in a petri dish filled with 20 mL DMEM medium.
2. Open the thoracic regions and isolate the hearts with blood vessels.
3. Gather the hearts in a petri dish filled with 5 mL DMEM medium.
4. Remove the atria and vascular tissue, wash the blood away, and transfer the hearts into a dish with fresh DMEM medium (2 mL).
5. Chop the hearts with a scissor into small pieces (as small as possible).
6. Collect cardiac fragments with a 10 mL pipette and transfer them into a 50 mL tube. Let the heart pieces sink down inside the pipette so that only a small volume of liquid (about 1 mL) is taken over to the tube.



2.4 Digestion with Trypsin

Incubate the heart fragments in 0.05 % trypsin / PBS– at 37 °C, as described in the following.

1. 10 min, 10 mL 0.05 % trypsin, discard supernatant.
2. 8 min, fresh 5 mL 0.05 % trypsin, collect the supernatant in 20% FCS medium (on ice).
3. Repeat step 2 until the tissue has been fully digested and you obtain a homogenous cell suspension. You can enhance and speed up the digestion by mechanic friction. For this, aspirate the suspension with a 5 mL pipette (in later steps with a 1 mL pipette).
4. Pass the collected cell suspension through a 100 µm nylon mesh cell strainer into a new 50 mL tube.
5. Centrifuge the cell suspension for 10 min at 800 rpm (or 110 g).
6. Discard the supernatant and resuspend the pellet in 1 mL 3% FCS medium.



2.5 Plating Cells onto the QT-Well Plate

Cells are plated in a high density onto the electrode. Therefore, the cell culture begins to beat spontaneously after 1–2 days in culture.

You will obtain about 3 million cells per egg, that is, the cell suspension should contain about 45 million cells in total.

1. Count the cells with a cell counter to estimate the cell density.
2. Pellet the cells at 1200 rpm (or 150 g) for 1.5 min.
3. Resuspend the cells at a density of 400 000–500 000 cells per 10 µl in 3% FCS medium.
4. Plate 10 µl cell suspension (equals 400 000–500 000 cells) directly into the well with an automatic 10 µl dispenser. Avoid applying a high pressure to the cell suspension and set the drop centered on the electrode. Do not use a multichannel pipette for plating the cells, as the tips are not precise enough to set the drop right onto the electrodes. Make sure that you press the pipette plunger only to the first stop, to avoid that the suspension spatters on the side of the well.
5. Wait 1 min until the cells have settled down. Make sure that the cell suspension does not dry out.
6. Carefully fill the wells with 300 µL 3% FCS medium (with an 8- or 12-channel pipette).
7. Incubate the QT-Well plate in an incubator at 37 °C and replace the medium daily. Place the MEA into a water-filled dish to prevent osmotic effects due to evaporation. It is ok to not replace the medium over the weekend.
8. Recording can be performed from two days after the preparation on until ten days after the preparation. Please note that cell properties change slightly over culture time. Thus, compare only cultures of approximately the same age.

3 Suggested MEA System

3.1 System Configurations

Depending on the throughput and the analysis requirements desired in your laboratory, various system configurations are recommended for the recording from cardiac myocytes.

- **MEA96MT System** / **QT-Screen**: These systems record from **96 well plates** with a **single electrode per well**. Whereas the MEA96MT system is based on MC_Rack and for a manual operation, the QT-Screen provides fully automated liquid handling and analysis. This is a high-end system for high content drug screening.

3.2 Microelectrode Arrays

Cardiac myocytes tend to form gap junction coupled cultures that are triggered by a single pacemaker.

Recommended MEAs include:

- **96 well QT-Well plates** for high throughput applications (for use with the MEA96MT System or QT-Screen system)

3.3 Recommended Amplifier Specifications

Though custom amplifiers with gain and bandwidth specified by the user are available, Multi Channel Systems recommend the following settings for this application.

- Lower cut-off frequency: **1 Hz**
  If you select an even lower value for the lower end of the bandwidth, slow signal drifts can disturb the recordings. A lower cutoff frequency of up to 10 Hz is recommended if you are not interested in Calcium currents, for example for safety screening or mapping of excitation spreading.
- Upper cut-off frequency: **3 kHz**
  Sufficient even for the rapid depolarization waveforms
- Gain: **1200**

4 References

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