



## Label-Free Study of the Global Cell Behavior during Exposure to Environmental Radiofrequency Fields in the Presence or Absence of Pro-Apoptotic or Pro-Autophagic Treatments

Alexandre Joushomme<sup>(1)</sup>, André Garenne<sup>(1)</sup>, Mélody Dufossée<sup>(2)</sup>, Rémy Renom<sup>(1)</sup>, Hermanus Johannes Ruigrok<sup>(1)</sup>, Yann Loick Chappe<sup>(1)</sup>, Anne Canovi<sup>(1)</sup>, Lorenza Patrignoni<sup>(1)</sup>, Annabelle Hurtier<sup>(1)</sup>, Florence Poulletier de Gannes<sup>(1)</sup>, Isabelle Lagroye<sup>(1,3)</sup>, Philippe Lévêque<sup>(4)</sup>, Noëlle Lewis<sup>(1)</sup>, Muriel Priault<sup>(2)</sup>, Delia Arnaud-Cormos<sup>(4,5)</sup> and Yann Percherancier<sup>(1,\*)</sup>

- (1) Univ. Bordeaux, CNRS, IMS, UMR 5218, F-33400 Talence, France; alexandre.joushomme@u-bordeaux.fr (A.J.); andre.garenne@u-bordeaux.fr (A.G.); remy330@msn.com (R.R.); hruigrok@sebia.com (H.J.R.); yann.chappe@u-bordeaux.fr (Y.L.C.); annabelle.hurtier@ims-bordeaux.fr (A.H.); florence.poulletier@ims-bordeaux.fr (F.P.d.G.); isabelle.lagroye@ephe.psl.eu (I.L.); noelle.lewis@u-bordeaux.fr (N.L.)
- (2) Univ. Bordeaux, CNRS, IBGC, UMR 5095, F-33000 Bordeaux, France; melody.dufossee@ibgc.cnrs.fr (M.D.); muriel.priault@ibgc.cnrs.fr (M.P.)
- (3) Paris Sciences et Lettres Research University, F-75006 Paris, France
- (4) Univ. Limoges, CNRS, XLIM, UMR 7252, F-87000 Limoges, France; philippe.leveque@unilim.fr (P.L.); delia.arnaud-cormos@unilim.fr (D.A.-C.)

Two main challenges hinder the complete description of low-level RF effects: (i) the lack of knowledge about low-level effective field parameters and (ii) the complexity of the living machinery, making it challenging to define which cellular or molecular processes are targeted or impacted by RF exposure. In the context of health-risk assessment, this double challenge can only be addressed by continuing the search for the biological effects of RF exposure at levels below the guidelines using innovative methods. In the last fifteen years, label-free techniques emerged, offering the possibility of interrogating a broad panel of drug molecules with comprehensive coverage in targets, pathways, networks, and cellular processes of native cells in real-time. These techniques are based on several reading modes, such as cellular impedance measurement (also called cell impedancemetry), resonant waveguide grating, or digital holographic microscopy, and they detect morphological changes in cells challenged with chemicals. Such techniques are powerful for revealing responses when there is little or no knowledge about the drug or stressor mechanism of action, and they are perfectly fit for bioelectromagnetics research. However, their use requires that cell-culture exposure to RF does not interfere with the read-out of the cell phenotype.

We here describe the use of two label-free techniques, cellular impedancemetry and Digital Holographic Microscopy (DHM), to assess the overall cellular response during RF exposure alone, or during co-exposure to RF and chemical treatments known to induce either apoptosis or autophagy. Two human cell lines (SH-SY5Y and HCT116) and two cultures of primary rat cortex cells (astrocytes and co-culture of neurons and glial cells) were exposed to RF using an 1800 MHz carrier wave modulated with various environmental signals (GSM: Global System for Mobile Communications, 2G signal), UMTS (Universal Mobile Telecommunications System, 3G signal), LTE (Long-Term Evolution, 4G signal), and Wi-Fi,) or unmodulated RF (continuous wave, CW). The specific absorption rates (S.A.R.) used were 1.5 and 6 W/kg during DHM experiments and ranged from 5 to 24 W/kg during the recording of cellular impedance. Cells were continuously exposed for three to five consecutive days while the temporal phenotypic signature of cells behavior was recorded at constant temperature. Statistical analysis of the results does not indicate that RF-EMF exposure impacted the global behavior of healthy, apoptotic, or autophagic cells, even at S.A.R. levels higher than the guidelines, provided that the temperature was kept constant.