MED64 The most sensitive microelectrode array system for *in vitro* extracellular electrophysiology

MED64 Protocol

Primary DRG Neuronal Culture

ALPHA MED SCIENTIFIC

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Alpha MED Scientific Inc.
Saito Bio-Incubator 209, 7-7-15, Saito-asagi,
Ibaraki, Osaka 567-0085, Japan
E-mail: support@med64.com
Website: http://www.med64.com

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1. Introduction

A Dorsal Root Ganglion (DRG) is a structure of the spinal cord where neuron cell bodies are clustered. These structures are located in the intervertebral foramina between vertebra. The neurons found in the DRG are pseudo-unipolar, afferent, sensory neurons which are responsible for sensing mechanical, thermal, chemical and noxious stimuli. The most commonly studied aspects of DRG neurons are mechanisms of nociception (pain receptors). The neurons of the DRG have three parts: 1) dendrites that receive information near the skin and relay activity to the cell body, 2) soma which integrates signals from the dendrites and 3) axon which relays the neuronal activity to somatosensory cortex. Because DRG neurons can be dissociated and grown in culture, it is possible to investigate DRG neuron function and electrophysiological properties using the MED64 in vitro micro-electrode array.

The MED64's broad acquisition bandwidth combined with superior signal to noise ratio affords impeccable extraction of basic electrophysiological variables. The combination of precise extraction of electrophysiological activity with the ease of culturing cells directly onto the MED Probe (Micro Electrode Dish) makes the MED64 ideal for pharmacological, drug safety screening, and basic scientific applications. The 64 electrodes on the MED Probe have the lowest impedance of any micro-electrode array, making the MED64 ideal for acquiring data from spontaneously spiking neurons or neurons that fire in response to drug application. The MED64 also has high capacitance electrodes, enabling the MED64 to deliver high stimulating current, which is essential for for evoked response studies.

The goal of this application note is to describe how to set up experiments with dissociated neuron cultures, acquire relevant data, and extract the data for presentation or publication. This material has been prepared by scientists with expertise in neuroscience and electrophysiology. A complete protocol for plating, culturing, and carrying out experiments on dissociated neuron cultures has been prepared based on the users' experience.

1-1. Acknowledgment

Alpha MED Scientific would like to thank the MED64 users that have shared their knowledge,:

Ikuro Suzuki, PhD - Tohoku Institute of Technology
Aoi Odawara - Tohoku Institute of Technology
Gong Cheng, MD- Senior Application Scientist, Alpha MED Scientific
Ryan Arant, PhD - Application Scientist, Alpha MED Scientific

1-2. Disclaimer

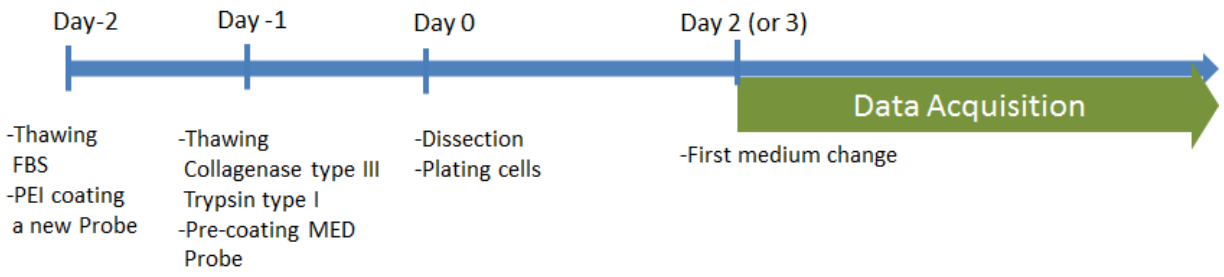
This application note is a summary of information shared by MED64 users and is to be considered marketing material. These methods have been developed, tested, and verified in the course of projects published in peer-reviewed literature. However, Alpha MED Scientific does not guarantee that the information written in this document is correct and is free from all liabilities. Please refer to the scientific literature for further insight on these techniques, as well as the MED64 and Mobius manuals for detailed instructions on use of the MED64 System.

2. Pretreatment of the MED Probes and plating DRG neurons

2-1. Material to be prepared

Items	Supplifer	Cat. No	Adjustment, Storage
Animal			
Wistar Rat (male)	Various		
Chemicals for coating			
Polyethyleneimine	Sigma	P3143	
Na2B4O7/10H2O	Sigma	S9640	
Ethanol (70%)	Various		
iMatrix-511 (Laminin)	Nippi	892011	Store at 4 degree.
Poly-D-Lysine (PDL)	Sigma	P1149	Dissolve wit sterilized water to 1mg/ml. Store at 4 degree
Chemicals for DRG culture medium			
MACS Neuro Medium	Miltenyi Biotec	130-093-570	Store at 4 degree. See page 10 for adjustment
MACS NeuroBrew-21	Miltenyi Biotec	130-093-566	Store at 4 degree. See page 10 for adjustment
L-glutamine	Gibco	25030081	Store at 4 degree. See page10 for adjustment
Pencillin-Streptomycin	Wako	168-23191	Store at -20 degree. See page 10 for adjustment
FBS	Invitrogen		Store at -20 degree. See page 10 for deactivation
NGF	Merch Millipore	01-125	Store at -20 degree
Chemicals for disection			
HBSS	Wako	084-08345	Store at 4 degree
10X HBSS	Sigma	H441	Store at 4 degree
Collagenase type III	Worthington	CLS3	Dilute with Neuro Medium to 2mg/ml, and filter it. Dispense 2ml to microtubes and store at -20 degree
Trypsin type I	Sigma	T8003	Dilute with HBSS to 10 mg/ml, and fiter it. Dispense 500 μl to microtubes and store at -20 degree
Trypsin inhibitor	Sigma	T9008	Ditule with HBSS x200. Dispense 150 μl to microtube and store at -4 degree
Percoll	GE Healthcare	17-0891-02	Store at 4 degree
Equipment			
MED Probe	Alpha MED Scientific	MED-P515A	
35mm culture dish	Various		
Conical tube (15, 50 ml)	Various		
Microtube (2.5ml)	Various		
Scissors (2sets)	Various		
Micro-Forseps	INOX	No 5	
Scalpel, Pipet, Petri dishes, kim wipes, waterbath, clean bench, CO2 incubator, Centrifuge, microscope			

2-2. Workflow for preparation



2-3. Pretreatment of the MED Probe

CAUTION:

Avoid contact with the electrodes in all of following procedures as they are extremely fragile.

Pretreatment of the MED Probe is the most critical step to culture neurons onto the electrodes. The surface of a new MED Probe is hydrophobic. Therefore, hydrophilization of the MED Probe is necessary to enhance the adhesion of neurons to the electrodes. If cells do not adhere to the electrodes, you will not be able to record from them. It should be noted that some coating agents can affect neural activity, viability, degree of neurite outgrowth, extent of migration, and longevity. Improper coating techniques can cause large-scale clumping and/or the death of neurons even if all other cell culture steps are performed properly. The following section contains recommendations for appropriate pretreatment of the MED Probes.

Sterilization

1. Rinse a new MED Probe with sterilized distilled water (SDW) at least three times. Rinse it with 70% ethanol several times (or immerse it in 70% ethanol for 15 minute), and then let it dry naturally on a clean bench. Higher-grade ethanol is recommended to avoid deposits of organic substances onto the MED Probe after drying.

2. Rinse the MED Probe with sterilized distilled water (SDW) at least three times, and then let it dry under ultraviolet irradiation for 15-30 min. Store and handle the MED Probe in a sterilized 90 mm petri dish.

Note:

Above steps are not always required, especially when reusing a MED Probe.

Pre-coating with PEI

The surface for the new MED Probe is hydrophobic. Thus, pre-coating with PEI is important for enhancing the hydrophilicity of the MED Probe's surface and preventing clumping of cells during their growth. While Pre-treatment with PEI is not typically required when re-using MED Probes, some MED Probes may need to be pre-treated again after several uses.

Note:

Pre-coating with PEI needs to be made on the day before plating cells.

1. Treat the surface of a MED Probe with 0.005%* Polyethyleneimine (PEI) in 25 mM borate buffer (pH 8.4) for one hour at room temperature. (Make sure the electrodes are covered by the PEI.)
   * If the insulating film of the MED Probe is acrylic, suitable concentration is 0.05%.
2. Aspirate the PEI and rinse the MED Probe with DSW 4 times.
3. Dry the MED Probe on a clean bench overnight.

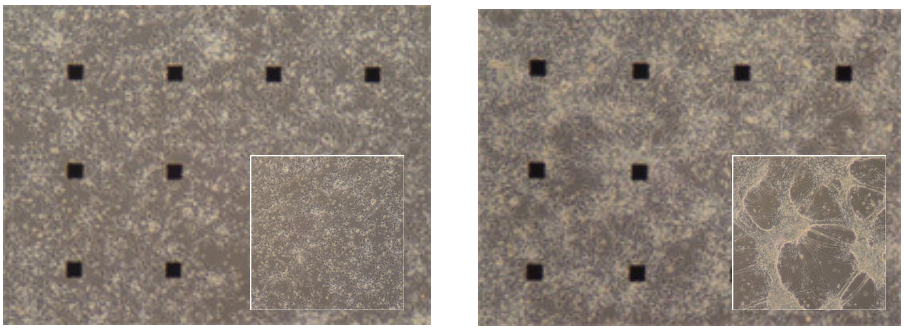


Figure 1. Neurons in 5 days after plating with PEI pre-treatment (left) and without PEI pre-treatment (right). Cells grew evenly on the MED Probe pre-treated with PEI (left).

Recipe -PEI solution-

<u>25 mM borate buffer</u>

Dissolve 25 mM Na2B4O7/10H2O (MW: 381.4, Sigma: S9640) in distilled water and adjust the pH to 8.4 with HCl. (500 ml borate buffer solution)

1) 4.768g Na2B4O7/10H2O in 450 ml distilled water.

2) Adjust pH to 8.4 with HCl.

3) Add distilled water to 500 ml.

<u>0.005% (or 0.05%) PEI (polyethyleneimine) solution</u>

Since a 50% PEI solution (Sigma: P3143) is so sticky, it is recommended to prepare a 1% stock solution first (1 ml 50% PEI to 49 ml 25mM borate buffer). This 1% stock solutions is then diluted to (0.005% or 0.05% PEI) for final use.

- The PEI solution can be stored in refrigerator up to 1 month.

Coating with Poly D Lysine and Laminin

Note:

Coating needs to be made on the day BEFORE plating cells.

1. Dilute the stored Poly D Lysine x100 with sterilized water to 10 μg/ml.
2. Rinse the MED Probe with PBS and fill the MED Probe with 1 ml of Poly D Lysine (10 μg/ml) and incubate for one hour at 37 degree/CO_2 5%.
3. Aspire the Poly D Lysine. Dry the MED Probe in a clean bench (for around one hour).
4. Dilute Laminin x200 with PBS (to 2.5 μg/ml).
5. When the MED Probe is dried, pour 1 ml of Laminin (2.5 μg/ml) into the MED Probe and leave the Probe in a refrigerator overnight.
6. Store the MED Probe in a refrigerator just before cells are plated.

CAUTION:

Be careful NOT to touch the electrodes.

Note:

The electrode surface should not repel water and should maintain moisture after the completion of this process.

2-4. Plating and culturing rat DRG neurons

Preparation

- Thaw the Collagenase type III and Trypsin Type I in a refrigerator (4 degree) on the day before dissection.
- All following procedures must be made on the day of dissection.
- Have the DRG culture neurons ready.

1. Fill a 35 mm culture dish with 3 ml of DRG culture medium. Warm it up in an incubator (5%CO_2) to 37 degree.
2. Dispense 4 ml of HBSS into a 15 ml conical tube and warm it up with a water bath at 37 degree.
3. Warm up the thawed Collagenase type III with a water bath at 37 degree.
4. Add 1.5 ml of HBSS to thawed Trypsin type I (2 ml in total). Warm it up with a water bath at 37 degree.
5. Take out the Trypsin Inhibitor from a refrigerator and leave it for 1-2 hours to warm to room temperature.
6. Make 40% Percoll solution (50 ml). compositions are:
   Percoll (20ml), 10xHBSS (1.8 ml), HBSS(28.2 ml)

   Dispense 6 ml of Percoll into a conical tube (50 ml) and leave it for 1-2 hours to warm up to room temperature.

Recipe -DRG culture medium-

Compositions for DRG culture medium (50 ml)

- Neuro Medium (Adjusted *) - 44.90 ml
- FBS (Deactivated *) - 5 ml
- NGF (diluted with Neuro Medium to 50 μg/ml) - 0.10 ml

* See Appendix (Page 10) for adjustment and deactivation.

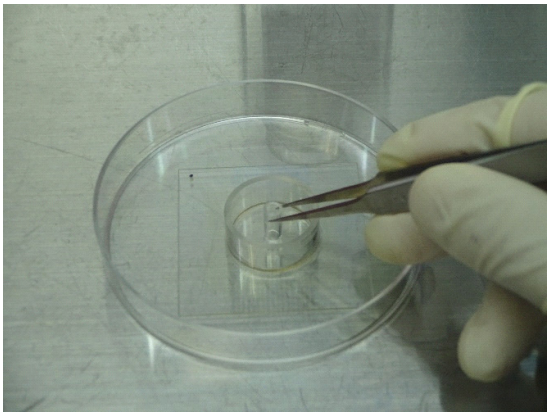
Dissection

1. Anesthetize a male 10-weeks-old Wistar Rat with Isofurane. Dissect carotid artery and cut along the dorsal region.
2. Excise spinal tissue (breast-loin).
3. Cut spinal canal with scissors and isolate spinal cord.
4. Excise nerve fiber bundles (one by one using a forceps). Transfer bundles to a 35 mm culture dish filled with DRG culture medium.
5. Remove nerve fiber to isolate ganglion. Doing this will increase the final density of DRG neurons.
6. Transfer the isolated ganglion into a microtube containing collagenase type III. Incubate with shaking in an incubator at 37 degree s Celsius for 2 hours.
7. Put the microtube containing the ganglion into a water bath (37 degrees Celsius) and leave it for 5 minutes. The ganglion will sink into the bottom of the microtube.
8. Aspirate as much supernatant as possible. (Be careful not to aspirate the ganglion.)
9. Add 2 ml of HBSS into the microtube. Pipet it gently several times and aspirate as much supernatant as possible.
    - Be careful not to aspirate the ganglion.
10. Repeat the #9 one more time.
11. Add 2 ml of Trypsin type I, and incubate it with a water bath (37 degrees Celsius) for 15 minutes.
12. Add Trypsin inhibitor (150 μl), and pipette it many times until the solution becomes cloudy and ganglions are dissociated.
    - It is important to repeat pipetting many times to increase neuron density.
13. Centrifuge the microtube at 1300 rpm for 5 minutes.
14. Aspirate 1 ml of supernatant, and then pipette it up and down several times.
15. Transfer the solution containing containing ganglia into the 50 ml conical tube containing Percoll.
16. Centrifuge the conical tube at 1300 rpm for 5 min.
17. Aspirate the supernatant.
18. Add the DRG culture medium (1 ml), and then pipet it several times.
19. Centrifuge at 1300 rpm for 5 min.
20. Aspirate the as much supernatant as possible. Add the DRG culture medium (0.3-1.0 ml) and pipet it.
    - For alternative method, please refer to:

      Production of dissociated sensory neuron cultures and considerations for their use in studying neuronal function and plasticity.
      Malin SA, Davis BM & Molliver DC. *Nature Protocols 2, 152-160 (2007)*

Plating DRG neurons onto the MED Probe

1. Take out the pre-coated MED Probe from a refrigerator and aspirate the Laminin.
2. Immediately (before probe surface dried) place a cloning ring around the electrodes in the MED Probe.
3. Gently transfer the cell suspension (100 μl) inside the cloning ring. Add the DRG culture medium (2 ml) outside the cloning ring.
4. Incubate with 5%CO_2 at 37 degrees Celsius for 2 hours.
5. Take out the Probe from the incubator and remove the cloning ring gently.
6. Change the DRG culture medium every 2-3 days. (Timing for the medium exchange can vary depending on condition for cells.)



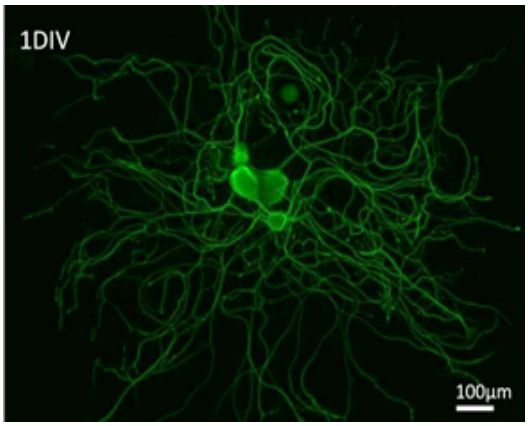
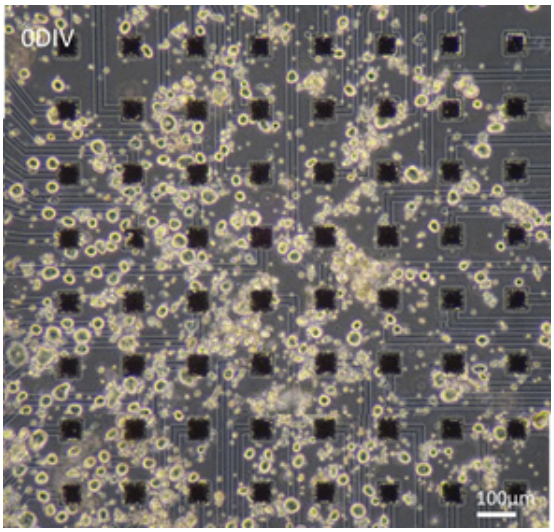


Figure 1 . [Left] Placing a cloning ring onto a MED Probe.
Figure 2. [Right] DRG neuron at day 1 in culture. (β-Tublin III fluorescent dyeing)



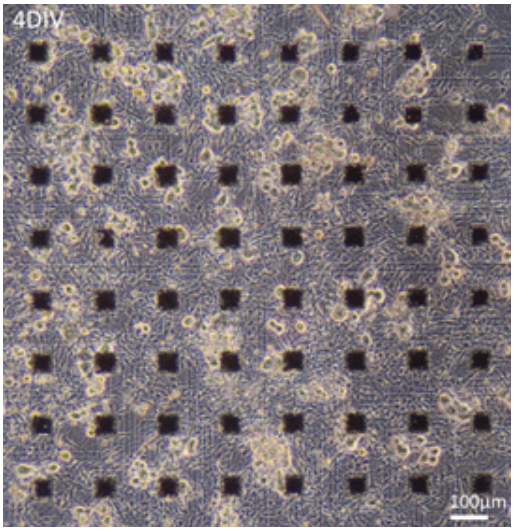


Figure 3. [Left] DRG neurons right after plating onto a MED Probe
Figure 4. [Right] DRG neurons at day 4 in culture.

2-5. Cleaning the used MED Probes

The MED Probe's electrical characteristics are best during the first use. High quality signals can be recorded and effective stimulation is possible with the MED64 System's MED Probes thanks to the electrode's characteristics, the lowest impedance in a commercially available microelectrode array. The electrode's impedance will increase with repeated use of the MED Probes due to damage in handling and/or residual cellular debris and coating materials. However, the MED Probes can be re-used if they are handled and cleaned very carefully. The following are protocols MED64 users recommend:

CAUTION:

ALWAYS avoid contact with the surface of the MED Probe to preserve the microelectrodes and insulation layer.

Trypsin method

1. Fill a used MED Probe with 0.25% Trypsin, and incubate at 37°C for 1 hour.
2. Rinse the MED Probe with PBS 3 times.
3. Rinse the MED Probe with sterilized distilled water (SDW) at least 3 times.
4. Fill the MED Probe with SDW, and store the MED Probe in a 90 mm petri dish.
5. Store the petri dish in refrigerator.

Bleaching method

CAUTION:

This method is available only for Platinum-Black electrodes-MED Probes. Do NOT use this method for CNT electrodes-MED Probes.

1. Carefully pour or pipette 1 ml of Bleach (Clorox®) into the MED Probe and leave it for about 15-30 seconds.
2. Aspirate and repeat step 1 three times. If the MED Probe is not clean after this, apply 2 more rinses and a longer exposure. (usually 1-2 minutes is enough, but as long as 15 minutes is acceptable.)

   CAUTION:

   Avoid exposing the MED Probe to Bleach over 20 minutes.
3. Rinse the chamber 5 times with double distilled water (DDW).
4. Allow it to dry or aspirate in a sterile hood.
5. Store the MED Probe (according to instructions) in a beaker with DDW in a cool dark area. (Please refer to the MED Probe product manual.)

2-6. Appendix

1) Adjustment for Neuro Medium

Chemicals to be prepared

Chemicals	Suppliser, Parts#
MACS Neuro Medium	Miltenyi Biotec, 130-093-570
MACS Neuro Brew-21	Miltenyi Biotec, 130-093-566
L-glutamine	Gibco, #25030081
Penicillin-Streptomycin	Wako, 168-23191

Adjusting Neural Medium (500 ml)

1. Put 40.7 ml of L-glutamine into a 50 ml conical tube.
2. Add 22 ml of Neuro Medium into the conical tube. Dissolve them.
3. Filter the Neuro Medium/L-glutamine solution.
4. Pour "20ml of the filtered solution" into the original bottle for the Neuro Medium.
5. Add Penicillin/Streptomycin (5 ml) and Neuro Brew-21 (10 ml) into the bottle and mix them very well.
6. Store it in a refrigerator.

2) Deactivation for FBS

1. Thaw frozen FBS in a refrigerator. (This can take 1-2 days).
2. Fill a water bath with water and set the temperature for 56 degrees Celsius. When the temperature reaches to 56 degree, put the FBS bottle into the water bath and incubate it for 30 min.
   - Avoid incubating at 56 degree OVER 30 min to avoid protein composition.
3. Take out the FBS bottle from the water bath. Remove water drops outside the bottle, and leave it in a room temperature for 15-20 min to cool it down.
4. Filter the cooled-down FBS.
5. Dispense the FBS to conical tubes and store them frozen.
   - Do the step 4-5 as quickly as possible. Leaving the bottle at room or higher temperature for a long time is not preferable.

3) Protocol - Capsaicin solution

Chemical to be prepared

Chemicals	Supplier, Parts#	Storage
Capsaicin	WAKO, 034-11351	4 degree
Dimethyl Sulfoxide (DMSO)	WAKO, 045-28335	Room
Tween 80	Kantokogaku, 40353-32	Room

Protocols for 10 mM Capsaicin solution (1 ml)

- Make the Capsaicin solution on the day of your experiment.

1. Put 3 mg of Capsaicin into a microtube.
2. Add 20 μl of DMSO and resolve it.
3. Add 20 μl of Tween 80. Mix it thoroughly.
    - It is recommended to use a P200 pipet with its tip cut due to the Tween 80's high viscosity.
4. Add 960 μl of DRG culture medium, and mix them very well.

Dilute the 10 mM with DRG culture medium as seen in the Figure 5 if lower-density is necessary.

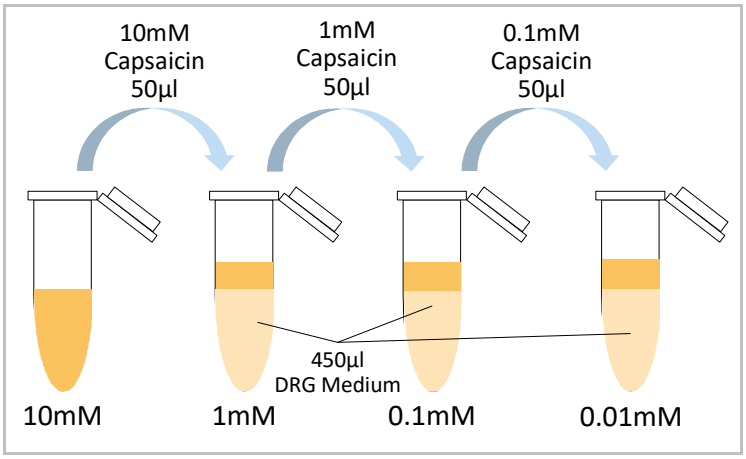


Figure 5.

3. Data acquisition

Cultured neurons are spontaneously active within 7-10 days after seeding, but initial activity can vary depending on culturing technique. Once the neurons begin firing spontaneously, activity can be recorded using the MED64. The recommended environment for recording from dissociated neurons is in the following section.

3-1. Recommended experimental environment

37°C is the typical recording temperature recommended for recording from dissociated neuron cultures However, the set temperature can vary depending on samples and nature of your experiments. To maintain the desired temperature, one of two methods are recommended.

1) Use of CO2 incubator

Place the MED Connector (MED-C03) inside a CO_2 incubator. Please note that incubators can introduce noise. Please refer to page 29-36 in the MED64 Handbook (vol1) to mitigate noise introduced by an incubator. Particularly, recordings can be compromised by noise introduced by the rapid temperature increases that occur as incubators power on. Wait until the desired temperature is stable before beginning acquisition. (It could take several hours depending on the incubator.)

Figure 9. Experiment with the MED Connector inside a CO2 incubator.

2) Use of the MED Heated Connector

The MED Heated Connector (MED-CP02H) heats the MED Probe chamber from the bottom. Below are recommendations for using the MED Heated Connector.

1. Do NOT place the MED Heated Connector in an environment where temperature changes frequently, for example in the proximity of an air conditioner or heater.

2. Cover the MED Probe to prevent ambient temperature fluctuation during acquisition.

3. It can take some time to reach the set-temperature. Power on the ThermoClamp™-1 (controller for the MED Heated Connector) connected to the MED Heated Connector at least 30 minutes before starting acquisition.

4. If/When the set-temperature is changed, wait for the temperature reported by the ThermoClamp™-1 controller to stabilize at the new temperature. It could take 5-30 minutes or more but is typically very fast.

3-2. Data acquisition

The MED Probe has 64 recording electrodes as well as 4 reference electrodes. The differences between the field potential acquired at the recording electrodes and the potential at the reference electrodes are measured by the MED64 System. Acquired signals are sent to the MED64 Head Amplifier through the MED Connector/MED Heated Connector. The raw signals are amplified by x10 by the Head Amplifier, and then amplified further and digitized with the MED64 Main Amplifier. We highly recommend reading the "Product manual" for each component, as well as the "MED64 Handbook", and "Mobius Tutorial" before using the MED64 System.

Required MED64 System Components

1) MED Probe
2) MED Connector (MED-C03) / MED Heated Connector (MED-CP02H) *1
3) MED64 Head Amplifier (MED-A64HE1S)
4) MED64 Main Amplifier (MED-A64MD1)
5) Acquisition PC
6) Mobius software (Mobius Spike Sorter or Mobius Spike Sorter with stim package)

*1. MED Heated Connector requires the ThemoClamp^{TM}-1 controller.

1. Sanitize the MED (Heated) Connector by cleaning with kim-wipe soaked with ethanol. (Don’t clean the contact pins in the Connector).

2. Mount the MED Probe containing the dissociated cells onto the MED (Heated) Connector.

CAUTION:

Clean the terminals on the outer portion of the MED Probe with a Kimwipe soaked in ethanol before mounting the MED Probe. Salt sediments can damage the contact pins on the MED (Heated) Connector.

3. Cover the MED Probe with a 35 mm petri dish or Perfusion Cap.

Available Mobius workflow templates

Data can be acquired immediately using the available Mobius workflow templates enumerated below (please refer to page 43, Chapter 3: Spike Sorter on the Mobius Tutorial):

1. Spontaneous_recording (Recording WITHOUT any real-time analysis).
2. Spike_recording (Recording with extracting spikes and their frequency analysis).
3. Spike_recording_filter (2 + filter).
4. Spike_recording_cluster (Recording with extracting, clustering spikes, and their frequency analysis).
5. Spike_recording_filter_cluster (4+filter).

Recommended acquisition settings

The following parameters are recommended to perform data acquisition and analysis of neuronal spikes:

Input Range (Maximum input signal level):	2.9 mV
Low cut freq (High pass filter) :	100 Hz
High cut freq (Low pass filter):	10000 Hz

Acquisition bandwidth can be narrowed to further decrease the baseline noise level.

4. Data analysis

The MED64 can easily record neuronal spikes from active neurons. The Mobius Spike Sorter package is available to extract and analyze neuronal spikes. Analyses as well as raw data can be exported for processing using other software packages.

4-1. Workflow templates available for analyzing neuronal spikes

The Mobius Spike Sorter package has 4 built-in analysis workflow templates available for analyzing neuronal spikes.

"Spike_frequency_analysis", "Spike_frequency_analysis_filter"

These workflow templates allow you to:

- Set thresholds.
- Extract spikes that go over the pre-determined thresholds.
- Counts spike frequencies for the extracted spikes.
- Graph the spike frequency chart.
- Export raw data
- Export waveform of extracted spikes and their time stamps.
- Export the spike frequency chart.

For the *"Spike_frequency_analysis_filter"* workflow template, all above analyses are performed on filtered data.

"Spike_sorting", "Spike_sorting_filter"

These workflow templates allow you to perform same analysis as above. However, the extracted spikes are clustered based on their waveform similarities. The spike frequency is computed independently for each cluster.

For the *"Spike_sorting_filter"* workflow template, all analyses are performed on filtered data.

4-2. Setting thresholds

All spike sorter workflow templates build in the [Extract Spikes Advanced] module, where thresholds are set by:

1. Typing numbers in the chart. (Top-left in the Figure 10)
2. Moving bars in the single channel display. (Top middle in the Figure 10)
3. Setting thresholds as SD (Standard Deviation) percentage. (Top right in the Figure 10)
4. Moving bars in the 64ch display. (Bottom in the Figure 10)

Spikes that go over the thresholds are extracted and their frequencies analyzed.

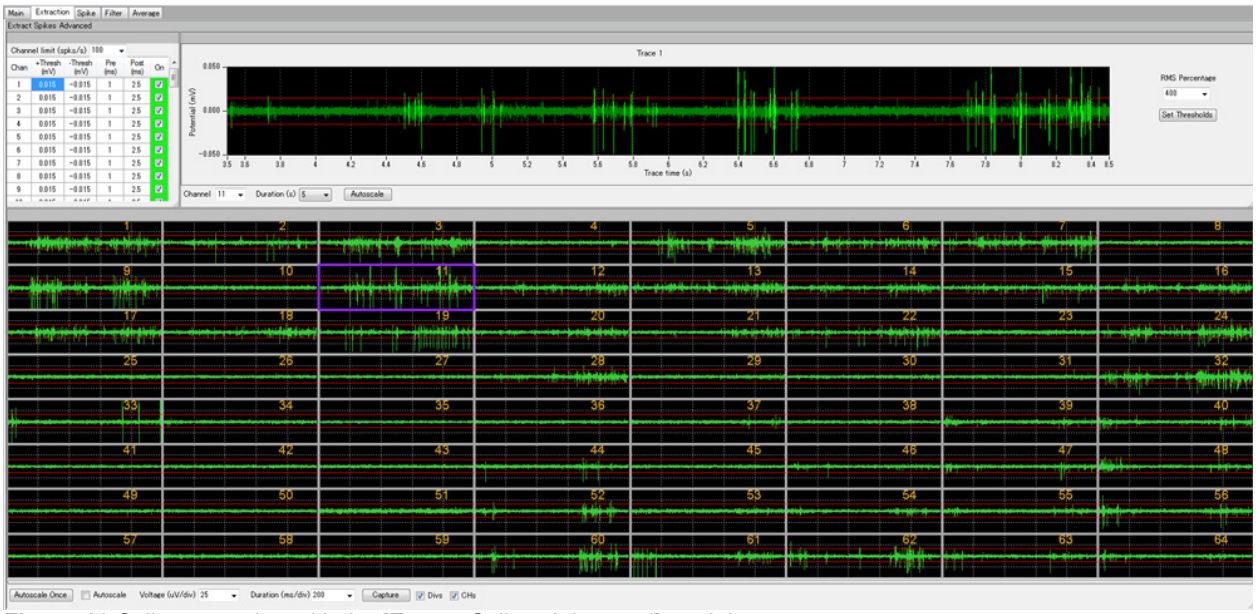


Figure 10 Spike extraction with the [Extract Spikes Advanced] module.

4-3. Spike frequency analysis

Mobius Spike Sorter's built-in "Spike_frequency_analysis_(filter)" workflow templates can extract neuronal spikes and analyze their spike frequencies during recording and/or post-acquisition. Time course of spike frequencies are computed and graphed. Please refer to page 63 in the Mobius Tutorial for detailed instructions.

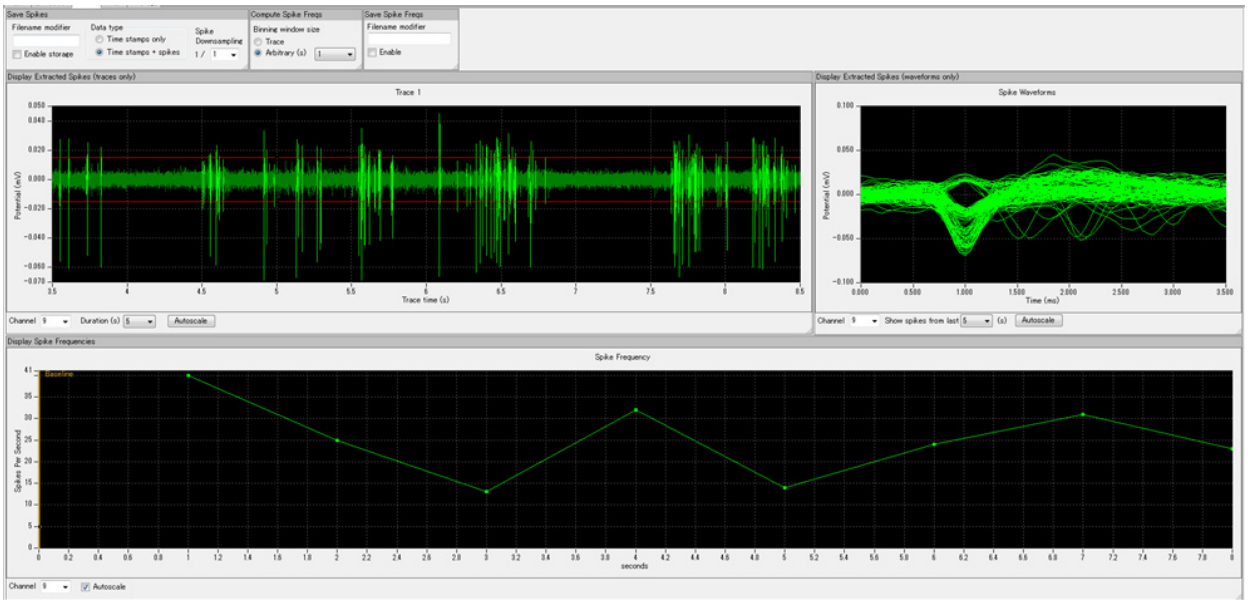


Figure 11. Spike frequency analysis using the *"Spike_frequency_analysis_filter"* workflow template. Spike frequencies for all extracted spikes are computed and graphed at each electrode.

4-4. Spike Sorting

The *"Spike_sorting_(filter)"* workflow templates extract neuronal spikes, sort them based on similarity of waveforms, and analyze their spike frequencies.

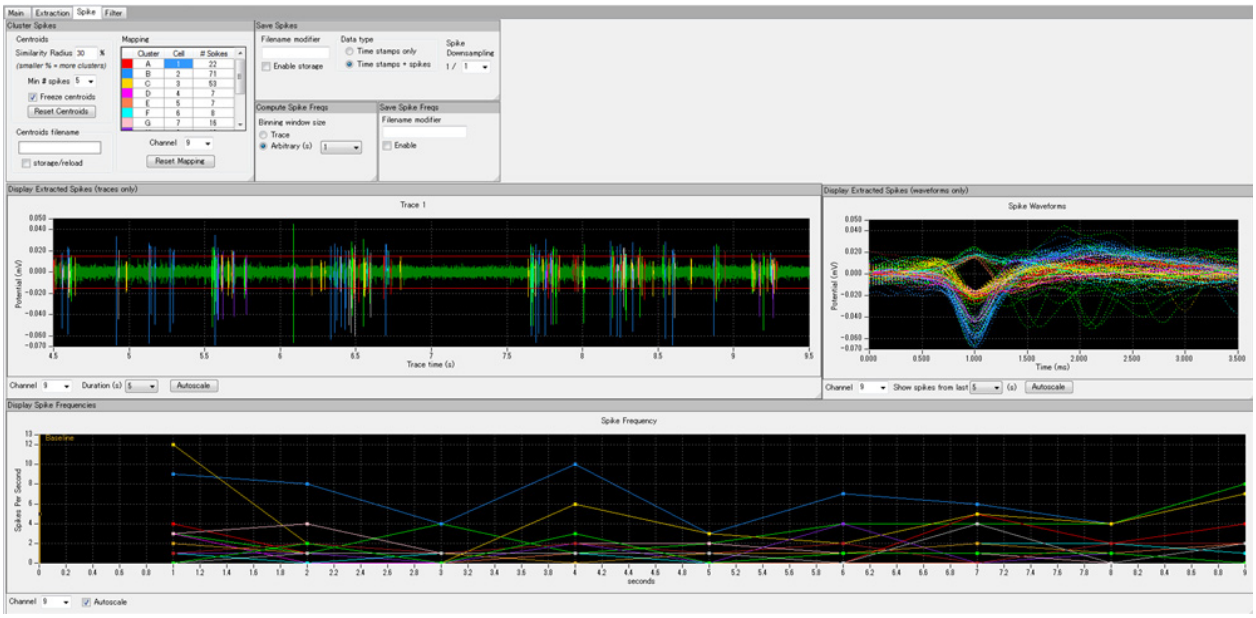


Figure 12. Spike sorting.

4-5. Exporting Data

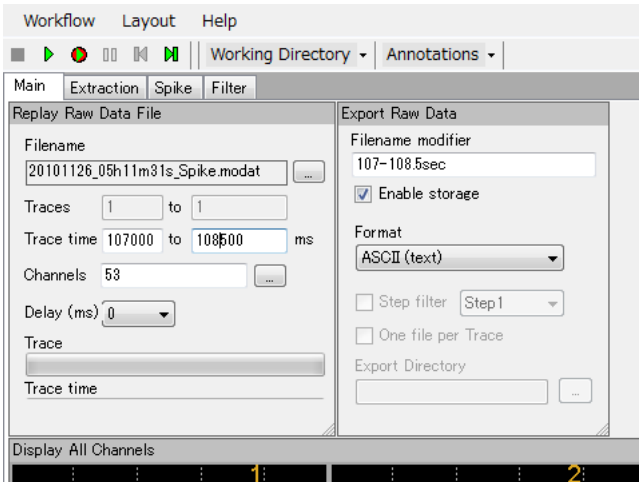
Mobius can export data in user friendly formats for analysis with other software.

Data to be exported	Module	File type
Raw data	Export Raw Data	Binary / ASCII
Time stamp for extracted spikes	Save Spikes	ASCII
Waveform for extracted spikes	Save Spikes	ASCII
Values for spike frequencies	Save Spike Freqs	ASCII
Waveform for centroids	Cluster Spikes	ASCII

Data is exported as ASCII (or binary for Raw data) when:

1) The check-box for the module is checked, and
2) Data is replayed with the Green/Red button.

Channels for exporting are selected via the channel selector in the [Replay Raw Data File]. Traces and times to be exported are also selected at the trace/time counter in the [Replay Raw Data File].



File Format Version	20071201
Session Start Time	2010/11/26 20:11:31 +09
time_ms	ch53_mV
107000	0
107000.05	-0.006836146
107000.1	-0.001953185
107000.15	-0.004394665
107000.2	-0.001953185
107000.25	0.000976592
107000.3	-0.005371258
107000.35	-0.008301035
107000.4	-0.002929777
107000.45	-0.002929777
107000.5	-0.004394665
107000.55	-0.007324442
107000.6	-0.000976592
107000.65	-0.001464888
107000.7	-0.007324442
107000.75	-0.003906369
107000.8	-0.002441481

Figure 13. [Left] Setting for exporting raw data with the Mobius (left). 107000-108500 ms of ch 53 are selected in the [Replay Raw Data File] module while ASCII is selected and Enable storage is checked in the [Export Raw Data] module. Data will be exported by clicking the green/red button.
[**Top-right]** A part of raw data exported by ASCII and displayed in Excel.
[**Bottom-right]** Waveform created by using Excel.

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Alpha MED Scientific Inc.
Saito Bio-Incubator 209, 7-7-15, Saito-asagi,
Ibaraki, Osaka 567-0085, Japan
Phone: +81-72-648-7973 FAX:+81-72-648-7974
http://www.med64.com support@med64.com

Manufactured by **Alpha MED Scientific Inc.**