

Standard Operating Procedure @ CNR- IREA

PROTOCOL	Cell cultures exposure to 4G LTE signal at 1950 MHz
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1. Purpose

This procedure describes the equipment and the protocol used for cell cultures exposure to electromagnetic field (EMF) at 1950 MHz, 4G LTE signal. The aim is to ensure consistency and compliance of the bioelectromagnetic experiments with good laboratory practices.

2. Equipments and Materials

- RF generator (Agilent, E4432B ESG-D series)
- One microwave amplifier (MALTD, AM38A-0925-40-43)
- Two bidirectional power sensors (Rohde & Schwarz, NRTZ43)
- One, –6 dB power splitter (Hewlett-Packard HP11667A)
- PC for remote control through a Labview program (National Instruments)
- One, cell culture incubator (Thermo Scientific Forma, Model 311)
- Three rectangular, short-circuited waveguides (WR430, 350mm long, SAIREM)
- Two coaxial-to-waveguide adapters (Maury Microwave R213A2; VSWR: 1.05)
- Three coaxial cables (SUHNER, SUCOFLEX SN233634 /4)
- 35 mm cell culture dish (Corning, cod. 430165)

- Three, four-layer customized plexiglass stands
- Three metallic slabs
- One plastic spacer

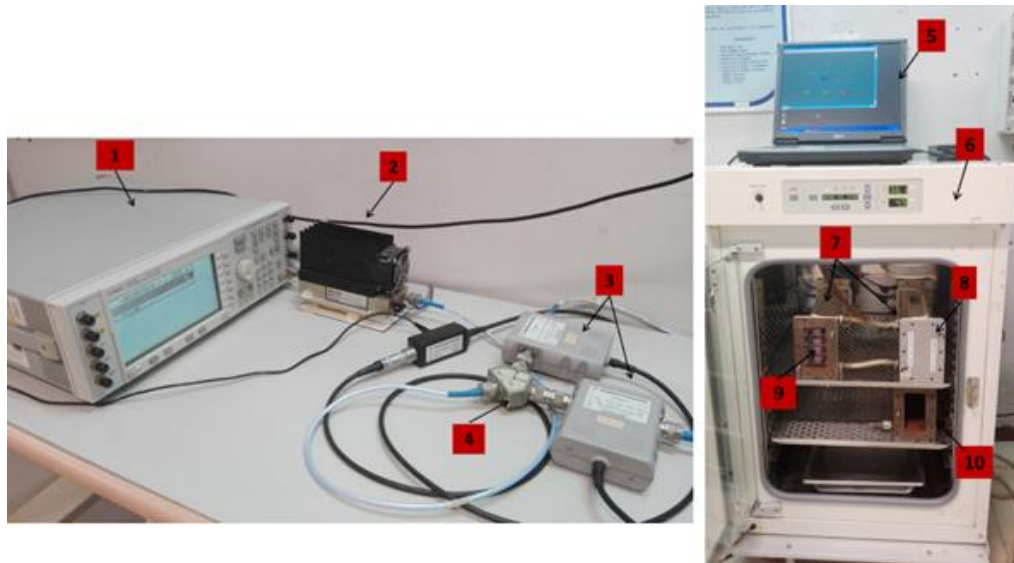


Figure 1. Exposure system setup. (1) RF generator, (2) microwave amplifier, (3) bi-directional power sensors (4) power splitter, (5) PC for remote control, (6) cell culture incubator, (7) waveguide for RF-exposure, (8) metallic slab for short-circuit, (9) four-layer Plexiglas stand loaded with cell cultures, (10) waveguide for sham-exposure.

3. Procedure

3.1. Preparation of cell samples

- Establish the following samples from the same batch of cells: incubator control, sham control, RF exposed at two SAR levels (0.3 and 1.25 W/kg), positive control. Label the cell culture dishes to decode each sample upon completion of the analysis for blind experiments: the operator who performs the analysis is not aware of the sample in hand.
- Locate the dishes on the Plexiglas stands (figure 2), insert the stands in each waveguide at the required distance from the short-circuit by using the plastic spacer and close each waveguide with the metallic slab. Perform this procedure two hours before starting exposure to allow the sample to acclimate in the waveguides.

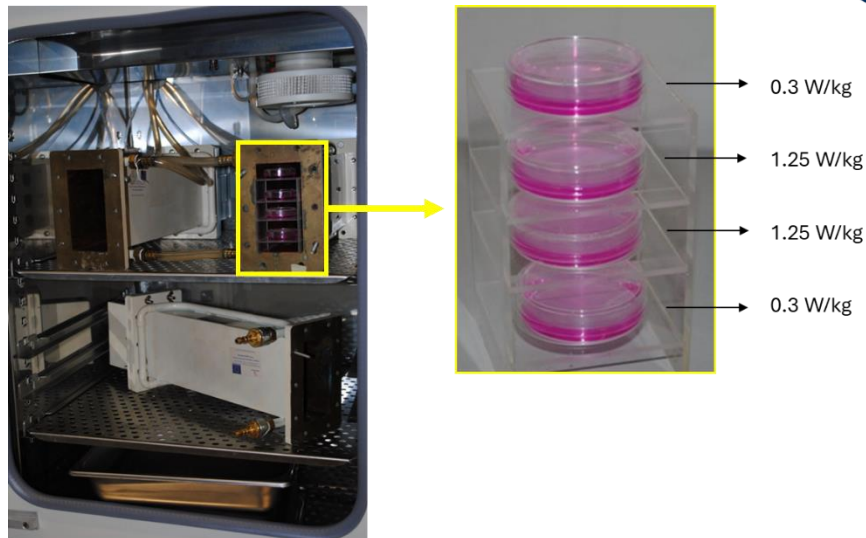


Figure 2. Plexiglas stand hosting cell culture dishes. A higher SAR value is obtained in the central samples with respect to the distal ones (4:1 SAR ratio).

3.2. RF exposure

- Switch on the RF generator and the PC
- (Optional) Open the NI-MAX program to verify that the generator and the two power meters are detected
- Connect to the Wi-Fi network
- Use the Matlab code to generate the LTE signal: launch Matlab 2019 on the PC and open the LTE LOADER program by following the path "Desktop→ Esposizione 4G→ LTE LOADER_ versione_1.0"
- Enter "LTE_LOADER_v1p0" and follow the instructions to build the signal
- Disconnect from the Wi-Fi network
- Start the Labview program for automated exposures by following the path "Desktop→ Esperimento_v4→Esperimento"
- Create a new experiment: select the folder in which the file containing the power measurements will be saved and set the file access mode as "replace or create"
- In the "MAIN" window, set the connection ports GPIBO::19::INSTR and COM3 under "Generator's name" and "Power meter's name", respectively
- Click on the "GENERATOR" field and set the frequency and amplitude, then click "start configuration" and finally "back"
- Click on the "POWER METER: ZEROING AND MONITORING" field, then "start", then "zero", then "quit" and finally "back"
- Set up the exposure conditions in the GUI: exposure duration, sampling interval, desired SAR (that of the central positions of the stands) and exposure starting time (figure 3)
- Click on the "START" field
- At the end of the exposure carefully remove the stands with the culture dishes from the waveguides

- Close the “experiment” program, switch off the PC and all the instruments
- Proceed with the harvest and the procedure for the biological assay by following the related SOP

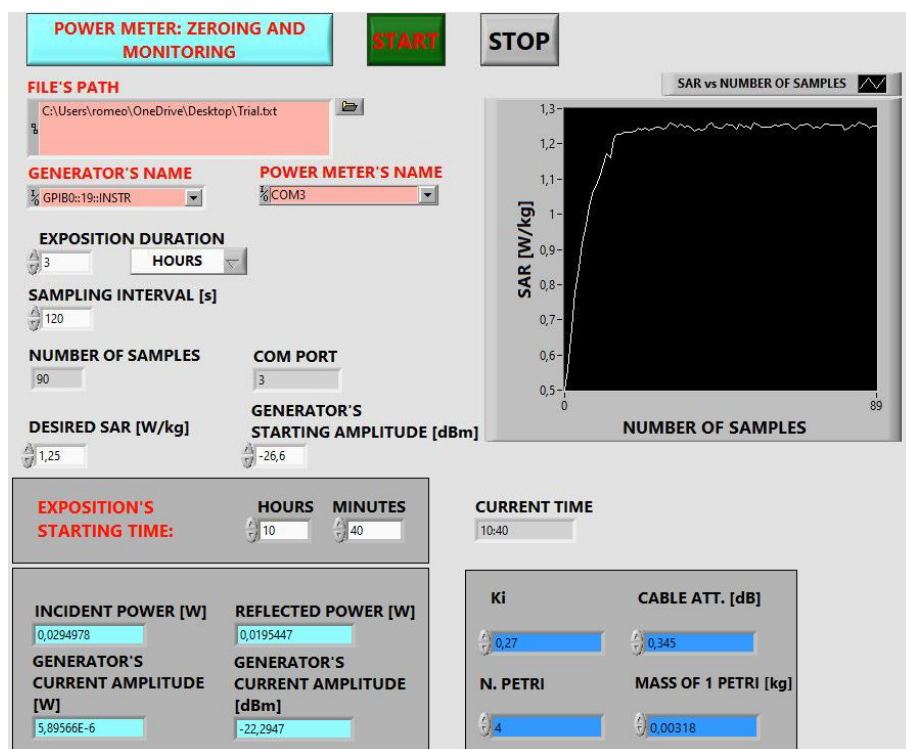


Figure 3. GUI of the Labview program for the control of the exposure setup.