iCell® GlutaNeurons Application Protocol for Multiwell-MEA

Measuring Neuronal Electrical Activity of iCell® GlutaNeurons (FUJIFILM Cellular Dynamics, Inc. (FCDI)) on the 24-well glass Multiwell-MEA System (Multi Channel Systems MCS GmbH).

Version 1.1: November 2018

1 Introduction

iCell® GlutaNeurons from FCDI are human glutamatergic-enriched neurons derived from induced pluripotent stem cells. They build fully differentiated, homogeneous cultures and are suitable for electrophysiological applications. The iCell® GlutaNeurons show stable spontaneous activity in burst-like activity patterns and display functional neuronal networks. It is recommended to perform experiments for compound testing between day 14 and 18 post thaw.

Multi Channel Systems MCS GmbH's microelectrode array (MEA) technology enables non-invasive, label-free measurements of local field potentials of electrically active cells including neurons, islets of Langerhans and cardiomyocytes and thus to decipher the function and dysfunction of the underlying ion channels.

iCell® GlutaNeurons can be cultured on MEA plates to form an electrically active neuronal network amenable to electrophysiological examination. Together, iCell® GlutaNeurons and the Multi Channel Systems MEA technology form an excellent, non-invasive platform for *in vitro* screening of compound efficacy and toxicity on human neuronal cells.

This Application Protocol describes how to handle iCell® GlutaNeurons for use on the Multi Channel Systems Multiwell system using the 24-well glass MEA plate.

2 Required Equipment, Consumables, and Software

The following equipment, consumables and software are required for the handling of the iCell® GlutaNeurons and the 24-well glass Multiwell-MEA plates.

Table 1: Overview of required equipment.

Item	Vendor	Catalog Number
Low Adhesion Pipette Tips 10 µl	Carl Roth	PC91.1
Multiwell-MEA System	Multi Channel Systems MCS	
Storage Box	Multiple Vendors	
37°C Water Bath	Multiple Vendors	
Biosafety Cabinet	Multiple Vendors	
Cell Culture Incubator	Multiple Vendors	
Centrifuge	Multiple Vendors	
Inverse Microscope	Multiple Vendors	
Hemocytometer or Automated Cell Counter	Multiple Vendors	
Pipettors 10, 100, 1000 µl	Multiple Vendors	

Table 2: Overview of required consumables.

Item	Vendor	Catalog Number
iCell® GlutaNeurons Kit, 01279	FUJIFILM Cellular Dynamics, Inc. (FCDI)	R1061 (≥1 x 10^6 cells) R1034 (≥6 x 10^6 cells)
50% Polyethyleneimine (PEI) Solution	Sigma-Aldrich	181978
Borate Buffer, 20X	Thermo Fisher Scientific	28341
1.5 ml and 50 ml Sterile Centrifuge Tubes	Multiple Vendors	
Trypan Blue Solution	Sigma-Aldrich	T8154
Laminin	Sigma-Aldrich	L2020
24-well Glass Multiwell-MEA Plates	Multi Channel Systems MCS	24W300/30G-288
BrainPhys Neuronal Medium	STEMCELL Technologies	05790

N-2 Supplement, 100X	Thermo Fisher Scientific	17502048
Penicillin-Streptomycin, 100X	Multiple Vendors	
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Multiple Vendors	
Sterile Distilled Water	Multiple Vendors	
0.22 µm Sterile Filter Unit	Multiple Vendors	

Table 3: Overview of required software.

Item	Vendor	Catalog Number
Multiwell-Screen v. 1.5.5.0 and above	Multi Channel Systems MCS	http://www.multichannelsystems.com/software/multiwell-screen
Optional further Analysis Software of your choice	Multiple Vendors	

3 Workflow

The iCell® GlutaNeurons are thawed into Complete BrainPhys Medium and plated in Complete BrainPhys Medium plus Laminin (dotting medium) into 24-well MEA plates, previously coated with 0.07% PEI solution. On day 1 and day 2 post-plating 50% of the used medium has to be changed with Complete BrainPhys Medium. During the subsequent culture 50% of the spent medium should be replaced every other day.

Table 4: Overview of workflow.

Days in culture	
Day -1	Coat MEA plate with 0.07% PEI
Day 0	Thaw and plate iCell® GlutaNeurons into MEA plate
Day 1	Replace 50% of spent medium
Day 2	Replace 50% of spent medium
Day 4+	Replace 50% of spent medium every other day

4 Preparations

4.1 Preparing the 0.07% PEI solution

1) Weight in a sufficient amount of 50% PEI solution.
2) Prepare a sufficient amount of borate buffer (1x) by diluting the borate buffer (20x) 1:20 in distilled water.
3) Dilute the 50% PEI solution in borate buffer (1x) to prepare a 0.07% PEI solution.
4) Vortex the 0.07% PEI solution to ensure that it is properly mixed.
5) Filter the 0.07% PEI solution through a 0.22 µm sterile filter unit.

4.2 Preparing the 24-well glass MEA plate

All steps need to be performed under sterile conditions.

1) Add 80 µl/well of the sterile 0.07% PEI solution to the 24-well glass MEA plate (Fig. 1) to coat the entire bottom of the wells.
2) Incubate for 1 h at 37°C.
3) Aspirate the PEI solution from the 24-well glass MEA plate and rinse the wells twice with >500 µl/well of D-PBS and once with >500 µl/well of sterile distilled water.
4) Air-dry the 24-well MEA plate with the lid removed in a laminar flow hood overnight. *It is crucial to let the MEA plate air-dry overnight to reach an optimal performance.*

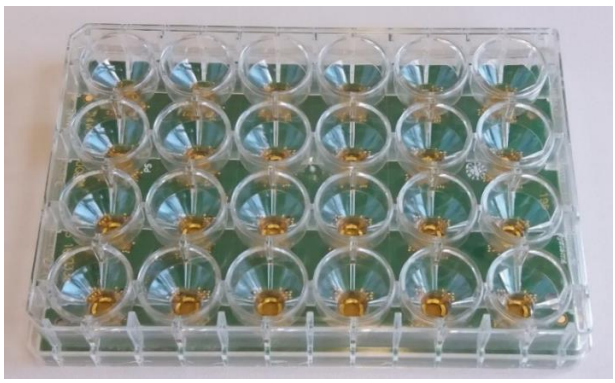

Fig. 1: 24-well glass Multiwell-MEA plate with PEDOT-coated gold electrodes.

4.3 Preparing the Medium

All steps need to be performed under sterile conditions.

1) Thaw iCell Neural Supplement B, iCell Nervous System Supplement and N2-Supplement at room temperature and Laminin in the refrigerator.
2) Transfer 95 ml of BrainPhys Neuronal Medium into a 100 ml sterile glass or plastic bottle.
3) Mix iCell Neural Supplement B by pipetting up and down.
4) Add 2 ml of iCell Neural Supplement B to the medium.
5) Mix iCell Nervous System Supplement by pipetting up and down.
6) Add 1 ml of iCell Nervous System Supplement to the medium.
7) Prepare the Complete BrainPhys Medium by adding 1 ml of N2-Supplement, 1 ml of Penicillin-streptomycin and 100 µl of a 1 mg/ml solution Laminin to the medium.
8) Filter the Complete BrainPhys Medium through a 0.22 µm sterile filter unit.
9) Store the Complete BrainPhys Medium at +4°C. The Complete BrainPhys Medium can be used up to 2 weeks.

Table 5: Components and volumes of Complete BrainPhys Medium.

Item	Volume	Final Concentration
BrainPhys Neuronal Medium	95 ml	-
iCell Neural Supplement B	2 ml	-
iCell Nervous System Supplement	1 ml	-
N-2 Supplement	1 ml	-
Penicillin-streptomycin	1 ml	-
Laminin (1 mg/ml)	100 µl	1 µg/ml

4.4 Preparing the Fill-up Medium

All steps need to be performed under sterile conditions.

1) Prepare an aliquot of Fill-up Medium with the volume that is needed to fill the wells containing iCell® GlutaNeurons and equilibrate this aliquot to room temperature.
2) The Fill-up Medium contains Complete BrainPhys Medium with a higher Laminin concentration of 33.3 µg/ml.

4.5 Preparing the storage box

1) Place a paper towel on the bottom of a storage box (Fig. 2) and add 10 - 15 ml of distilled water to moisten the paper towel.
2) Put the lid of a common culture plate in the storage box to avoid that the bottom of the MEA plate gets wet.
3) Loosely place the lid of the storage box on top of it.

Fig. 2: Exemplary storage box with paper towel that can hold 3 Multiwell-MEA plates.

5 Thawing iCell® GlutaNeurons

All steps need to be performed under sterile conditions. Also see the iCell® GlutaNeurons User's Guide.

1) Equilibrate the Complete BrainPhys Medium to room temperature for 1 h.
2) Prepare the dotting medium with 900 µl Complete BrainPhys Medium and 100 µl Laminin to reach a final Laminin concentration of 100 µg/ml.
3) Quickly transfer cryopreserved iCell® GlutaNeurons from the vapor phase of liquid nitrogen storage tank directly to a 37°C water bath (avoid submerging the cap). Ideally use a Mr. Frosty™ or a similar device for the transfer or alternatively transport the cryovial on dry ice.

   Attention: *Avoid keeping the cryovial longer than 10 min on dry ice.*

4) Thaw the vial until only a small ice clump is visible (**2 minutes**).
5) Gently transfer the cell suspension to a 50 ml centrifuge tube using a 1000 µl pipette.

   Attention: *Avoid repeated pipetting of the cell suspension.*

6) Rinse the empty iCell® GlutaNeurons cryovial with 1 ml of Complete BrainPhys Medium to recover remaining cells from the cryovial.
7) Add the 1 ml of Complete BrainPhys Medium with the recovered cells drop-wise (~1 drop / 2 sec) to the cell suspension in the 50 ml centrifuge tube. Carefully swing the centrifuge tube back and forth while adding the medium to mix the cell suspension and reduce the osmotic stress on the cells.
8) Gently add additional 8 ml of Complete BrainPhys Medium drop-wise (~1 drop / 1 sec) to the 50 ml centrifuge tube, while gently swirling the tube back and forth, to reach a total volume of 10 ml.

9) Gently mix the cell suspension prior to cell counting. Remove a 20 µl sample of cells suspension to count the cells and confirm viability using a hemocytometer (using trypan blue exclusion to identify viable cells) or an automated cell counter.
10) Centrifuge the cell suspension at 400 x g for 5 min at room temperature.
11) During centrifugation, count the cells and calculate the final volume of dotting medium needed to resuspend the cell pellet to 24 x 10^6 viable cells / ml using the number of viable cells.
12) Aspirate the supernatant, being careful not to disturb the cell pellet. Tilt the centrifuge tube, while aspirating the supernatant.
13) Add the calculated volume of dotting medium to the cell pellet to resuspend the neurons to a density of 24 x 10^6 viable cells / ml.
14) Gently mix the cell suspension by carefully swirling the centrifugation tube back and forth.
15) Transfer the cell suspension to a sterile 1.5 ml centrifuge tube.

6 Plating iCell® GlutaNeurons into the MEA plate

All steps need to be performed under sterile conditions.

1) Gently mix the cell suspension by swirling the 1.5 ml centrifugation tube.
2) Plate iCell® GlutaNeurons on the pre-coated MEA plate by dispensing 5 µl droplets of the cell suspension (120,000 cells / well) in the center of the electrode field of each well (Fig. 3).

   Attention: *Even though gold electrodes are robust try to avoid touching the electrode area to prevent damage. Ideally form a drop on the tip of the pipette and place onto the electrode field.*

3) Put the lid on the MEA plate and place it in the storage box (Fig. 4) and store it in a cell culture incubator at 37°C and 5% CO_2 in a humidified atmosphere for 1 h to allow the cells to attach to the plate.

4) Gently add 300 µl of Fill-up Medium down to the side of the wells of the MEA plate. Carefully place the tip at the edge between bottom and side of the wells and add the medium, slowly lifting up the pipette during dispensing.

   Attention: *Adding the medium too quickly will dislodge the adhered cells.*

5) Place the MEA plate in the storage box and transfer the storage box into the incubator (avoid major vibrations). Culture the iCell® GlutaNeurons at 37°C and 5% CO_2 in a humidified atmosphere.

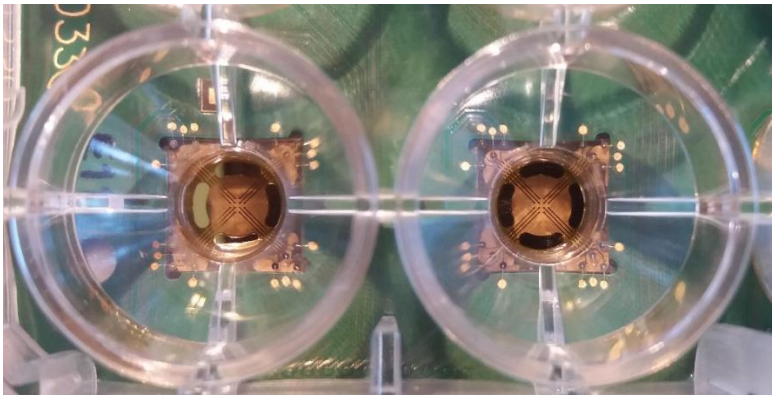

Fig. 3: Close-up view of two wells of a 24-well glass Multiwell-MEA plate. Note the 12 electrodes in the center of the well that are surrounded by 4 reference electrodes. Cells ideally are only plated in the center on the recording electrodes without covering the reference electrodes.

Fig. 4: Exemplary storage box with moistened paper towel, holding 3 Multiwell-MEA plates.

7 Maintaining iCell® GlutaNeurons in the MEA Plate

All steps need to be performed under sterile conditions.

1) Prepare an aliquot of Complete BrainPhys Medium with the volume that is needed for the medium change and equilibrate the aliquot to room temperature.
2) On day 1 and day 2 post-plating, change 50% of the medium (150 µl).
3) Maintain the neurons in the MEA plate by changing 50% of the spent medium every other day.
4) Culture the neurons in a cell culture incubator at 37°C, 5% CO_2 in a humidified atmosphere.
5) It is recommended to perform experiments for compound testing between day 14 and 18 post-plating.

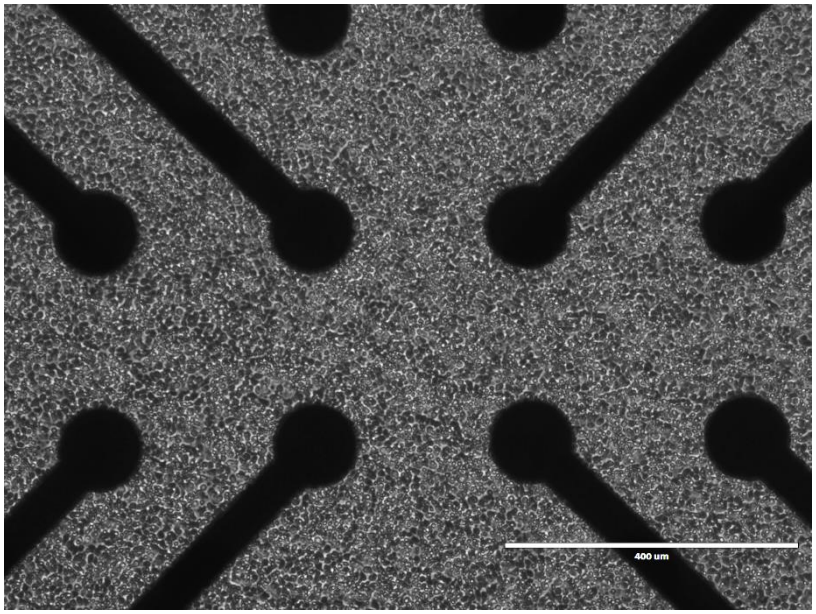

Figure 5: GlutaNeurons on an electrode field of the 24-well MEA in a density of 120k per 5 µl on day 1 post-plating. (Scale bar: 400 µm)

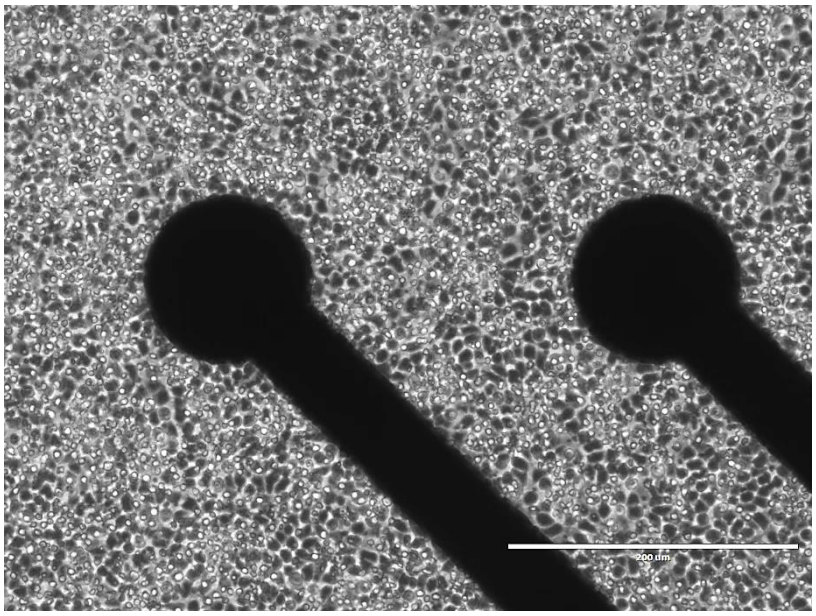

Figure 6: GlutaNeurons on an electrode field of the 24-well MEA in a density of 120k per 5 µl on day 1 post-plating. (Scale bar: 200 µm)

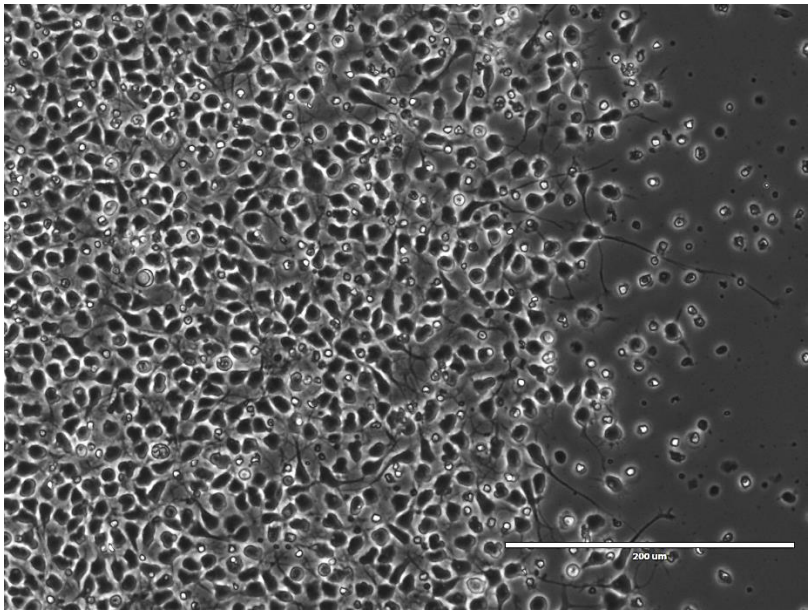

Figure 7: GlutaNeurons on an electrode field of the 24-well MEA in a density of 120k per 5 µl on day 1 post-plating. There are already neurites present. The picture was taken at the outer part of the well, where the density of the cells is lower. (Scale bar: 200 µm)

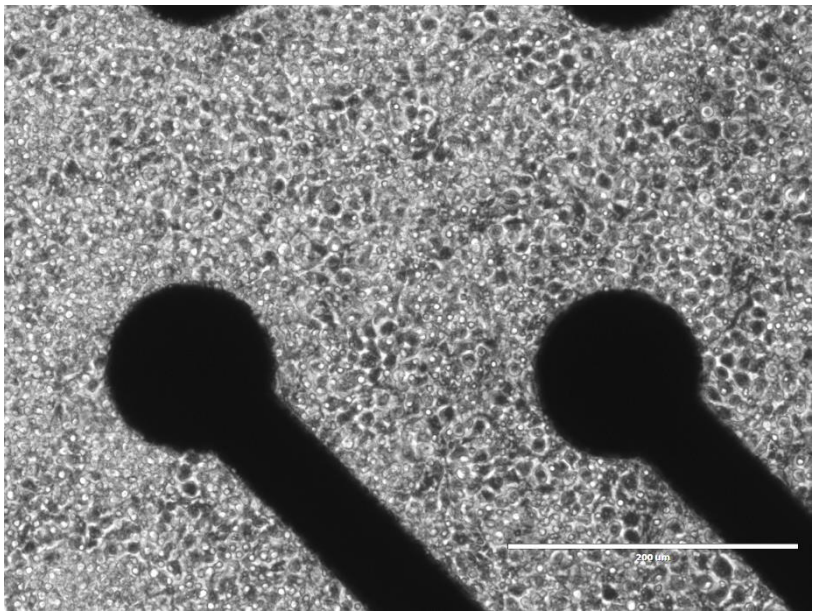

Figure 8: GlutaNeurons on an electrode field of the 24-well MEA in a density of 120k per 5 µl on day 14 post-plating. (Scale bar: 200 µm)

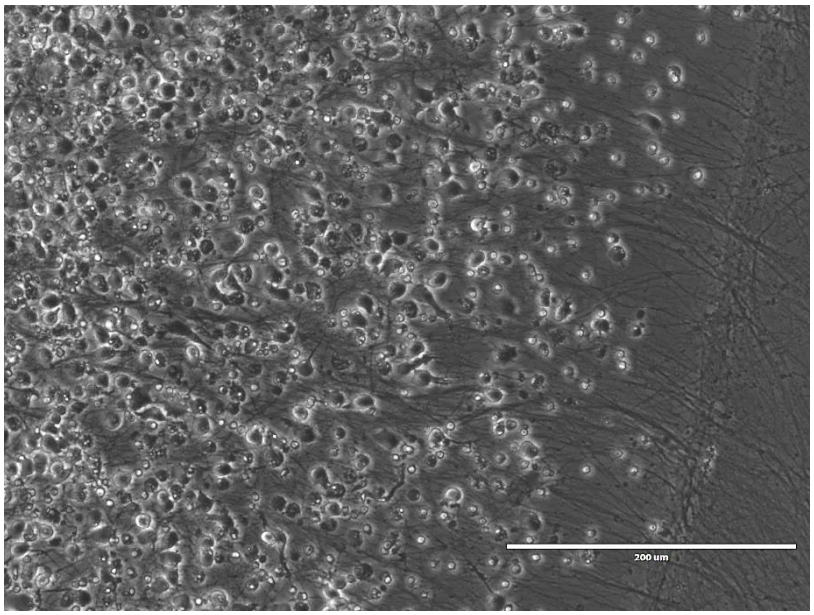

Figure 9: GlutaNeurons on an electrode field of the 24-well MEA in a density of 120k per 5 µl on day 14 post-plating. The picture was taken at the outer part of the well, where the density of the cells is lower. (Scale bar: 200 µm)

8 Data Acquisition

The acquisition of electrical activity of the iCell® GlutaNeurons on the Multiwell-MEA system should be executed according to the relevant manuals of MCS. The following procedure details the preparations before starting a recording of neuronal activity.

1) Turn on the MCS Multiwell-MEA system and start the Multiwell-Screen Software.
2) Wait until the temperature of the device has reached 37°C.
3) Dry the bottom of the MEA plate with a paper towel, before placing the MEA plate into the Multiwell-MEA system. Humidity on the electrodes might affect the quality of the recording by increasing the background noise.
4) Place the MEA plate in the device and choose your settings for the recording.
5) Let the cells rest for 10 min, before starting the recording.
6) Start the data acquisition.

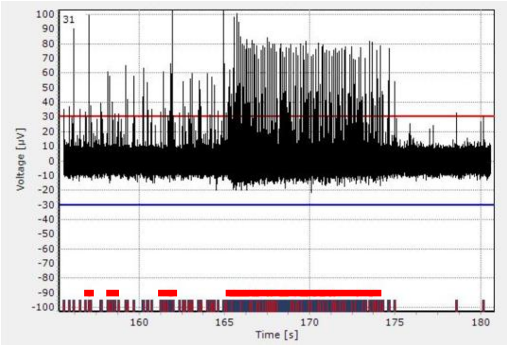

Figure 10: Local field potentials grouped in bursts on day 12 post-plating.

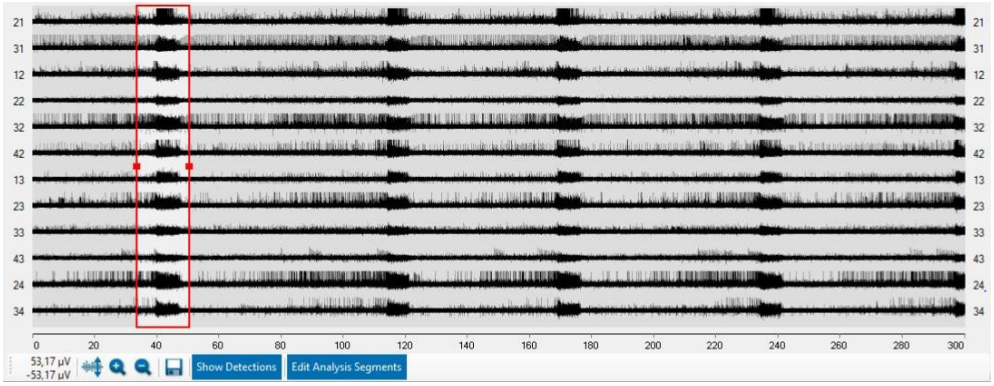

Fig. 11: Voltage traces of local field potentials of one well at day 12 post-plating. The red box marks synchronous bursts on all 12 electrodes. (Time scale in [sec])

9 Summary

iCell® GlutaNeurons can be reanimated from cryopreservation and plated directly into MEA plates where they exhibit the expected spontaneous neuronal electrical activity and establish neuronal networks. The procedures presented in this application protocol point out the ease of using FCDI's iCell® GlutaNeurons on the Multi Channel Systems MCS Multiwell-MEA system. The combination of these two products provides an *in vitro* system for acquiring electrical activity of human neuronal cells with a higher throughput.

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