1. Objective

This protocol aims to evaluate the biological effects of exposure to a **700 MHz electromagnetic field (RF)** on two different cell types:

- **Primary rat astrocytes** (Gibco),
- **Human SH-SY5Y neuroblastoma cells (ATCC)**.

The experimental design includes:

- **Two exposure durations**: 1 hour (short) and 24 hours (prolonged),
- **Three SAR levels**: SHAM (0 W/kg), 0.08 W/kg (low), 4 W/kg (high),
- **Two post-exposure analysis times**: **immediately** after exposure or **24 hours later (D+1)**.

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2. Cell Material and Culture Conditions

Cell types:

- **Primary rat astrocytes** – Gibco (Thermo Fisher Scientific),
- **SH-SY5Y human neuroblastoma cells** – ATCC.

Culture support:

- **35 mm Petri dishes**, compatible with the exposure setup,
- Each dish contains **3 mL of culture medium**.

Seeding and incubation:

- Cells are **seeded 48 hours before exposure** to reach **70–80% confluency** at the time of the experiment.
- Cultures are maintained at **37°C**, under **5% CO_2** in a humidified atmosphere.

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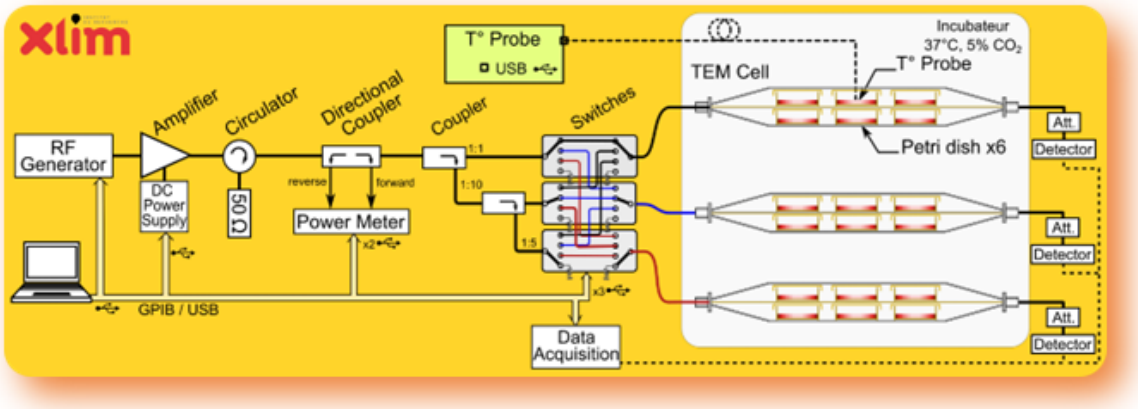
3. Exposure System and Conditions



- **Frequency**: 700 MHz
- **Specific Absorption Rate (SAR)** levels:
  - **SHAM**: 0 W/kg (no RF emission),
  - **Low SAR**: 0.08 W/kg,
  - **High SAR**: 4 W/kg.
- **Exposure durations**:
  - **1 hour** or **24 hours**, depending on the experimental condition.

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4. Experimental Design

Each condition is performed using **6 independent biological replicates (n = 6)**.

Cell Type	SAR (W/kg)	Exposure Duration	Analysis Time	Replicates
Astrocytes (Gibco)	0 / 0.08 / 4	1 h / 24 h	Immediate / D+1	n = 6
SH-SY5Y (human)	0 / 0.08 / 4	1 h / 24 h	Immediate / D+1	n = 6

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5. Post-Exposure Biological Analyses

Cells are analyzed by **flow cytometry**, either **immediately** after RF exposure or **24 hours later** (D+1), depending on the condition.

Assays performed:

- **Mitochondrial oxidative stress**:

    - Detection using **MitoSOX Red** (mitochondrial ROS marker).
- **Apoptosis**:
    - Staining with Annexin V (early/late apoptosis and necrosis).
- **Cell viability**:
    - Assessed by **Sytox Blue**
- **Cell proliferation**:
    - Tracked using **CellTrace™**.

Each sample is analyzed for a minimum of **10,000 events**, with **6 biological replicates per condition** to ensure statistical robustness.