ISTITUTO ITALIANO DI TECNOLOGIA
OPTICAL NANOSCOPY

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UNIVERSITÀ DEGLI STUDI DI GENOVA

SLG Functionalized MEA for Enhanced Detection of Neural Network Development

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Scientific Background

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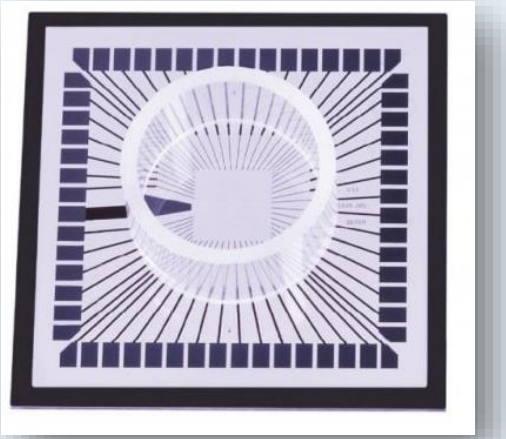
Microelectrode Array (MEA)

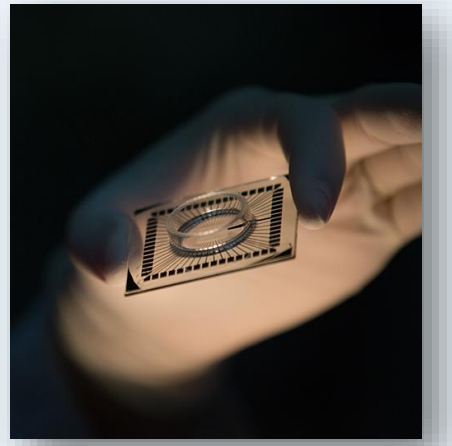
Application of MEAs

- **Electrophysiology** tool
- Extensive ***in vitro*** studies
- Recording of the **spontaneous activity** of **primary neuronal networks**
- Recordings used as an **assay** for **network performance** in applied settings



Description

- Consists of a discrete number of metal electrodes integrated on a **solid substrate** (glass or silica)
- Planar gold, titanium and platinum are the most common **electrode** materials
- Encapsulated by a glass ring to perform **cell cultures** on the chips



Problems Faced/Drawbacks

Low signal-to-noise ratio (SNR)

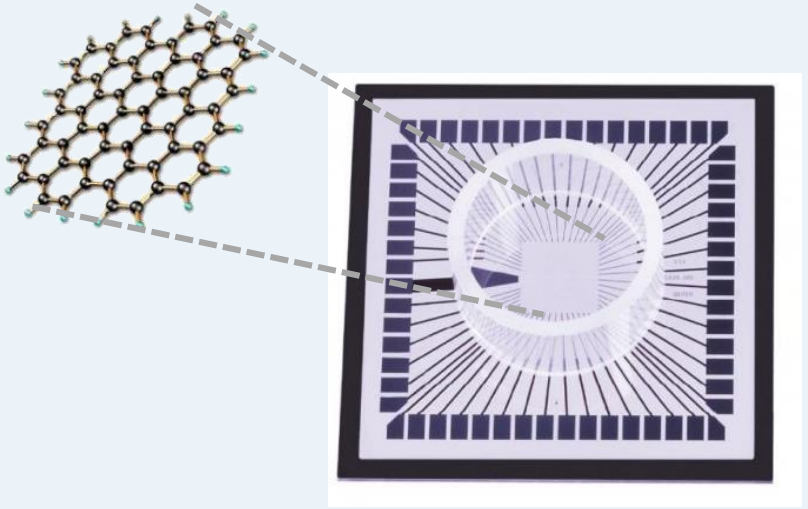
Improvements

- Chemical functionalization of the electrodes
- Topographical modification increasing roughness
- Fabrication of porous electrodes
- Graphene MEAs consisting of graphene electrodes

Objectives

- Exploring the interplay between the **carbon based interface** and **neuronal networks** during the **complete developmental phase** at **whole network scale**

Role of Single Layer Graphene (SLG) in (MEAs)? ? ?



- **SLG** grown by **chemical vapor deposition** on Cu foil may be considered extremely **favorable** in the field of **biosensor development**

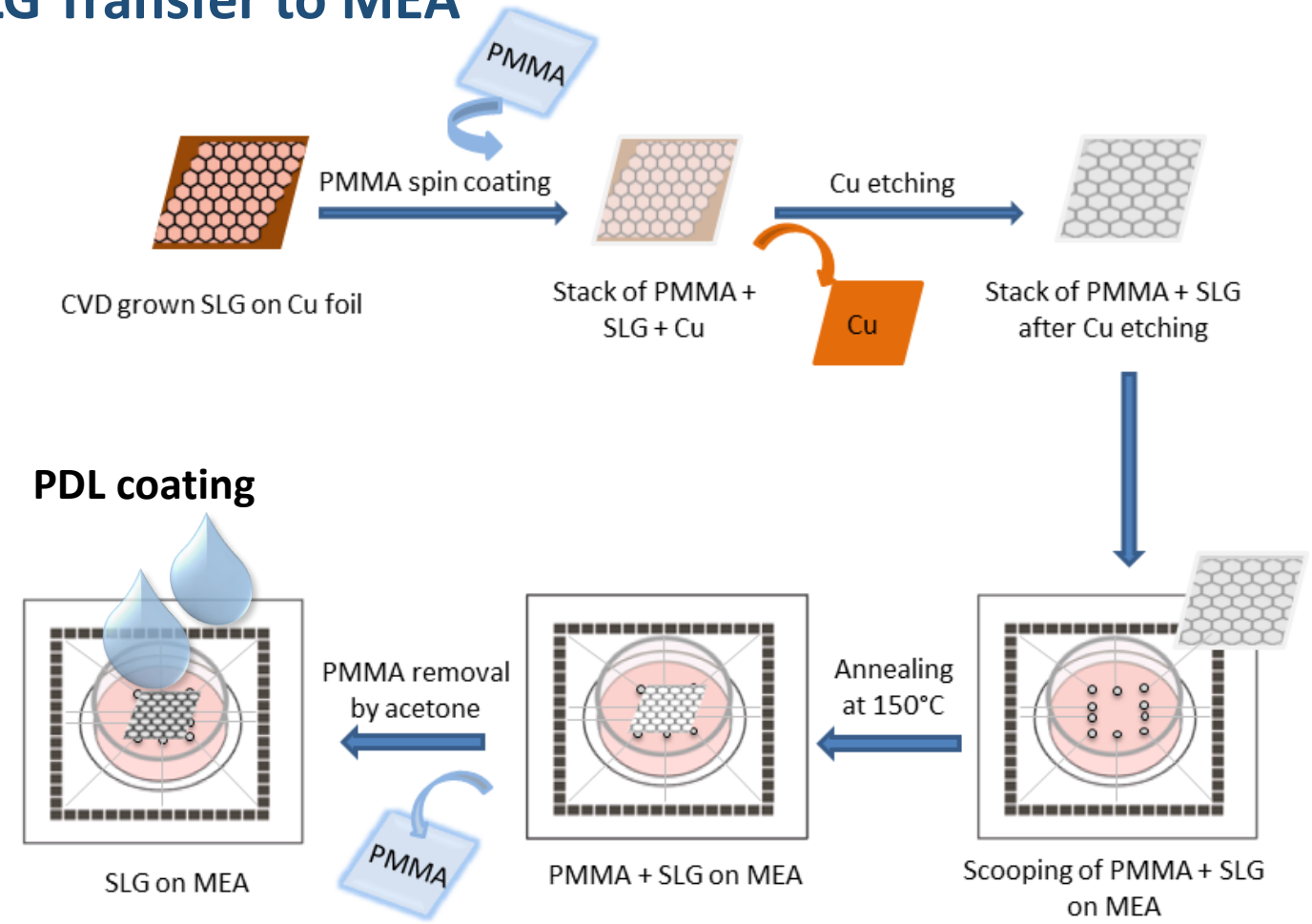
- ✓ **transparency**
- ✓ **scalability**
- ✓ **convenient transfer onto any substrate, including flexible ones**

Large Grain SLG (LG-SLG)

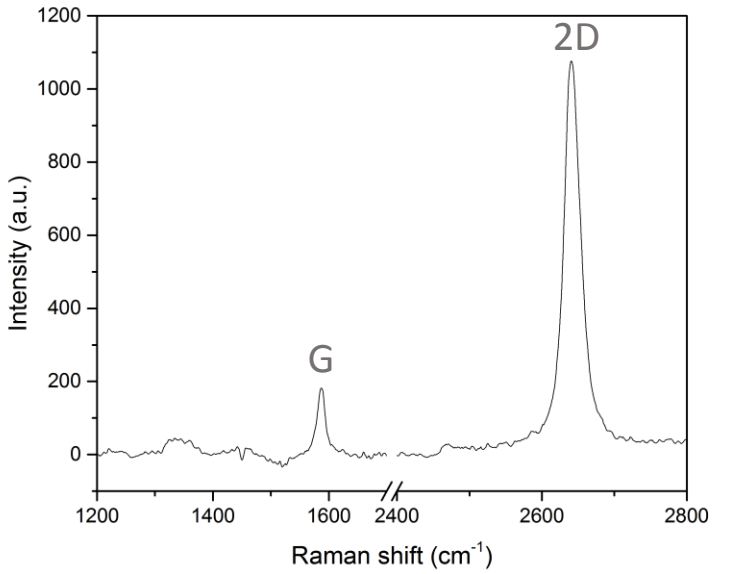
Methodology

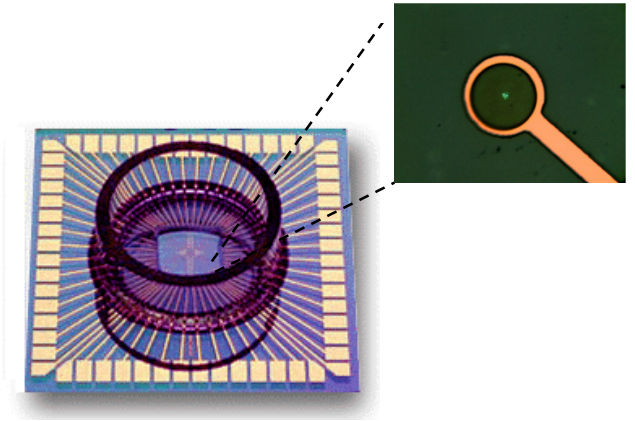
Methodology

SLG Transfer to MEA



Raman Characterization





PDL = poly-D-lysine

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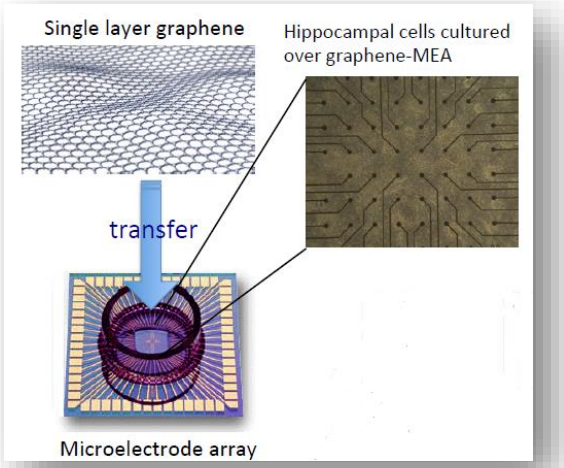
Methodology

Cell Culture → **MEA Recordings** → **Spike & Burst Detection** → **Immunolabeling & Image Analysis**

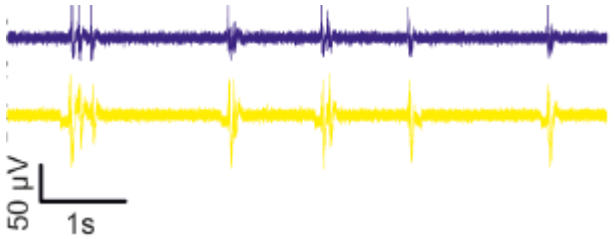
Dissociated neuronal cultures (**hippocampi of 18-day old embryonic rats**)

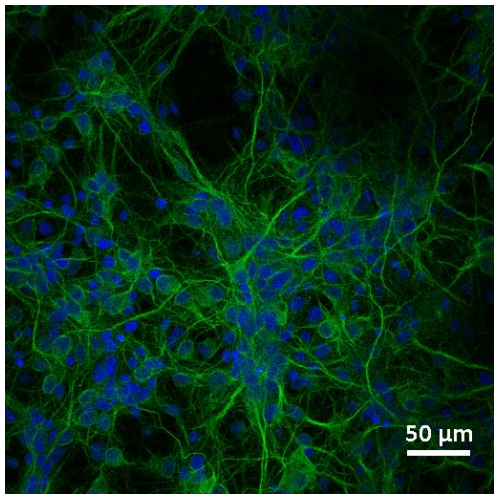


Electrophysiological activity monitored and recorded for **90'** at **7 different days in vitro (DIV)** (from DIV7 to DIV25)

Spikes: **neuronal** signals consisting of short electrical pulses (**action potentials**)

Bursts: consist of packages of spikes over a few milliseconds





Results

Confocal Microscopy on Immunolabeled Samples

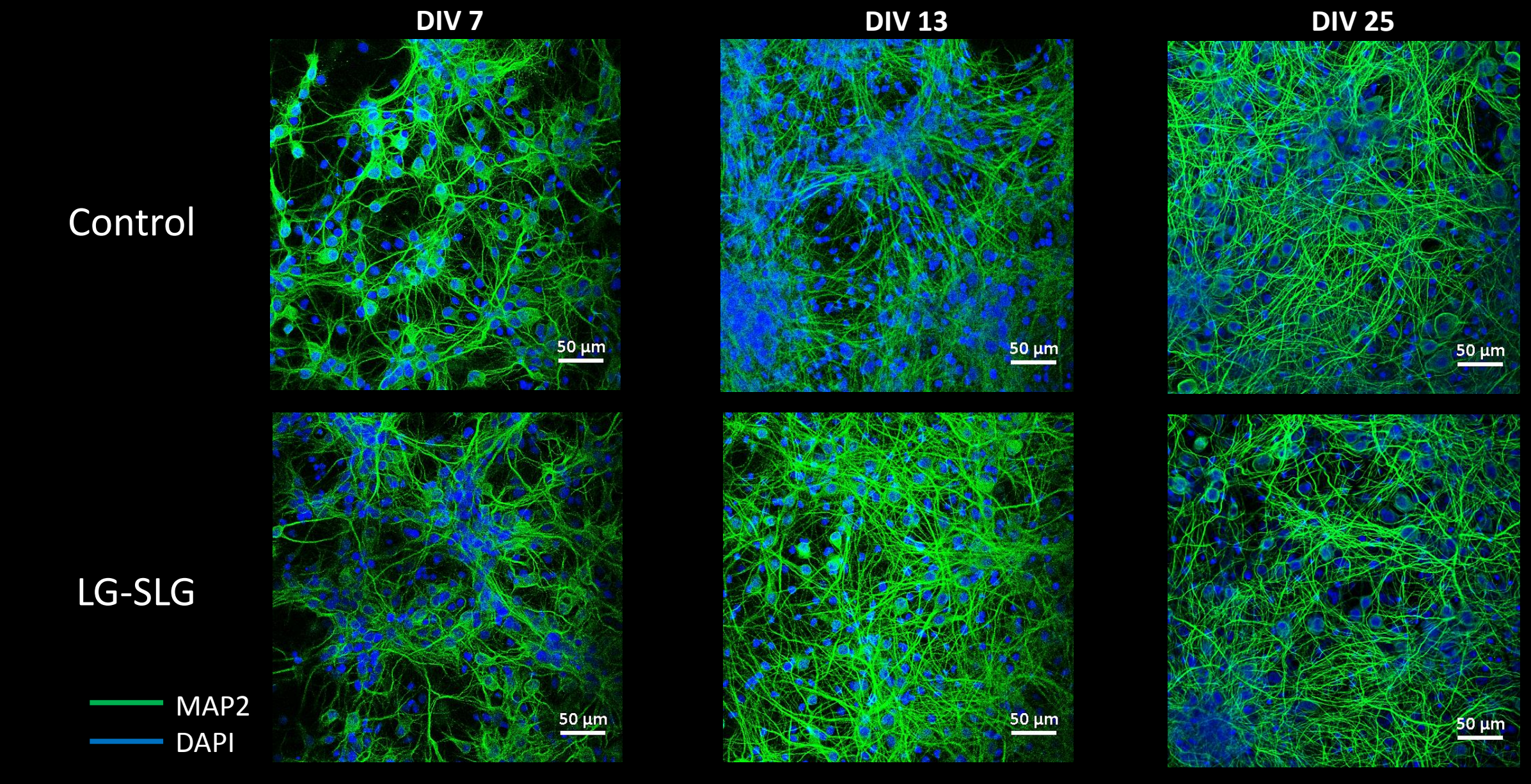
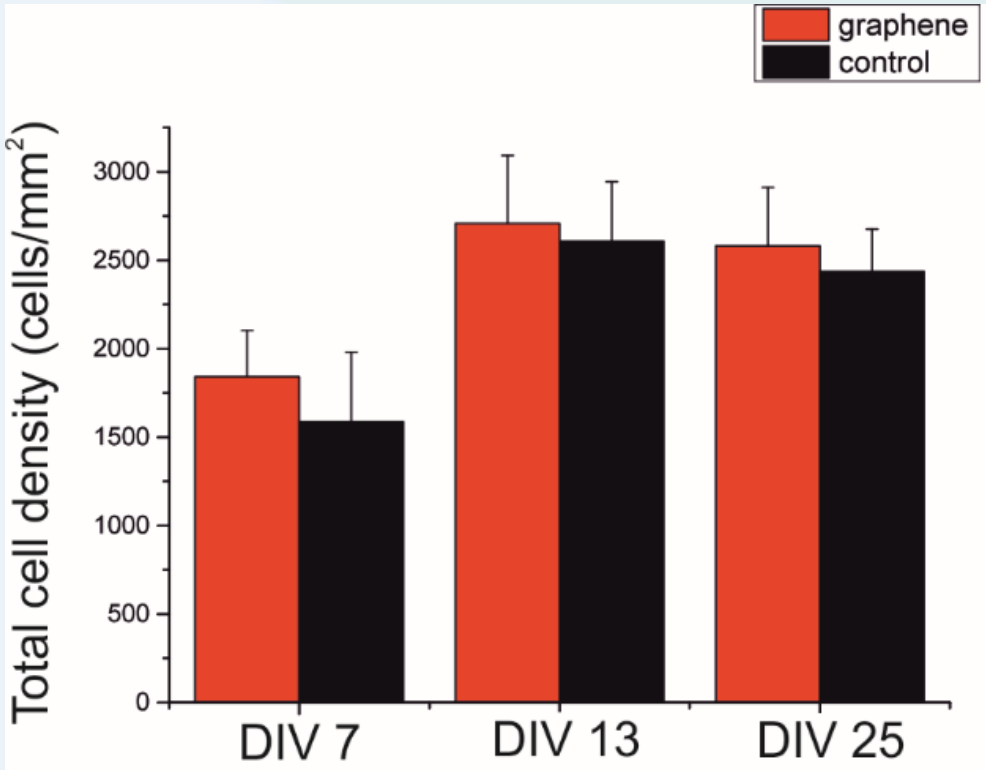
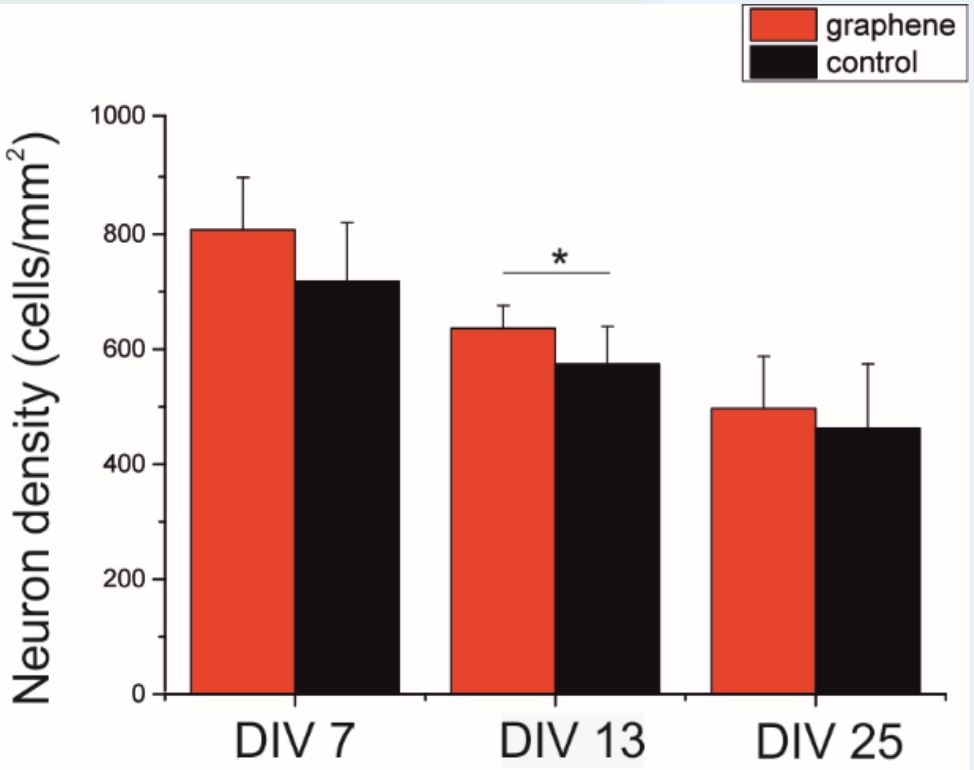


Image Analysis

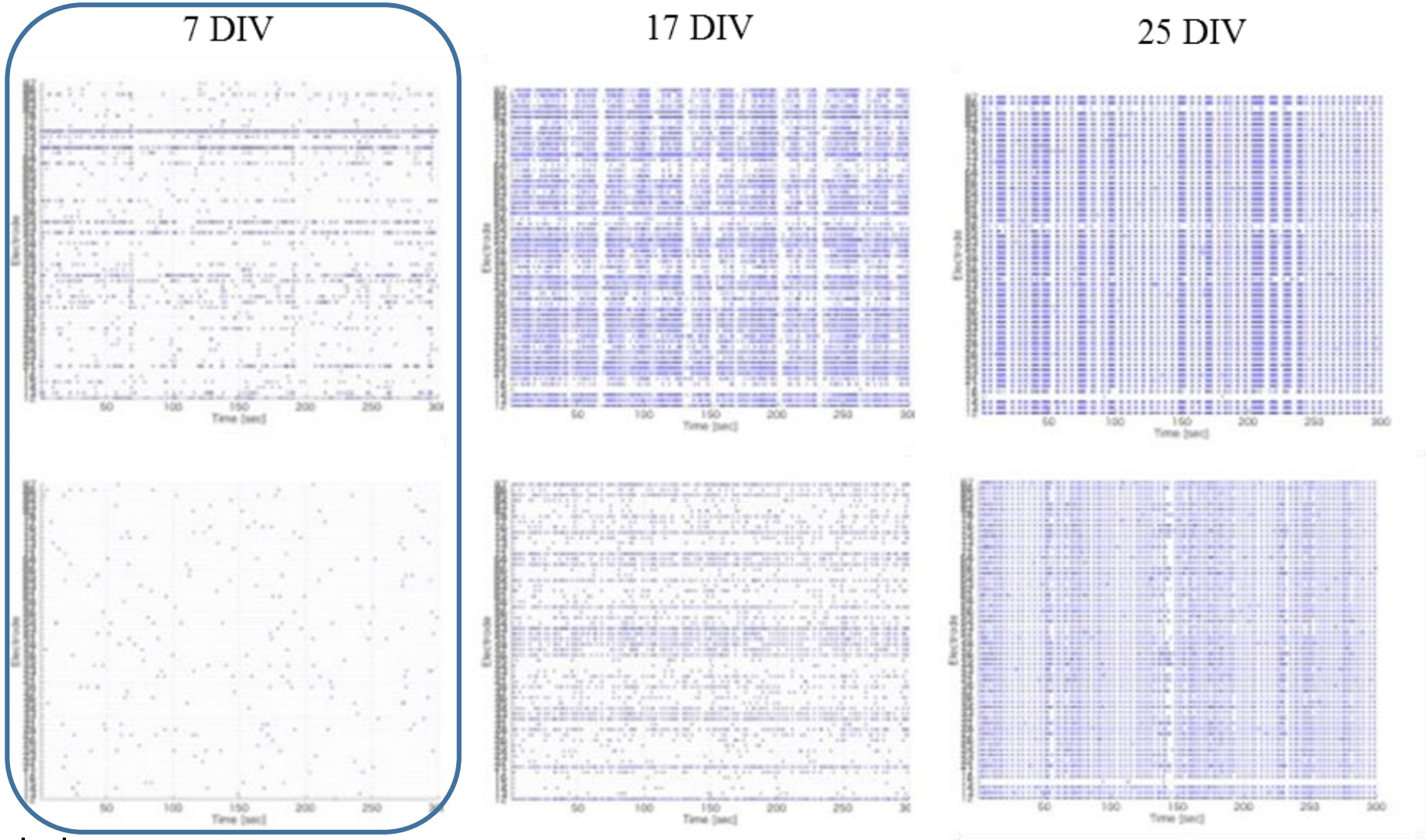




- ✓ Comparable morphology of healthy cells of the neuronal network on LG-SLG and control substrates
- ✓ Total cell density ↑ during the development of both LG-SLG and control cultures
- ✓ Higher number of neurons on LG-SLG

Analysis of Neuronal Networks Activity

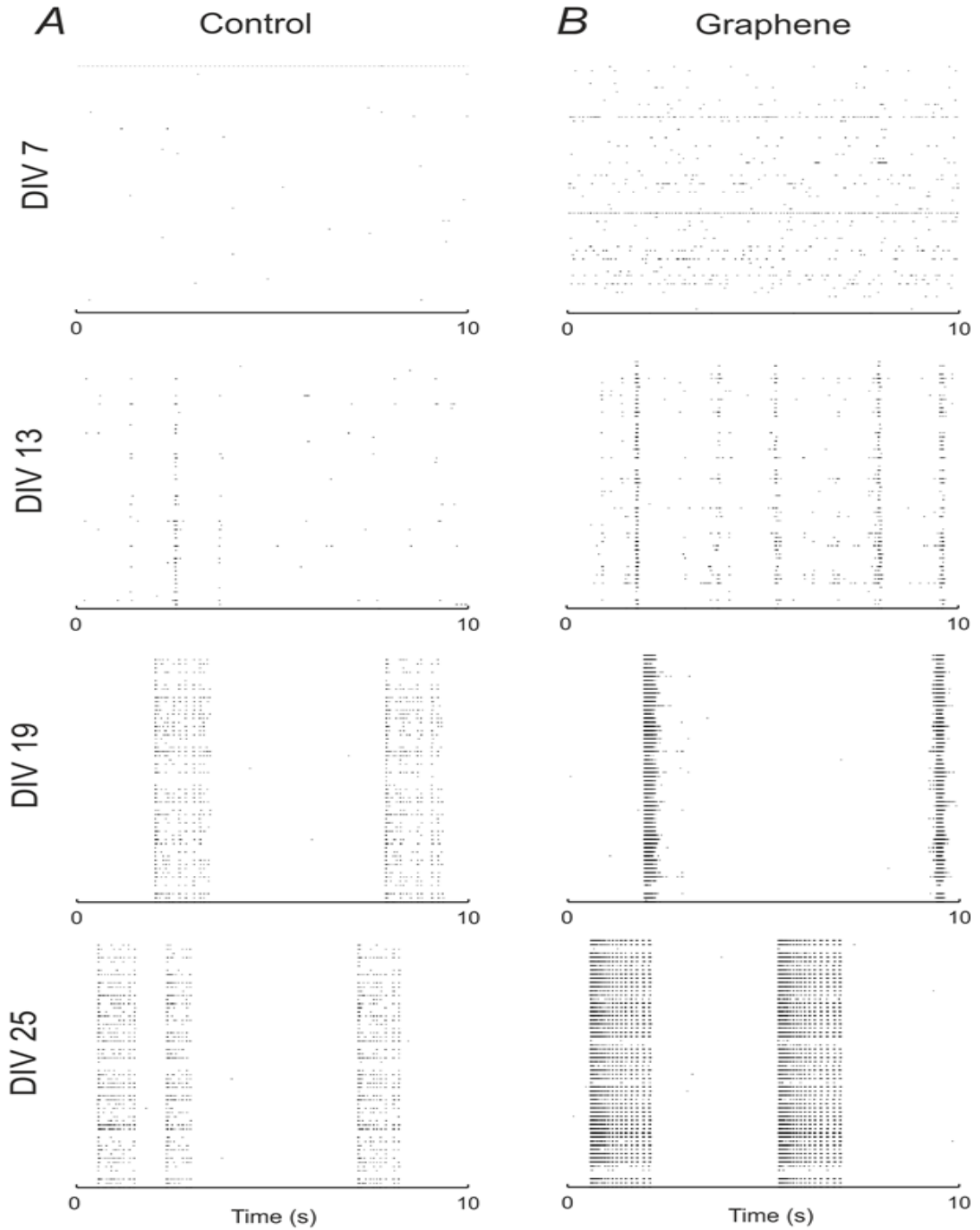
Raster Plots



DIV: developmental phase

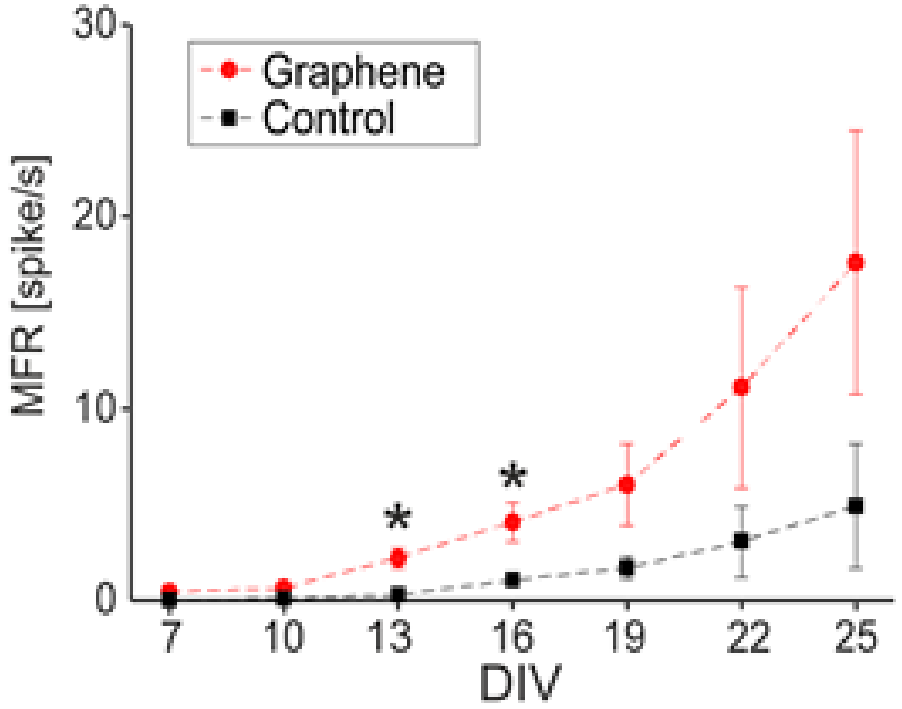
El Merhie A., Ito D. et al., Sensors and Actuators B: Chemical, *submitted*

10-s Raster Plots

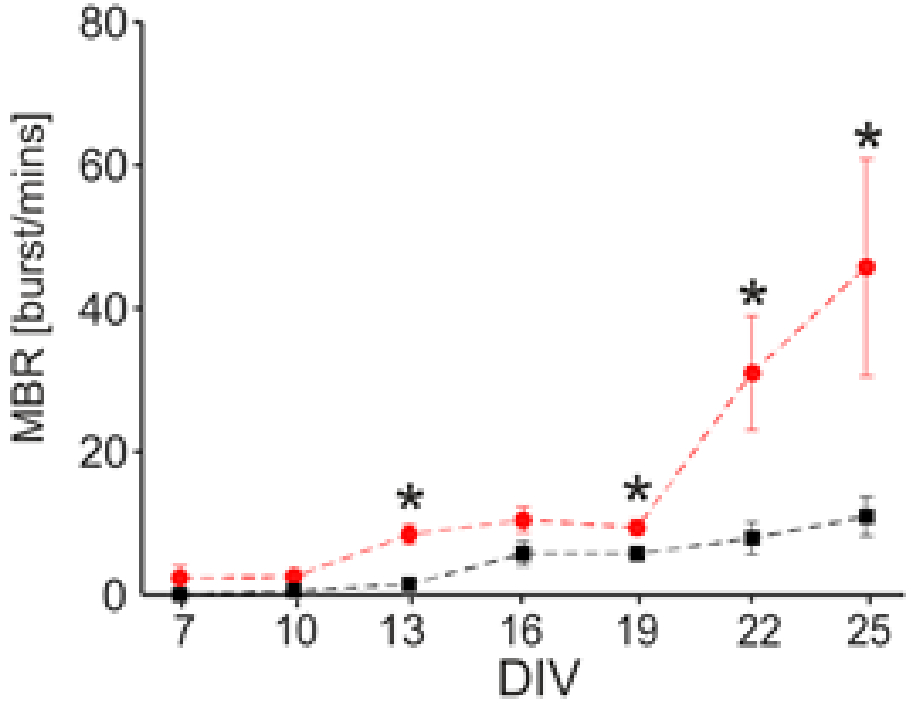


Analysis of Neuronal Networks Activity

Mean firing rate (spikes/s)



Mean bursting rate (burst/min)



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Conclusions & Perspectives

- ✓ **Long-term development** of **neuronal networks** on **LG-SLG interface** from the first week *in vitro* up to complete network maturation

- ✓ No major morphological differences with respect to control have been detected (healthy cultures)

- ✓ The higher survival rate, the higher number of adhered cells and firing activity → LG-SLG devices are compatible with physiological functionality of neuronal network → **provide an improved detection capability** (due to a better neuron/substrate coupling)

- ✓ **Neuronal network activity** was detected **earlier** on **LG-SLG** and a **more synchronous** behavior of the network was recorded

→ Results are in agreement with **single neuron synaptogenesis study** on **SLG** *versus* **glass** substrates by **patch clamp** (*Keshavan et al, Acta Biomaterialia, 2017*)

THANK YOU for Your Attention!

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