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CRYO System for studying bioimpedance properties of biological tissue and fluid during cryosurgical operation

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Abstract. This paper presents a cheap and compact system designed to study the impedance properties of the examined objects. This system can be used in medicine to carry out imaging of biological tissue during the cold for operations. One of the main problems of cold application in medicine is the difficulty in determining the boundaries and depth of the cryogenic effect. This problem arises due to the individual characteristics of the tissue, different cooling rates and high temperature gradients during freezing. In order to increase the accuracy in determining the boundaries and depth of the cryogenic effect, the impedance properties of liquid and muscle tissue have been studied. Based on the data obtained from the developed system for the study of impedance properties, based on the AD5933 impedance meter, the obtained data were analyzed at temperatures ranging from minus 18 to plus 25 degrees Celsius in the frequency range from 1 to 100 kHz.

1. Introduction

Automated The Cryosurgery is a method of using ultra-low temperatures to destroy and remove unhealthy tissues with various benign or malignant formations. This method is widely used in many medical fields, from warts removal (Figure 1) to internal tissue ablation, such as kidney ablation (Figure 2) [1].

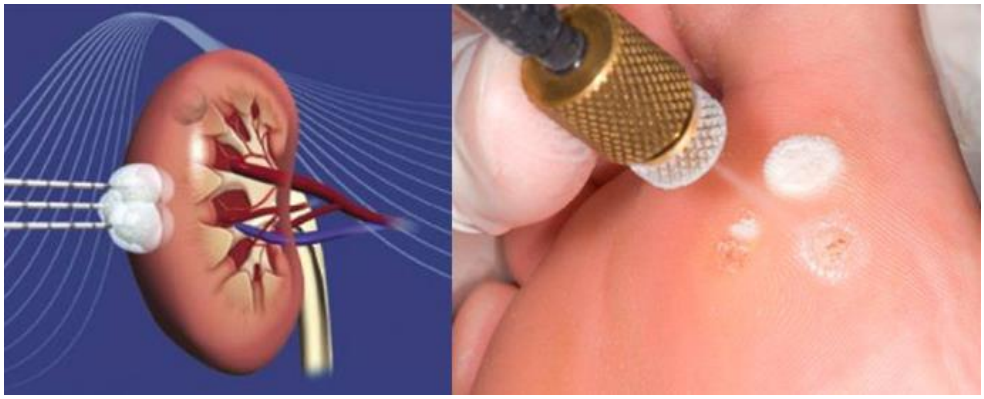

Figure 1. Kidney cryoablation (left) and wart removal (right).

Cryosurgical method of tissue removal has a number of advantages, among the main ones are: bloodlessness, low invasiveness, as well as low cost of surgery. Although attempts have been made to

use cold methods of tissue removal everywhere, but there is no widespread use of cryosurgery, except for the removal of skin formations such as papillomas, warts, moles. It would seem that there are no obstacles to using this method everywhere. However, there are a number of limitations that prevent the use of cryosurgery widely [2].

One of the main drawbacks of cryosurgery is the difficulty in determining the boundaries and depth of cryosurgery. This difficulty arises from the peculiarities of the structure and heterogeneity of biological tissue. Directly in the cryogenic zone, the fabric temperature is either close to the coolant temperature (liquid nitrogen or carbon dioxide is most often used), but after a few millimeters, the temperature starts to rise sharply, as shown in Figure 2. Uncertainty arises from the fact that it is unknown how deeply the freezing of the tissue occurred.

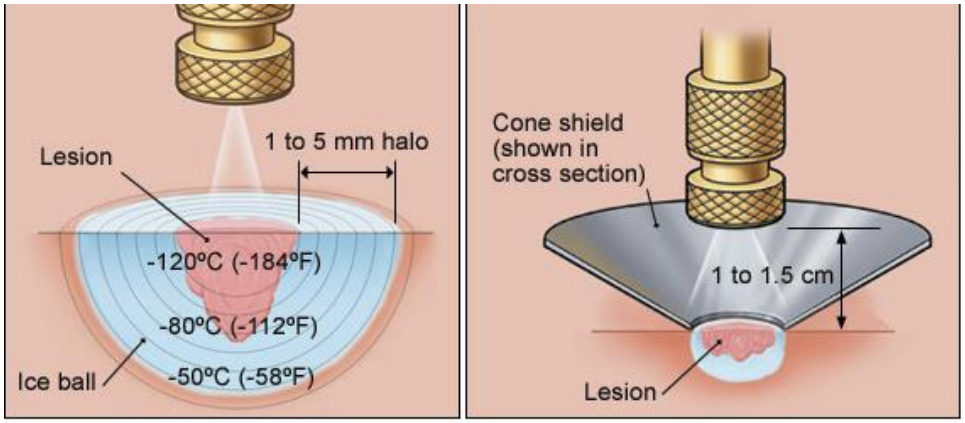

Figure 2. Timed spot freeze technique used to treat a malignancy (possibly a small basal cell cancer), demonstrating freeze ball formation and the 5-mm treatment margins necessary to achieve a temperature of −50°C and, thus, the required depth of 4 to 5 mm (left). Liquid nitrogen spray application using a timed spot freeze technique and an open cone shield to direct the liquid nitrogen. The spray nozzle is positioned approximately 1 to 1.5 cm above the target lesion (right) [3].

Nowadays there are several methods of biological tissue imaging, such as

- Magnetic resonance imaging (MRI). This method is the most accurate, but it imposes a number of limitations. Intense magnetic field imposes extreme requirements not only on the tomograph, examination room and staff, but also on the equipment that can be used directly inside the room. MRI scans are also quite expensive. Cryosurgical apparatus elements usually contain metal and cannot be used during an MRI scan.
- Computed tomography (CT). The cost of a CT scan is comparable to an MRI scan. This method also affects the patient with ionizing radiation.
- Ultrasound tomography. To date, this method is the most rapidly developing, but when freezing the biological tissue is heterogeneous. This results in noises that interfere with the precise determination of the boundaries and depth of the cryoprobe during ultrasound imaging [4].

Electrical impedance tomography (EIT) is proposed as a solution to improve the accuracy of boundaries and depth of cryo-influence. The essence of the given method consists that passing small electric current through investigated object, it is possible to define distribution of electric current. Also, by changing the frequency of the signal, the path of electric current flow changes. Frozen in biological tissue, impedance increases sharply, which is a reliable indicator of tissue frozen. This method has several advantages, including low cost of the procedure, high accuracy compared to empirical methods, absence of soreness during the procedure and safety, which is provided by a low level of current flowing through the tissue under study [5-7].

Today there are many expensive devices, such as Medtronic (CryoFlex system), AtriCure (Cryo ICE system), Metrum Cryoflex (Cryo-S Classic system), as well as simple cheap analogues, such as

CryoIney-401 [8-11]. However, all these systems are united by the absence of any systems that allow to visualize the boundaries and depth of the cryo-reaction. To correct this shortcoming, the CRYO system was developed, which allows to study bioimpedance properties of biological tissue. In the future, it is planned to use the developed CRYO system together with the existing cryosurgical devices during cryosurgical operations.

2. Devices for measuring the impedance of fluid and biological tissue and their characteristics

There are some important features of measuring the impedance of biological objects compared to measurements in other areas. The main limitations of measurement are more related to the peculiarities of biological tissue structure. The following features can be identified that arise during impedance studies:

1. Polarization of electrode, fluid and biological tissue. As a rule, this feature causes the isolation drift (zero mixing) during the measurement, which reduces the accuracy of the measurement [12].

2. The heterogeneous cooling rate of the electrode, liquid and muscle tissue, which causes the area around the electrode to freeze faster than the adjacent cooled area to be cooled.

3. The surface of the muscle tissue will form a liquid between the electrode-tissue contact, which will also freeze faster than the adjacent areas.

4. Homogeneity of the investigated object. There may be different layers or areas (e.g. fatty tissue) in the cooled area where the cooling rate may be different. As a result, the area of cryoinfluence will be heterogeneous.

Due to the described features it is necessary to use specialized equipment that takes into account the peculiarity of the measurements.

In contrast to the previously presented system [13], a precision impedance converter based on the AD5933 chip of Analog Devices was used [14, 15]. Features of this solution are several factors: the ability to set the frequency of impedance measurement up to 100 kHz with an accuracy of 0.1 Hz, a wide range of impedance measurement, lying in the range from 100 to 1000 ohms, the ability to determine the active, reactive component of the impedance, as well as the definition of phase shift. Separately, we would like to highlight the built-in software (AD5933 Beta Version REV1.0), supplied with evaluation boards (EVAL-AD5933), which allows you to quickly move to direct measurements without the long process of developing an electronic board device. The main and main window of the supplied software is shown in Figure 3.

The measuring cell is used as a measuring chamber, on the inner edge of which silver electrodes are located (Figure 3) [16, 17].

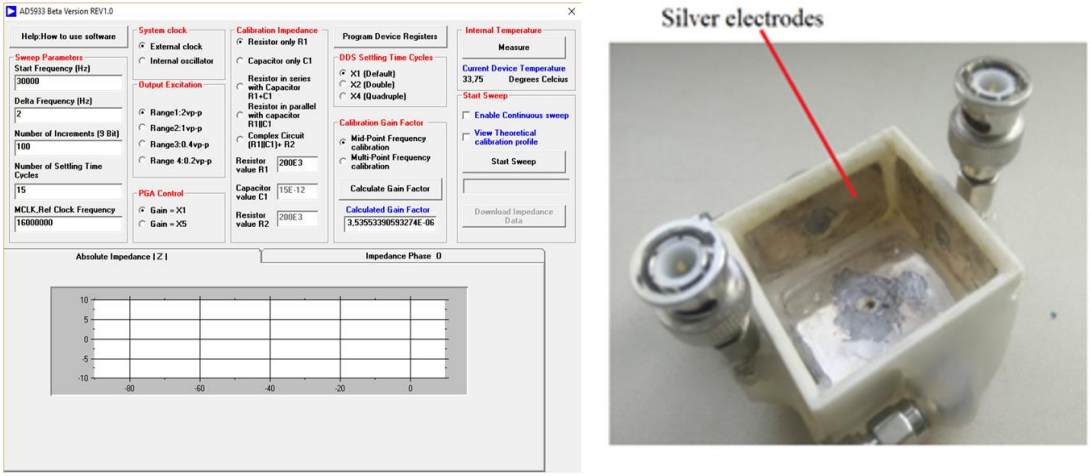

Figure 3. Main program window (left) and measuring cell (right).

For correct work with the impedance meter, it is necessary to properly configure the parameters of the AD5933 chip (Figure 3). First of all it is necessary to set the parameters specified in the "Sweep Parameters" section: initial frequency, step of frequency measurement and number of measurements. The other most important parameters are the "Calibration Impedance" section. The correct way to calibrate the impedance must be selected. The impedance is calibrated by measuring the known resistance and capacitance values of the resistors and capacitors set beforehand. The value of the gain coefficients is calculated using the "Calculate Gain Factor" command. After pressing the "Start sweep" button, the impedance is measured.

3. Calibration

One of the preconditions for accurate measurements is the process of calibrating the impedance meter, because the accuracy of the measurements depends on the calibration process [18, 19]. The calibration process is as follows: the feedback loop (*Rfb*) and the measurement loop (*Rcal*) are set to a known resistance value. The resistance *Rfb* is calculated according to the formula:

$$Rfb = \frac{\left(\frac{AVdd}{2} - 0{,}2\right) Zmin}{Vpk} * \frac{1}{GAIN}$$

where, *AVdd* - supply voltage of analogue measuring network, *Vpk* - amplitude value of measuring signal (see Figure 3, "output excitation" parameter), *Zmin* - lower limit of measured impedance, *GAIN* - gain of output amplifier.

The value of *Rcal* is calculated by the formula:

$$Rcal = \frac{(Zmin + Zmax)}{3}$$

where, *Zmin* – lower limit of the measured impedance, *Zmax* - upper limit of the measured impedance.

4. Resistance measurements

As a test of the calculated values of *Zmin*, *Zmax* and error calculations, we have already measured the known resistance values in the range from 24 Ohm to 201 kOhm. At *Rfb* = 60 Ohm and *Rcal* = 743 Ohm, *Zmin* is 82 Ohm and *Zmax* is 2150 Ohm. The results of the measured values are presented in Table 1. Resistances were measured with a HoldPeak HP-890CN digital multimeter [20].

Table 1. Resistance measurement error, at *Zmin* = 82 Ohm, *Zmax* = 2150 Ohm, *Rfb* = 60 Ohm, *Rcal* = 743 Ohm.

Known resistance (Ohm)	Measured resistance (Ohm)	Measurement error (%)
24	46.2	92.5
107	107	0
1990	1988	0.1
3270	3234	1.1
5500	5345	2.8
8600	8166	4.8
20540	14286	30.4
41300	18969	54.1

The measurement error increases sharply when measuring the impedance beyond the range between *Zmin* and *Zmax*. When measuring the bioimpedance, it is necessary to calibrate the measurement impedance so that the measured impedance lies in the range between *Zmin* and *Zmax*. If

it is necessary to carry out the measurement outside the calibration range, it is necessary to recalibrate the measurement.

5. Fluid impedance measurements

The experiments were devoted to the study of fluid impedance in the frequency range from 2 kHz to 100 kHz. Purified water was used as the test samples. The temperature of the test samples varied from minus 18 to plus 27 degrees Celsius.

The highest impedance value occurs during complete freezing of the measuring cell. After a few seconds, the electrodes start to heat up and a thin layer of liquid is formed between the electrode-ice contact (the interval between 0 and 60 seconds shown in Figure 4). At this point, there is a sharp decrease in impedance, which is shown in Figure 3. Further on, the ice starts to heat up and melt, which leads to further reduction of impedance. The largest drop in impedance occurs during the beginning of ice melting (the interval between 1 and 4 minutes shown in Figure 5).

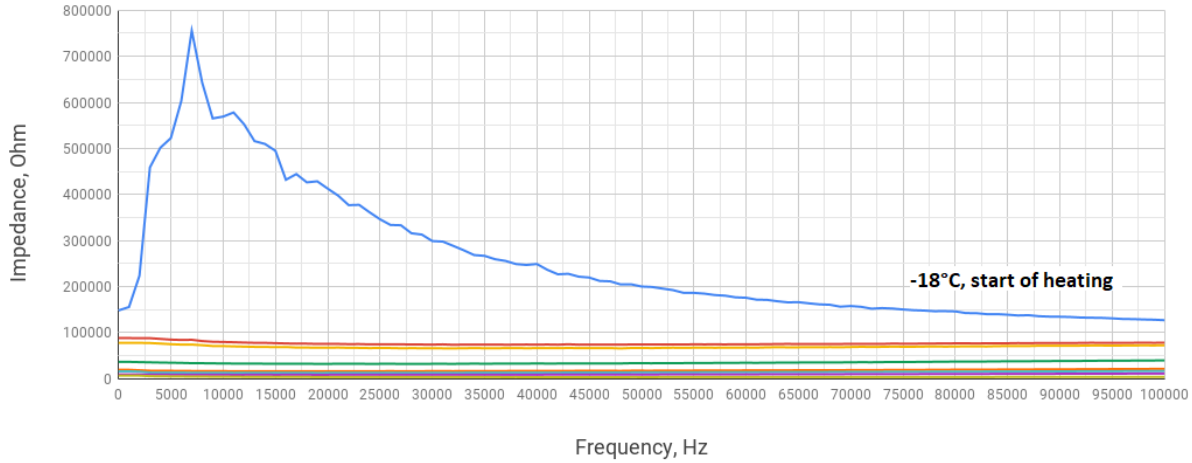

Figure 4. Bioimpedance spectrum of liquid. Y axis - impedance in Ohms, X axis - frequency in Hertz.

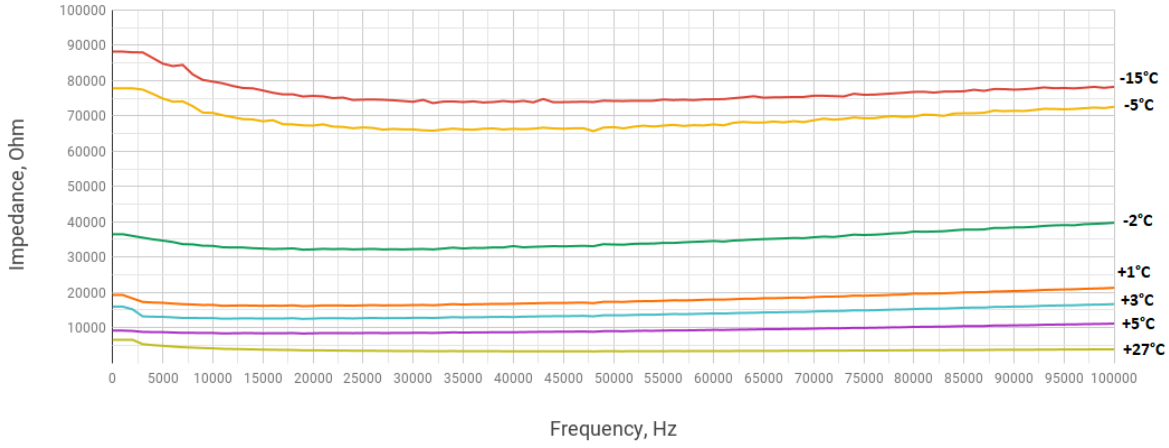

Figure 5. Bioimpedance spectrum of liquid. Y axis - impedance in Ohms, X axis - frequency in Hertz.

6. Muscle tissue impedance measurements

Muscle tissue impedance measurements were carried out in the range from 2 kHz to 100 kHz. The muscle tissue of the pig was used as the test sample. The temperature of the studied object varied in the range from minus 20 to plus 10 degrees Celsius.

In contrast to liquids, a sample with muscle tissue heats more evenly than ice. For this reason, there is no sharp drop in impedance in the first minute after the beginning of the experiment. As the temperature in the frozen muscle tissue increases, the impedance begins to decrease sharply. In 10 minutes after the beginning of the experiment the surface layer of muscle tissue begins to melt, the temperature increases to minus 2 degrees. After the beginning of the melting process, the reduction of impedance is no longer so intensive. On 40 minutes after the beginning of experiment the investigated sample melts, on 60 minutes it is heated up to plus 10 degrees (see Figures 6 and 7).

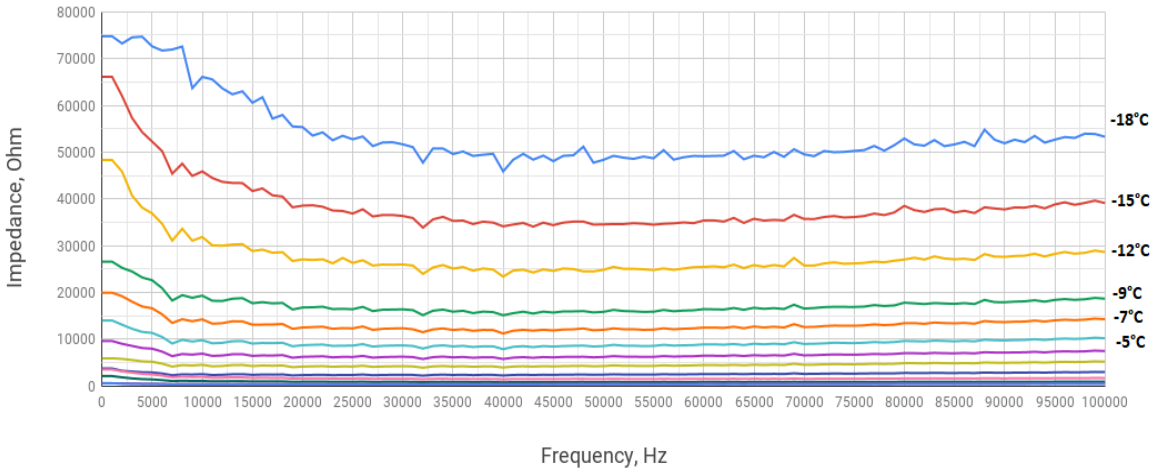

Figure 6. Bioimpedance spectrum of muscle tissue. Y axis - impedance in Ohms, X axis - frequency in Hertz.

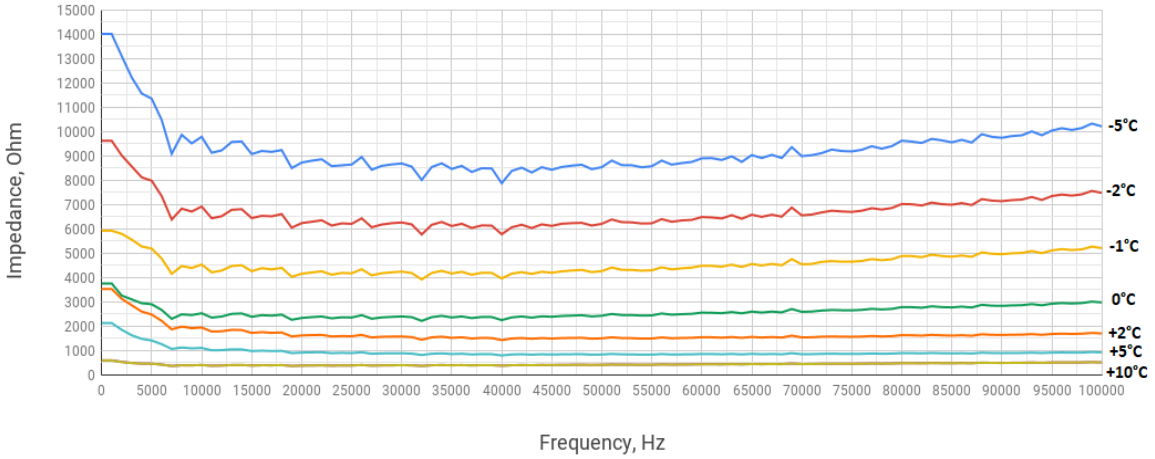

Figure 7. Bioimpedance spectrum of muscle tissue. Y-axis is impedance in Ohms, X-axis is frequency in Hertz.

7. Related work

An early version of the system was presented in the current work, which allows to study the properties of the system by means of impedance measurements. Although the system has been developed to measure impedance with high accuracy, the accuracy of the measurement increases sharply when approaching the *Zmin* and *Zmax* boundaries. In the future, we hope to significantly improve the developed prototype. To increase the number of channels, to increase the accuracy of measurements, to add a visualization system that allows not only to analyze the spectra of the objects under study, but also to visualize the boundaries of the cryo-effect during spot freezing. It is also planned to extend the

frequency range of measurement from 100 kHz to MHz units. To extend the frequency range, the AD5933 chip will need to be replaced with a digital-to-analogue converter and analog-to-digital converter chip. This solution will significantly increase the time of obtaining the spectrum of the signal, to expand the possibilities of the visualization system.

8. Conclusion

In this paper, a laboratory prototype of a CRYO installation was presented - a system for measuring the impedance based on the AD5933 chip of Analog Devices. With the help of the developed prototype, the properties of the fluid and muscle tissue of the pig were investigated. When both samples are heated, the impedance decreases sharply. The greatest reduction occurs during the first minutes of heating and during the transition from solid (ice) to liquid standing. Thus, it was experimentally confirmed the possibility of using the impedance meter based on the AD5933 chip for use in electrical impedance tomography for the study of biological objects. The developed prototype allows to determine impedance, active and reactive resistance. The advantages of the solution include a sufficiently high accuracy of impedance measurement, compactness of the finished device. It is also necessary to adjust the circuitry, as calibration can be done by software methods.

In the course of experimental studies the dependencies and properties of the bioimpedance spectrum of aqueous solution and pig's muscle tissue at temperatures from minus 18 to plus 10 (muscle tissue) and 27 (liquid) degrees Celsius have been studied. The experiment can be divided into several stages:

1. Initial phase. The measuring chamber has not yet started to heat up and defrost. The impedance is the maximum value.
2. Ice is heated and a thin layer of liquid is formed between the electrode-ice contacts, which leads to a sharp decrease in impedance. For the liquid, melting begins as early as 1 minute after the experiment, for muscle tissue, one minute after the start of heating. The graph is almost linear over the entire frequency range. The impedance is in the range from 30 to 85 kOhm.
3. Active ice melting phase. The ice starts to heat up. The temperature becomes close to the melting point. For liquid, the impedance decreases from 85 to 10 kOhm, for muscle tissue from 85 to 1 kOhm.
4. The phase transition of water in the sample under study from ice to liquid. For the liquid, the impedance decreases from 10 to 3 kOhm, for muscle tissue from 1 to 300 Ohm.
5. Further heating to room temperature. At this stage, all the ice has already melted and the temperature of the liquid gradually increases to room temperature. At the moment, the impedance is practically unchanged.

Acknowledgments

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