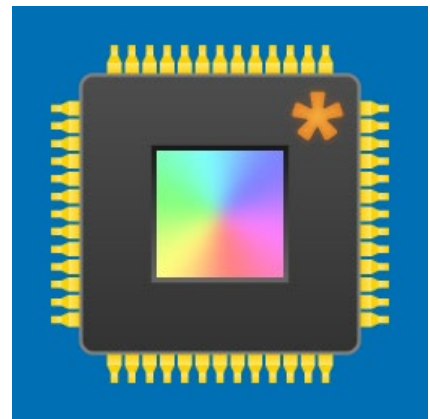


# **CMOS-MEA Application Note: Recording of light-induced spiking of retinal ganglion cells from *vertebrate retinae***

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Multi Channel Systems MCS GmbH

Aspenhaustraße 21  
72770 Reutlingen  
Germany

Phone +49-71 21-909 25 - 0  
Fax +49-71 21-909 25 -11

[sales@multichannelsystems.com](mailto:sales@multichannelsystems.com)  
[www.multichannelsystems.com](http://www.multichannelsystems.com)

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# 1 Introduction

## 1.1 About this Application Note

The intention of the MEA Application Notes is to show users how to set up experiments with a MEA-System based on typical applications that are used worldwide.

The documents have been written by or with the support of experienced MEA users who like to share their experience with new users.

In this document we describe the preparation and interfacing of vertebrate retina for CMOS-MEA recordings. Similar proceedings can be used for mouse, rat, rabbit or primates.

## 1.2 Acknowledgements

Multi Channel Systems would like to thank all users who shared their experience and knowledge with us, especially Dr. Günther Zeck and his group members from the *NMI* Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany.

Dr. Günther Zeck  
NMI Natural and Medical Sciences Institute  
Phone 07121 51530-807  
Fax 07121 51530-62  
[guenther.zeck@nmi.de](mailto:guenther.zeck@nmi.de)  
Markwiesenstrasse 55  
72770 Reutlingen  
Germany

## 2 Material

### 2.1 Animals

Experiments on CMOS-MEAs have mostly been performed using *ex vivo* retina from healthy, wild-type C57Bl/6J retina or from photoreceptor-degenerated rd1 or rd10 mouse retina [1, 2]. CMOS-MEA recordings using SD rat retina [3], New Zealand white rabbit [4] and non-human primate have been performed as well. Small changes in the retina preparation may be considered; however, the interfacing and recording follows the same general rules.

### 2.2 Technical Equipment

#### Dissection

- Dissection microscope
- Forceps: 55 and 5
- Surgical eye scissors
- Scalpel
- Coating for MEA (e.g. PLL)
- Electrolyte solution

#### Recording

- [CMOS-MEA5000-System-E](#) including a peristaltic pump, perfusion and floor heating
- CMOS-MEAs with either 16 or 32  $\mu\text{m}$  electrode spacing ([CMOS-MEA16-SCA](#) or [CMOS-MEA32-SCA](#)). Spike sorting shows a better performance using CMOS-MEAs with 16  $\mu\text{m}$  spacing while a larger field of view can be investigated when using 32  $\mu\text{m}$  spacing.
- Carbogen (5%  $\text{CO}_2$ , 95%  $\text{O}_2$ )
- Ag/AgCl [Reference Electrodes](#)
- An up-to-date computer (recommended: Windows 10 Pro, SSD 256 GB, i7 core, 32GB RAM)

#### Stimulation (optical/electrical)

- Upright microscope or customized light stimulation system
- Light sources to stimulate the retina (i.e. micro OLEDs like CoolLED System, LED display (Fig. 3))
- Electrical stimulation is available within the CMOS-MEA5000-System

### 2.3 Chemicals and Media

- Cleaning Solution (e.g. 5% Tickopur R60)
- Poly-L-Lysine (1 mg/ml)
- Ames' medium or equivalent electrolyte solution, prepare the medium as indicated by [Sigma](#). Use Carbogen (95 %  $\text{O}_2$ , 5 %  $\text{CO}_2$ ) to maintain the appropriate pH.

## 3 Methods

### 3.1 Preparations

- Make sure the CMOS-MEA surface is clean and hydrophilic prior to coating. A hydrophilic surface can be achieved by cleaning the chip with a warm detergent solution. For example, use 5 % Tickopur at 80 °C for at least two minutes. Rinse with distilled water after Tickopur treatment.
- Prior to the interfacing of the retina the planar CMOS-MEA surface shall be coated with poly-L-lysine (1 mg/ml). A drop of 20-50  $\mu\text{l}$  can be deposited onto the surface. Coat for at least 20 minutes, afterwards rinse the chip surface with electrolyte solution.
- Aerate the Ames' medium with Carbogen to adjust the pH.

## 3.2 Dissection

The dissection is performed under infrared or under dim red illumination to minimize photo pigment bleaching.

### 3.2.1 Removing the Eye-Cups

1. Euthanize the animal according to institutional guidelines.
2. Both eyes are excised with large straight forceps (see Fig. 1 A on the right) or scissors.

### 3.2.2 Retina Isolation

All following steps are carried out in Ames' medium or equivalent electrolyte solution aerated with Carbogen at room temperature. The dissection is performed under a dissection microscope.

1. Remove tissue around the eye ball.
2. Perforate the eye-cup with an injection cannula (Fig. 1 B).
3. Open the eye-cup along the *ora serrata* using a fine eye scissor or a surgical eye scissor. Use the perforation hole as an access point for the scissors (Fig. C).
4. Remove the lenses with fine straight forceps.
5. Dissect a piece of eye-cup that fits the size of your MEA (Fig. 1 D).
6. Detach the retina from the RPE with fine curved forceps (Fig. 1 E).
7. Clear the vitreous from the retina carefully. When the retina lies out flat, the removal of the vitreous most likely has been successful.

The last step is very critical. Insufficient removal of the vitreous body will result in low-quality recordings. Be careful not to damage the retina in this step.

### 3.2.3 Preparing and Transferring the Retina onto the CMOS-MEA

1. Transfer the retinal sample with a plastic dropper and Ames' medium into the CMOS-MEA chamber.
2. Make sure the retina is well flattened on top of the sensor area so it makes a good contact. Make sure that the edges are not crumbled below the retina. This is the crucial step, which determines your experimental outcome. Take your time.
3. Optional steps are to remove the medium after a successful placement and additionally flattening the retina by removing the last drops of medium with a filter paper. Make sure not to touch the retina with the filter paper.
4. Afterwards fill the chamber with medium again.

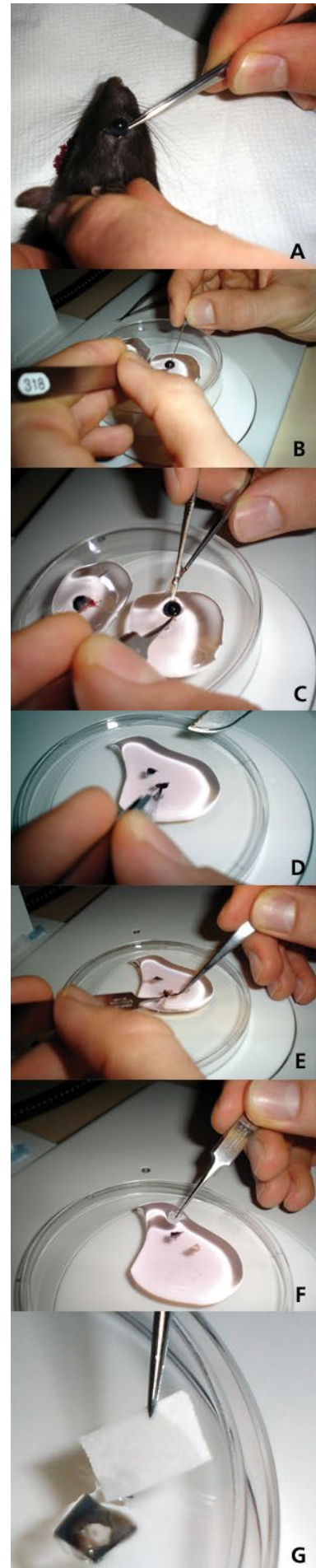
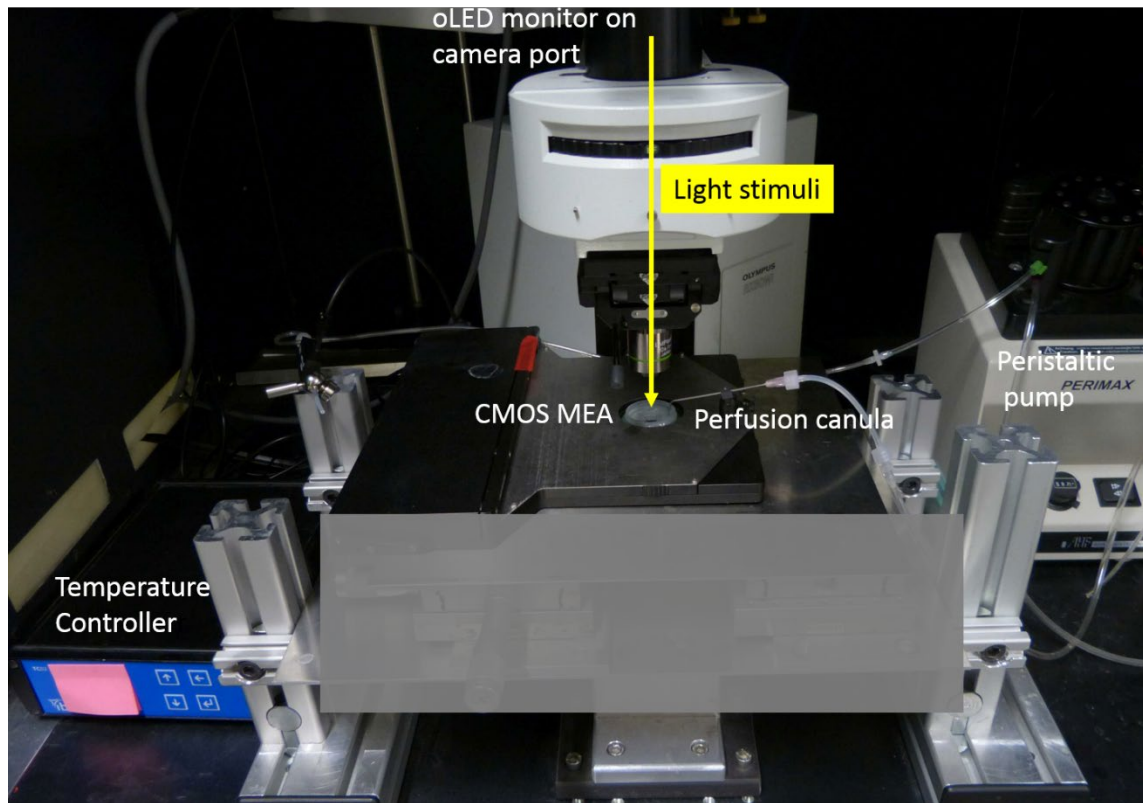


Fig. 1

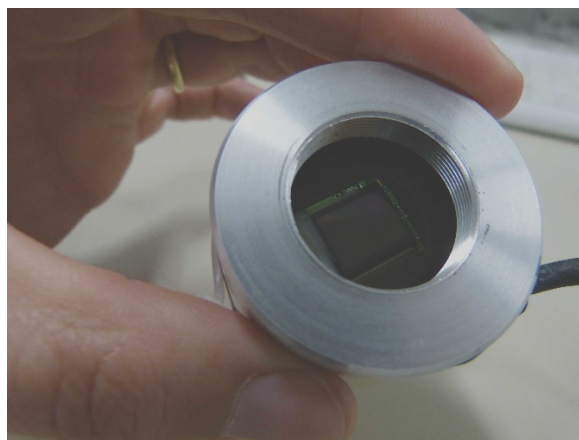
### 3.3 Recording and Stimulation Setup

1. Insert the CMOS-MEA with the retinal sample into the CMOS-MEA amplifier.
2. Start the perfusion at a flow rate of  $\sim 3$  ml/min with carbonated Ames' medium. Let the retina equilibrate with running perfusion for at least 10 minutes.
3. Monitor the spiking activity. When the retinal activity is stable, your experiment may be started.



**Fig. 2: Experimental setup used for light stimulation of the *ex vivo* retina on CMOS-MEA.**

A typical experiment can last for several hours. Make sure that perfusion and temperature remain stable. Ensure stable light conditions around the setup. Light stimulation may be applied from the top. In the example above, a miniature oLED monitor mounted on the camera port of the microscope is used. See reference [4] for details.



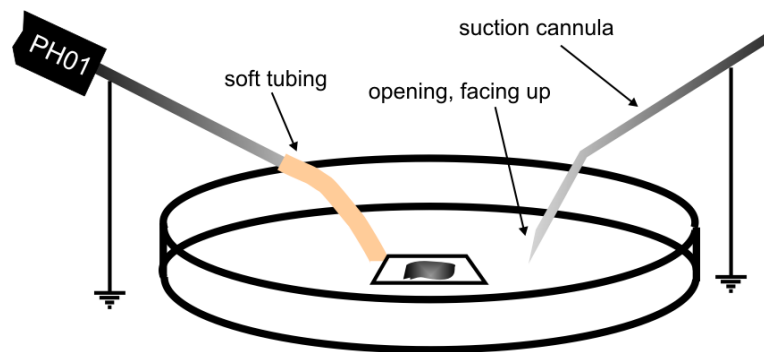
**Fig. 3: Miniature monitor for retina stimulation.**

### 3.4 Perfusion and Noise

Low frequency fluctuations are most often caused by the perfusion. Shortly switch off the pump to see whether the fluctuations disappear if the pump is off. **50 Hz noise** can also be caused by the perfusion, but is independent of the pump running or not.

Perfusion in and out should contain a piece of **metal** that can be connected to the amplifiers ground to remove 50 Hz noise. The easiest way is to use a bend cannula for suction. The opening of the cannula should be positioned in a way that it always sucks air and liquid at the same time, possibly resulting in a constant slurping noise. This prevents the fluid level from going up and down, which also causes noise.

See a suggested perfusion setup below.



**Fig. 4: Suggested perfusion setup.**

If you experience low frequency noise from the perfusion, try to optimize the suction as described above. Additionally, a **droplet isolator** can be used to interrupt the fluid flow between pump and recording chamber. The PPS2 pump from MCS is already equipped with droplet isolators. A 10 Hz high pass filter in the software can also be used to remove low frequency fluctuations in the baseline, if the amplitude is not larger than maybe 200  $\mu\text{V}$ .

50 Hz noise can be caused by the perfusion or by external noise sources. Remove perfusion in and out from the bath. If the noise persists, check for **external noise sources**, like microscope lamp, power supplies close by and so on. If the noise is caused by the perfusion, check the grounding of the perfusion in and out. If the magnetic perfusion holders ([MPH](#)) from MCS are used, the perfusion should already be grounded via the magnet.



### 3.5 System Configuration

The CMOS-MEA amplifier allows recording all 4225 sensors or selected subsets (ROIs) from one CMOS array. Be aware that CMOS data files will be huge. 20 or 25 kHz **sampling rate** is recommended for spike recordings. Slow local field potentials can be sampled with lower sampling rates. In any case, only a few minutes of continuous recording will already result in several GB of data, so design experiments with care. It is possible to restrict recording to regions of interest, which can be selected manually or automatically, based on detected activity. Alternatively, only spike cutouts can be recorded. For details, see the CMOS-MEA5000-System [manual](#).

Suggested **filter settings** for recording spikes only would be 100 Hz to 3 kHz, for slow local field potentials 0.1 Hz to 3 kHz. However, filtering can always be done offline, so recording broad band data is usually the safest option, and won't influence the data volume. Be aware, though, that a low high pass filter setting may result in a fluctuating baseline, and problems with online spike detection.

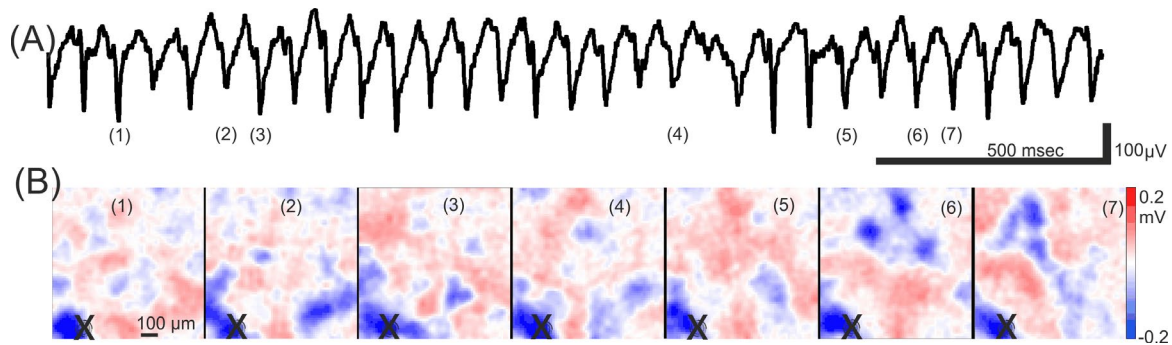
Concerning the **computer**, we recommend Windows 10 Pro, SSD 256 GB, a 1 TB HDD, a Core i7 CPU and a minimum of 16GB RAM, ideally 64 GB.

Both **CMOS-MEA array** versions (16  $\mu\text{m}$  and 32  $\mu\text{m}$  sensor pitch) are well suited for retina recordings. For retina recordings the recording chamber may be selected sufficiently large. Given that the retina recording is performed as an acute experiment with perfusion, we recommend the chambers optimized for laminar flow ([CMOS-MEA16-SCA](#) or [CMOS-MEA32-SCA](#)), in combination with an external Ag/AgCl pellet as ground electrode.

## 4 Sample Recordings

### 4.1 Local Field Potentials in Photoreceptor-degenerated Retinae

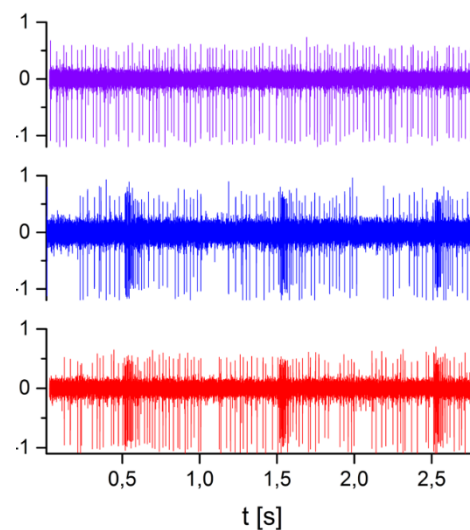
The following figure is adapted from the study by Menzler & Zeck [2]. Local field potentials are routinely recorded in adult (> post-natal day 60) retinas of rd1 or rd10 mouse. Note that for these recordings the filter settings should be HP 0.1 Hz and LP 3 kHz. Currently for the LFP analysis custom written software (Python or Matlab) is required.



**Fig. 5: Electrical imaging of local field potentials in the ganglion cell layer of blind mouse retina. Figure adapted from [5]. (A) Low-pass filtered trace recorded by one electrode of the CMOS-MEA. The numbers in parentheses correspond to the electrical images shown in (B). Continuous recording of the LFP signal is shown here for several seconds only; whereas the CMOS-MEA5000-System allows for minute-long recordings. (B) Spatially filtered electrical images at the selected time points. The local field potentials originate from the same location (sensor marked by an "X").**

### 4.2 Light Induced Spiking Activity

The spikes depicted below were recorded from one CMOS sensor upon presentation of **light stimuli** with different wave length. The light was flashed on at time 0.5 sec / 1.5 sec and 2.5 seconds. For spike recordings the filter settings should be HP 100 Hz and LP 3 kHz. Spiking activity can be analyzed with the CMOS-MEA-Tools, see later chapters.



**Fig. 6: Spiking activity upon presentation of different light stimuli (from top to bottom: deep blue (405 nm), blue (470 nm) and red light (640 nm). The light was flashed on at time 0.5 sec / 1.5 sec and 2.5 seconds.**

## 5 Spike Sorting on CMOS-MEAs

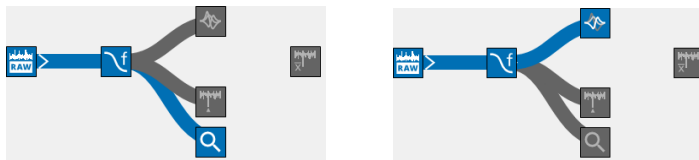
### 5.1 Theoretical Background

The spike sorting of CMOS-MEA data is based on the PhD thesis of Christian Leibig and the paper by Leibig, Wachtler & Zeck [6]. The underlying concept of the spike sorting algorithm is a modified version of “**Independent Component Analysis**” (ICA). This method retrieves the source signals (i.e. the action potentials of one neuron) from recorded signals, which contain mixtures originating from different sources (i.e. neurons). For a detailed description of the spike identification and subsequent sorting please see the [CMOS-MEA5000-System manual](#).

### 5.2 Workflow

The workflow for spike sorting is:

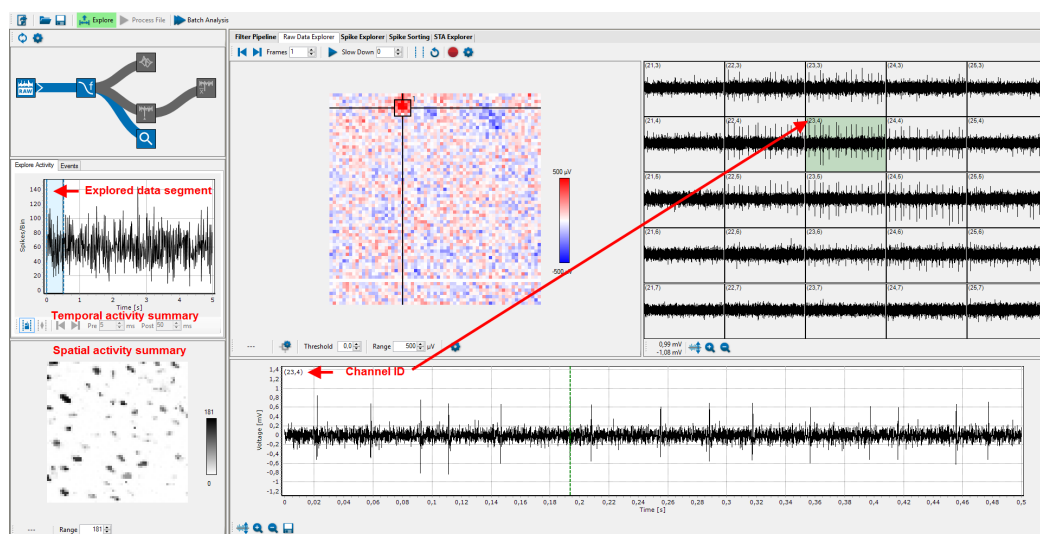
- Generate an **Activity Summary** to localize activity in time and space.
- Inspect the file with the “**Explore**” function and the Raw Data Explorer.
- Test and apply **filter** parameters, if applicable, on a segment of the data (Explore).
- Activate Spike Sorter tool, possibly adjust parameters. Start the sorting process with “**Process File**”, which might take some time.
- Inspect sorted units and **export** results.



Details are described in the chapters below.

### 5.3 Unsupervised Automated Spike Sorting

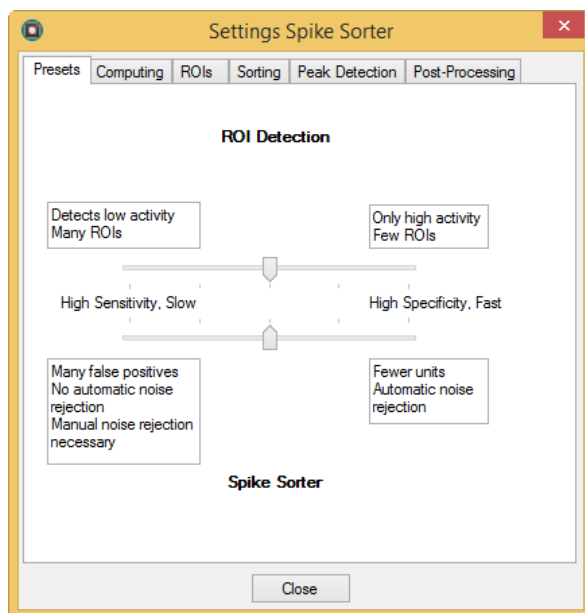
Cell identification is performed using CMOS-MEA-Tools as described in [6] and in the CMOS-MEA5000-System manual. The following pictures show some screenshots exemplifying the spike sorting process. Upon loading the recorded file, the Raw Data Explorer allows for a brief inspection of the signals.



**Fig. 7: Raw traces processed using CMOS-MEA-Tools. The extracellular voltages recorded at one given timepoint are presented in the central image (red-blue color coded). The trace of one selected sensor is shown in the lower part. A set of 5x6 selected electrodes is shown in the right part of the figure.**

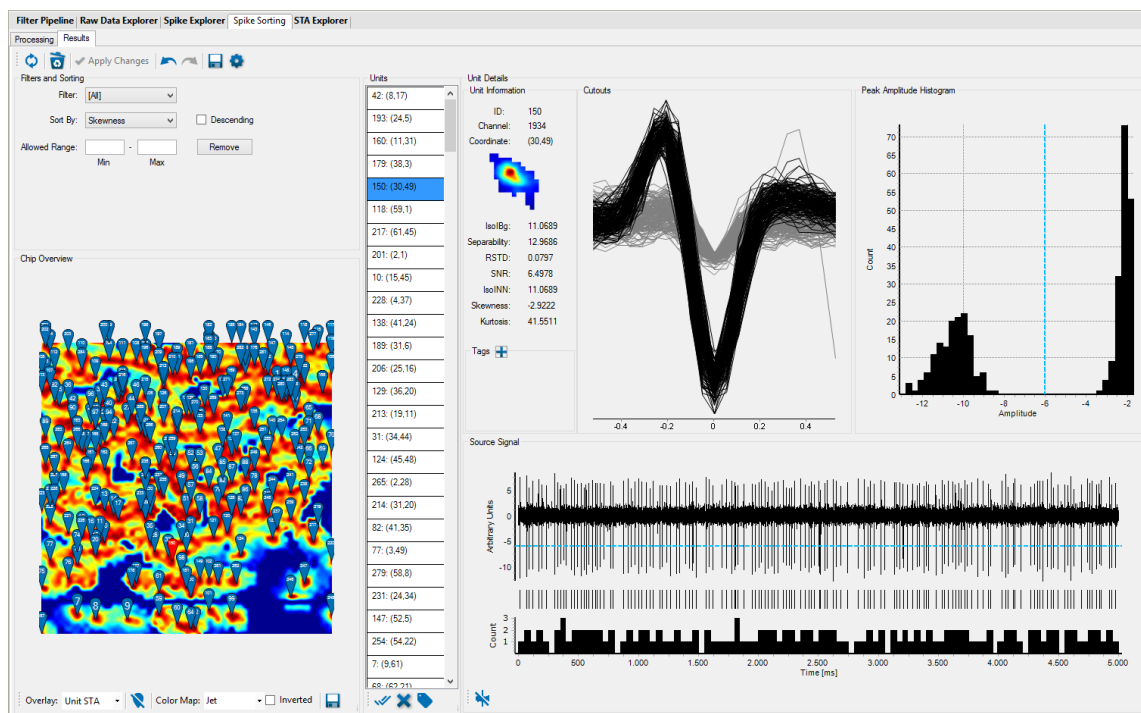
Appropriate **filters** may now be added using the filter pipeline. The filtered recording can then be passed through the spike sorter. Spike sorting should be performed using a powerful desktop or server. We recommend at least an i7 processor and 64 GB of RAM. For large files it's possible to distribute the workload among several PCs in a network with the **Remote Sorting Client** function. Please see manual for details.

If necessary, all parameters of the sorting algorithm can be individually adjusted. For example, the "fraction of coincident spikes" sets the maximal fraction of spikes which can occur in two spike trains at identical time points (including a small-time shift set in the "Max Shift" parameter). The default parameter setting of 50 % means that the two spike trains are assigned to two cells, if less than half of the spikes occur simultaneously. For wild-type retina, this parameter might be decreased. However, for most purposes a simple adjustment of the **sorting sensitivity** with the provided slider interface is sufficient.



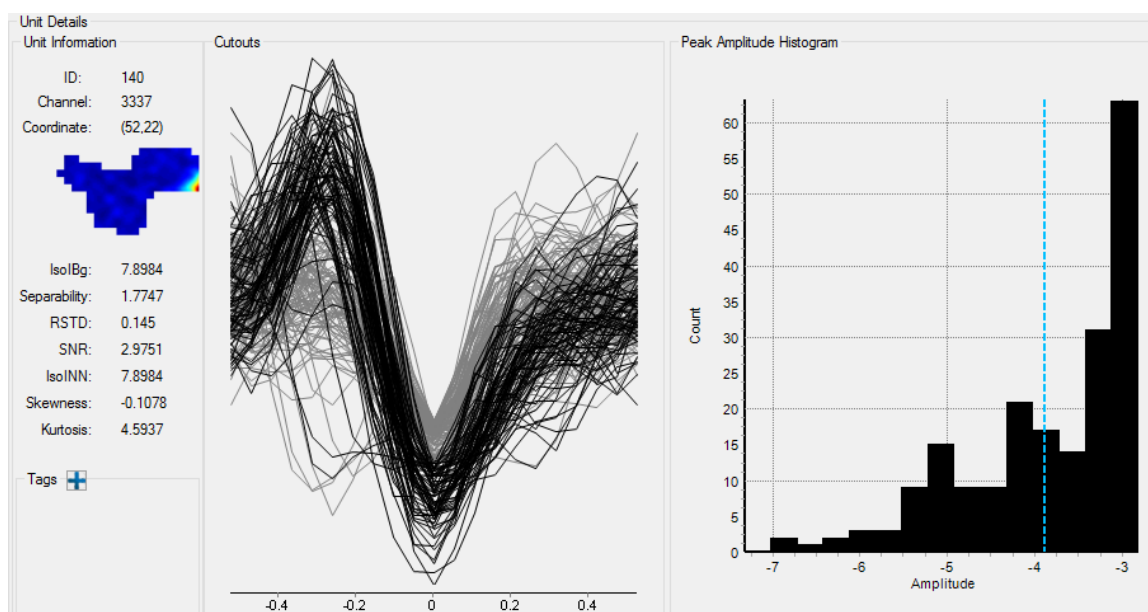
**Fig. 8:** With a simple slider interface, the sorting sensitivity can be adjusted.

We recommend starting with the default settings. Manual inspection of the sorted results reveals if the sorter properly separated signals from noise. (Fig. 9).



**Fig. 9: Result of the spike sorting.** In the middle part a list of all identified units (cells) is shown. On the left side the spatial positions of these units are visualized. On the right part, one selected unit is shown in detail including the quality measures of the sorting result.

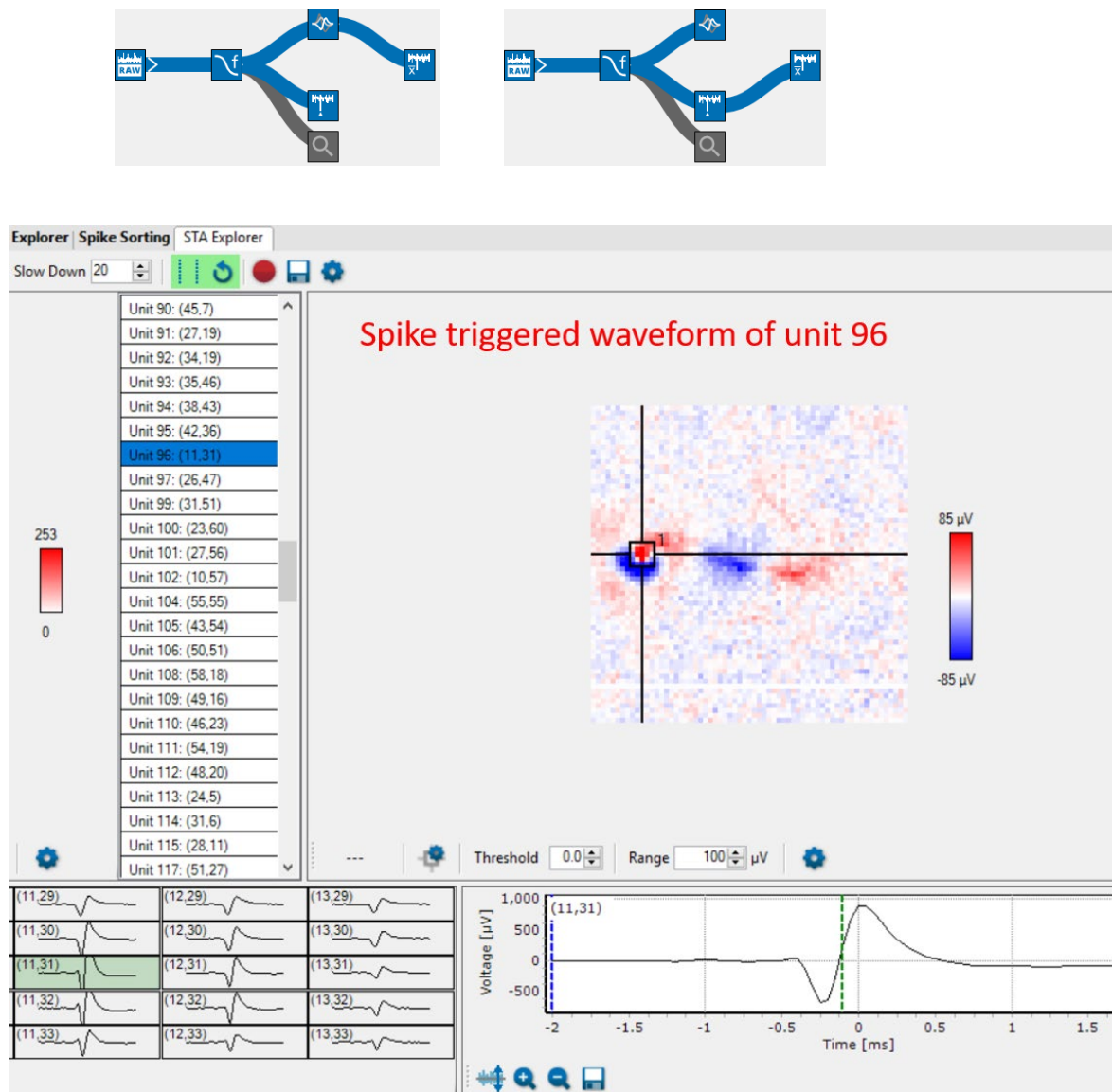
The sorted units can be manually checked and sorted by tags. Units with poor sorting results may be removed. Sorted units can be **exported** in ASCII or HDF5 format for further processing.



**Fig. 10: Unit with poor sorting result.** There is no clear separation of noise and signal in the Peak Amplitude Histogram, like for the unit shown in Fig. 9.

## 5.4 Tracing Activity on the CMOS-MEA

**Spike Triggered Averages (STA)** can be used to extract small signals, like action potentials propagating along an axon, which are normally hidden in the noise. The STA tool uses spikes detected on **one selected channel** as reference. For each detected event on the reference channel, data from all channels for a selected time frame plus/minus a few milliseconds around each event is averaged. This means that reoccurring events are pronounced, while random noise is reduced. As a result, small signals become detectable, and propagating signals can be visualized. Sorted units or unsorted spikes can be used as source for the STA. Just click on the STA icon to change the source.



**Fig. 9: STA with sorted units on channel 11, 31 as reference. With these STAs, the propagation of action potentials along the axon can be visualized.**

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