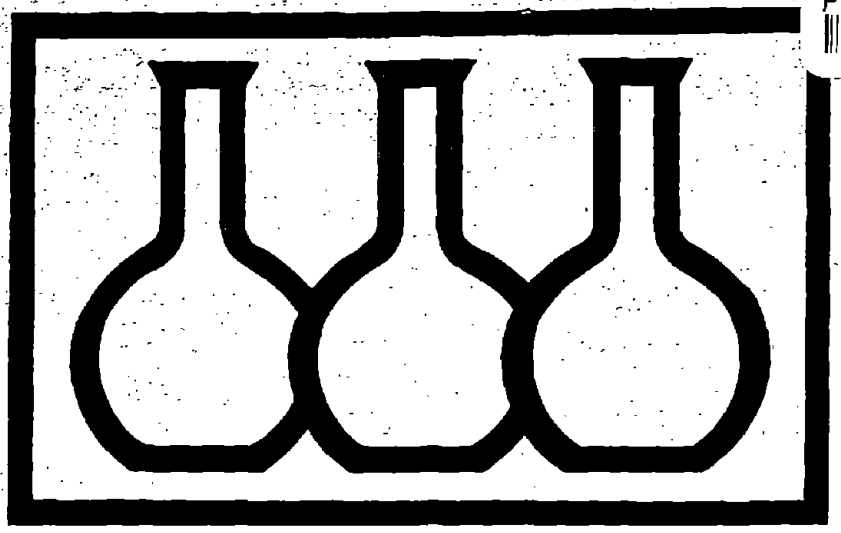

NIOSH RESEARCH REPORT

RF Cell Culture Irradiation System with Controlled Temperature and Field Strength

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE / CENTER FOR DISEASE CONTROL
NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH

RF CELL CULTURE IRRADIATION SYSTEM WITH CONTROLLED TEMPERATURE AND FIELD STRENGTH

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Interagency Agreement NIOSH-IA-75-30

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June 1977

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NIOSH Project Officer: David L. Conover, Ph.D.

DHEW (NIOSH) Publication No. 77-182

ABSTRACT

This report describes a unique RF cell culture irradiation system with controlled temperature and field strength designed under contract for the National Institute for Occupational Safety and Health, which was used in determining human RF exposure thresholds in the frequency range D.C. to 100 MHz. The irradiation system can produce electric field strengths up to 100 V/cm from D.C. to 1000 MHz in a 5-ml sample of culture medium. The culture medium temperature can be controlled and measured up to 100 MHz by monitoring the feedline impedance, which is dependent on culture medium temperature. Constant temperatures below 37° C can be maintained at field strengths in excess of 25 V/cm. The information that can be obtained with use of this system is needed to fill information gaps for standards criteria development.

This report was submitted by the USAF School of Aerospace Medicine in fulfillment of Interagency Agreement NIOSH-IA-75-30 under the sponsorship of the National Institute for Occupational Safety and Health.

ACKNOWLEDGMENTS

We wish to acknowledge the USAF School of Aerospace Medicine (USAFSAM), Aerospace Medical Division (AFSC), Brooks Air Force Base, Texas, for jointly funding this research with NIOSH under Interagency Agreement NIOSH-IA-75-30. We wish to express our gratitude to Dr. James W. Frazer*, USAFSAM, and to Dr. Arthur W. Guy, University of Washington, for their approval and assistance in publishing this report.

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INTRODUCTION

The analysis of data in many past experiments involving the effects of electromagnetic (EM) fields on cell cultures, blood samples, and solutions containing micro-organisms raised questions concerning the exact magnitude of the fields and the exact temperature within the solutions during exposure (1-9). Often the samples had been placed in EM fields of known strength and power density but, due to the complex shapes of the vessels holding the samples, the actual fields acting on the cells or organisms and the maximum temperatures in the sample were unknown. This made it difficult in many cases to determine whether any noted effects were specifically due to the fields, or simply due to a temperature rise. Also, attempts at measuring the temperature or fields within the sample by conventional methods can produce perturbations that may significantly modify the results of the experiment.

This report describes a method for exposing such preparations that allows precise field and temperature control within the same sample over a frequency range from dc to 100 MegaHertz (MHz). It appears that the system may easily be used for exposure frequencies as high as 1 GigaHertz (GHz). A power source capable of providing a net power of 600 Watts (W) to the system can allow it to operate with electric field strengths as high as 100 Volts/cm (V/cm) in a 5-ml sample producing a maximum specific absorption rate (SAR) of approximately 2×10^5 W/kg. A heat exchanger used in the system allows a steady-state temperature of below 37° C to be maintained in the sample while exposing it continuously to continuous wave (CW) field strengths exceeding 25 V/cm, or SAR levels of 8×10^3 W/kg.

At lower field strengths, the temperature may be held constant at any desired level over a wide range. At higher field strengths, a duty cycle less than one must be used to prevent overheating of the sample. Electrical impedance of the sample's container can be continuously monitored during exposure, and used as a direct measure of dielectric properties and temperature of the sample. The latter information can be used as a feedback signal to regulate the power source in order to maintain a constant temperature in the sample under varying exposure and cooling conditions. The system is useful for determining if effects observed in in vitro biological specimens exposed to EM fields are athermal or thermal in nature.

A number of samples can be run in series in the exposure system by placing the samples in specially designed sterilizable and reusable containers which become part of the exposure system when they are attached to the coaxial transmission line. Due to the unique design of the system, teflon-insulated thermocouples can be inserted in the culture medium without producing field enhancements which commonly occur around the sharp edges of any metal objects placed in radiofrequency (RF)-irradiated samples. Such field enhancements have been known to cause artifacts in research results.

Accurate dosimetry can be maintained during exposure through measurements made on the system with a vector voltmeter. Complete dosimetry information can be obtained from the measurements in a matter of seconds through the use of a programmable Hewlett-Packard 65 hand-held calculator.

The following sections describe the design of the system, the methods of dosimetry (theoretical formulations), the methods for determining properties of the fields in the exposed sample (operation of the system), and some typical dosimetry results (see tables) in exposing culture samples.

DESIGN OF THE SYSTEM

In most of the past research involving the exposure of biological specimens to EM fields, the specimens were contained in test tubes and culture dishes when they were exposed to plane wave or waveguide fields. The effects of source configuration, object shape, and object size were often ignored. Therefore, estimation of the actual amount of absorbed power for these cases could be in error by several orders of magnitude, leaving the relationships between dose and biological effects somewhat in doubt. Transmission line methods provide the convenience of exposing small specimens under limited space conditions where the absorption can be well-quantified in terms of incident power or transmission line parameters measured by inexpensive instruments. Unfortunately, past utilizations of these techniques for exposing biological fluid specimens have been plagued by problems resulting from the inadequate design of the transmission line and the method of coupling it to the sample.

Our first attempt to design such an exposure system was not spared these problems. The first design, however, was valuable in providing us with considerable insight and information concerning the type of problems that one must face in the exposure of in vitro preparations under sterile, environmentally-controlled, and known EM field conditions. Thus, to better elucidate these problems, the unsuccessful design will be briefly discussed before the discussion of the final successful design.

THE INITIAL EXPOSURE SYSTEM DESIGN

Three major considerations had to be given to the design of a cell culture exposure system: 1) the fields within the sample should be easy to quantify, 2) the temperature changes of the sample should be controllable under exposure conditions, and 3) the exposure had to be carried out under sterile conditions without contacting the preparation with any toxic substances.

Figure 1 illustrates the first attempt in designing an exposure system to quantify the incident power and the power absorbed by a cell culture sample. An RF power source was used to provide power through a matching network and coaxial-cable transition to a standard EIA 3-1/8-inch-diameter coaxial transmission line used for holding the cell culture sample, as detailed in the sketch in Figure 2.

The advantage of using the large diameter coaxial line is that it is a standard transmission line which is readily available with matched loads, has directional couplers, and permits transitions with smaller diameter coaxial line components. Incident and reflected power in the line can easily be measured with standard laboratory equipment. The impedance at any point in the line can be measured over a broadband by means of sampling

loops and probes coupling the transmission line voltages and currents to a vector voltmeter.

It was originally felt that a culture sample holder could be placed anywhere in the line so as to expose it to any field impedance controlled by the line termination. It was expected that the sample could be maintained at a constant temperature by circulating a cooling fluid through a heat exchanger surrounding the culture. The coaxial transmission line exposure system was very attractive from the standpoint of being operable over a large frequency range from dc up through 1 GHz or more. It also was relatively easy to fabricate various transitions and hardware for such configurations. By using swept power sources leveled by the sampling probes placed close to the culture sample holder, one can expose a sample to a wide range of frequencies while maintaining constant electric fields.

The culture container (i.e., sample cell) in the original design contained an annular ring designed to partially fill the cross-sectional area in the coaxial line, as shown in Figure 2. The culture container proper consisted of an annular ring divided into twelve compartments. The sample holder was surrounded with cooling chambers such that the coolant could be circulated along the bottom and sides of the annular culture container. This was achieved most effectively by circulating the coolant through every other subcompartment of the annular ring. The dielectric properties of the saline solution coolant were adjusted to match those of the culture. The entire chamber could be represented electrically by sections of transmission line and lumped capacitances.

It was envisioned that by measuring the incident and reflected powers to the sample and the line impedance, one could calculate very closely, the electric and magnetic fields in the culture medium. It was expected that a large number of environmental and exposure conditions could be set up by proper adjustment of circulating fluid temperatures and input power. Impedance conditions could be varied by choice of the termination at the end of the transmission line. With such an exposure apparatus, it was expected that one could obtain the desirable high field levels in the culture with relatively low input power sources. The non-metallic portions of the culture container were constructed of rexolite plastic.

Initial tests on the system indicated the following problems.

1) The input impedance to the culture ring was so high that it was difficult to couple significant power to the culture medium. Since the conductivity of the culture medium was high and the dielectric constant of the plastic cell walls was low, the major voltage drop across the culture holder occurred across the dielectric framework, circumferentially surrounding the culture and the cooling solution. Although increased coupling could be accomplished by placing the culture cell in a high impedance portion of the transmission line, very high voltages were required between the inner and outer conductors to provide the necessary coupling to the highly reactive capacitive load, thereby increasing the complexity of the dosimetry.

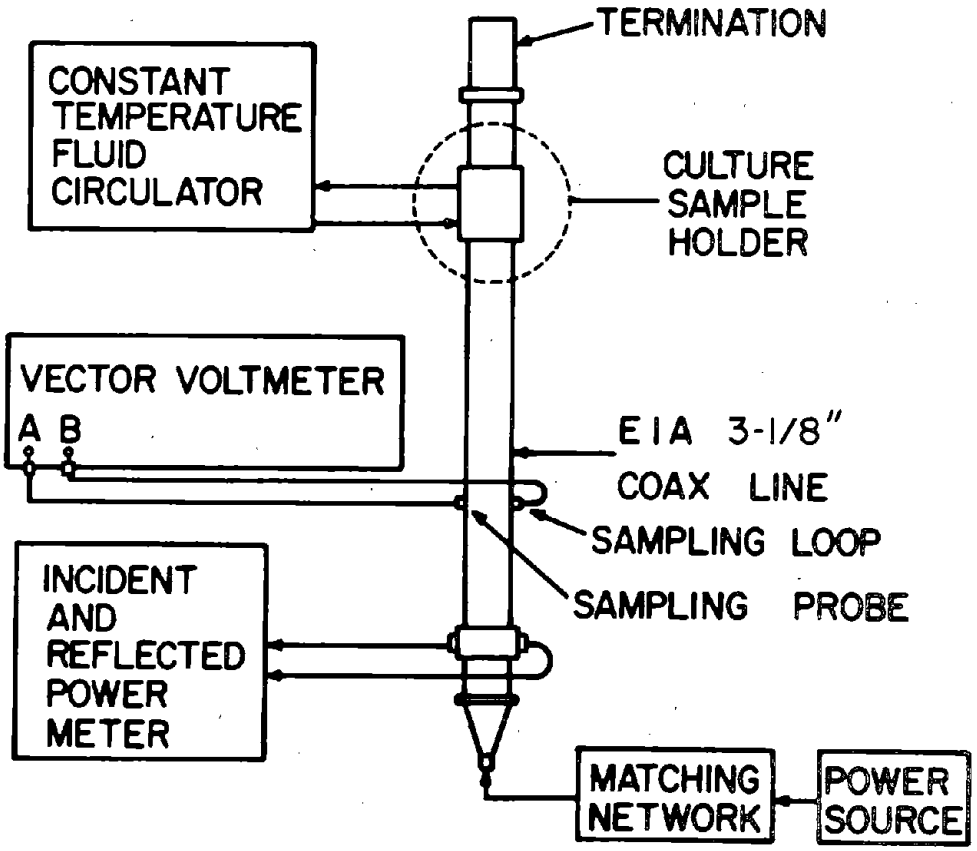


Figure 1. Initial design of transmission line cell culture exposure system

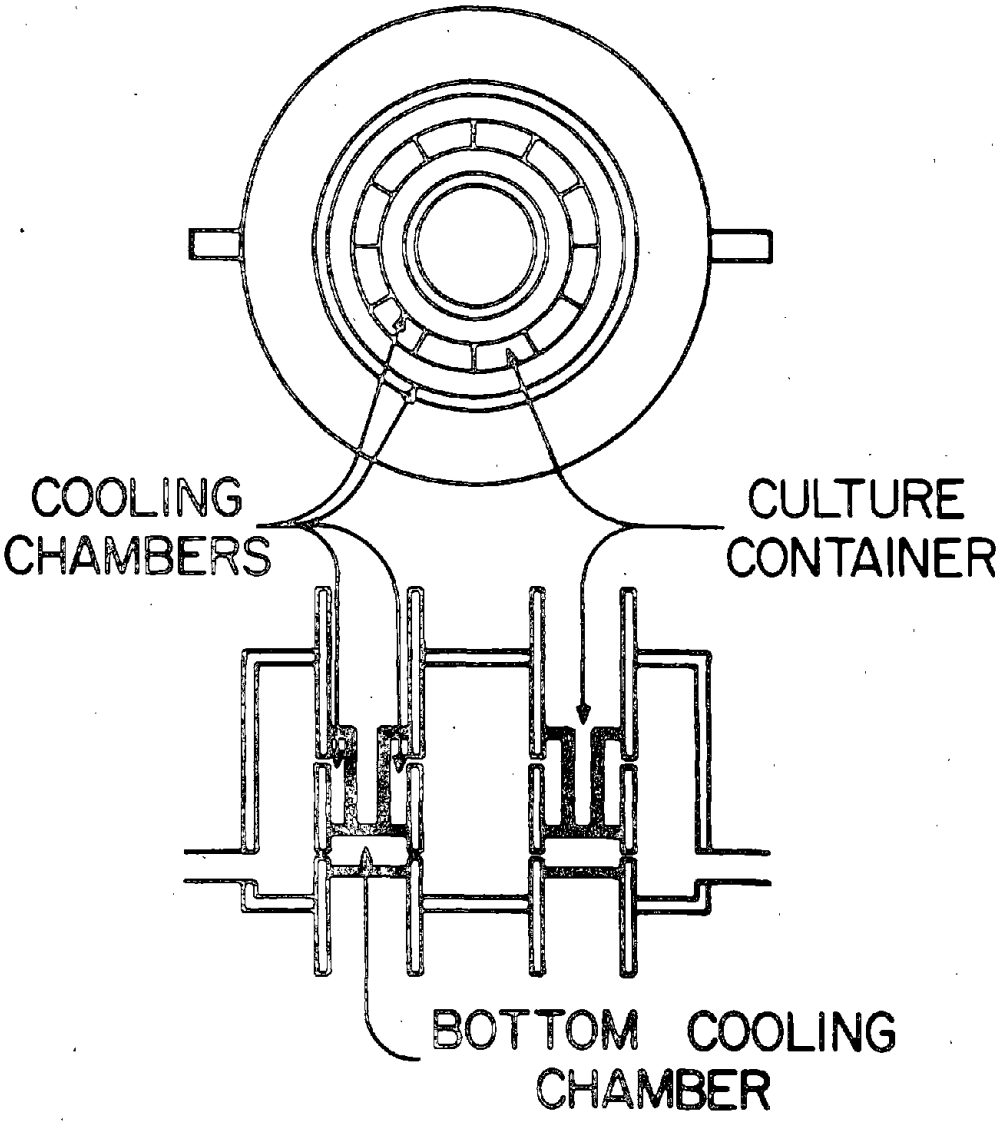


Figure 2. Initial design of culture exposure chamber

2) Considerably more power was absorbed by the coolant than the culture, reducing the efficiency of the system. 3) Under high field strength exposure conditions, the temperature of the culture was significantly higher than that of the coolant, and since the electrical conductivity of the liquids was temperature-sensitive, the distribution of the electric field between the various chambers became complex functions of related temperatures between the coolant and the culture samples, further complicating the dosimetry. 4) The difference in coefficients of expansion between the plastic and metal walls made it difficult to maintain liquid-tight seals between each section. 5) It was impossible to conveniently sterilize the system by steam autoclaving without damaging the plastic framework.

Based on the experience gained with the first unsuccessful system and various attempts to modify it to eliminate the various problems, a much improved system was developed, as discussed below.

THE FINAL DESIGN

Figure 3 illustrates the final design of the assembled sample holder and transmission line heat exchanger. The device is designed to connect to the coaxial waveguide by standard flange connectors. The sample container proper, illustrated in Figure 4, consists of two concentric stainless steel ring walls with a flat annular teflon ring bottom. The teflon bottom forms a liquid-tight seal with the stainless steel rings since it is compressed against the bottom edges of the rings by special brass retainer clamps soldered under pressure against the teflon to the stainless steel rings. The culture medium in the sample container is protected from contamination by an annular ring teflon cap placed at the top of the sample holder. A smaller removable teflon cap is placed on the teflon ring cover to relieve air pressure as the cover is placed over the ring after it is filled with culture medium. The cap also allows access to the culture for thermocouple temperature measurements.

The construction details of the transmission line, heat exchanger housings and coolant fittings are shown in Figure 5. The construction details of the dielectric centering support and clamps for securing the sample holder cup in place are shown in Figure 6. The sample holder may be clamped in place in the heat exchanger housing, as shown in Figure 3, with sufficient force such that a liquid-tight seal is formed by compressing whole ring seals between the coaxial housing and the flanges at the top of the sample holder. At the same time, the bottom of the sample holder is pressed against a phosphor bronze washer between it and the coaxial housing, forming a good electrical contact and becoming part of the coaxial transmission line.

The washers are designed with spring fingers cut in the periphery facing the exterior of the coaxial housing, as shown in Figure 6, to insure even electrical contact around the periphery of the rings. The thin 6-mm thick walls of the metal rings and the 8-mm thick teflon film forming the culture container floor allow very effective heat transfer between the cell culture and a silicon oil coolant circulating through the coaxial heat exchanger. The circulating fluid is allowed to pass through the rexolite plug and up into the cooling chamber adjacent to the inner ring where it flows

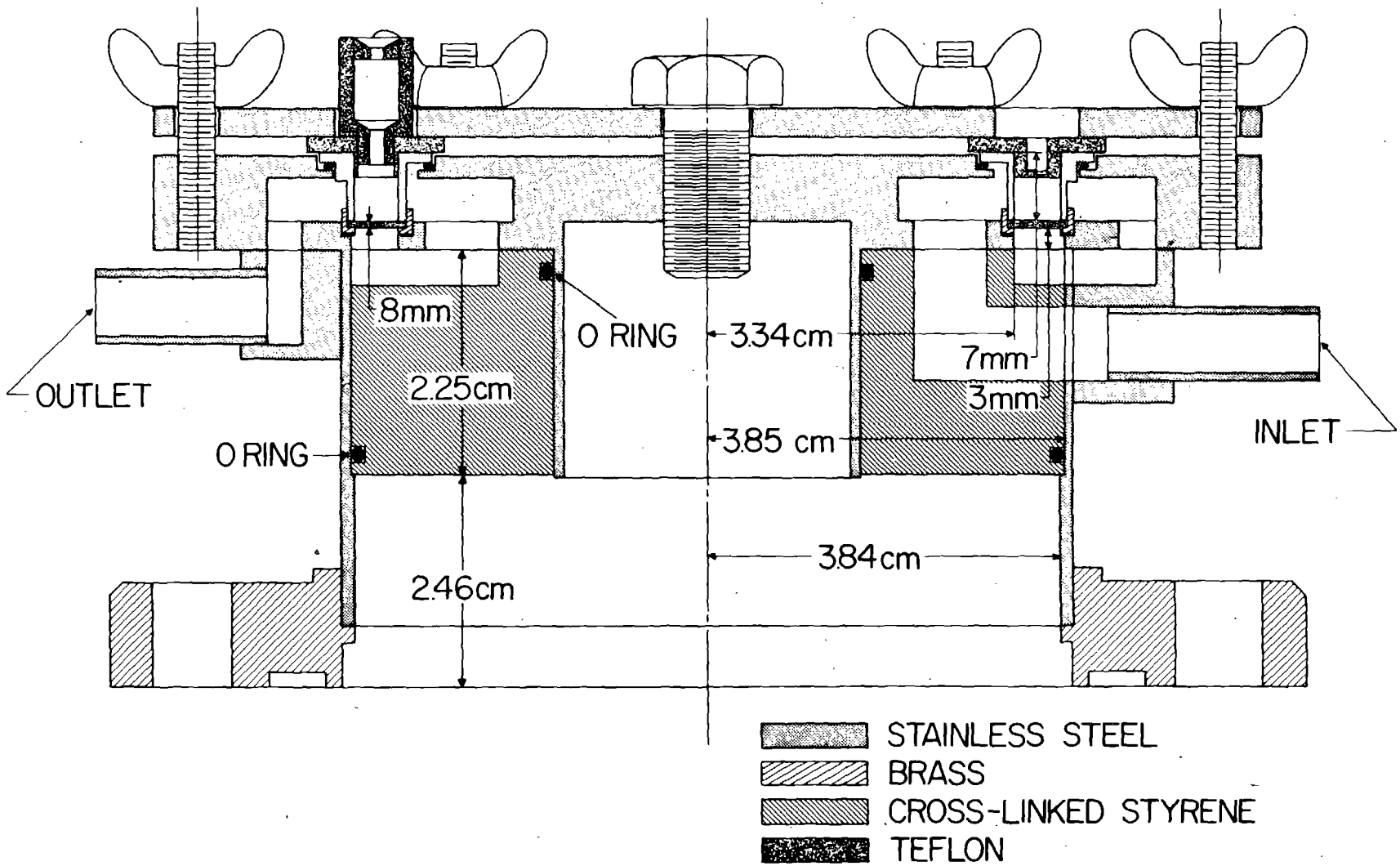


Figure 3. Cross sectional view of assembled transmission line cell culture sample holder and heat exchanger

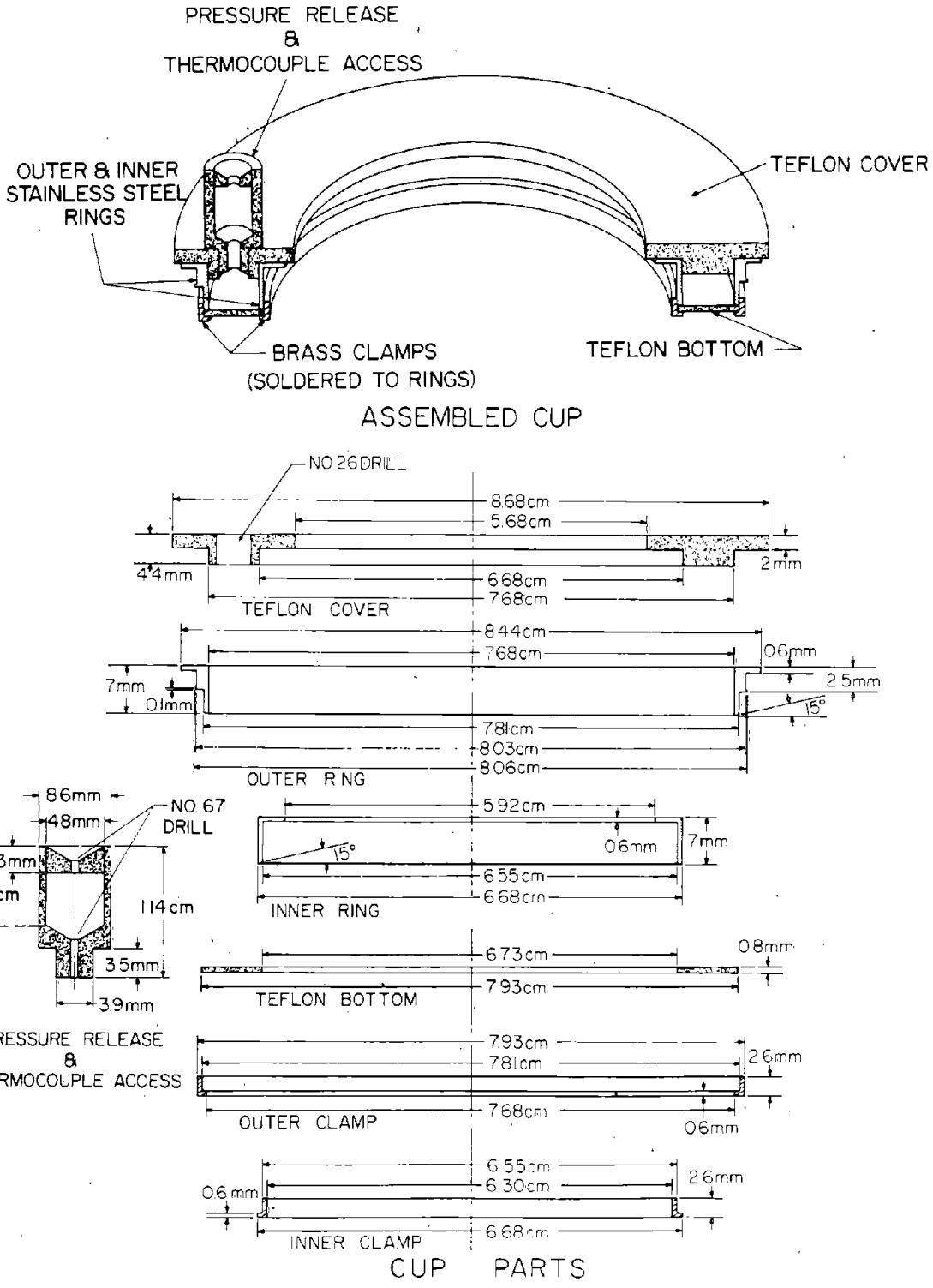


Figure 4. Cross section of assembled cell culture sample cup and exploded view of disassembled components

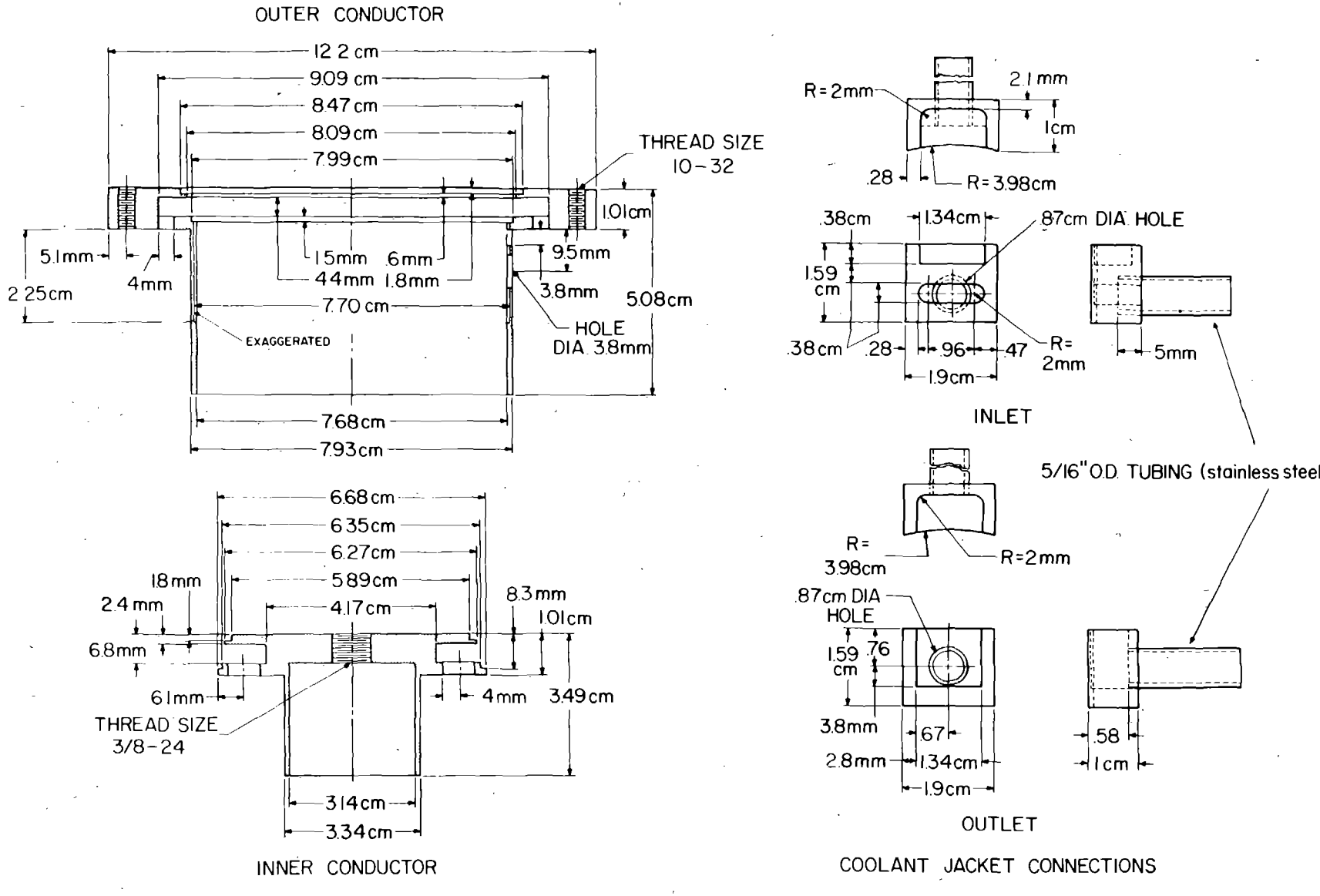


Figure 5. Construction details of transmission line, heat exchanger housings and coolant fittings

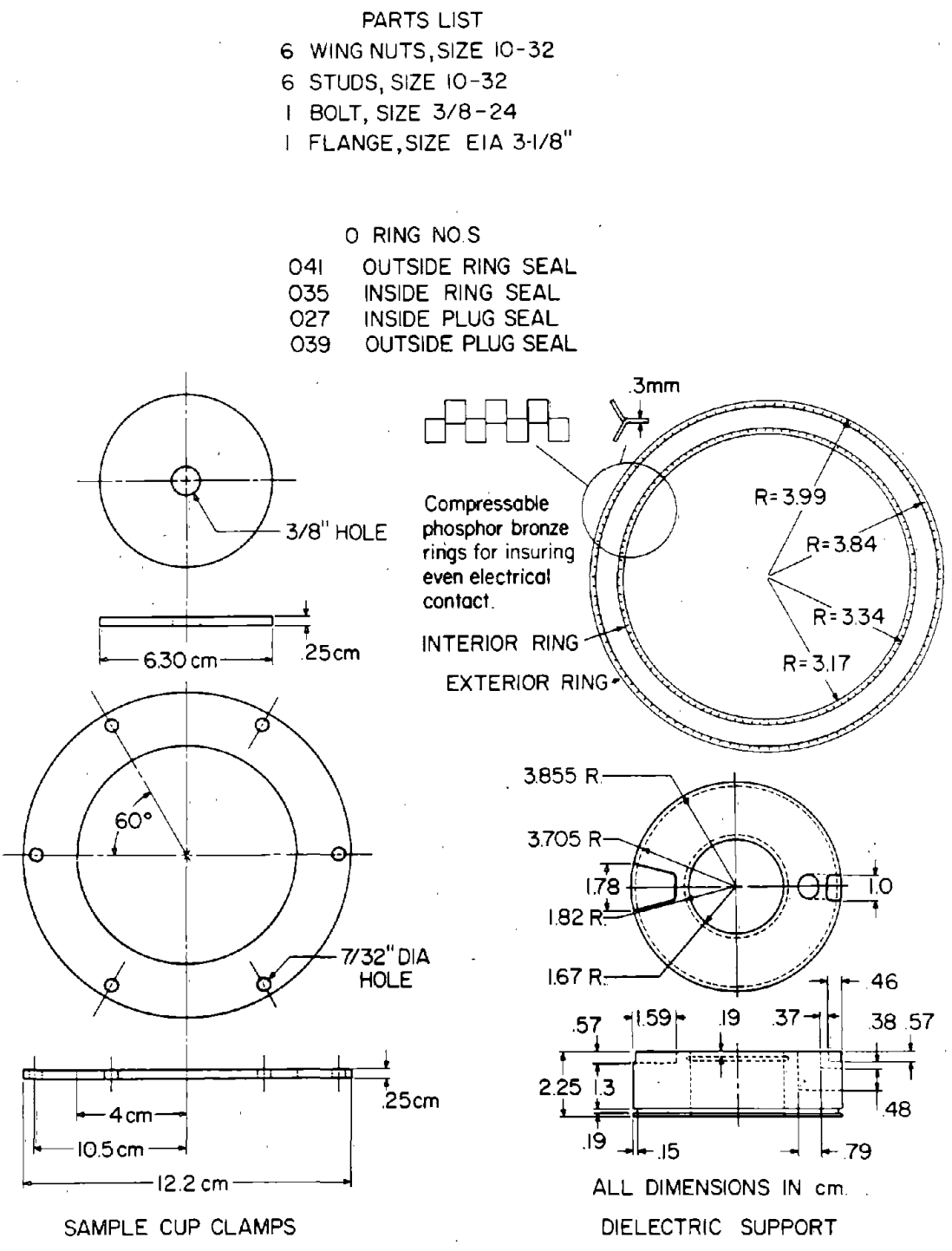


Figure 6. Construction details of dielectric centering support and clamps for securing sample holder cup

circumferentially in two opposite directions to a port in the opposite side. From there it is channeled into the annular space below the teflon bottom of the culture container where it flows circumferentially in two opposite directions toward another port on the opposite side. From there the fluid is directed up into the cooling chamber adjacent to the outer ring of the culture cell where it continues the same flow pattern to an exit port to the heat exchanger.

A number of sample holders were fabricated to allow a number of exposures to be done serially in a short time. A sample holder can be easily removed from the heat exchanger housing and replaced by another sample holder simply by removing the bolts holding the stainless steel clamps that press the sample holder in place. Figure 7-a photographically illustrates the disassembled components of the device and Figure 7-b illustrates a completely assembled system with the clamps in place.

The device electrically consists of several sections of coaxial transmission lines of different characteristic impedances, as illustrated geometrically in Figure 8. The bottom section is a 50 ohm (Ω) line of standard 3-1/8-inch-diameter solid coaxial transmission line. In this line we can establish a reference plane where the line impedance may be measured with a vector voltmeter through a magnetic loop and an electric probe. The first discontinuity of the dielectric sample holder device appears at a distance ℓ_ρ = 10.03 cm from the reference plane. This characteristic impedance of the line at this point changes to 31 Ω since it is dielectrically-loaded with a rexolite plug of length ℓ_d = 2.25 cm for structural strength and to provide passageways for the liquid coolant of the same dielectric constant. The next 0.27-cm long section with a characteristic impedance of 5.58 Ω has a larger diameter center conductor and is dielectrically-loaded with the silicon heat exchanger oil and the teflon ring. The final section of line has the same cross-section as the latter but is dielectrically-loaded with a biological sample of depth, t. Since the electrical conductivity of the biological solution is high, (from 75 to 1.5 S/m, where S is ohm^{-1}),the input and characteristic impedance of this section of coaxial line is much lower than the radiation impedance beyond. Thus, for all practical purposes, the loaded line can be treated as an open-ended transmission line, as verified in the next section.

In operation the device is connected to a power source, a circulating constant temperature liquid source and instrumentation, as shown in Figure 9. EM power from the source is connected to the large diameter coaxial feed section through a directional coupler for monitoring incident and reflected power at the tapered transition. A matching device may be placed in the large diameter feed section to transform the line impedance at that point to the 50 Ω impedance of the feed system. The matching device can actually be placed anywhere in the system, depending on the method used. In the experiments described in this report, a matching network was used between the generator and the directional coupler. The impedance of the line at a fixed distance below the sample can easily be measured by sampling the electric field or voltage with a probe and the magnetic field or current by a shielded loop. Voltages proportional to these parameters are monitored by a vector voltmeter or network analyzer designed for the appropriate frequency range used. The construction details of the loop and probe are given in Figure 10. The impedance and

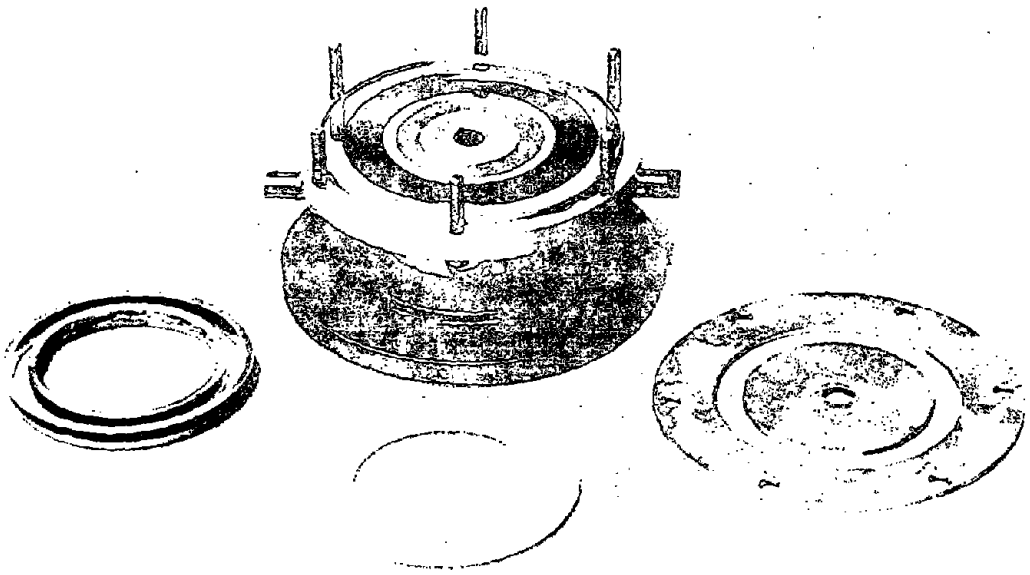


Figure 7-a. Photograph of disassembled cell culture exposure device. Left to right foreground, sample cup, teflon cap for sample cup, and clamps for holding cup in transmission line housing. Background transmission line housing and heat exchanger.

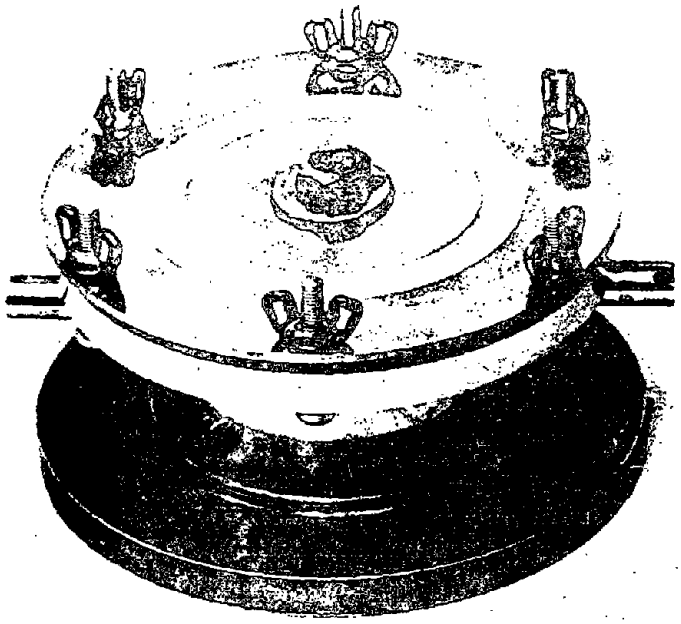


Figure 7-b. Assembled cell culture exposure device

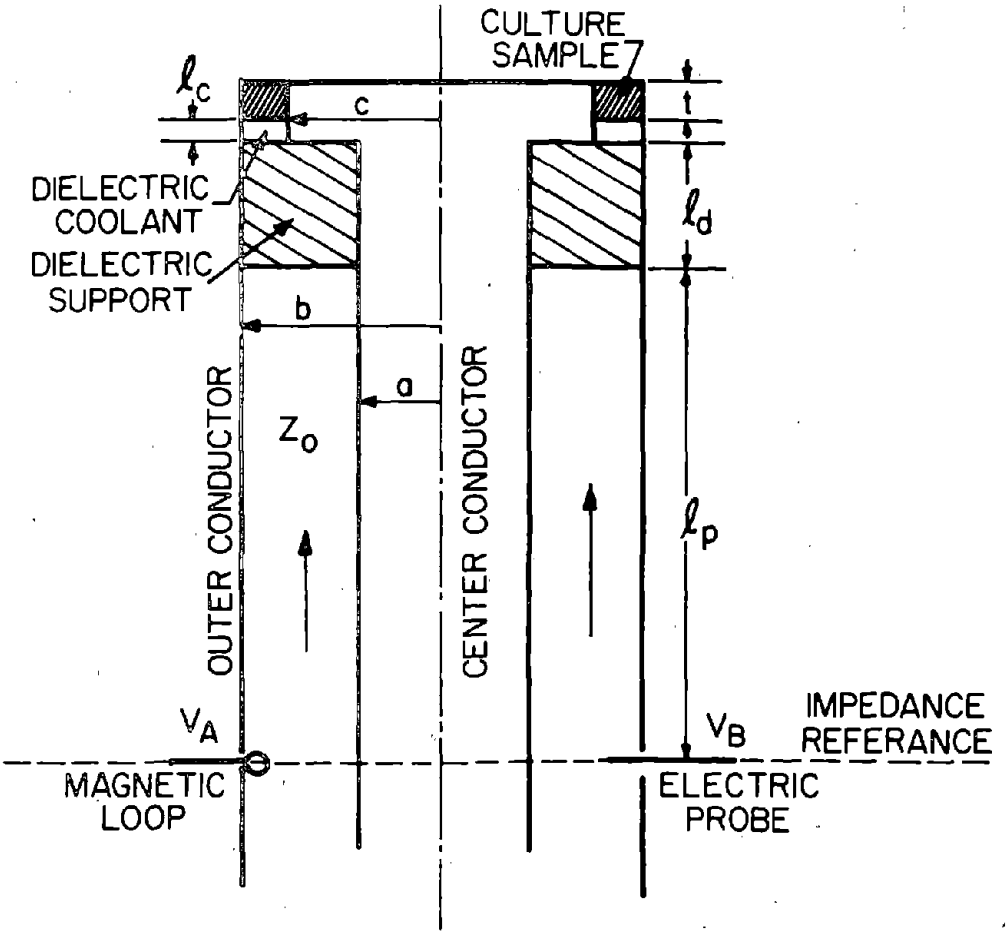


Figure 8. Simplified geometric representation of cell culture transmission line exposure system

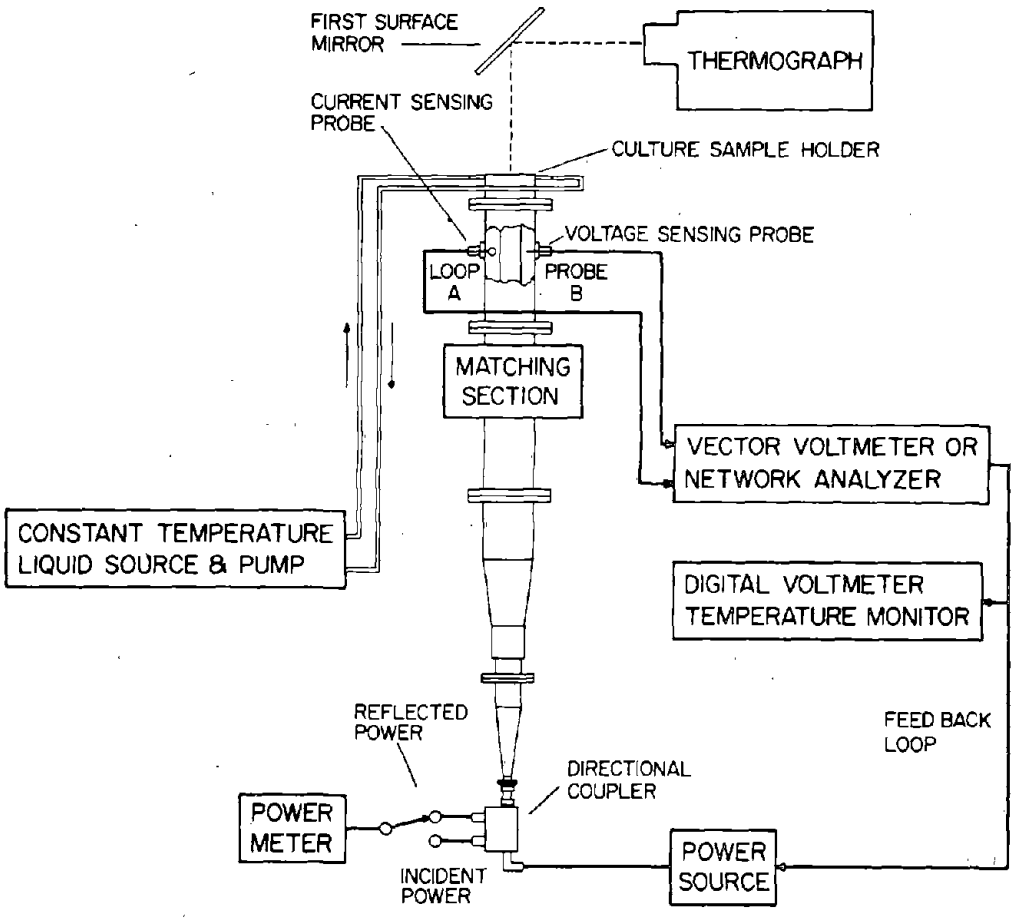
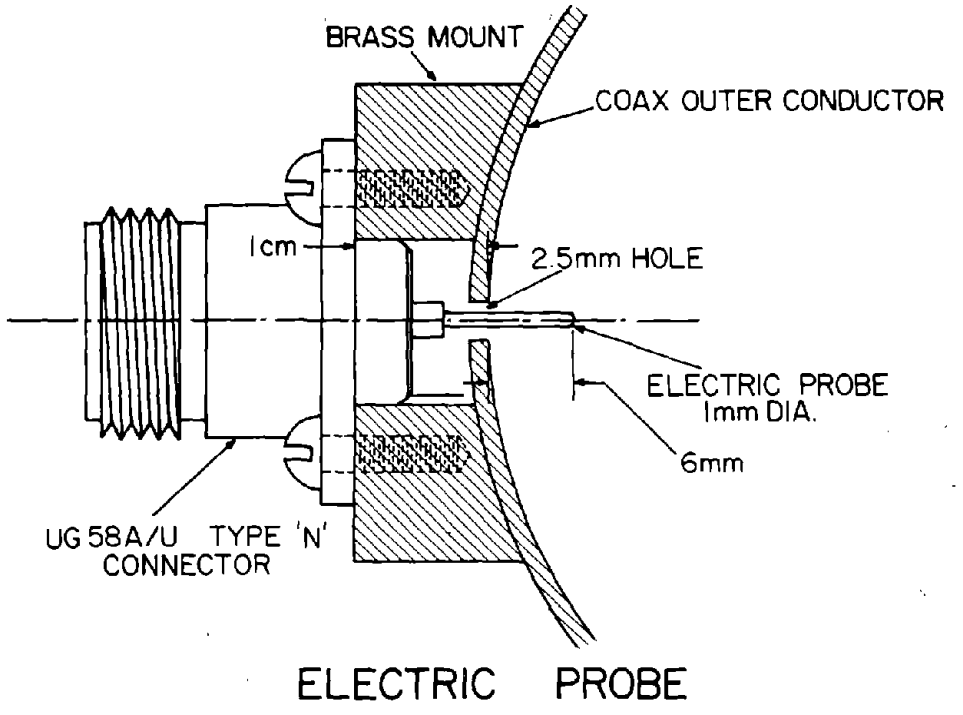


Figure 9. Complete system for exposing cell cultures to EM fields

dielectric constant of the sample and fields within the biological solution can easily be calculated from standard transmission line equations. The step discontinuity impedance, due to the transition in center conductor size, is large compared to the 3.3 Ω sample impedance, so the former may be ignored in the calculations. The low impedance provides significant advantage in reducing leakage fields and allows the use of an electrically insulated thermocouple to be inserted into the sample with negligible field coupling between it and the sample, thereby eliminating field perturbations within the culture sample.

The entire set of equations have been programmed on a standard programmable hand calculator. This permits conversion of the vector voltmeter readings to impedance and dielectric properties of the sample. The electric field strength and SAR within the sample can also be calculated. The system is initially calibrated using a known input power and a 50 Ω or a short to replace the sample holder and housing. The conductivity of the biological solution varies linearly with temperature (2.44×10^{-2} S/m° C) as measured with the system. This can be used as a basis for an on-line monitor and a control of the sample temperature for frequencies below 100 MHz.

Figure 11 illustrates photographically the system in operation. On the table top is the signal generator supplying a reference signal through an attenuator and power splitter for calibrating the vector voltmeter probes. An output is supplied to a counter at the shelf at the top left and to a 5-W power amplifier directly above. Above the power amplifier is a linear amplifier capable of supplying up to 600 W of power (requiring amplification over the 5-W input routinely used for field output which can be supplied by an available 100-W amateur radio transmitter, not shown). At the top right is a 1-kW match box for matching the power source to the exposure system. Directly under the match box is a temperature-controlled unit for circulating coolant through the culture heat exchanger. The assembled exposure device, transmission line, and directional coupler are standing vertically in the rack at the right in the photograph. At the right of the rack is a vector voltmeter with a digital temperature reading device (left) and a digital power meter (right) sitting on its top. The voltages V_A at the loop and V_B at the probe, as well as the phase angle θ between them, can be monitored on the vector voltmeter. Two sets of sampling probes are placed in the transmission line to cover different frequency bands. The temperatures of the coolant at the input and output of the culture heat exchanger and in the cell culture can be observed on the digital power meter. The digital power meter is used to monitor the incident and reflected power to the system.



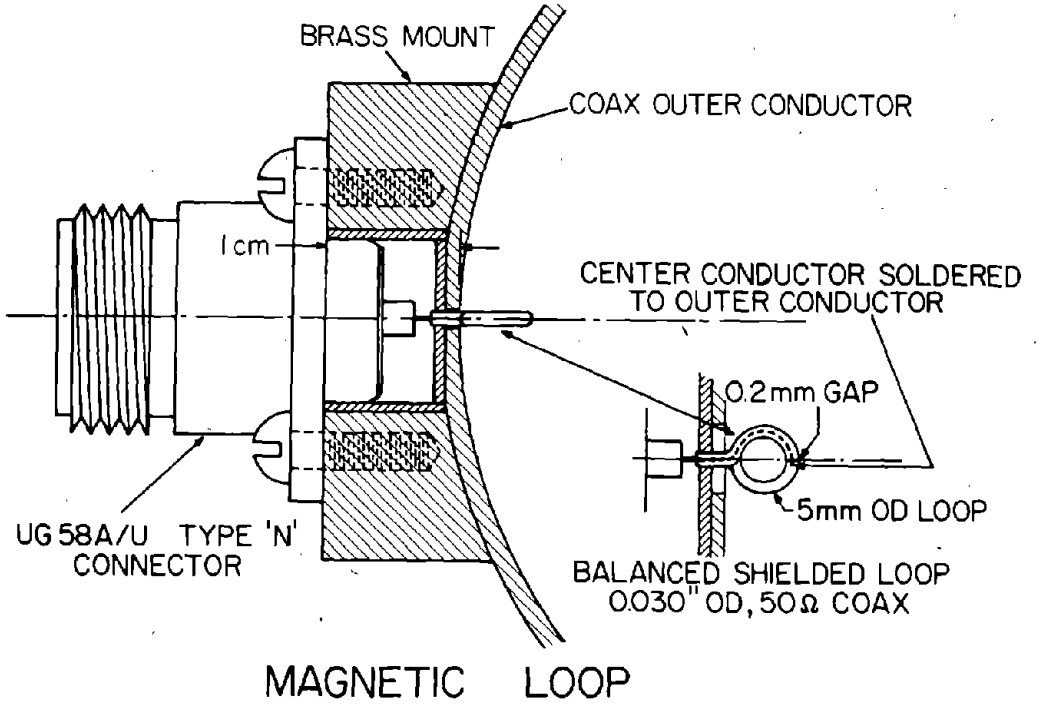


Figure 10. Construction details of electric probe and magnetic loop for sampling transmission line voltages

THEORETICAL FORMULATIONS

Transmission line equations can easily be used to determine the impedance, the dielectric properties, the fields, the specific absorption rate (SAR), and the total absorbed power of the exposed culture from simple measurements of the impedance at some reference point in the line. For purposes of transmission line analysis of the system, we may refer to the simplified sketch of the hardware in Figure 8 and the equivalent transmission line circuitry shown in Figure 12.

Figure 8 shows that the system consists basically of sections of coaxial transmission line. The first section connecting the source to the culture sample holder is standard 3-1/8-inch-diameter line of 50 Ω impedance containing both magnetic and electric probes for measuring the line impedance at a reference point placed at a distance ℓ_p from the sample holder assembly. The next section of line is of the same cross-section but it is loaded with rexolite dielectric material of thickness ℓ_d which maintains the structural stability of the line and provides a pathway for the coolant. Above the dielectric, the center conductor of the line is expanded to within 0.5 cm from the outer conductor. This section of line is divided into two sections, one of length ℓ_c carries a dielectric coolant with the same dielectric properties as the rexolite section, and the other contains the culture sample of depth t.

Figure 12 illustrates the electrical properties of the line. From left to right on the figure is the generator and a reference point containing probes for measuring transmission line voltage, V_p, current, I_p, and impedance, Z_p, normalized to 50 Ω. The figure illustrates the locations where impedances Z_d, Z_c, and Z_s (normalized to 50 Ω) at the dielectric, coolant, and culture interfaces, respectively, may be observed. The characteristic impedance of the standard line $Z_{o\ell}$ is 50 Ω, and of the dielectric-filled line is $Z_{o\ell}/\sqrt{\varepsilon_d}$, where $\varepsilon_d = 2.59$ and is the dielectric constant of the rexolite support. The impedance of the line with the larger-diameter center conductor after the transition is Z_{os} without the coolant present, and $Z_{os}/\sqrt{\varepsilon_d}$ with the coolant. Simplicity in analysis is obtained by maintaining a coaxial transmission configuration throughout the system.

The culture holder itself consists of a section of low impedance transmission line with large-diameter inner and outer conductors. This configuration has the advantage of maintaining relatively uniform fields within the culture medium, as well as presenting such a low impedance to the source that the radiation resistance and impedance of the annular slot at the surface of the culture can be ignored. We can demonstrate the validity of this assumption and also show that the junction capacitance in the transition from a standard feed coaxial cable to the culture field can be ignored.

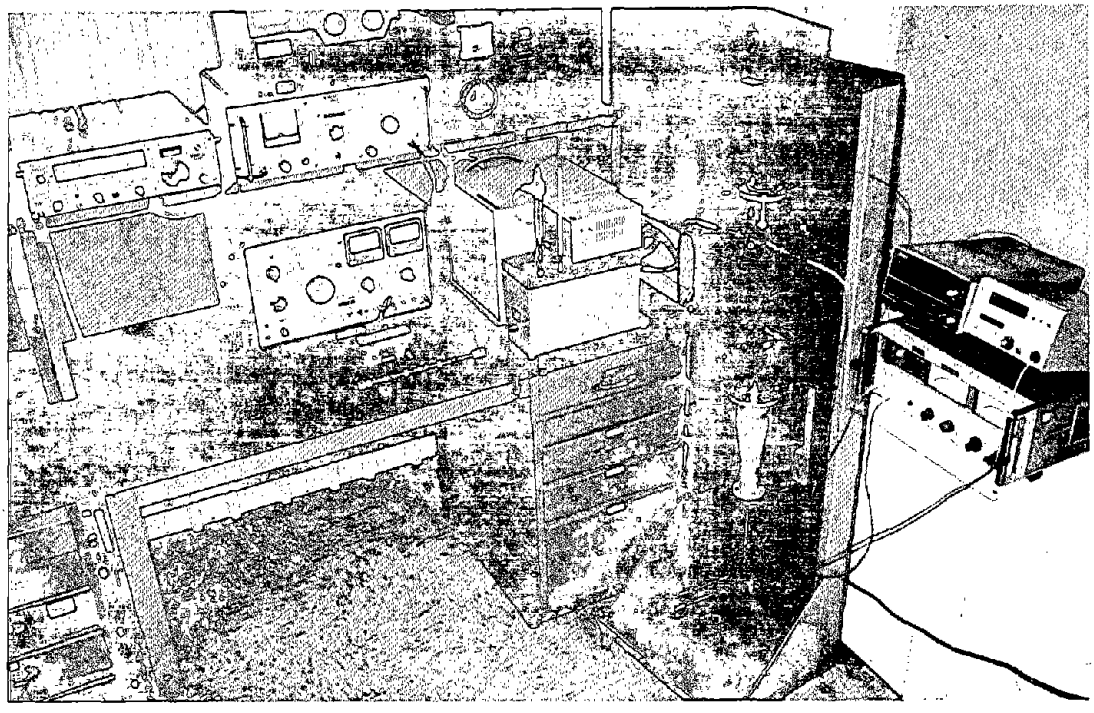


Figure 11. Photograph of cell culture exposure system and associated equipment.

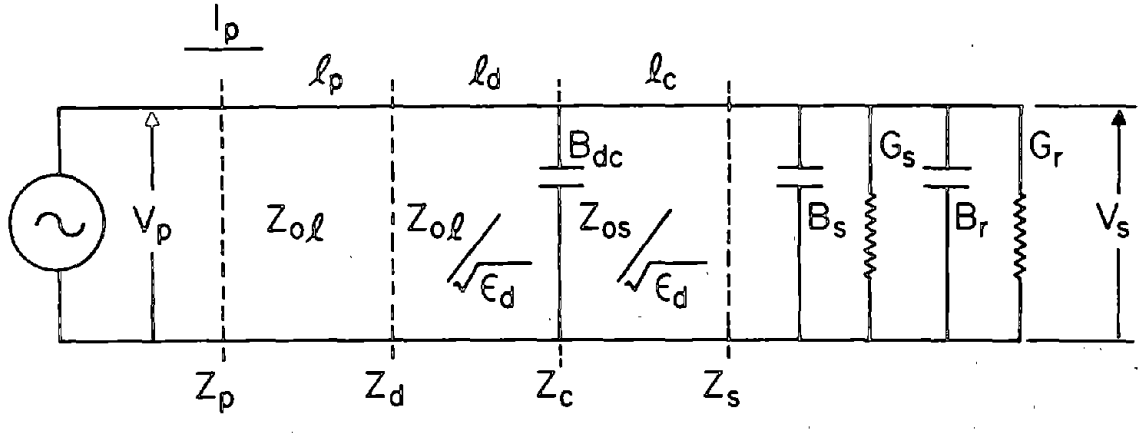


Figure 12. Equivalent transmission line and electric circuit of culture exposure system

Referring to the equivalent circuit in Figure 12, we may calculate the radiation admittance $G_r + jB_r$ based on a coaxial line with the same annular aperture size radiating into a semi-infinite space. According to Marcuvitz (10),

$$\frac{G_r}{Y_o} \simeq \frac{2}{3} (\ln \frac{b}{c})^{-1} [\frac{\pi^2 (b^2 - c^2)}{\lambda^2}] \tag{1}$$

$$\frac{B_r}{Y_o} \simeq \frac{8(b + c)}{\lambda \ln b/c} [E(\frac{2\sqrt{bc}}{b + c}) - 1] \tag{2}$$

where $b = 3.84$ cm is the outer radius
$c = 3.34$ cm is the inner radius of the transmission line
λ is the wavelength
$Y_o = \frac{1}{Z_{os}} = [60 \ln b/c]^{-1} = 0.121$ is the characteristic admittance of the transmission line, and
$E(\frac{2\sqrt{bc}}{b + c})$ is a complete elliptical integral of the second kind.

At 10 MHz, $G_r \simeq 8.96 \times 10^{-12}$ S and $B_r \simeq 1.42 \times 10^{-4}$ S and at 100 MHz, $G_r \simeq 8.96 \times 10^{-8}$ S and $B_r \simeq 1.42 \times 10^{-3}$ S.

The admittance of the transmission line loaded with culture can be estimated by assuming that it is terminated by an open circuit. Thus,

$$Y_s = G_s + jB_s = \frac{1}{Z_s} = \frac{1}{-j Z_{os} (\varepsilon_s^*)^{-\frac{1}{2}} \cot k(\varepsilon_s^*)^{\frac{1}{2}} t} . \tag{3}$$

Since the line is electrically short,

$$Y_s \simeq j\omega \frac{2\pi\varepsilon_o\varepsilon_s^* t}{\ln b/c} \tag{4}$$

where ω is the angular frequency
ε_o is the permittivity of free space, and
$\varepsilon_s^* = \varepsilon' - j \frac{\sigma}{\omega\varepsilon_o}$ is the complex dielectric constant of the culture.

Thus, for typical values of $\varepsilon' = 80$ and $\sigma = 1.55$ S/m at 10 MHz (assuming a 5-ml culture sample ($t = 0.433$ cm)) the calculated values are $G_s = .299$, and $B_s = 9 \times 10^{-3}$.

Since G_s is constant with frequency, and B_c increases directly with frequency, these values by far exceed the values of G_r and B_r up to 100 MHz and beyond. A similar analysis from Marcuvitz (11) also shows that the junction admittance B_{dc} is negligible compared to B_r, so straightforward transmission line equations may be used to analyze the system.

In order to determine the relationship between the voltages V_a and V_b, measured at the loop and probe of the transmission line and the fields in the culture, we must first establish the relation between the voltages measured at the probe and the actual voltage V_p and current I_p in the transmission line at that point. These relations can be characterized by two constants C_z and C_v defined by

$$Z_p = \frac{V_P}{I_p} = C_z \frac{V_B}{V_A} \tag{5}$$

and

$$V_p = C_v V_B . \tag{6}$$

The constant C_z may be determined by terminating the line with a matched load and observing the voltage V_{BL} and V_{AL} at the probe and loop. We may then obtain the relation

$$Z_p = 1 = C_z \frac{V_{BL}}{V_{AL}} \tag{7}$$

or $$C_z = \frac{V_{AL}}{V_{BL}} .$$

If the incident power is adjusted to a known value p_{in}

$$V_p = \sqrt{50\ p_{in}} = C_v V_{BL} \tag{8}$$

or $$C_v = \frac{\sqrt{50\ P_{in}}}{V_{BL}} .$$

The constants may also be obtained by terminating the line with a short and observing the probe and loop voltages V_{BS} and V_{AS} to obtain the relation

$$Z_p = j \tan k\ell_p = C_z \frac{V_{BS}}{V_{AS}} \tag{9}$$

or $$C_z = j \frac{V_{AS}}{V_{BS}} \tan k\ell_p \tag{10}$$

and

$$V_p = j\ 2\sqrt{50\ P_{in}} \sin k\ell_p = C_v V_{BS} \tag{11}$$

or $$C_v = \frac{j\ 2\sqrt{50\ P_{in}} \sin k\ell_p}{V_{BS}} . \tag{12}$$

After the constants C_z and C_v are obtained, the exposure conditions may be obtained from the measurements of V_A and V_B for a given situation. For example, the impedance and voltage at the reference plane is obtained from equations (9) and (11).

The normalized impedance at thenext reference plane is obtained from the equation

$$Z_d = \frac{Z_p - \tan k\ell_p}{1 - jZ_p \tan k\ell_p} \tag{13}$$

and the voltage at the same plane is obtained from

$$V_d = \frac{V_p}{\cos k\ell_p + jZ_d^{-1} \sin k\ell_p} . \tag{14}$$

We may normalize the impedance, Z_d, to the characteristic impedance of the succeeding section of transmission line by dividing it by $\sqrt{\varepsilon_d}$. Then, with this impedance and the electrical length of the line, $\ell_d\sqrt{\varepsilon_d}$, substituted in the appropriate places in equations (13) and (14), we may calculate Z_c and V_c. The process may be repeated, taking into account the changes in characteristic impedance, until the impedance Z_s of the culture sample and voltage V_s across the sample are obtained. The dielectric constant of the sample can then be obtained from equation (4) by letting $Y_s = Z_s^{-1}$ and solving for ε^*. The field in the cell culture may be obtained from

$$E = \frac{V_s}{r \ln b/c} \tag{15}$$

where r is the radial distance from the axis of the center conductor into the sample.

The specific absorption rate (SAR) is obtained by

$$SAR = 10\ \sigma\ E^2\ W/kg . \tag{16}$$

The total absorbed power is obtained from

$$W \simeq 10\ t\ \sigma \int_A E^2\ dA = \frac{\sigma t V_s^2}{2.22} . \tag{17}$$

Since the impedance of the voltage sensing probe can be affected slightly by the transmission line impedance at that point, it is wise to calibrate the system with a terminating impedance that provides an impedance at the probe reference position that is nearest in value to that expected under the actual culture exposure conditions. Since it was shown above that the culture impedance is very small compared to the 50 Ω characteristic impedance of the transmission line, $Z_s = \frac{1}{G_s} \simeq 3.34\ \Omega$, it appears that the short termination at the position of the sample would provide an impedance Z_p at the probe positions that would be closer in value to the normal operating impedance than obtained with either a load termination or a short termination in the 50 Ω section of transmission line. For this case, the constants C_z and C_v may be obtained from

$$C_z = \frac{V_{AS}}{V_{BS}} \tan k\ell'_p \tag{18}$$

$$C_v = \frac{2\sqrt{50\ p_{in}\ W}}{V_{BS}} \sin k\ell'_p \qquad (19)$$

where $\tan k\ell'_p = Z_{ps}$ and Z_{PS} is the impedance at the probe reference plane calculated by successive applications of impedance transformation Equation (13) with $-\ell$ substituted for ℓ.

We begin with a short circuit termination at the culture position and complete the transformation at the probe reference position.

The above equations have been programmed on a Hewlett-Packard 65 hand calculator. The programs tabulated in the Appendix consist of the program EM 02-A-D for calibrating the system using either a load or a short, or a combination of each, as a termination of the 50 Ω transmission line. EM 02-A may be used along with the calibration program to calculate the real and imaginary parts of the dielectric constant and the conductivity of the sample. Program EM 02-B may be used to calculate the impedance of and the voltage across the sample. Program EM 02-C may be used along with the previous program for determining the minimum and maximum fields; dielectric properties; minimum, maximum, and average specific absorption rate (SAR); and total absorbed power in the sample. Program EM 02-D may be used to predict the vector voltmeter readings from a given dielectric constant and conductivity of the sample. It also may be used to determine the normalized impedance at the reference probe location in the coaxial cable by successive applications of equations (13) and (14) in the reverse order with $-\ell$ substituted for ℓ. Program EM 02-E may be used for calibrating the system with a short placed in the location of the bottom interface of the culture sample at the location of the sample holder. Complete instructions on the use of the programs are given in the Appendix.

OPERATION OF THE SYSTEM

Calibration of the system is accomplished by feeding a power of 1 W incident to the input of the transmission line and recording the vector voltmeter readings with the load or short terminations. These values are then placed in the hand calculator using the proper calibration program. Either the load or short termination is then replaced with the actual sample and the coolant is applied to the heat exchanger. Then, with the proper setting of the input power and the coolant temperature, one can set up a wide range of exposure conditions of various field strengths and culture material temperatures. The proper setting of the coolant and the input power for a given exposure temperature and field level in the culture can be achieved through experience in using the system.

At lower field exposure conditions, electromagnetic-field-induced temperature change is quite small and the temperature of the culture can be set at any desired level simply by setting the heat exchanger temperature control. When exposing the culture to high electric fields, there will be significant heating of the culture material. The coolant temperature must be set much lower to maintain the final steady-state temperature at the desired level below 37°C. It should be recognized that for high-electric-field-strength exposure conditions, in order to provide sufficient cooling to maintain maximum temperature in the culture below 37°C, a significant temperature gradient will exist in the culture material with relatively low temperatures against the stainless steel walls and maximum temperature in the center of the culture. The calculator programs may be used to predict the errors in dosimetry due to the expected inaccuracies of the vector voltmeter readings. With the HP 8405A vector voltmeter in the frequency range 1 to 100 MHz, the expected accuracy in voltage measurement is ± 2% and in angle measurements is ± 3° or more, depending on conditions of use. If we consider a culture sample with dielectric constant $\varepsilon' = 81$ and electrical conductivity $\sigma = 1.5$ exposed to 14 MHz fields, we can predict the vector voltmeter readings by the calculator programs EM 02-D, as shown on the first row in Table 1. We can then determine the values of culture properties predicted by the calculator program EM 02-A with the maximum expected variations in the vector voltmeter, as shown in the remaining rows of data in the table.

One may note from Table 1 that the predicted values of the angle of the culture impedance and the dielectric constant varies over a wide range with only slight deviations of the vector voltmeter readings. This is due to the fact that the imaginary part of the dielectric constant is very high compared to the real part, making the predicted value of the latter and the smaller angle of the impedance very susceptible to the small changes in measured transmission line impedance Z_p at the reference plane. This is of no consequence to the accuracy of the culture dosimetry, however, since the value of ε'' is the dominant factor implying that the culture impedance is nearly a pure resistance. As seen in the table, all parameters important to

Table 1. Effect of Vector Voltmeter Error on Dosimetry Parameters (Assuming culture solution with $\varepsilon' = 81$, $\sigma = 1.5$ ($\varepsilon'' = 1928$ at 14 MHz and $\ell p = 10.03$ cm))

Vector VM Error			Vector VM Indications			Calculated Dosimetry Parameters							
ΔV_A %	ΔV_B %	$\Delta\Theta$ (deg)	V_A	V_B	Θ	Mag Z ohms	Arg Z (deg)	ε'	σ S/m	V Volts	E_{max} V/cm	Avg SAR W/kg	W Watts
0	0	0	1.54	2.99	-63.5	3.34	-2.40	81	1.5	0.850	1.83	43.3	0.217
0	0	3	1.54	2.99	-60.5	3.24	0.562	-1.95	1.55	0.826	1.77	42.1	0.211
0	0	-3	1.54	2.99	-66.5	3.43	-5.28	173	1.45	0.875	1.88	44.4	0.222
2	0	0	1.57	2.99	-63.5	3.27	-3.02	104	1.53	0.851	1.83	44.2	0.221
-2	0	0	1.51	2.99	-63.5	3.40	-1.77	58.6	1.47	0.850	1.82	42.4	0.212
0	2	0	1.54	3.04	-63.5	3.40	-1.77	58.6	1.47	0.867	1.86	44.2	0.221
0	-2	0	1.54	2.93	-63.5	3.27	-3.04	104	1.53	0.834	1.79	42.4	0.212
-2	2	3	1.51	3.04	-60.5	3.38	1.82	-6.08	1.48	0.843	1.81	42.1	0.211
2	-2	-3	1.57	2.93	-66.5	3.31	-6.51	221	1.51	0.859	1.84	44.4	0.222

proper dosimetry are relatively stable with the small variations of vector voltmeter impedance. At higher frequencies where the value of ε' becomes more important in affecting dosimetry, the variations become progressively less severe so there is no problem over the entire operating bandwidth of the system. Tables 2, 3, and 4 illustrate typical recorded and calculated data for a 5-ml sample of cell culture exposed to 14, 30, and 100 MHz fields, respectively. The data sheets are used for recording the sample number, the date and time of exposure, the exposure period, operating frequency, and the expected electric (E) field in the sample and measurements of temperature, incident power, reflected power, and reference probe voltages. Various parameters are measured initially when the fields are first turned on, and finally, prior to turning the fields off, in order to show any changes that may have occurred during the exposure period. The temperatures in the coolant entering and exiting the chamber are measured, as well as the maximum temperature of the culture, by means of thermocouples. The sample-to-probe distance is recorded and the method of calibration for both impedance measurements and voltage measurements in the culture are designated by S for a short and L for a load, or CS for the cup short. Calculations are made with the hand calculator of the impedance, conductivity, and dielectric constant of the culture, as well as the voltage across the culture. Calculations are also made of the maximum and minimum fields within the culture and the average, the maximum, and the minimum SAR in the sample. The maximum SAR occurs adjacent to the inner ring and the minimum SAR occurs near the outer ring. The total absorbed power, based on the measured voltage and impedance of the culture, is also recorded and may be compared to the net power input as measured for the complete system.

Tables 2 through 4 indicate that for nominal field strengths of 5 V/cm in the culture, the maximum culture temperature is no more than $1^{\circ}C$ higher than the coolant temperature, indicating that the temperature is relatively uniform throughout the culture. For these exposure conditions, the SAR is greater than 300 W/kg. This would correspond to a very high SAR for in vivo exposure conditions and would result in significant temperature rises and possible tissue damage in exposed animals or humans. In the in vitro exposure system, however, it can be seen that the temperatures can be held to relatively constant normal levels with very small gradients for the same internal fields. Therefore, the system is useful in determining whether an effect from an exposure is due to a temperature change or specifically related to electric field effects. Since the impedance of the sample is very low, it is also being subjected to high magnetic field strengths far exceeding those normally associated with an internal electric field of 5 V/cm.

Table 5 illustrates culture exposure data for a relatively high level of exposure corresponding to 20-22 V/cm in the sample. Note that under these conditions the total power absorbed by the sample is on the order of 35 W and the temperature is increased approximately $33^{\circ}C$ above the coolant temperature due to the heating of the culture by the electromagnetic fields. The SAR for this case exceeds by two orders of magnitude the SAR normally produced in typical in vivo exposures. The system, therefore, provides a means for exposing living cells to field levels far in excess of that which could be applied to living animals, but under temperature conditions not exceeding normal body temperature. Thus, the flexibility of the system in controlled experiments for seeking athermal effects is well-demonstrated.

Table 2. Culture exposure data (5 ml sample)

MEASUREMENTS

Sample No. 4 Date 13 April 1976 Time started 1700

Time exposed 20 min. Frequency 14 MHz

Expected E field in sample 5 V/cm

Incident Power	Reflected Power		Net Power
Initial 39.3 dbm	38.2 dbm		1.90 W
Final _____ dbm	_____ dbm		_____ W
Vector VM Data	V_A(mV)	V_B(mV)	θ (deg)
Initial	4.4	8.85	-57.4
Final	_____	_____	_____
Temperature (°C)	coolant in	coolant out	culture (max)
Initial	36.3	36.2	37.3
Final	_____	_____	_____

Sample to probe distance 10.03 cm

Calibration Method Z CS V CS

CALCULATIONS

Mag Impedance	Arg Impedance	Conductivity (S/m)	Dielectric Constant
Initial 3.10 Ω	3.42 deg	1.61	-110
Final _____ Ω	_____ deg	_____	_____
Sample Voltage	Maximum E Field		Minimum E Field
Initial 2.33 V	4.99 V/cm		4.34 V/cm
Final _____ V	_____ V/cm		_____ V/cm
SAR (W/kg) Average	Maximum		Minimum
Initial 349	402		304
Final _____	_____		_____

Total Absorbed Power (W) Initial 1.74 Final _____

Table 3. Culture exposure data (5 ml sample)

MEASUREMENTS

Sample No. 3 Date 13 April 1976 Time started 1630

Time exposed 20 min. Frequency 30 MHz

Expected E field in sample 5.0 V/cm

Incident Power	Reflected Power		Net Power
Initial 38.4 dbm	37.2 dbm		1.67 W
Final _____ dbm	_____ dbm		_____ W
Vector VM Data	V_A (mV)	V_B (mV)	θ (deg)
Initial	8.9	11.3	-39.2
Final	_____	_____	_____
Temperature (^oC)	coolant in	coolant out	culture (max)
Initial	36.2	36.3	37.2
Final	_____	_____	_____

Sample to probe distance 10.03 cm

Calibration Method Z ____ V ____

CALCULATIONS

Mag Impedance	Arg Impedance	Conductivity (S/m)	Dielectric Constant
Initial 3.06 Ω	-0.267 deg	1.64	4.58
Final _____ Ω	_____ deg	_____	_____
Sample Voltage	Maximum E Field		Minimum E Field
Initial 2.14 V	4.60 V/cm		4.00 V/cm
Final _____ V	_____ V/cm		_____ V/cm
SAR (W/kg) Average	Maximum		Minimum
Initial 300	346		262
Final _____	_____		_____

Total Absorbed Power (W) Initial 1.50 Final _____

Table 4. Culture exposure data (5 ml sample)

MEASUREMENTS

Sample No. 2 Date 13 April 1976 Time started 1600

Time exposed 20 min. Frequency 100 MHz

Expected E field in sample 5 V/cm

Incident Power	Reflected Power		Net Power
Initial 38.4 dbm	36.24 dbm		2.71 W
Final _____ dbm	_____ dbm		_____ W
Vector VM Data	V_A (mV)	V_B (mV)	θ (deg)
Initial	27.3	27.5	-14.3
Final	_____	_____	_____
Temperature (°C)	coolant in	coolant out	culture (max)
Initial	36.3	36.2	37.0
Final	_____	_____	_____

Sample to probe distance 10.03 cm

Calibration Method Z CS V CS

CALCULATIONS

Mag Impedance	Arg Impedance	Conductivity (S/m)	Dielectric Constant
Initial 3.34 Ω	1.314 deg	1.50	-6.89
Final _____ Ω	_____ deg	_____	_____
Sample Voltage	Maximum E Field		Minimum E Field
Initial 2.39 V	5.14 V/cm		4.46 V/cm
Final _____ V	_____ V/cm		_____ V/cm
SAR (W/kg) Average	Maximum		Minimum
Initial 343	396		299
Final _____	_____		_____

Total Absorbed Power (W) Initial 1.72 Final _____

Table 5. Culture exposure data (5 ml sample)

MEASUREMENTS

Sample No. 1 Date 4 March 1976 Time started 1000

Time exposed 20 min. Frequency 14 MHz

Expected E field in sample 25 V/cm

Incident Power	Reflected Power	Net Power
Initial 51.7 dbm	50.5 dbm	35.7 W
Final _____ dbm	_____ dbm	_____ W

Vector VM Data	V_A(mV)	V_B(mV)	θ (deg)
Initial	19.0	36.5	-63.4
Final	_____	_____	_____

Temperature (°C)	coolant in	coolant out	culture (max)
Initial	0.0	0.8	32.9
Final	_____	_____	_____

Sample to probe distance 10.03 cm

Calibration Method Z CS V CS

CALCULATIONS

Mag Impedance	Arg Impedance	Conductivity (S/m)	Dielectric Constant
Initial 3.15 Ω	-4.07 deg	1.59	161
Final _____ Ω	_____	_____	_____

Sample Voltage	Maximum E Field	Minimum E Field
Initial 10.2 V	21.9 V/cm	19.0 V/cm
Final _____ V	_____ V/cm	_____ V/cm

SAR (W/kg) Average	Maximum	Minimum
Initial 6590	7600	5750
Final _____	_____	_____

Total Absorbed Power (W) Initial 32.9 Final _____

Figure 13 illustrates the measured conductivity of a culture sample consisting of 20% primate blood plasma and 80% MEM (Monolayer) Earle's Base culture medium, as a function of temperature in the system with an incident power level of 1 W. Note that the conductivity as determined for the three frequencies, 14, 30, and 100 MHz, varies linearly with temperature (2.44×10^{-2} S/m°C). This variation can be used as an on-line monitor and control of the sample temperature after an initial value is measured with a thermocouple without the application of fields. Then, prior to turning the fields on, the thermocouple can be removed and the analog output of the vector voltmeter with proper scaling may be displayed directly as a temperature reading on a digital voltmeter, or it may be used as a feedback signal for maintaining the power for constant temperature while a sample is simultaneously being irradiated and cooled. This provides a very fast-acting temperature-measureing and temperature-controlling device since the change in the vector voltmeter output is instantaneous with any change in medium temperature. The system can also be calibrated directly by removing the teflon cap from the culture container and observing the culture surface temperature directly by a remote temperature sensing device such as a thermograph. The temperature can be measured, however, by using a teflon-coated thermocouple placed in the culture without any danger of enhancing the fields since, compared to the low 3 Ω impedance of the culture sample, the impedance between the thermocouple would be many, many orders of magnitude higher, limiting any thermocouple current to negligible values.

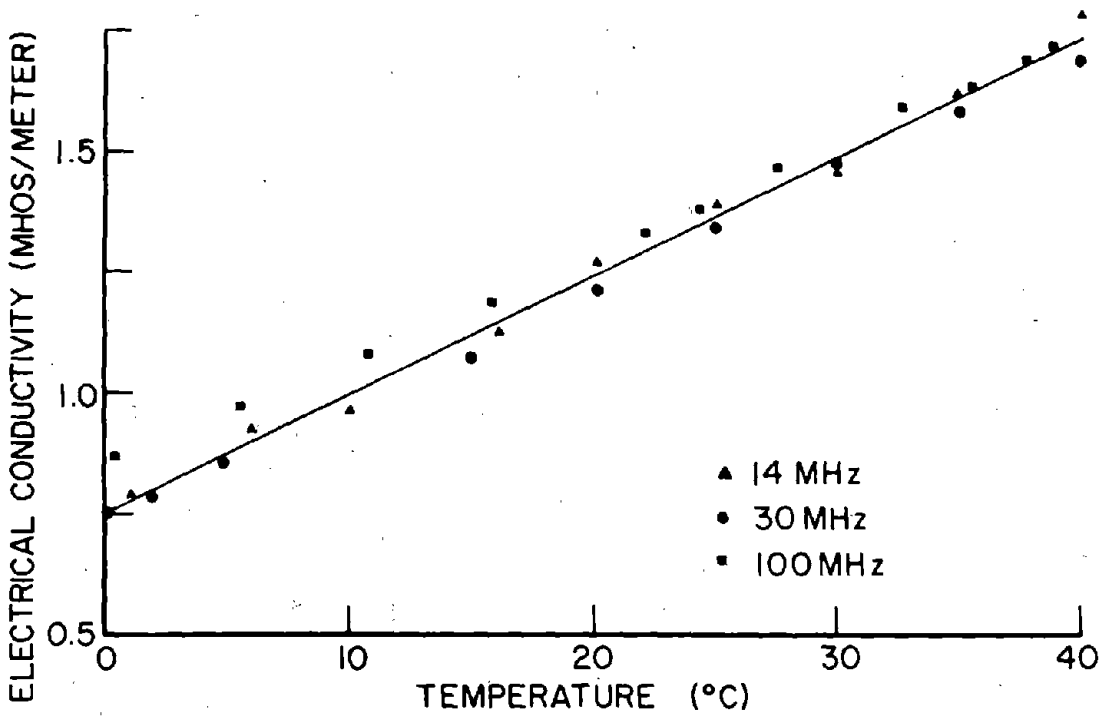


Figure 13. Temperature dependence of measured electrical conductivity of cell culture system (20% primate blood plasma, 80% MEM (Monolayer). Earle's base culture medium)

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APPENDIX

CALCULATOR PROGRAMS

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Title: Coaxial Culture Exposure System (Calibration) EM02 A-D Page 1 of 1

SWITCH TO W/PRGM. PRESS f PRGM TO CLEAR MEMORY.

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS
g		l_p in y, t in x
deg		
DSP		
3		
STO5		
R↓		
STO4		
R/S		
LBL		f in w, V_{BL} in z
A		V_{AL} in y, θ_L in x
x⇄y		V_{AL}
R↓		θ_L
x⇄y		V_{BL}
STO7		
R↑		V_{AL}
÷		V_{BL} / V_{AL}
STO1		Mag C_z^{-1}
R↓		θ_L
STO2		Arg C_z^{-1}
R↓		f
.		
0		
1		
2		.012
×		k (deg/cm)
STO3		
7		
.		
0		
7		
1		
RCL7		V_{BL}
÷		C_V
STO8		
RTN		
LBL		f in w, V_{BS} in z
B		V_{AS} in y, θ_S in x
x⇄y		V_{AS}
R↓		θ_S
x⇄y		
STO7		
R↑		
÷		
R↑		
.		
0		
1		
2		
×		
STO3		k (deg/cm)

KEY ENTRY	CODE SHOWN	COMMENTS
R↓		V_{BS} / V_{AS}
RCL3		k
RCL4		l_p
×		$k l_p$
f		
tan x		$\tan k l_p$
÷		Mag C_z^{-1}
STO1		
R↓		θ_S
9		
0		90
−		Arg C_z^{-1}
STO2		
RCL3		k
RCL4		l_p
×		
f		
sin x		$\sin k l_p$
2		
f		
$\sqrt{x}$		$\sqrt{2}$
×		$\sqrt{2} \sin k l_p$
1		
0		
×		$10\sqrt{2} \sin k l_p$
RCL7		V_{BS}
÷		C_V
STO8		
RTN		
LBL		
C		V_{BL} in x
7		
.		
0		
7		
1		
x⇄y		
÷		
STO8		C_V
RTN		

35

REGISTERS

- R_1 Mag C_z^{-1}
- R_2 Arg C_z^{-1}
- R_3 k
- R_4 l_p
- R_5 t
- R_6
- R_7 V_{BL} / V_{BS}
- R_8 C_V
- R_9

LABELS

- A Load Cal.
- B Short Cal.
- C L.S. Cal.
- D
- E
- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9

FLAGS

- 1
- 2

TO RECORD PROGRAM INSERT MAGNETIC CARD WITH SWITCH SET AT W/PRGM.

Title Coaxial Culture Exposure System (Calibration) EM02A-D

Programmer A. W. Guy Date 2/15/76

HP-65 USER INSTRUCTIONS

Coax Culture Exp (Cal.) EM02A-D
L.Cal S.Cal LSCal

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	Load Program			
2	Key in probe to (dielectric) short distance	l_p (cm.)	Enter	l_p (cm.)
3	Key in sample thickness	t (cm.)	R/S	l_p (cm)
	Go to any of following options			
	I OPTION A.- Calibrate with 50Ω load			
1	Key in frequency	f (MHz)	Enter	f (mHz)
2	Key in Vector VM B voltage	V_{BL} (mV)	Enter	V_{BL} (mV)
3	Key in Vector VM A voltage	V_{AL} (mV)	Enter	V_{AL} (mV)
4	Key in Vector VM Angle	Θ_L (deg)	A	C_V
5	Load Appropriate Program EM02			
	A, B, D in forward position			
	II OPTION B.- Calibrate with Short			
1	Key in frequency	f (mHz)	Enter	f (mHz)
2	Key in Vector VM B voltage	V_{BS} (mV)	Enter	V_{BS} (mV)
3	Key in Vector VM A voltage	V_{AS} (mV)	Enter	V_{AS} (mV)
4	Key in Vector VM angle	Θ_S (deg)	B	C_V
5	Load Appropriate Program in			
	forward direction EM02 A, B, or D			
	III OPTION C - calibrate			
	impedance with short and voltage			
	with load			
1.	Carry out option B to step 4			
2.	Key in Vector VM B voltage for load		C	C_V
3.	Load Appropriate program			
	in forward direction			

Title Coaxial Culture Exposure System (ϵ^*) EM02A Page 2 of 2

SWITCH TO W/PRGM PRESS [f] [PRGM] TO CLEAR MEMORY

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS
RCL 2		V_D in z, V_A in y, θ in x
−		Arg C_z^{-1}
R↓		Arg z_p
÷		V_A
RCL 1		V_B / V_A
÷		Mag C_z^{-1}
R↑		Mag z_p
x⇄y		Arg z_p
RCL 4		Mag z_p
B		l_p
x		$z_d \sqrt{\epsilon_d}$
3		
.		
6		
2		$l_d \sqrt{\epsilon_d}$
B		
R↓		$z_c \sqrt{\epsilon_d}$
5		
.		
9		
7		$z_{or} z_{os}^{-1}$
x		$z_{or} z_{os}^{-1} z_c \sqrt{\epsilon_d}$
.		
6		
0		$l_c \sqrt{\epsilon_0}$
B		
÷		$z_{or} z_{os}^{-1} z_s$
9		
1/x		$z_{or}^{-1} z_{os} z_s^{-1}$
f st x		$z_{or} z_{os}^{-1} z_s$
.		
1		
.		
÷		$z_{or} z_s$
STO 7		
CLR x		
RCL 3		k
RCL 5		t
x		kt
f		
tan x		$\tan kt$
÷		
f⁻¹		
R→P		ϵ'' in x, $-\epsilon'$ in y
RCL 3		k
2		
1		
6		
÷		
x⇄y		ϵ''

KEY ENTRY	CODE SHOWN	COMMENTS
x		σ
f st x		ϵ''
R↑		$-\epsilon'$
CHS		ϵ'
RTN		
LBL		Transmission line
B		Impedance transf.
RCL 3		k
x		kl
f		
tan x		$-\tan kl$
R↓		z_1 (z at Ref. 1)
f⁻¹		
R→P		Re z_1
R↑		$\tan kl$
Enter		
STO 6		
R↑		
STO 7		Im z_1
x		Im $z_1 \tan kl$
1		
+		Re Den. = [1 + Im $z_1 \tan kl$]
R↓		$\tan kl$
x		Re $z_1 \tan kl$
x⇄y		Re z_1
RCL 7		Im z_1
R↑		
STO 7		Re Den.
R↓		Im z_1
RCL 6		$\tan kl$
−		Im Num. = [Im z_1 − $\tan kl$]
x⇄y		Re Num.
f		
R→P		Mag Num.
R↑		Im Den.
RCL 7		Re Den.
f		
R→P		Mag Den
x⇄y		Arg Den
R↑		Arg Num
+		Arg z_2
x⇄y		Mag Den
R↑		Mag Num.
x⇄y		Mag Den.
÷		Mag z_2 (z at Ref 2)
1		
.		
6		
1		$\sqrt{\epsilon_d}$
RTN		

REGISTERS

R1 Mag C_z^{-1}

R2 Arg C_z^{-1}

R3 k

R4 l_p

R5 t

R6 $\tan kl$

R7 Im z_1

R8 Re Den.

R9 C_v

R0

LABELS

A
B
C
D
E
0
1
2
3
4
5
6
7
8
9

FLAGS

1

2

LIFT USER

TO RECORD PROGRAM INSERT MAGNETIC CARD WITH SWITCH SET AT W/PRGM

Title Coaxial Culture Exposure System (ϵ^*) EM02A Page 1 of 1

Programmer A.W. Guy Date 2/7/76

HP-65 USER INSTRUCTIONS

Coax Culture Exp. (ϵ^*) EM02A

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	Load Program in Reverse direction (EM02A-D) and calibrate according to instructions for EM02A-D			
2	Load Program in forward direction		RTN	
3	Enter Vector VM V_B	V_B (mV)	RTN Enter	V_B (mV)
4	Enter Vector VM V_A	V_A (mV)	Enter	V_A (mV)
5	Enter Vector VM θ	θ (deg)	R/S	ϵ'
6	Read dielectric constant in x			ϵ'
7	Read loss factor		R↓	ϵ''
8	Read electrical conductivity		R↓	σ (S/m)
	For entering new data with same freq f and probe-short distance ℓ_p begin with step 3. For new sample thickness t store in register 5 and begin with step 3. Begin with 1 for any other changes			

Title Coaxial Culture Exposure System (Z,V) EM02B Page 1 of 1

SWITCH TO W PRGM PRESS f PRGM TO CLEAR MEMORY

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS
		V_B in Z, V_A in y, θ in x
RCL 2		Arg C_z^{-1}
−		Arg Z_p
R↓		V_A
÷		V_B / V_A
RCL 1		Mag C_z^{-1}
÷		Mag Z_p
x⇄y		V_B
RCL 8		C_v
x		
STO 5		
R↓		Mag Z_p
RCL 4		l_p
B		Z_d
1		
.		
6		
1		$\sqrt{\epsilon_d}$
x		$Z_d\sqrt{\epsilon_d}$
3		
.		
6		
2		$l_d\sqrt{\epsilon_d}$
B		$Z_c\sqrt{\epsilon_d}$
5		
.		
9		
7		$Z_{0\ell} Z_{0s}^{-1}$
x		$Z_{0\ell} Z_{0s}^{-1} Z_c\sqrt{\epsilon_d}$
.		
6		
0		$l_c\sqrt{\epsilon_d}$
B		$Z_{0\ell} Z_{0s}^{-1} Z_s\sqrt{\epsilon_d}$
5		
.		
2		$Z_{0s}(\epsilon_d)^{-\frac{1}{2}}$
x		$Z_{0\ell} Z_s$
RTN		
LBL		Transformation of
B		Z and V in Xmission line
RCL 3		k
x		kl
f		
tanx		tan kl
R↓		Z_1 (ref 1)
f⁻¹		
R→P		Re Z_1
R↑		tan kl
Enter		
STO 6		
R↑		Im Z_1

KEY ENTRY	CODE SHOWN	COMMENTS
STO 7		
x		Im Z, tan kl
1		
+		Re Den = [1 + Im Z, tan kl]
R↓		tan kl
x		Re Z, tan kl
x⇄y		Re Z_1
RCL 7		Im Z_1
R↑		
STO 7		Re Den
R↓		Im Z_1
RCL 6		tan kl
−		Im Num = [Im Z, tan kl]
x⇄y		Re Num
f		
R→P		Mag Num
R↑		Im Num
RCL 7		Re Den
f		
R→P		Mag Den
x⇄y		Arg Den
R↑		Arg Num
+		Arg Z_2
RCL 6		tan kl
R↑		Mag Num
R↑		Mag Den
÷		Mag Z_2 (ref 2)
STO 7		
÷		tan kl / Mag Z_2
f⁻¹		
R→P		Re [tan kl / Mag Z_2]
x⇄y		Im [tan kl / Mag Z_2]
1		
+		1 + Im [tan kl / Mag Z_2]
f		
R→P		Mag $\left[1 + j\frac{\tan kl}{Z_2}\right]$
RCL 6		tan kl
f⁻¹		
tanx		kl
f		
cos x		Cos kl
x		Mag $\left[\cos kl + j\frac{1}{Z_2}\sin kl\right]$ V_1 (ref 1)
RCL 5		
x⇄y		V_2 (ref 2)
÷		
STO 5		
R↑		Arg Z_2
STO 6		
RCL 7		Mag Z_2
RTN		

REGISTERS	
R0	
R1	Mag C_z^{-1}
R2	Arg C_z^{-1}
R3	k
R4	l_p
R5	V
R6	tan kl, Arg Z_2
R7	Im Z_1, Re Den, Mag Z_2
R8	C_v
R9	

LABELS: A B C D E 0 1 2 3 4 5 6 7 8 9

FLAGS: 1 2

TO RECORD PROGRAM INSERT MAGNETIC CARD WITH SWITCH SET AT W/PRGM

Title Coaxial Culture Exposure System (Z,V) EM02B Page 1 of 1

Programmer A. W. Guy Date 2/8/76

HP-65 USER INSTRUCTIONS

Coax Culture Exp (Z,V) EM02B

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	Load Program in Reverse direction and calibrate according to instructions for EM02A-D			
2	Load Program in forward direction			
3	Enter Vector VM V_B	V_B (mV)	RTN Enter	V_B (mV)
4	Enter Vector VM V_A	V_A (mV)	Enter	V_A (mV)
5	Enter Vector VM θ	θ (deg)	R/S	Mag Z (Ω)
6	Read magnitude of impedance			Mag Z (Ω)
7	Read Angle of impedance		R↓	Arg Z (deg)
8	Read Voltage across sample		R↓	V (volts)
	For entering new data with same frequency f and probe-short distance l_p begin with step 3. For new sample thickness t store in register 5 and begin with step 3. Begin with 1 for any other changes			

Title: Coaxial Culture Exposure System (ϵ^*, E, SAR, W) EM02C Page 1 of 1

SWITCH TO W/PRGM | PRESS | f | PRGM | TO CLEAR MEMORY

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS
Enter		t in x
Enter		
RCL6		Avg $Z_{os} Z_{os}^{-1} Z_s \sqrt{\epsilon_d}$
RCL7		Mag $Z_{os} Z_{os}^{-1} Z_s \sqrt{\epsilon_d}$
1		
.		
6		
1		$\sqrt{\epsilon_d}$
÷		Mag $Z_{os} Z_{os}^{-1} Z_s \sqrt{\epsilon_d}$
9		
1/x		
RCL3		k
R↑		t
x		kt
1st x		t
R↓		kt
f		
tan x		tan kt
÷		
f -1		
R→P		$\epsilon''_{in x}$, $-\epsilon'_{in y}$
RCL3		k
x		$k\epsilon''$
2		
1		
6		
÷		σ
STO6		
R↓		$-\epsilon'$,
CHS		ϵ'
STO7		
R↓		t
STO		
9		
A		$\sigma_{in x}$, $\epsilon'_{in y}$
RTN		
LBL		Display σ
A		(in x) and ϵ' (in y)
RCL7		ϵ'
RCL6		σ
RTN		
LBL		Calculate E
B		Field
RCL5		$\sqrt{V}$
.		
5		
3		
6		
÷		E_{min} (V/cm)
RCL5		

KEY ENTRY	CODE SHOWN	COMMENTS
.		
4		
6		
6		
÷		E_{max} (V/cm)
RTN		
LBL		Calculate SAR
C		
B		E_{max}
x≷y		E_{min}
E		min SAR (W/kg)
x≷y		E_{max}
E		max SAR (W/kg)
RCL5		$\sqrt{V}$
E		$10\sigma V^2$
3		
.		
9		
9		
x		Avg. SAR (W/kg)
RTN		
LBL		Calculate total
D		Power W
RCL5		$\sqrt{V}$
E		$10\sigma V^2$
RCL		
9		t
x		
2		
2		
.		
2		
÷		W (watts)
RTN		
LBL		Power and SAR
E		sub routine
f -1		
√x		E^2 or V^2
RCL6		
x		
1		
0		
x		
RTN		$10\sigma E^2$ or $10\sigma V^2$

REGISTERS

R1 Mag C_z^{-1}

R2 Arg C_z^{-1}

R3 k

R4 l_p

R5 V

R6 Avg $\frac{Z}{\sigma}$

R7 Mag $\frac{Z}{\epsilon'}$

R8 C_v

R9 t

LABELS

A σ, ϵ'

B E

C SAR

D W

E

0

1

2

3

4

5

6

7

8

9

FLAGS

1

2

USER CODE

TO RECORD PROGRAM ON HP MAGNETIC CARD WITH SWITCH SET AT W/PRGM

Title Coaxial Culture Exposure System (ϵ^*, E, SAR, W) EM02C Page 1 of 1

Programmer A. W. Guy Date 2/15/76

HP-65 USER INSTRUCTIONS

Coax Culture Exp (ϵ^*, E, SAR) EM02C
ϵ^* E SAR W

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	Calculate Z and V by Programs			
	EM02A-D and EM02B per instructions			
2	Load Program EM02C in forward direction			
3.	Enter sample thickness	t (cm)	RTN R/S	σ (S/m)
4.	Calculate Dielectric properties		A	σ (S/m)
5.	Read electrical conductivity			σ (S/m)
6.	Read dielectric constant		R↓	ϵ'
7.	Calculate Fields		B	E_{max} (V/cm)
8.	Read maximum E Field			E_{max} (V/cm)
9.	Read minimum E Field		R↓	E_{min} (V/cm)
10.	Calculate SAR		C	SAR_{avg} (W/kg)
11.	Read Average SAR			SAR_{avg} (W/kg)
12.	Read maximum SAR		R↓	SAR_{max}
13.	Read minimum SAR		R↓	SAR_{min}
14	Calculate total absorbed Power		D	W (watts)
	σ and ϵ', E, SAR, and W may be calculated in any order. For new thickness begin with 3.			

Title: Coaxial Culture Exposure System (V_B/V_A, θ) EM02D Page 1 of 1

SWITCH TO W/PRGM. PRESS [f] [PRGM] TO CLEAR MEMORY.

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS
		σ in y, ϵ' in x
CHS		$-\epsilon'$
x⇄y		σ
2		
1		
6		
x		
RCL 3		
÷		ϵ''
f		
R→P		Mag ϵ^*
RCL 3		k
RCL 5		t
x		kt
f		
tanx		tankt
x		Mag ϵ^* · tankt
9		
1/x		$Z_{oL} Z_{os}^{-1} Z_s$
1		
.		
6		
1		
x		$Z_{oL} Z_{os}^{-1} Z_s \sqrt{\epsilon_d}$
.		
6		
0		$l_c \sqrt{\epsilon_d}$
B		$Z_{oL} Z_{os}^{-1} Z_c \sqrt{\epsilon_d}$
5		
.		
9		
7		$Z_{oL} Z_{os}^{-1}$
÷		$Z_c \sqrt{\epsilon_d}$
3		
.		
6		
2		$l_d \sqrt{\epsilon_d}$
B		$Z_d \sqrt{\epsilon_d}$
1		
.		
6		
1		$\sqrt{\epsilon_d}$
÷		Z_d
RCL 4		l_p
B		Mag Z_p
STO 6		
x⇄y		
STO 7		Arg Z_p
RCL 2		Arg C_z^{-1}
+		θ
x⇄y		Mag Z_p

KEY ENTRY	CODE SHOWN	COMMENTS
RCL 1		Mag C_z^{-1}
x		V_B / V_A
RTN		
LBL		Transformation of
B		Impedance
RCL 3		k
x		kl
f		
tanx		tankl
R↓		Z_2 (Ref 2)
f^{-1}		
R→P		Re Z_2 in x, Im Z_2 in y
R↑		tankl
Enter		
STO 6		
R↑		
STO 7		Im Z_2
x		Im Z_2 tankl
CHS		
1		
+		Re Den = [1 − Im Z_2 tankl]
R↓		tankl
x		Re Z_2 tankl
CHS		− Re Z_2 tankl
x⇄y		Re Z_2
RCL 7		Im Z_2
R↑		
STO 7		Re Den
R↓		Im Z_2
RCL 6		tankl
+		Im N = [Im Z_2 + tankl]
x⇄y		Re Num
f		
R→P		Mag Num
R↑		Im Den
RCL 7		Re Den
f		
R→P		Mag Den
x⇄y		Arg Den
R↑		Arg Num
+		Arg Z_1
x⇄y		Mag Den.
R↑		Mag Num.
x⇄y		Mag Den.
÷		Mag Z_p
RTN		

REGISTERS	
R0	Mag C_z^{-1}
R1	
R2	Arg C_z^{-1}
R3	k
R4	l_p
R5	t
R6	tankl
R7	Im Z
R8	Re Den
R9	Mag Z_p

FLAGS	
1	
2	

TO RECORD PROGRAM INSERT MAGNETIC CARD WITH SWITCH SET AT W/PRGM

Title Coaxial Culture Exposure System ($V_B/V_A, \theta$) EM02D Page 1 of 1

Programmer A. W. Guy Date 2/15/76

HP-65 USER INSTRUCTIONS

Coax Culture Exp ($V_B/V_A, \theta$) EM02D

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS		OUTPUT DATA/UNITS
1	Load Program in Reverse direction and calibrate according to instructions for EM02A-D				
2	Enter Program EM02D (forward)				
3	Enter electrical conductivity	σ (S/m)	Enter		σ (S/m)
4	Enter dielectric constant	ϵ'	R/S		V_B/V_A
5	Read Vector VM volt. ratio				V_B/V_A
6	Read Vector VM angle		R↓		θ (deg)
7	Read Impedance - probe position		RCL	6	Mag Z_p
8	Read Impedance Angle - probe position		RCL	7	Arg Z_p
	To enter new values at same freq., sample thickness, and probe-sample distance begin with step 3. Otherwise begin with step 1				

Title: Coaxial Culture Exposure System (Cup Short Calib.) EM02E Page 1 of 1

SWITCH TO W/PRGM | PRESS f | PRGM | TO CLEAR MEMORY

HP-65 PROGRAM FORM

KEY ENTRY	CODE SHOWN	COMMENTS: ℓ_p in y, f in x
9		
deg		
Dsp		
3		
.		
0		
1		
2		
X		
STO 3		k
R↓		
STO 4		
.		
4		
4		
3		
STO 5		t
R/S		V_{BS} in Z, V_{AS} in y
LBL		θ_S in x
A		
STO 2		θ_S
R↓		V_{AS}
x⇄y		V_{BS}
STO 8		
÷		V_{AS}/V_{BS}
STO 1		
RCL 3		k
.		
6		
0		$\ell_c\sqrt{\epsilon_d}$
X		$k\ell_c\sqrt{\epsilon_d}$
f		
tanx		$\tan k\ell_c\sqrt{\epsilon_d}$
5		
.		
9		
7		Z_{OS} or Z_{OS}^{-1}
÷		$Z_c\sqrt{\epsilon_d}$
3		
.		
6		
2		$\ell_d\sqrt{\epsilon_d}$
B		$Z_d\sqrt{\epsilon_d}$
1		
.		
6		
1		$\sqrt{\epsilon_d}$
÷		Z_d
RCL 4		ℓ_p
B		Z_p

KEY ENTRY	CODE SHOWN	COMMENTS
STO 7		Mag Z_p
f⁻¹		
tanx		$k\ell_p$
f		
Sinx		$\sin k\ell_p$
2		
0		
0		
f		
√x		
X		
RCL 8		V_{BS}
÷		C_V
STO 8		
RCL 1		V_A/V_B
RCL 7		Mag Z_p
X		
9		
1/x		Mag C_Z^{-1}
STO 1		
RCL 2		θ_S
9		
0		90
−		
STO 2		Arg C_Z^{-1}
RTN		
LBL		Impedance transfr.
B		subroutine
RCL 3		k
X		$k\ell$
f		
tanx		$\tan k\ell$
STO 6		
x⇄y		Z_2
+		$Z_2 + \tan k\ell$
1/x		Z_2
RCL 6		$\tan k\ell$
X		$Z\tan k\ell$
CHS		$-Z\tan k\ell$
1		1
+		$1 - Z\tan k\ell$
÷		Mag Z_1
RTN		

REGISTERS	
R1	V_A/V_E Mag C_Z^{-1}
R2	θ_S Arg C_Z^{-1}
R3	k
R4	ℓ_p
R5	t
R6	$\tan k\ell$
R7	Mag Z_p
R8	V_{BS} C_V
R9	

LABELS	
A	
B	
C	
D	
E	
0	
1	
2	
3	
4	
5	
6	
7	
8	
9	

FLAGS	
1	
2	

TO RECORD PROGRAM INSERT MAGNETIC CARD WITH SWITCH SET AT W/PRGM

Title Coaxial Culture Exposure System (Cup Short Calib) EMOZE Page 1 of 1

Programmer A. W. Guy HP-65 USER INSTRUCTIONS Date April 13, 7

Coax Culture Exp. (Cup Short C.) EMOZE

STEP	INSTRUCTIONS	INPUT DATA/UNITS	KEYS	OUTPUT DATA/UNITS
1	Load Program			
2	Key in probe to dielectric distance	ℓ_p (cm)	Enter	ℓ_p (cm)
3	Key in frequency	f (MHz)	R/S	
4	Key in VM Voltage B	V_{BS} (mV)	Enter	V_{BS} (mV)
5	Key in VM Voltage A	V_{AS} (mV)	Enter	V_{AS} (mV)
6	Key in Vector VM angle	θ_S (deg)	A	
7	Load Appropriate Program in forward direction			

☆ U.S. GOVERNMENT PRINTING OFFICE: 1977— 757-057/6734

BIBLIOGRAPHIC DATA SHEET	1. Report No. NIOSH-77-182	2.	3. Recipient's Accession No. PB274793
4. Title and Subtitle RF CELL CULTURE IRRADIATION SYSTEM WITH CONTROLLED TEMPERATURE AND FIELD STRENGTH			5. Report Date June 1977 6.
7. Author(s) Arthur W. Guy			8. Performing Organization Rept. No.
9. Performing Organization Name and Address University of Washington School of Medicine Bioelectromagnetics Research Laboratory Seattle, Washington 98195			10. Project/Task/Work Unit No. 11. Contract/Grant No.
12. Sponsoring Organization Name and Address National Institute for Occupational Safety and Health 4676 Columbia Parkway Cincinnati, Ohio 45226			13. Type of Report & Period Covered 14.

15. Supplementary Notes

Interagency Agreement NIOSH-IA-75-30

16. Abstracts

A unique RF cell culture irradiation system with controlled temperature and field strength is described, which was used in determining human RF exposure thresholds in the frequency range D.C. to 100 MHz. The system can produce electric field strengths up to 100 V/cm from D.C. to 1000 MHZ in a 5-ml sample of culture medium. The culture medium temperature can be controlled and measured up to 100 MHz by monitoring the feedline impedance, which is dependent on culture medium temperature. Constant temperatures below 37° C can be maintained at field strengths in excess of 25 V/cm. The information that can be obtained with the use of this system is needed to fill information gaps for standards criteria development.

17. Key Words and Document Analysis. 17a. Descriptors

Electromagnetic radiation
Radiofrequency power
Temperature control
Radiobiology
Dosimetry
Radiation protection

17b. Identifiers/Open-Ended Terms

Nonionizing radiation

17c. COSATI Field/Group 06/J

18. Availability Statement	19. Security Class (This Report)	21. No. of Pages
Release unlimited	UNCLASSIFIED	55
	20. Security Class (This Page) UNCLASSIFIED	22. Price PCA04-A01