Design and Dosimetric Characterization of a Broadband Exposure Facility for In Vitro Experiments in the Frequency Range 18–40.5 GHz

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A novel exposure facility for exposing cell monolayers to centimeter and millimeter waves (18–40.5 GHz) used by future 5G mobile communication technology and similar applications has been developed. A detailed dosimetric characterization of the apparatus for frequencies of 27 and 40.5 GHz and 60 mm petri dishes, used in a presently ongoing study on human dermal fibroblasts and keratinocytes, was carried out. The exposure facility enables a well-defined, randomized, and blinded application of sham exposure and exposure with selectable values of incident power flux density, and additionally provides the possibility of continuous monitoring of the sample temperature during exposure while it does not require significant deviations from routine in vitro handling procedures, i.e. petri dishes are not required to be placed inside waveguides or TEM cells. Mean specific absorption rate (SAR) values inside the cell monolayer of 115 W/kg (27 GHz) and 160 W/kg (40.5 GHz) per watt antenna input power and corresponding transmitted power density (St) values at the bottom of the cell monolayer of 65 W/m² (27 GHz) and 70 W/m² (40.5 GHz) per watt antenna input power can be achieved, respectively. For reasonable amounts of harvested cells (80% of petri dish bottom area), the variation (max/min) of SAR and St over the cell monolayer remains below 3.7 dB (27 GHz) and 3.0 dB (40.5 GHz), respectively. Bioelectromagnetics. 2022;43:25–39. © 2021 Bioelectromagnetics Society.

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INTRODUCTION

The worldwide rollout of the fifth generation of cellular mobile phone networks (5G) has recently started. Present 5G networks operate at frequencies close to the ones already used by earlier radio communication technologies, typically below 3.8 GHz (frequency range 5G-FR1). Substantial research efforts concerning the question about potential adverse health effects of such radiofrequency (RF) electromagnetic fields (EMF) have been made in recent decades, and the outcomes have served as the basis for summaries and conclusions of several expert panels [International Agency for Research on Cancer IARC, 2013; Scientific Committee on Emerging and Newly Identified Health Risks SCENIHR, 2015].

However, future 5G installations and devices will also use significantly higher frequencies in the range 24–28 GHz and around 40 GHz, respectively (frequency range 5G-FR2). A recent review of published papers in the frequency range 6–100 GHz clearly pointed out that the available...
studies do not provide adequate and sufficient information for a meaningful safety assessment, or for the question about possible non-thermal effects of RF-EMF above 6 GHz [Simkó and Mattson, 2019]. Hence, there is obviously a need for high-quality research concerning potential non-thermal effects of RF-EMF in the frequency range used by future 5G technology.

As a consequence, the German Federal Office for Radiation Protection funded an in vitro study to

**Fig. 1. Schematic block diagram of the exposure system.**
investigate the potential effects of RF-EMF at future 5G-FR2 frequencies on human dermal fibroblasts and keratinocytes.

Several concepts of radiofrequency exposure systems for cell cultures have been published in the past. While for frequencies up to approximately 2.2 GHz, beside a few others (mainly transverse electromagnetic [TEM] cell [Ivaschuk et al., 1997; Nikoloski et al., 2005; Schönborn et al., 2000, 2001] and waveguide-based concepts [Schuderer et al., 2004a,b] have been realized), these approaches are typically not feasible for millimeter-wave (mmW) frequencies due to geometric limitations, i.e. the space inside TEM cells and waveguides for mmW is too small for typical petri dish sizes.

For exposing cell cultures in the mmW frequency range, the approach of placing the petri dishes at a certain distance from the aperture of a horn antenna is commonly used. For frequencies between 42 and 61 GHz, several such systems, including their dosimetric characterization for relatively small cell culture dishes (≤35 mm), have been described in the past [Zhadochov et al., 2008, 2009, 2012; Zhao and Wei, 2005].

This paper describes the design and dosimetric characterization of a new broadband exposure apparatus used for frequencies in the range 18–40.5 GHz, particularly developed for the requirements of the abovementioned study concerning potential effects of RF-EMF at future 5G-FR2 frequencies on human dermal fibroblasts and keratinocytes.

**MATERIALS AND METHODS**

**Requirements for the Exposure Apparatus**

In addition to the general requirements of well-characterized and reproducible exposure conditions, software-controlled blinded application of exposure, and seamless monitoring of all relevant exposure parameters throughout the experiments, the following particular requirements could be derived from the study protocol: (i) exposure of cell monolayer at the bottom of 5 ml culture medium inside a 60 mm polystyrene (PS) petri dish CLS430196 (the inner diameter of this dish is approximately 50 mm) (Corning Life Sciences, Amsterdam, The Netherlands); (ii) exposure inside incubator(s) with constant environmental conditions of 37°C and saturated humidity; (iii) exposure at selectable frequencies (26 or 40.5 GHz); (iv) incident power flux density up to 100 W/m²; (v) freely selectable exposure duration; (vi) sham-exposed samples shall be exposed simultaneously to exposed ones; (vi) contin-
uous temperature monitoring of exposed and sham-exposed samples during the experiments; and (vii) minimum deviation from routine workflow of cell experimental procedures (e.g. avoiding laborious or poorly reproducible positioning of petri dishes inside the exposure apparatus).

**Overview of the Dual Incubator—Dual Sample Approach**

Based on the requirements listed above, the concept depicted in Figures 1 and 2 was chosen. Each of two identical incubators MCO-80IC-PE (PHC Europe, Etten-Leur, The Netherlands) contains one...
of two identical exposure sets (exposure set A and exposure set B).

Each of the exposure sets contains two identical RF branches (consisting of horn antennas, directional couplers, and diode detectors for forward and reverse power measurements), allowing for simultaneous and identical exposure of two 60 mm petri dishes positioned in a corresponding sample holder made of Teflon (polytetrafluoroethylene, PTFE) at a certain distance $d$ to the apertures of the horn antennas. At the start of each experiment, exposure is randomly assigned and automatically applied to one of the two exposure sets (incubators) by the corresponding control software, while the experimenters are kept blind to the applied condition.

One of the two petri dishes (containing 5 ml culture medium) in each exposure set always serves exclusively for temperature measurements using fiber optic temperature probes (for details, see below). The other petri dish contains the cell cultures that will be further biologically evaluated after exposure. Using this approach allows for a continuous temperature monitoring of the sample temperature without the risk of infections of the cell culture due to insertion of the temperature probes.

### Broadband System Using Horn Antennas

Due to the fact that the exposure system should be suitable for the frequency range from below 26.5 GHz to above 40 GHz, standard waveguide components could not be used, i.e. signal transmission from the signal generator SC5520A (SignalCore, Round Rock, TX) to the antennas is accomplished by low-loss coaxial cables (type UFB142A; Micro-Coax, Pottstown, PA). The nominal output frequency range of the signal generator is 100 MHz–40 GHz; however, it allows operation of up to 41.5 GHz with degraded output power. Therefore, the

### TABLE 1. Relevant Properties of the Materials Considered in the Computational Model

|                  | $f$ [GHz] | Rel. permittivity $\varepsilon_r$ | Conductivity $\sigma$ [S/m] |
|------------------|-----------|-----------------------------------|-----------------------------|
| Sample holder (PTFE) | 27.0      | 2.05                              | 0.05                        |
| Petri dish (PS)   | 27.0      | 2.54                              | 0.25                        |
|                  | 40.5      | 2.54                              | 0.25                        |
| Setup case (PP)   | 27.0      | 2.25                              | 0.25                        |
|                  | 40.5      | 2.25                              | 0.35                        |
| Radomes (styrofoam) | 27.0 | 1.50                              | 0.001                       |
|                  | 40.5      | 1.50                              | 0.001                       |
| Culture medium    | 27.0      | 35.2                              | 55.5                        |
|                  | 40.5      | 20.6                              | 72.2                        |
| Cell monolayer    | 27.0      | 24                                | 32                          |
|                  | 40.5      | 16                                | 42                          |
signal from the signal generator can be optionally pre-amplified (pre-amplifier type RLNA33G50GA, RF-Lambda, San Diego, CA) via switches Switch 1a and Switch 1b (type RFSPDT40EMC-T, RF-Lambda) for frequencies above 40 GHz (see Fig. 1). Via Switch 2 (same type as switches 1a and 1b), the RF signal is then fed to either branch A (incubator A) or branch B (incubator B), controlled by the control software developed under LabView 2016, and running on a PXIe 8821 Controller (National Instruments, Austin, TX). The samples inside the incubator of the other branch serve as sham-exposed controls. In the branch connected to the RF source, the signal is amplified by a power amplifier (type RFLUPA18G45G32-CDK, RF-Lambda) before it is fed to the corresponding exposure set inside the incubator(s). The system controller, signal generator, RF switches, and amplifiers are mounted in the central electronics cabinet (Figs. 1 and 2).

Each of the identically built-up exposure sets consists of a robust case made of polypropylene (PP) in which the required RF components are mounted (Fig. 3). The RF signal coming from the output of the power amplifier is fed via a two-way power splitter (type RFLT2W1840, RF-Lambda) and directional couplers (type RFDC8G40G15, RF-Lambda) to the two broadband horn antennas BBHA 9170 (Schwarzbeck Mess-Elektronik, Schönau, Germany). Each of the horn antennas was covered by a semi-spherically shaped radome made of low-density Styrofoam (outer diameter 150 mm, shell thickness 15 mm). As power sensors for measuring forward and reverse power at each antenna input, diode detectors DC 2158 A (Analog Devices, Wilmington, MA) were used. As all the RF components show frequency-dependent variations of their properties (stray parameters) in the order of 0.2–0.5 dB, they were selected and arranged in a way that deviation from symmetry between the different branches was minimized at the frequencies of interest in the present study (see uncertainty evaluation section).

Considering RF power losses within the microwave components inside the exposure set, it can be derived that up to approximately 7.5 W RF power will be dissipated inside the incubator at the highest possible exposure level (100 W/m² incident power flux density at the bottom of the petri dish/cell monolayer). As this may lead to noticeable systematic differences in temperature control behavior of the two incubators, which may endanger the blinding of the study, DC current-controlled heating pads are installed in each exposure set, which generate an equivalent heat load inside the exposure set, which does not receive any RF power, i.e. during sham exposure.

![Fig. 6. Situations during the validation measurements in air, above the antennas (covered by the radom), in the plane of the petri dish bottom without the sample holder (a), with the sample holder in place (b), and inside the culture medium (c).](image)

![Fig. 7. Temperature measurement points in the center (C), front (F), back (B), and left side (L) of the petri dish are considered for thermal validation of the numerical model. The sensitive tip of the fiber optic temperature sensors was at a 1 mm distance to the bottom of the petri dish. The fiber optics temperature sensors (blue) were fed through the cover of the petri dish via rubber grommets (black) and were kept in place by glue.](image)
On top of the PP-case a sample holder made of PTFE allows for reproducible positioning of two 60 mm petri dishes by deepenings fitted to the dish diameter. At the bottom side in the region of the petri dish, the sample holder has a cone jacket shape to minimize wave reflections (Figs. 3 and 4).

### Continuous Monitoring of Sample Temperature

At higher exposure levels, significant temperature elevations inside the cell cultures must be expected. To separate between thermal and possible non-thermal effects, and to allow for thermal control experiments, it is therefore an essential requirement to reliably assess the temperature elevation inside the cell cultures during the experiments. Placing temperature probes into the cell cultures that are biologically evaluated, however, is not possible due to the risk of infections. Therefore, a dual sample approach was chosen in which one of two identically exposed petri dishes containing culture medium is exclusively used for temperature measurements and serves as a proxy for the temperature inside the biologically evaluated cell culture (Figs. 1 and 2). For these temperature measurements, a fiber optic temperature measurement

| Material          | Mass density [kg/m$^3$] | Spec. heat constant [J/kg K] | Therm. conductivity [W/m K] |
|-------------------|-------------------------|-----------------------------|-----------------------------|
| Petri dish (PS)   | 1050                    | 1400                        | 0.17                        |
| Culture medium    | 1000                    | 4181                        | 0.56                        |

**Fig. 8.** Results of the validation of the numerical antenna model based on measurements of the electric field strength at 150 mm distance to the plane of the antennas’ aperture without the sample holder (see Fig. 6a). Results for 1 W radiated power per antenna. See Figure 5 for the origin of coordinate axes $x$ and $y$. The arrows indicate the respective position of the petri dishes. Standard uncertainty of E-field measurement was ±13.6%.

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system FOTEMP OEM Plus with sensor type TS5/2 (both from Polytec, Waldbronn, Germany) was used, with the temperature-sensitive probe tip placed at 1 mm distance from the inner surface of the bottom of the petri dish. In order to achieve a distance of as low as 1 mm between the temperature-sensitive crystal at the probe tip and the inner surface of the petri dish, the original protection cover of the probe tip had to be shortened at the front end and resealed. After that, temperature probes were recalibrated. The resulting standard uncertainty of the temperature measurements is lower than ±0.6 °C and ±0.1 °C in terms of absolute temperature and relative temperature changes, respectively. Although fiber optic temperature sensors have some disadvantages concerning response time and sensitivity compared to micro thermocouples [Zhadobov et al., 2017], they provide a reasonable proxy for the sample heating during exposure under the conditions of the present study, i.e. controlled continuous wave exposure, and at the same time are more robust in view of their repeated use in the laboratory routine workflow.

Dosimetric Analysis and Characterization

The dosimetric analysis, optimization, and characterization of the exposure setup was based on numerical computations using the Finite Differences in Time Domain (FDTD) solver of the simulation platform Sim4Life V5.2 (Zurich MedTech, Zurich Switzerland) after validation of the computational model. The computational model consisted of the horn antenna (fed by 50 Ω voltage source), all PP parts of the case, the PTFE sample holder, the petri dish

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TABLE 3. Comparison of Simulation and Measurements Based on Incident E-field Statistics at a Circular Area (r = 22 mm) in the Plane of the Petri Dish Bottom

|               | 27 GHz |       | 40.5 GHz |       |
|---------------|--------|-------|----------|-------|
|               | Sim.   |       | Measurements |       |
|               | A1, B1 | A2, B2 | A1, B1, A2 | B2    |
| Maximum [V/m] | 241    | 236   | 231       | 226   | 238   | 242   | 210    | 215   | 217   | 210  | 220  | 221  |
| Mean [V/m]    | 191    | 191   | 185       | 189   | 192   | 199   | 175    | 176   | 162   | 165  | 168  | 172  |
| Minimum [V/m] | 161    | 157   | 151       | 145   | 163   | 165   | 130    | 133   | 150   | 148  | 151  |      |
| Percentage of dish bottom area within 90% of max. | 8.1 | 7.2 | 7.8 | 6.0 | 8.2 | 7.5 | 31 | 31 | 5.7 | 6.5 | 5.0 | 6.7 |
| within 80% of max. | 32 | 29  | 21 | 24 | 23 | 27 | 85 | 86 | 20 | 21 | 22 | 22 |
| within 70% of max. | 70 | 66  | 53 | 56 | 52 | 56 | 97 | 97 | 61 | 65 | 62 | 63 |
| within 60% of max. | 100 | 100 | 83 | 89 | 87 | 84 | 100 | 100 | 85 | 87 | 84 | 85 |
| within 50% of max. | 100 | 100 | 100 | 99 | 99 | 99 | 100 | 100 | 94 | 95 | 94 |      |
| Max/Min [dB]  | 3.50   | 3.54  | 3.69     | 3.85  | 3.29 | 3.33 | 4.17  | 4.17 | 3.21 | 3.04 | 3.86 | 3.31 |
| Max/Mean [dB] | 2.02   | 1.83  | 1.93     | 1.55  | 1.87 | 1.70 | 1.58  | 1.74 | 2.54 | 2.09 | 2.34 | 2.18 |

Standard uncertainty of E-field measurement was ±13.6%.

It was found that it still shows reasonable sensitivity in the frequency range 18–41 GHz. Therefore, the probe was calibrated at frequencies of 27 and 40.5 GHz in free space in the far-field of a horn antenna (probe axis in radiation direction) in a reference field (uncertainty ± 25%, k = 2). In addition, its isotropy and linearity error were obtained in the range of electric field strengths of interest. Based on these calibration and linearity data, measurements of the electric field strength were carried out with the probe (mounted on a computer-controlled three-axis positioning system) in the air in front of the antenna aperture in the plane of the bottom of the petri dish (both with and without the sample holder in place; Fig. 6a and b). Moreover, electric field strength measurements inside the petri dish filled with culture medium were carried out in planes parallel to the dish bottom in planar measurement grids with a step size of 1 mm and with the probe tip at a distance of 1 mm to the dish bottom (Fig. 6c). Due to the lack of calibration data for the EX3DV4 probe immersed in the culture medium, these measurements do not allow the determination of absolute SAR values; however, they can be used to estimate the relative SAR distribution, i.e., the SAR pattern inside the medium.

The thermal validation was carried out based on temperature measurements during exposure (100 W/m² incident power flux density), at four locations (Fig. 7) inside the culture medium; the fiber optic temperature measurement system (probe tips positioned at 1 mm distance to the dish bottom) and corresponding simulations used the thermal solver of the Sim4life simulation platform. Table 2 summarizes the thermal material properties used for the simulations. The environmental temperature during the validation measurements was 25 °C. Convective heat transfer coefficients of 10, 15, and 20 W/m²K, corresponding to a typical range for the
free air convection, were considered during the simulations. Due to the particular design of the sample holder (Fig. 4), the contact area between the petri dish and sample holder is less than 5 mm², i.e. heat conduction from the petri dish to the sample holder was considered negligible. Hence, the sample holder was not considered in the thermal simulation.

RESULTS

Validation of the Numerical Model

Figure 8 shows a comparison of measurements and simulations in terms of electric field strength along two orthogonal axes parallel to the E-field vector (E-plane) and parallel to the H-field vector (H-plane), respectively. The distance d to the antennas’ aperture was 150 mm and the input power was 1 W per antenna. In the main radiation direction of the antennas, i.e. at (x, y) = (−190 mm, 0 mm) and (190 mm, 0 mm) for 27 GHz, and at (x, y) = (−190 mm, −35 mm) and (190 mm, −35 mm) for 40.5 GHz, there is reasonable agreement between measurement and simulation within ±1 dB (for the origin of coordinate axes x and y, see Fig. 5). It is pointed out that, while at 27 GHz the antennas’ main radiation direction is obviously perpendicular to the plane of the antenna aperture (z-axis), the used antennas show two lobes that are tilted with respect to the z-axis at 40.5 GHz, which is a characteristic of the used antenna. The nominal frequency range of the used antenna type, according to its datasheet, is specified with “15-26.5 GHz, usable up to 40 GHz”, i.e. the typical expected (single lobe) pattern is

![Figure 8: Comparison of measurements and simulations in terms of electric field strength along two orthogonal axes.](image)

Fig. 8. Comparison of measurements and simulations in terms of electric field strength along two orthogonal axes. The distance d to the antennas’ aperture was 150 mm and the input power was 1 W per antenna. In the main radiation direction of the antennas, there is reasonable agreement between measurement and simulation within ±1 dB. It is pointed out that, while at 27 GHz the antennas’ main radiation direction is obviously perpendicular to the plane of the antenna aperture, the used antennas show two lobes that are tilted with respect to the z-axis at 40.5 GHz.
achieved up to 26.5 GHz, but the antenna shows reasonable matching and gains up to even 41 GHz. However, above 26.5 GHz the antenna no longer shows the typical single lobe pattern expected from horn antennas when used in their optimum frequency range. At a distance of 150 mm, the absolute maximum electric field strength at 40.5 GHz occurs at $y = -35$ mm. Hence, the optimum position of the petri dishes in the $x/y$ plane is

![Graph](image)

**Fig. 11.** Comparison of computed and measured temperature elevation $\Delta T$ (standard uncertainty $\pm 0.1 \, ^\circ\text{C}$) at four points inside the culture media (1 mm above the bottom of the petri dish) for 100 W/m$^2$ incident power flux density. For positions of temperature sensors, see Figure 7. The measurements were carried out at 25°C room temperature and free air convection. Shown simulation results correspond to a convective heat transfer coefficient of 15 W m$^{-2}$K$^{-2}$.

**TABLE 4.** SAR Statistics in Cell Culture (Monolayer), at 27 GHz, Normalized to 1 W Accepted Antenna Input Power, for Various Sizes of the Elliptical Harvesting Area, Defined By Semi-Axes $a$, $b$

| Semi-axis $b$ [mm] | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 |
|-------------------|----|----|----|----|----|----|----|----|
| Cell yield [%]    | 92 | 88 | 84 | 81 | 77 | 73 | 69 | 65 |
| Maximum SAR [W/kg]| 162| 162| 162| 162| 162| 162| 162| 162|
| Mean SAR [W/kg]   | 111| 112| 114| 115| 117| 119| 121| 123|
| Minimum SAR [W/kg]| 68.8| 69.6| 69.6| 69.6| 72.3| 72.6| 78| 86.7|
| Fraction of cells exposed to [%] | >90% v. Max | 8.3 | 8.6 | 9.1 | 9.5 | 10 | 10.5 | 11 | 12 |
|                     | >80% v. Max | 20 | 21 | 22 | 23 | 25 | 26 | 27 | 29 |
|                     | >70% v. Max | 52 | 54 | 57 | 60 | 63 | 66 | 70 | 74 |
|                     | >60% v. Max | 68 | 71 | 75 | 78 | 83 | 86 | 91 | 96 |
|                     | >50% v. Max | 87 | 89 | 90 | 93 | 96 | 99 | 100 | 100 |
| Max/Min [dB]       | 3.71 | 3.66 | 3.66 | 3.66 | 3.49 | 3.47 | 3.14 | 2.70 |
| Max/Max [dB]       | 1.64 | 1.58 | 1.52 | 1.46 | 1.39 | 1.32 | 1.25 | 1.18 |

SAR = specific absorption rate.

*Relative of entire circular inner petri dish bottom area with radius 25 mm.
at $(x, y) = (\pm 190 \text{ mm}, 0 \text{ mm})$ for 27 GHz and at $(x, y) = (\pm 190 \text{ mm}, -35 \text{ mm})$ at 40.5 GHz.

To further validate the numerical model of the setup with respect to the field incident to the bottom of the petri dishes, electric field strength measurements were carried out with a grid step of 1 mm along the circular area corresponding to the petri dish bottom $(d = 150 \text{ mm})$, when the sample holder was in place (see Fig. 6b). Figure 9 shows a comparison of measurements and simulations in

| Semi-axis $b$ [mm] | 24 | 23 | 22 | 21 | 20 | 19 | 18 | 17 |
|-------------------|----|----|----|----|----|----|----|----|
| Cell yielda [%]   | 92 | 88 | 84 | 81 | 77 | 73 | 69 | 65 |
| Maximum $S_t$ [W/kg] | 94 | 94 | 94 | 94 | 94 | 94 | 94 | 94 |
| Mean $S_t$ [W/kg]  | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 |
| Minimum $S_t$ [W/kg] | 40 | 40 | 40 | 40 | 40 | 41 | 44 | 49 |
| Fraction of cells exposed to [%] | 8.7 | 9.0 | 9.4 | 9.9 | 10 | 11 | 12 | 12 |

*Relative of entire circular inner petri dish bottom area with radius 25 mm.

| SAR statistics in cell monolayer @ 40.5 GHz, 1 W |
|-----------------------------------------------|
| Harvesting area in the shape of circle segment $(R, x)$ |

| $R/x$ [mm] | 24/24 | 24/20 | 24/19 | 24/18 | 23/23 | 23/20 | 23/19 | 23/18 |
|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Cell yielda [%] | 92 | 88 | 87 | 86 | 85 | 84 | 81 | 80 |
| Maximum SAR [W/kg] | 211 | 211 | 211 | 211 | 211 | 211 | 211 | 211 |
| Mean SAR [W/kg] | 159 | 161 | 162 | 163 | 162 | 163 | 164 | 165 |
| Minimum SAR [W/kg] | 66.7 | 85.4 | 97.7 | 108 | 67.1 | 85 | 99 | 112 |
| Fraction of cells exposed to [%] | 6.8 | 7.1 | 7.2 | 7.3 | 7.5 | 7.6 | 7.7 | 7.9 |

*Relative of entire circular inner petri dish bottom area with radius 25 mm.

Table 5. Statistics of Transmitted Power Density $S_t$ at the Bottom of the Cell Monolayer, at 27 GHz, Normalized to 1 W Accepted Antenna Input Power, for Various Sizes of the Elliptical Harvesting Area, Defined By Semi-Axes $a, b$

Table 6. SAR Statistics in Cell Culture (Monolayer), at 40.5 GHz, Normalized to 1 W Accepted Antenna Input Power, for Various Sizes of the Harvesting Area in the Shape of a Circle Segment, Defined by Parameters $R$ and $x$
terms of electric field strength along the two orthogonal central axes through the petri dishes in E- and H-planes. Table 3 compares measurements and simulations based on statistics of incident electric field strength in the circular area corresponding to the bottom of the petri dish within a radius of 22 mm around the center (measurements at a larger radius are not possible due to the finite thickness of the field probe).

As a further validation step, measurement and simulation data for the SAR pattern inside the culture medium were compared and showed reasonable agreement (Fig. 10).

TABLE 7. Statistics of Transmitted Power Density $S_t$ at the Bottom of the Cell Monolayer at 40.5 GHz Normalized to 1 W Accepted Antenna Input Power for Various Sizes Of The Harvesting Area in the Shape of a Circle Segment Defined by Parameters $R$ and $x$

| $S_t$ statistics at the bottom of cell monolayer @ 40.5 GHz, 1 W | Harvesting area in shape of circle segment ($R, x$) |
| --- | --- |
| $R/x$ [mm] | 24/24 | 24/20 | 24/19 | 24/18 | 23/23 | 23/20 | 23/19 | 23/18 |
| Cell yield$^a$ [%] | 92 | 88 | 87 | 86 | 85 | 84 | 81 | 80 |
| Maximum $S_t$ [W/kg] | 92 | 92 | 92 | 92 | 92 | 92 | 92 | 92 |
| Mean $S_t$ [W/kg] | 67 | 69 | 69 | 69 | 70 | 70 | 70 | 70 |
| Minimum $S_t$ [W/kg] | 27 | 35 | 40 | 45 | 27 | 35 | 41 | 46 |
| Fraction of cells exposed to [%] | >90% | >80% | >70% | >60% | >50% |
| v. Max | 5.8 | 5.9 | 6.0 | 6.1 | 6.2 | 6.4 | 6.5 | 6.6 |
| | 32 | 33 | 34 | 34 | 35 | 35 | 36 | 36 |
| | 68 | 70 | 71 | 73 | 70 | 75 | 76 | 77 |
| | 90 | 92 | 94 | 95 | 93 | 95 | 96 | 98 |
| | 95 | 98 | 99 | 100 | 96 | 98 | 100 | 100 |
| Max/Min [dB] | 5.29 | 4.19 | 3.58 | 3.10 | 5.27 | 4.19 | 3.52 | 2.96 |
| Max/Mean [dB] | 1.34 | 1.26 | 1.24 | 1.21 | 1.25 | 1.20 | 1.18 | 1.16 |

$^a$Relative of entire circular inner petri dish bottom area with radius 25 mm.

TABLE 8. Uncertainty Budget for the Exposure Apparatus

| Uncertainty contributor | $U$ [±%] | Dist. | Div. | $U_{stand}$ [±%] |
| --- | --- | --- | --- | --- |
| Validity of the numerical model | | | | |
| Calibration of miniature E-field probe ($E^2$) | 12.5 | N | 1.0 | 12.5 |
| Isotropy of miniature E-field probe ($E^2$) | 3.5 | R | 1.7 | 2.0 |
| Probe positioning during validation measurements ($E^2$) | 8.0 | R | 1.7 | 4.7 |
| Non-linearity (after correction) | 2.5 | R | 1.7 | 1.4 |
| Maximum deviation between measurement and simulation in the region of the petri dish (mean value of $E^2$), considering imperfect symmetry of RF branches | 16.5 | N | 1.0 | 16.5 |
| Calibration of power detectors | 5.0 | N | 1.0 | 5.0 |
| Temperature drift of power detectors | 1.0 | R | 1.7 | 0.6 |
| Numerical uncertainty (Computational grid, FDTD algorithm) | 5.0 | N | 1.0 | 5.0 |
| Dielectric material parameters | | | | |
| Medium/Monolayer (±10% $\varepsilon_r$, ±20% $\sigma$) | 3.5 | N | 1.0 | 3.5 |
| PE-Case, PS-Petri dish, PTFE-Sample holder (±10% $\varepsilon_r$, ±20% $\sigma$) | 3.0 | N | 1.0 | 3.0 |
| Other | | | | |
| Mechanical accuracy of setup | 2.5 | N | 1.0 | 2.5 |
| Remaining drift of RF components (signal generator, amplifier), after 15 min warm-up | 5.0 | R | 1.7 | 2.9 |
| Combined standard uncertainty | | | | |
| Expanded uncertainty (k = 2) | 23.2 | | | 46.4 |

Dist. = Probability Distribution; Div. = Divisor; $U$ = Uncertainty; $U_{stand}$ = Standard Uncertainty.
Temperature Elevation Inside the Culture Medium

Figure 11 shows a comparison of measured and computed temperature elevation inside the cell culture medium, obtained at four locations (see Fig. 7) 1 mm above the bottom of the petri dish for an incident power flux density of 100 W/m². Again, a reasonable agreement between measurements and numerical computations could be achieved, underpinning the validity of the numerical model with respect to the steady-state of the temperature elevations. It should be noted that the temperature elevations in the order of approximately 2.5 °C indicated in Figure 11 have been measured at the laboratory at room temperature (i.e., outside the incubator) for system validation purposes only. The temperature elevations observed during the experiments (samples inside the incubator at 37 °C) are much lower due to the cooling effect of the lateral airflow inside the incubator (Fig. 2). The maximum observed sample temperature elevation at 100 W/m² during the experiments was approximately 1.6 °C, dropping to values lower than 1 °C within a few seconds after RF is switched off. Such low-temperature differences between the sham and exposed samples are not detectable by the experimenter when taking the samples out of the incubator after exposure (usually at least 10–20 s after RF was switched off).

Absorption Inside Cell Monolayer

Tables 4 and 5 summarize the computed statistical data for the distribution of SAR inside the cell monolayer and transmitted power density $S_t$ at the bottom of the cell monolayer for a frequency of 27 GHz, respectively. The SAR data is based on the mean value of the two voxel layers representing the cell monolayer in the numerical computations. Tables 6 and 7 show equivalent data for a frequency of 40.5 GHz. The obviously asymmetrical pattern at 40.5 GHz is caused by the tilted antenna pattern at this frequency (see Fig. 6 and corresponding text). Based on these data, appropriate areas for harvesting the cells can be selected, depending on the acceptable variations of SAR and $S_t$, respectively.

Uncertainty Analysis

Table 8 summarizes the uncertainty budget of the exposure facility. The different uncertainty terms were obtained as follows: Calibration uncertainties of the field probe and power detectors were obtained from the accredited calibration laboratory in which the calibrations were carried out (Seibersdorf Laboratories, Seibersdorf, Austria). Isotropy and non-linearity uncertainty of the field probe was experimentally determined after calibration using the same reference field. Probe positioning uncertainty was evaluated by repeated measurements using constant field conditions, and temperature drift of power detectors was determined for the temperature ranges 20–22 °C (validation measurements in the laboratory) and 36–38 °C (experiments inside incubator) with the higher value reported in Table 8. Uncertainty of dielectric properties of the medium was taken from the corresponding measurement report (Schmid & Partner Engineering), and the uncertainty of dielectric properties of the monolayer and the materials of the case and other mechanical components were estimated from the literature and datasheets. Finally, the uncertainty figures due to mechanical tolerances of the setup and the drift of RF components were experimentally determined. The expanded uncertainty of ±46% suggests that, in the case of experiments using multiple exposure levels, an exposure contrast of at least a factor of three between adjacent exposure levels shall be applied in order to allow for a reliable exposure-response analysis.

The suppression of unwanted exposure under sham conditions is limited by the isolation of Switch 2 (see Fig. 1), which is specified to be at least 50 dB. Hence, for a nominal incident power density of 10 W/m², unwanted exposure under sham conditions is lower than 1 mW/m².

DISCUSSION AND CONCLUSION

A novel exposure facility for in vitro studies on cell monolayers, concerning possible effects of radiofrequency exposure in the frequency range 18–40.5 GHz has been developed, and a detailed dosimetric characterization of the apparatus for frequencies of 27 and 40.5 GHz and 60 mm petri dishes, as used in a presently ongoing study on human dermal fibroblasts and keratinocytes was carried out. The design of the exposure facility provides the possibility of continuous monitoring of the sample temperature during exposure and does not require significant deviations from routine in vitro handling procedures, i.e. petri dishes are not required to be placed inside waveguides or TEM cells. Mean SAR values inside the cell monolayer of 115 W/kg (27 GHz) and 160 W/kg (40.5 GHz) per watt antenna input power and corresponding transmitted power density values at the bottom of the cell monolayer of 65 W/m² (27 GHz) and 70 W/m² (40.5 GHz) per watt antenna input power can be achieved, respectively. For reasonable amounts of harvested cells (80% of petri dish bottom area), the variation (max/min) of SAR and $S_t$ over the cell monolayer remains below 3.7 dB (27 GHz) and 3.0 dB (40.5 GHz), respectively.
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AUTHOR CONTRIBUTIONS

G.S. and R.H. technically implemented the basic idea of A.L.’s design and performed all simulation calculations and measurements in the laboratory in Seibersdorf. I.G., V.M., and K.D. performed extensive experiments in the laboratory in Bremen to validate the exposure parameters under real conditions in the incubator.

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