HERBERT FRÖHLICH, FRS

A physicist ahead of his time

A Centennial Celebration of his Life and Work

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Coupling of Fröhlich-Modes as a Basis of Biological Regulation

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8.1. Introductory Remarks

One of us (F-AP) remembers a discussion with Herbert Fröhlich, over 20 years ago, about the question as to what experiments have to be performed in order to decide whether electromagnetic radiation from and within biological systems displays a high degree of coherence. Fröhlich expressed his conviction that one cannot expect that some kind of *experimentum crucis* will solve this problem, but rather that the increasing frequency of experimental findings will one day suffice for a general acceptance of this property. Actually there is now a manifold of indications for 'coherent excitation' of living systems such that one can no longer avoid taking seriously this basic idea of an extraordinary coherence in living systems. It invites consideration of realistic models as well as suitable experimentation: for a review see Hyland (Hyland, 2002).

In 1983, at a conference on Biophysics in Vulcano (Sicily) under the guidance of Fröhlich, discussions about coherent microwaves and coherent visible radiation ('biophotons') gave rise to the basic question as to how the different modes in a living system may be coupled – if at all – and precisely how the extremely wide range of frequencies, covering at

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least 20 decades, is involved in the various biological functions. These frequencies range from a lower limit of 10^{-5} Hz, represented by circadian rhythms, through brain waves (10^2 Hz), up to oscillations of biomolecules, organelles and membranes, muscle activities, and even to vision. At the same time, it becomes more and more evident that, in all ranges, the sensitivity of the 'biological detectors' is limited only by the uncertainty relation (Smith, 1989, Eccles 1993), thus indicating already rather remarkable properties of macroscopic quantum coherence (Sassaroli *et.al.*1998).

Biological systems are governed by rather opposing influences. On the one hand, external influences may get modelled by a heat bath, since, so long as no 'informational' couplings are at play, the outside world is no different from the surroundings of dead materials. Although there is still the question as to whether the heat radiation of the body is really only 'heat', the existence of such radiation is certainly not incompatible with this point of view. On the other hand, food supply, which is necessary for the living state, and the extremely high sensitivity due to external and internal 'informational' couplings point to extraordinary physical properties of open living systems that are not allied to perfect equilibrium. Herbert Fröhlich's model [F134][2] takes account of equilibrium properties by its inclusion of the Bose-Einstein-Statistics, but at the same time recognises the 'openness' of biological systems by including non-thermal energy supply in terms of variable chemical potential $\mu(\varepsilon)$, where ε is the quantum energy of the mode under consideration. At the same time, he shows that a high degree of coherence can be established by Bose-condensation-like long-range interactions of single modes (Fröhlich-modes) when the chemical potential $\mu(\varepsilon)$ of these extraordinary oscillations approaches there the quantum energy ε. As a consequence, considerably high occupation densities ($f(\varepsilon) > 1$) are the realised above the threshold. To some extent, Fröhlich's model offers an elementary physical

[2] This denotes reference (134) in the Complete Bibliography of H. Fröhlich, at the end of this volume; similarly, [Fx] is reference (x) in the Bibliography.

description of Prigogine's dissipative structures (Prigogine, 1976) far away from thermal equilibrium. Despite a lot of attempts to explain hitherto unexplained biological functions such as cancer, brain activities, cell growth, and enzymatic reactions, the model has not yet been generally accepted by biologists, perhaps due to its too fundamental character and the difficulties biologists have in transforming elementary physics into concrete biological phenomena. A further difficulty concerns the usual separation of chemical and physical knowledge by neglecting the fact that chemical reactions are governed by physical laws and not *vice versa* (see also Hyland, 2002).

The approach of a part of our group (F-AP, WK, YY and others) (Popp *et.al.*, 1979, 1992, 1993, 1998, 2002a, 2002b, 2004) is completely in line with Herbert Fröhlich's ideas, with the inessential exception that our work is focussed on the optical range ('biophotons') instead of microwaves. The entropy is studied at its absolute maximum ($f(\varepsilon)$ = constant), and is minimized by shrinking the degree of freedom *via* mode couplings; last but not least, the degree of coherence is investigated experimentally by using photocount statistics (PCS), revealing proof of *i*) a rather high degree of coherence, *ii*) the non-thermal character of the spectral distribution, *iii*) mode couplings in the optical range, and *iv*) the identification of some basic biological phenomena that are based on these properties. In this way, the chemical potential, together with the degree and nature of the coherence – *i.e.* whether it is quantum or classical – assume the role of essential order parameters of the biological system, completely different from the usual role of photon intensity. Nevertheless, rather small aberrations from 'chaotic' interactions may provide strong and essentially non-linear behaviour as soon as the time scales of biologically relevant interactions become macroscopically large. The extraordinarily high degree of coherence and the broadband character of modes constitute a basis for the so-called multiplicative organization principle in biology which is fundamental to the psycho-physical (Weber-Fechner) law of physiology. Already it is clear that, for reasons of saturation, a non-linear effect must be active in the living state, whereby the over-excitation leads to a decline in occupation inversion by emission. This may happen significantly even at low occupation. The modes arrive at constant phase relations (Popp, 2005) that persist over the dimensions

of cells and cell populations, in consequence of the small volume of cells compared to the coherence volumes of at least 10^3 cm^3.

The extraordinary high polarization of biological matter which has been repeatedly emphasized by Fröhlich is a permanent source of non-linear interactions where the permittivity can reach abnormally high values that are strongly dependent on the frequency of the modes under investigation. The non-linear interactions may here induce additional resonance frequencies (super- and sub-harmonics), the creation of sub-harmonics being possibly accompanied by amplification effects. Today, parametric systems are well known also in technical systems. In living tissue, all the provisions of these effects, including inhomogeneous, but specific, distributions of quasi-free charges are permanently and comprehensively available over the whole spectral range from optical frequencies (electronic excitations) down to ELF, with resonances ranging from single biomolecules up to the whole body.

In order to highlight this situation by bridging the gap between fundamental physics and biological phenomena, let us return to a rather exemplary question of Erwin Schrödinger.

8.2. Schrödinger's Question and Realistic Answers

Schrödinger's question addresses the surprising fact that during cell division no error occurs in the distribution of the molecules, which are exactly partitioned into two equal fractions by the daughter cells. Fig. 1 shows the completely developed spindle apparatus of a fish (Corregonus) in mitosis (Darlington and Lacour, 1960). If 'random walk' theory governs cell growth, one can estimate that about 10^5 out of 10^{10} molecules should be located at incorrect positions. However, no error can be discovered at all. One might suggest that some kind of crystallization takes place, such as a freezing process of the biomolecules, in which the binding energies are, on the one hand, not far from the mean thermal energy (so as to be able to provide high enough flexibility) but, on the other hand, exceed it sufficiently, in order to stabilize the local positions of the molecules under investigation. However, this process would require an extraordinarily high local accuracy and temporal regulation of the distribution of binding energies. More likely is the presence of a force

which repels molecules that are in erroneous positions back to their correct place.

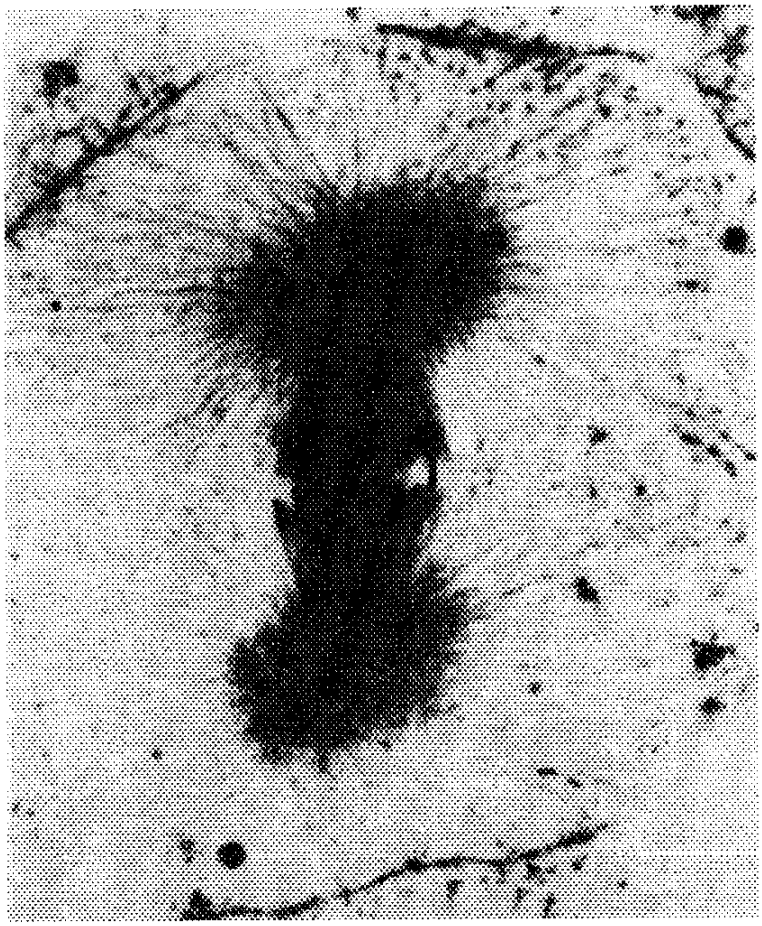

Figure 1. Completely developed spindle apparatus of a fish cell (Corregonus) in mitosis (Darlington, 1960).

The solution of finding such a source of force is easy to obtain. If one calculates the cavity resonator waves of a single cell (Popp, 1979), one gets (1) a number of relatively stable modes that cover the range of visible light (at least between 200 and 800 nm), (2) a set of orthogonal functions that by superposition can describe every kind of electromagnetic potential, including, in particular, that necessary to account for the 'mitotic structure shown above, whereby molecules are piloted into their correct positions. Both statement (1) and statement (2) are independent of, whether one considers conducting or dielectric cavities of cylindrical or ellipsoid shape. Most decisive, in order to get modes into the optical range, is the

volume, which must be about 10^{-9} cm^3. Table 1 presents a set of modes calculated for a right circular cylindrical cavity of radius R and the length d, of volume 10^{-9} cm, where R/d =0.25 for optimal storage capacity.

Table 1: Modes in a right-circular cylindrical cavity (see text, Popp 1979)

TE mode mnp	TM mode mnp	wavelength λ/nm
111		690
	010	574
112		571
	011	546
	012	481
113		462
211		438
	013	410
212		402
114		379
	110	360
213		358
011	111	353
	014	349
012	112	333.5
311		323
115		318

Fig. 2 displays the electric field pattern of a TM_{11} mode at about 350 nm, which just fits the condition of stabilizing the mitotic figures of Fig. 1. Consequently, a rather satisfying, and to our knowledge, the most natural answer to Schrödinger's question that has been so far advancd is that cavity resonator waves exist in cells, which are stable enough to regulate, by satisfying the boundary conditions, both spatial and temporal organization of cell growth and cell division. If this answer is correct, photons in the range of at least 200 to 800 nm should exist in cells which (1) are correlated to cell division rate, (2) have a rather high degree of coherence, and (3) are 'away' from thermal equilibrium. We called them 'biophotons' in order to distinguish them from ordinary bioluminescence, and to express their quantum character. In the last 30 years, we have shown evidence of their existence and have carefully studied most of their

properties. Some review papers about techniques, existence, properties, and applications are listed in the bibliography (under Popp *et al.* in the list of references). Biophotons are still subjected to intensive investigations.

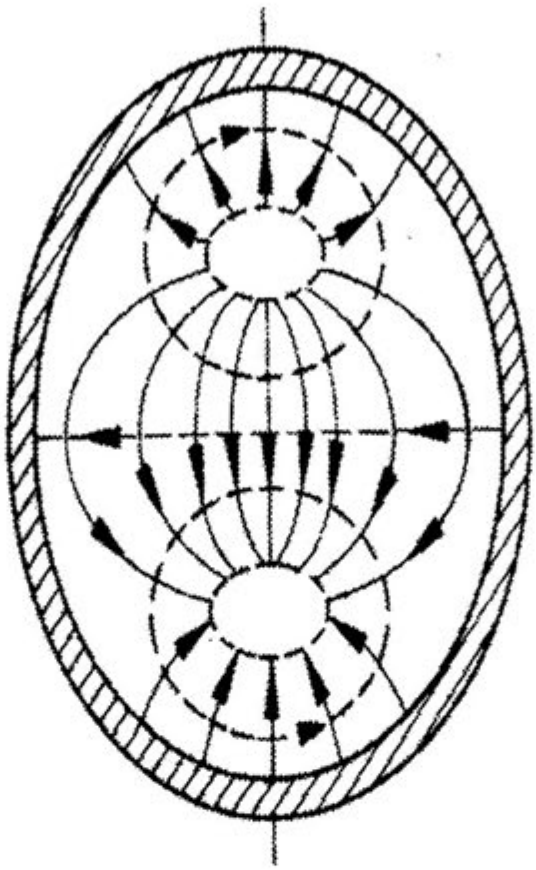

Figure 2. Electric field of a TM11 cavity mode in a right circular (elliptical) cavity. Comparison with Fig.1 shows that the stability of mitotic figures can be explained in terms of the high storage capacity for cavity resonator waves. For cells their wavelenghts are in the optical range. They are the sources of biophotons (see text and references).

On a rather basic level of consideration, Herbert Fröhlich´s model of coherent radiation in biological systems itself provides a most valuable answer to Schrödinger´s question. It concerns the *coupling* of modes, since just this could solve the crucial problem of regulation and coordination of many synchronous, different biological functions in the living state. The regulation of cell division, for example, involves a continuous transition between perfectly matched modes. Decisive parameters of this coherent organization are the phase relations, together with the interplay of frequencies of the interacting modes.

A key of understanding these connections is (1) the chemical potential of the Fröhlich-modes and (2) the frequency condition of coherent fields according to R.Glauber (Glauber, 1969). Following Fröhlich, the Bose-condensation-like accumulation of a mode takes place as soon as the chemical potential, perhaps surprisingly $\mu(\varepsilon)$ approaches the quantum energy ε of the mode under study. Glauber requires the coupling of the modes of a coherent field ('standing waves') by the general condition

$$\sum_i \varepsilon_i = \sum_k \varepsilon_k \qquad (1),$$

where i and k run over a definite, but arbitrarily selected, group of N and M modes, respectively. We can rewrite this condition in terms of Boltzmann factors, $f_l(\mu_l,\varepsilon_l) = \exp((\mu_l-\varepsilon_l)/(kT))$, as follows:

$$W = \Pi_i f_i = \Pi_k f_k \qquad (2)$$

If Equ. (1) is valid, Equ. (2) can be satisfied for all N and M modes. This is then certainly the situation in the case of a stabilized coherent field that approaches thermal equilibrium, where all μ are zero. On the other hand, for an ideal open system, Eqs. (1) and (2) are certainly satisfied for all $\mu_l = \varepsilon_l$, where $l = i, k$. This is the case for all Fröhlich-modes. As a consequence, one may consider that Fröhlich-modes are a branch of modes that works as a kind of hub system for establishing and connecting in a dynamical way all different modes that fulfil the actual boundary conditions of the biological system under study. Glauber's condition and Fröhlich's chemical potential allow perfect synchronization and regulation of biological functions by providing the suitable activation frequency and by controlling timings through the correct phase relations between the coupled coherent excitations associated with the processes under study. It is easy to show that by satisfying Equ. (1), and in view of homeostatic regulation that keeps the total energy constant, every mode can be created and annihilated by at least two other modes, such as, for instance, those involving sub-harmonics, superharmonics, or even beat frequencies. Indeed, there is even evidence of squeezed states in living matter (Popp et.al, 2002).

It will be shown that there are experimental results in favour of this kind of mode coupling and that fundamental properties of biological systems – such as, for instance, the multiplicative organizational principle of the basic Weber-Fechner-law – can be truthfully explained in terms of this model. At the same time, we show that the conversion of energy into different modes is not confined to the step-down dissipation from higher to lower mode energies (*i.e.* subharmonics), but can move also upwards (*i.e.* superharmonics, or coherent multiphoton processes of different modes of lower energy – *see* (Swain, 2006)).

Actually, there are two main streams of thought that suggest macroscopic quantum mechanical effects are associated with the living state. One is Herbert Fröhlich's suggestion that one would expect coherent excitations in the microwave region of the spectrum due to nonlinear couplings of biomolecular dipoles – a suggestion that is perhaps more theoretically developed than experimentally investigated; for brevity, we will refer to microwave photons in such a state as 'Fröhlich photons'. The other line of thought is Fritz Popp's observation that the photocount statistics of ultraweak bioluminescence (biophotons) around the visible region of the spectrum, as well as certain other data, suggest a coherent electromagnetic component linked to the living state. Here it is important to note that one should not forget that there are numerous reactions – in particular oxidative ones – that are part of the normal chemistry that goes on in living things, which will give rise to *in*coherent photoemission. Such a traditional contribution is always to be expected, but here we are considering the rather remarkable, spectrally flat (highly coherent) distribution that seems to be associated with life. We will refer to visible photons of this kind as 'Popp photons'.

8.3. From Frohlich photons to Popp photons: a model for up-conversion

Fröhlich's original model was based on phenomenological rate equations representing a set of harmonic oscillators labelled by an index i of various frequencies, ω_k, each excited with n_k quanta coupled to each other. In order to be able to couple these oscillators, and still have conservation of energy, the oscillators are also coupled to a heat bath at some temperature

T, which provides or accepts whatever energy is needed. In addition, an external pump source was included which could drive the oscillators.

Fröhlich showed that under rather general conditions one could, even at high temperatures, for a sufficiently strong external pump, find a macroscopic excitation of just one oscillator – the one with the lowest energy $\hbar\omega_0$ – at the expense of the other modes. It was this macroscopic quantum state, which is reminiscent of lasing or of Bose-Einstein condensation, which has been identified as possibly characteristic of the living state. While Fröhlich came upon this sort of mechanism while thinking about the very large electric fields across the membranes of living cells, it is important to keep in mind that this could occur multiple times in different systems in the same living organism, and one should be open to the idea of multiple Fröhlich state appearing in various contexts. That being said, if one imagines the dipoles in question to be large biological molecules, one is naturally led to frequencies in the microwave region, and it is far from clear what, if anything, this has to do with biophotons.

Before prematurely dismissing any connection on the grounds of the large difference in energy scales between Fröhlich and Popp photons, let us recall that large up-conversions are, in fact, well-known, although rarely treated in the standard texts. For example, consider a light bulb running from a 1.5 V battery. There are two natural energy scales involved in this system. One is 1.5 V – the total energy that an electron can give up as it crosses the filament of the bulb, and the other is zero (!) – the frequency of the input current (which is DC here). Even the 1.5 V is rather optimistic, since one expects an electron to undergo many collisions as it passes through the filament, each time giving up only a small fraction of an eV. Nevertheless, common experience shows that there is strong emission in the visible spectrum of several electron volts. Here we have an example of a macroscopic nonlinear system that, even without coherence, manages to couple many low energy quanta into one higher energy quantum of, say, blue light. A first response on hearing this argument is to trivialize it, saying 'Well, isn't that just blackbody radiation?' The answer, of course, is yes, but whenever one treats the approach to blackbody radiation one has to imagine complicated couplings between many oscillators, which one subsumes into statements

like 'one lets the oscillators exchange energy until the most probable distribution (*via* maximizing entropy) is reached'. With this in mind, it may come as less of a surprise that a highly organized system may be more efficient at large up-conversions.

Let us now slightly generalize Fröhlich's model (J. Swain, August 2005 – talk at the Fröhlich Symposium, IIB, Neuss, Germany) by adding one more term to his rate equations. This will be a coupling of the whole Fröhlich system (which is a large number of oscillators all coupled together with the lowest energy oscillator coherently excited) to one external oscillator of frequency Ω. The system is already coupled to an entire heat bath of oscillators, so this is just one more oscillator. The difference is that the entire heatbath of oscillators in Fröhlich's model are constrained to have their energy thermally distributed at a temperature, whilst the additional oscillator is taken not be a part of a thermal ensemble. Thinking in concrete terms, this can be an oscillator corresponding to the QED vacuum, its occupation number representing the number of photons in that state. It could also be an oscillator corresponding to the excitation energy for some chemical reaction, or some other process. Such an additional coupling outside the original Fröhlich model will drain energy from the system, which we can compensate for by an increase in pumping so as to not lose the coherent Fröhlich excitations.

Now let us consider the probability that N Fröhlich photons of frequency ω_0 can couple to a Popp photon of frequency Ω ($= N\omega_0$). Here is it useful to think of Feynman diagrams that represent quantum mechanical amplitudes for such photons to couple. Consider then a line that diagrammatically represents a charged particle in a living system; the particle can be an electron, or a dressed quasiparticle. In keeping the Fröhlich's original work, which stressed the generality of a possible mechanism, rather than details of a specific model, we will refer to this as an electron, keeping in mind that generalizations are possible. Diagrams contributing to the process we want have N incoming photon lines and one outgoing photon line. The amplitude for the process has a factor of electron charge (e) for each photon, and the transition probability is obtained by *i*) adding all diagrams with all permutations of incoming photons, *ii*) finding the squared magnitude of the resulting complex

quantity, and *iii*) multiplying the result by the amount of phase space available for the outgoing photons. In general, this is a difficult problem to work out, and one expects (assuming that different diagrams have different phases, and are thus unlikely to constructively interfere) a result of order α^n, where $\alpha = e^2/\hbar c$ is the fine structure constant – a pure number roughly equal to 1/137. Clearly, coupling of a large number n of photons will be very unlikely, and it is this sort of intuition which tends to make one immediately dismiss large up-conversions as impossible or unlikely.

The Fröhlich state, however, is a special one in that it is a coherent state in which all the photons carry the same energy. In fact, as a coherent state, it has not got a well-defined number of photons at all, but for the following we can consider projecting out a certain number, N, by the requirement that N must be combined in order to get the single photon of frequency Ω [Details will appear elsewhere (Swain, 2006).] Now we have N indistinguishable photons coming in and $N!$ diagrams, all of which must be counted; all of these have the same numerical value, and thus interfere constructively. This is nothing more than Bose-symmetrization for N indentical incoming photons. Now the original naïve estimate of a rate proportional to α^N must be replaced by $\alpha^N N!$. For small N, the $N!$ term is negligible compared to the rapid falloff in powers of the fine structure constant, but for N of about 400, $\alpha^N N!$ becomes unity and the factorial term begins to dominate, and the perturbative concepts become unreliable. As N goes to infinity, the probability of this process grows without bound, and violates unitarity, signalling that one has pushed perturbation theory beyond its limit. This sort of breakdown of perturbation theory is, in fact, to be expected, since it is well-known that the perturbative QED expansion is divergent; in fact, it is still quite mysterious that the results of low order perturbation theory are as good as they are! It should be noted here that one cannot extend the argument to suggest the outgoing photon energies would grow without limit. With perturbation theory breaking down one would need additional non-perturbative effects to set in and enforce unitarity. Qualitatively, however, one would expect a low probability for a few photons to couple, a large probability for about 400, and a flattening off thereafter with increasing numbers.

In all honesty one must be aware that here one is really using the structure of perturbation theory only as a guide, with no attempt at a full non-perturbative calculation. Illuminating though it is, a non-perturbative treatment is in preparation (Swain, 2006), which suggests a similar result from a different point of view.

A similar factor of $N!$ was anticipated on phenomenological grounds by Popp (Slawinski &. Popp, 1987), but here finds its expression rooted in a microscopic model linking Fröhlich and Popp photons. Rather remarkably, the factor of of around 400 derived above is about what one would want to get from Fröhlich frequencies in the microwaves to visible Popp photons! The picture that emerges then, subject to more (nonperturbative) theoretical work and, of course, experimental verification is the following:

a) There are at least two types of quantum electromagnetic systems associated with the living state. One is in the microwaves frequency range as suggested by Fröhlich, and one is around the visible region as suggested by Popp.,

b) There is a coupling of these two systems that is natural within the context of known physics, assuming that the Fröhlich mechanism takes place.

c) The model presented above is predictive in that it suggests coherent excitations in the microwave region followed by small excitations at integer multiples of the Fröhlich frequency, which decrease in intensity but rise again at about 400 times the Fröhlich frequency, after which they level off, and eventually fall in order to preserve unitarity. Exact details are beyond perturbation theory, and require different mathematical methods. The physics involved is, however, experimentally accessible, at least over part of the frequency range, with the THz region still being quite challenging in terms of instrumentation.

There is even a degree of reversibility in the system, and one can imagine coupling what we have so-far considered as an outgoing photon mode to an external visible photon that could, in turn, be down-converted into Fröhlich photons. This offers the possibility of influencing Fröhlich dynamics inside a cell *via* visible photons injected from outside, as well as the possibility of a long-range coupling of internal cellular Fröhlich dynamics between cells *via* visible Popp photons.

One might well ask what use such a setup with two such disparate frequency ranges is in living things. An immediate suggestion is that living things require a large range of energy quanta to drive various reactions. This is akin to going shopping, where one needs to make purchases using a wide range of sums of money. In order to ensure the correct energy for each reaction (correct change, in the monetary analogy), one would do well to store one's energy (money) in small quanta (small coins). There is a smallest sensible quantum to store (smallest coin to keep), which is set by what quantity can be had or lost for essentially nothing. For a biological system which is bathed in thermal quanta this energy is kT (a penny in the money analogy since nobody really cares about getting or losing one). Storing energy in microwaves ensures that one can be very close to just about any amount of energy that would be needed to drive an electronic transition (chemical reaction) as needed. Storing in lower energy excitations makes no sense, and higher frequencies would, not be expected (perhaps surprisingly) to couple as easily to the required energy scale. Even a degree of automatic specificity is expected, since a reaction which is all set to go but for the energy required constitutes a resonance (extra phase space) into which the outgoing quantum can couple. This is complementary to Fröhlich's 'selective long-range dispersion forces between large systems' [F150]. The use of single photons as part of cell-to-cell signalling is also fascinating, and the sort of system described here could allow for a high degree of selectivity with little cross-talk by choosing slightly different optical frequencies for different communications. Of course, what nature actually does can only be decided experimentally, and this is likely to provide even more surprises!

8.4. A medical application : Electromagnetic Man and Regulation Diagnostics

That the coherence condition is of practical significance can be demonstrated by the fundamental psycho-physical law of physiology, called, after its inventors, the Weber-Fechner-law. It states that the relation between the intensity of a signal and its sensorial uptake does not work linearly, but logarithmically. At the same time, physiological values

like blood pressure, pulse frequency, tolerability of medicaments or simple physiological parameters, such as skin resistance, do not follow a random (Gaussian) frequency distribution but an lognormal one (Gebelein & Heite, 1950; Zhang and F.A.Popp, 1994; Klimek, 2004). This is demonstrated, for instance, in Fig.3.

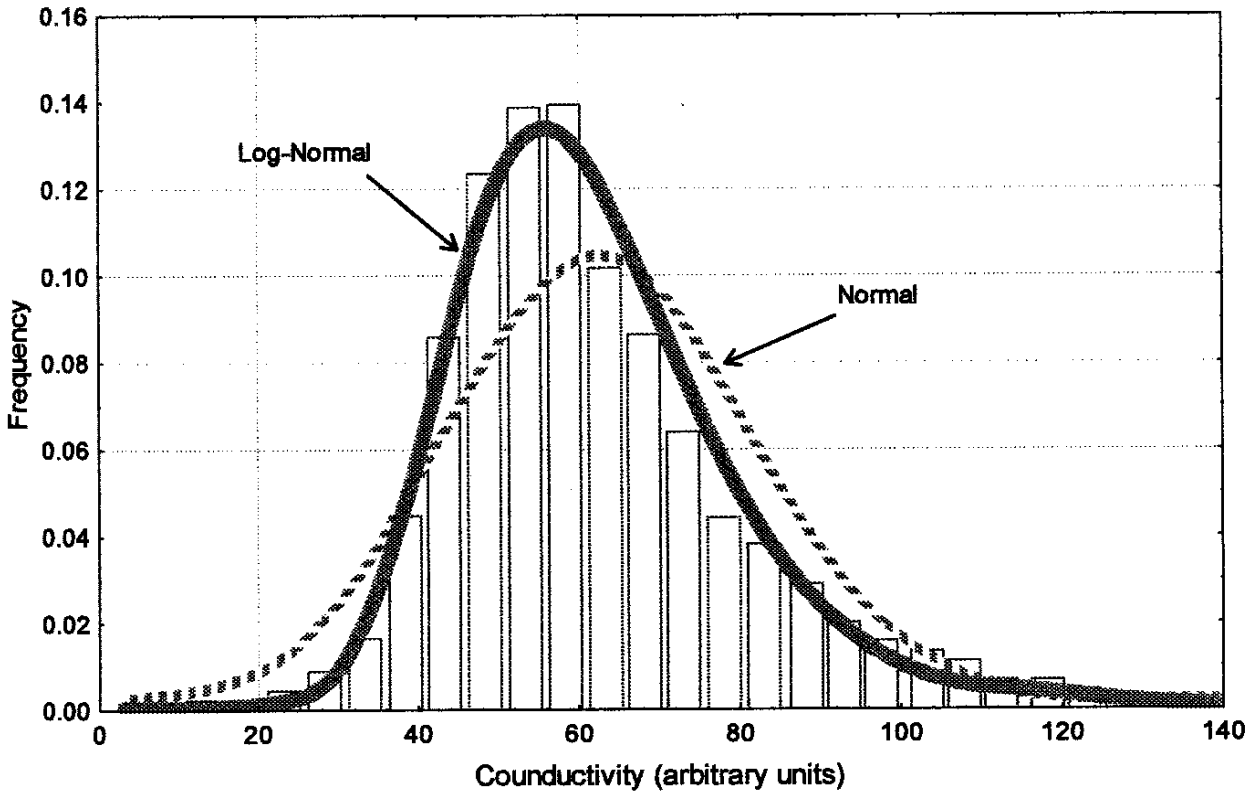

Figure 3. The conductivity values of the human skin (or the resistance values) are not distributed according to a Normal (Gaussian) distribution, but follow for healthy people a Log-normal distribution. Fig. 3 displays the measurements of 20 000 values on the skin of healthy people.

If the probabilities of occupation numbers of different modes were additive and random, one would get a Gaussian distribution of the coupled physiological parameters. However, in view of the multiplicative organizational principle we have, according to Eq. (2)

$$\log W = \sum \log f_i = \sum \log f_k \qquad (3)$$

Consequently, stochastic fluctuations of the f_i yield a Gaussian distribution for *log* f_i, which means that the frequency distribution of

physiological functions follows a lognormal-distribution instead of a Gaussian one. From whole-body biophoton measurements, correlations between biological rhythms and biophoton emission are known already some years ago (Cohen, 1997).

Fig 4. displays an actual example of measurements of 200 values on the skin of a rather healthy man.

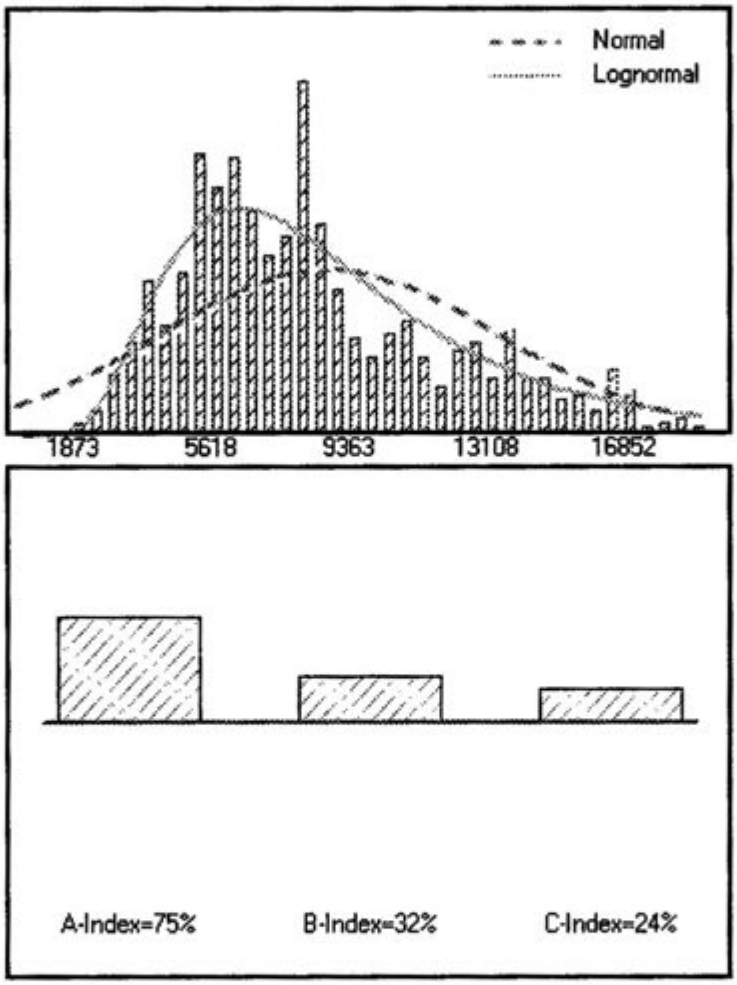

Figure 4. Distribution of 200 conductivity values of the skin of a rather healthy man. The full line is the best approximated Log-normal distribution, the dashed one the best approach of a Gaussian distribution. The A-index is a measure of the agreement of the Log-normal distribution, the B-index a measure of the disagreement of the Gaussian distribution and the C-index concerns A/B.

In addition, if biophotons are coherent and connected to physiological functions, the mode coupling between the log-normally distributed

resistance values and the photon should provide significant correlations. Fig.4 shows evidence of this connection.

A surprising, but according to these considerations. not unexpected new, discovery is then the recent observation that biophoton emission of the human body is significantly correlated to the electrical parameters of the body's skin (Fig.5). This holds not only for the resistance (conductivity-) values, but even for the agreement (or otherwise) of the distribution function of skin-resistance values with (or from) either a Gaussian or lognormal distribution.

A subject (a girl) has been subjected to measurements of biophoton emission, and, at the same time, to measurements of skin-resistance values, where both hands were subjects of the measurements of (1a) spontaneous biophoton-emission (SE) and (1b) delayed luminescence (DL) in complete darkness, as well as (2a) 500 hundred skin-resistance values (R), and (2b) distribution functions of skin resistance values (A and B). The value A is a measure of the agreement of the frequency of skin resistance values to a lognormal distribution, whilst B measures the deviation of this distribution function from a Gaussian function. The measurements were performed over every day from 10 a.m. to 4 p.m., every 2 hours for 2 weeks. Fig.5a displays the original values of SE, separated for left and right hand; Fig.5b shows the same for the Fourier coefficients (periodogram) of Fig. 5a. It turns out that not only are the (expected) correlations of all the parameters SE, DL, R, A and B between left- and right-hand and between SE and DL- values observable, but – and this is most astonishing – but also that there are significant correlations between SE + DL values and R, A, and B parameters. Table 2a and Table 2b quantitatively demonstrate the correlation coefficients within and between the different groups of measurements.

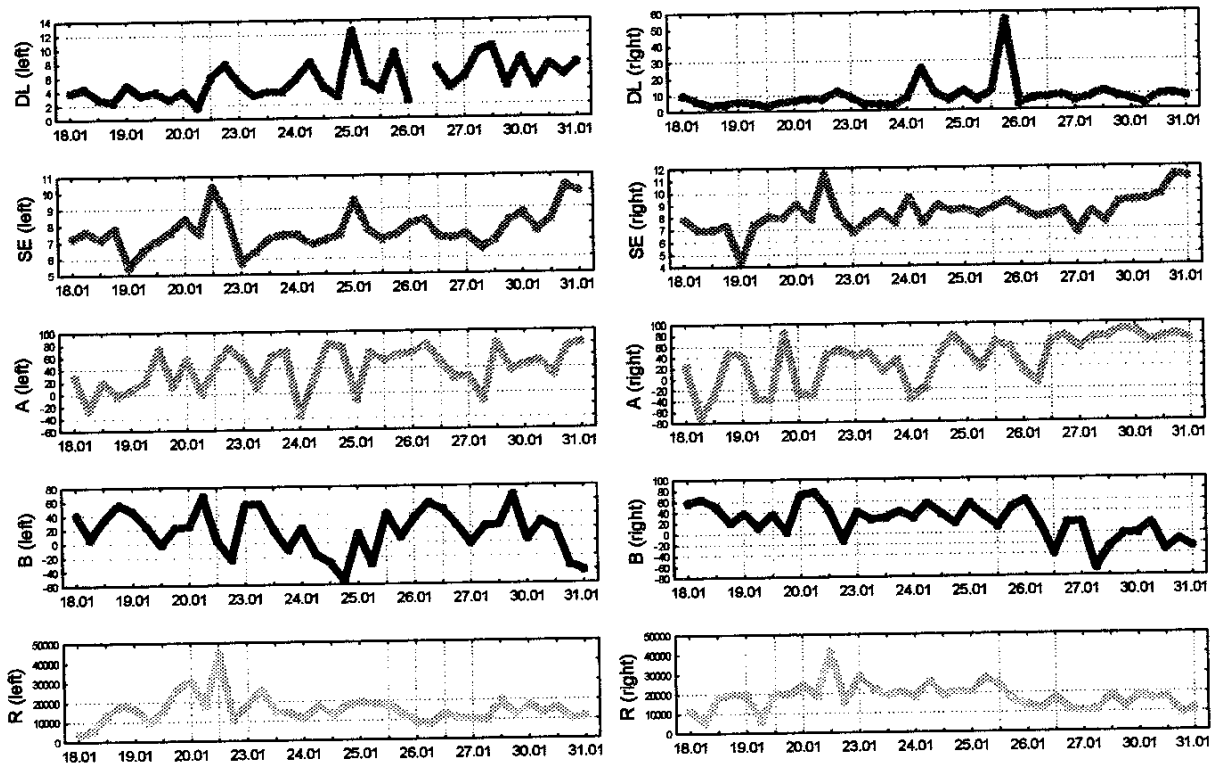

Figure 5a: The temporal course of measurement results of biophoton emission (SE, spontaneous photon counts per second, left hand and right hand, SE-li Hi, SE-re Hi), delayed luminescence (DL in counts per 50 ms, left hand and right hand, DL-liHi, DL-reHi), A-values as a measure of the agreement of the lognormal distribution of resistance values of the hands with the actually measured distribution function of left and right hand (Ali, Are), B-values as a measure of the disagreement of the Gaussian distribution of the resistance values of left and right hand (Bli, Bre) and the mean values of the resistance of both hands (Rli and Rre). The measurements were performed every two hours over three weeks. Table 2a displays the correlation coefficients between all these measurements.

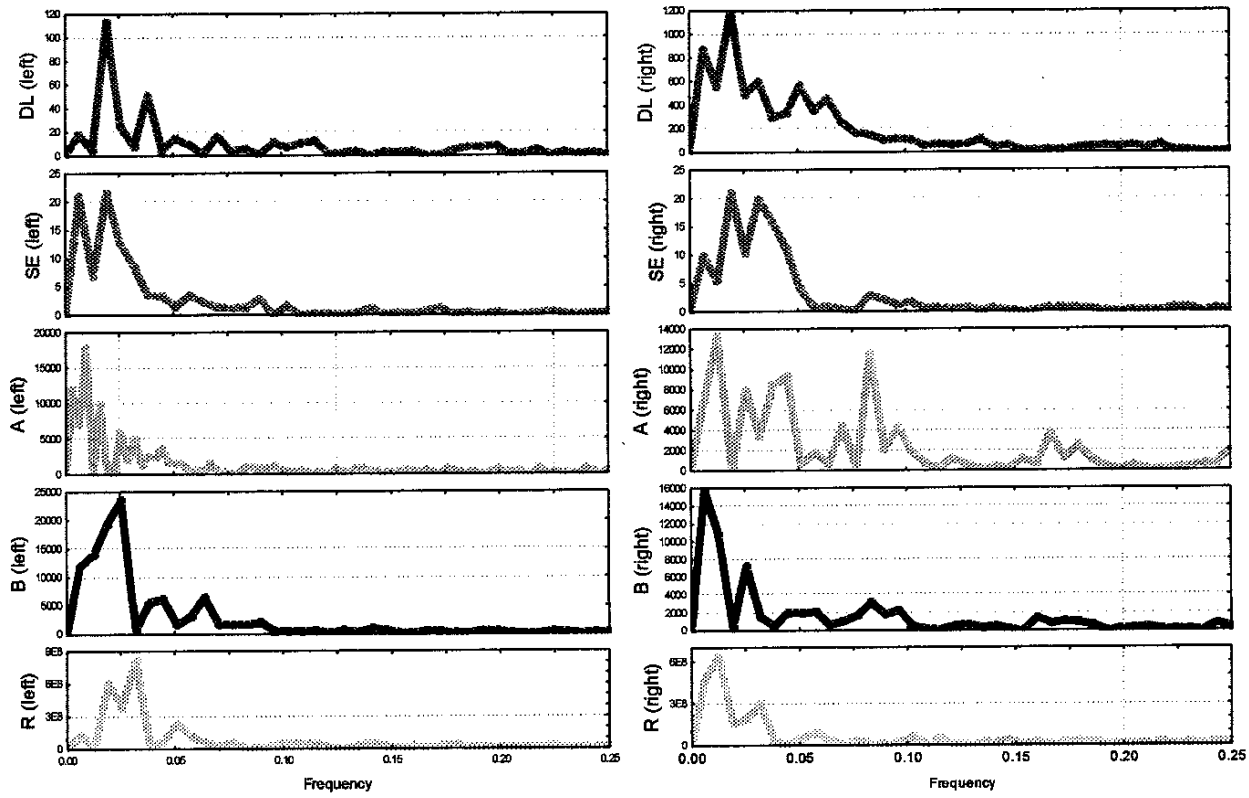

Figure 5b. displays the periodogram of all these measurements, and Table 2b the corresponding correlations coefficients.

Table 2a: Correlation coefficients between biophoton emission and skin-resistance values.

	SE-li HI	SE-re HI	DL-li HI	DL-re HI	Ali	Are	Bli	Bre	Rli	Rre
SE-li HI	1.00	0.79	0.29	0.00	0.23	0.21	-0.41	-0.10	0.23	0.06
SE-re HI	0.79	1.00	0.24	0.14	0.32	0.28	-0.37	-0.26	0.19	0.09
DL-li HI	0.29	0.24	1.00	0.47	0.03	0.36	-0.28	-0.41	-0.02	-0.02
DL-re HI	0.00	0.14	0.47	1.00	0.10	0.10	-0.14	0.10	-0.06	0.01
Ali	0.23	0.32	0.03	0.10	1.00	0.32	-0.48	-0.15	0.06	0.17
Are	0.21	0.28	0.36	0.10	0.32	1.00	-0.09	-0.64	0.08	0.00
Bli	-0.41	-0.37	-0.28	-0.14	-0.48	-0.09	1.00	0.13	0.06	0.00
Bre	-0.10	-0.26	-0.41	0.10	-0.15	-0.64	0.13	1.00	0.18	0.30
Rli	0.23	0.19	-0.02	-0.06	0.06	0.08	0.06	0.18	1.00	0.84
Rre	0.06	0.09	-0.02	0.01	0.17	0.00	0.00	0.30	0.84	1.00

Table 2b: Correlation coefficients between the periodogram of biophoton emission and skin-resistance values.

	P-SE-liHi	P-SE-reHi	P-DL-liHi	P-L-reHi	P-Ali	P-Are	P-Bli	P-Bre	P-Rli	P-Rre
P- SEliHi	1.00	0.80	0.72	0.91	0.85	0.44	0.85	0.69	0.68	0.70
P-SEreHi	0.80	1.00	0.74	0.82	0.73	0.51	0.68	0.39	0.81	0.53
P- DLliHi	0.72	0.74	1.00	0.74	0.70	0.22	0.67	0.16	0.58	0.25
P-DLreHi	0.91	0.82	0.74	1.00	0.92	0.47	0.80	0.61	0.73	0.69
P-Ali	0.85	0.73	0.70	0.92	1.00	0.30	0.62	0.51	0.71	0.66
P-Are	0.44	0.51	0.22	0.47	0.30	1.00	0.57	0.69	0.17	0.60
P-Bli	0.85	0.68	0.67	0.80	0.62	0.57	1.00	0.66	0.53	0.65
P-Bre	0.69	0.39	0.16	0.61	0.51	0.69	0.66	1.00	0.21	0.86
P-Rli	0.68	0.81	0.58	0.73	0.71	0.17	0.53	0.21	1.00	0.43
P-Rre	0.70	0.53	0.25	0.69	0.66	0.60	0.65	0.86	0.43	1.00

The results confirm again evidence of the following results:

- The biophoton field is almost fully coherent and – as a consequence – is strongly coupled to all physiological functions.
- It represents the regulatory activity not only from all the chemical reactivity in single cells, but performs the regulatory activity even over the whole body.
- In this holistic function, it displays all the biological rhythms of the body.
- In turn, the measurements of the electric parameters of the skin provide a powerful tool for looking through the window of biological regulation.
- Regulatory activities of the body are not stable functions of the electromagnetic fields within the body, but are subjects of permanent rhythmical, oscillatory and coherent field amplitudes.
- On this basis, powerful tools of both diagnostic and therapeutic methods can be developed. One example is a new kind of diagnosis called 'Regulation Diagnosis', which is already used in serious medical applications.

To conclude this *Festschrift* contribution, we will present evidence of mode coupling in the transfer of biophotonic energy down to the 10^{-1} to the 10^{-3} Hz range.

8.5. Couplings between optical and $10^{-1} - 10^{-3}$ Hz Modes in Biology

While studying biophoton emission from the developing eggs and embryos of a bone fish (*Misgurnus fossilis L.*) and from the monolayer cell cultures, we noticed that, in most cases, the average intensity of biophoton emission from the living samples did not exceed that of the control ones (quartz cuvettes with water or with cultural medium, but *without* biological objects). On the other hand, when represented in terms of Fourier patterns, the differences became obvious and, moreover, manifested a clear *specificity* that is allied to the stage of development and/or dependence upon physiological conditions. Let us give a more detailed account of these results.

Materials and techniques

1. Fish (*M. fossilis L.*) eggs were obtained by hormonal stimulation of females, artificially fertilized and allowed to develop in tanks with tape water at room temperature. For photonic measurements, about 50 eggs or embryos at a given stage of development were put into quartz cuvette and mounted within the photomultiplier (PMS) chamber. This was a single photon counting device equipped with a cooled (-25°C) EMI 9558QB photomultiplier tube. The cathode has a diameter of 44 mm, and is sensitive in the range between 200 and 800 nm. The average quantum efficiency in this range was approximately 10%. A required temperature could be set up within PMS chamber with a precision of $\pm$ 0,1^0 C;for the fish embryos, it was maintained at 20$\pm$ 0,1^0 C. The dwell time was 0.1s, and the total number of measurements in most cases was 6000 (corresponding to a 10 min period).

Biophoton emission has been measured for:

a) samples at different developmental stages, starting from non-fertilized eggs up to newly hatched larvae,
b) embryos subjected, immediately before a start of measurements to a gentle pressure by a coverglass mounted within a quartz cuvette.

2. For cell culturing, we mostly used the mouse C3H10T1/2 fibroblast cells, and, in some experiments, also mouse cardyomyocytes. Cell suspensions were prepared by trypsinization, and were seeded as a

monolayer onto the internal surface of the quartz walls of rectangular flattened cuvettes of size 48 mm (horizontal), 22 mm (vertical) and 2 mm transversal (opening). Cuvettes were filled with Dulbecco's Minimal Essential Medium (DMEM), supplemented with 10% fetal calf serum and buffered with 10 mM N-2-hydroxyethylpiperazine-N'- 2 ethanesulphonic acid (HEPES) pH 7.4. The total amount of the cells seeded onto a cuvette wall ranged from 2 to $6x10^5$. For seeding the cells, the cuvettes were oriented horizontally, and within an hour (after proper cell attachment) the cuvettes were reoriented in such a way that the cell-containing wall took a vertical position. This orientation has been maintained during the entire measurement period, which lasted from 10 to 100 min. Measurement conditions were the same as previously, with exception of temperature (kept at $37 \pm 0{,}1^0$ C) and duration of measurements (up to 60,000 cases).

Biophoton emission has been measured:

c) for intact cell monolayers in different times after seeding;
d) immediately after returning the samples from cold shock conditions (4^0C) back to 37^0C;
e) after administration to 0,05 mg/ml FGF-1 (fibroblast growth factor);
f) after transfer to a hunger medium (phosphate buffer solution);
g) after addition to the cultural medium one of the following *cytoskeletal drugs*:
cytochalasine D (10μg/ml), disrupting actin microfilaments;
colchicine (10^{-4} M), disrupting microtubules;
trypsin / EDTA 0,025% (which in these concentrations disrupts cell-cell contacts and indirectly contacts-associated microfilaments.

Fourier spectra of different length time series have been constructed with the use of STATISTICA 6.0 program. The results have been represented as following:

1. Ordinary Fourier spectra (FS), in terms either of periodograms or spectral densities, for brief enough successive time periods, consisting of 1000, 2000 or 8000 successive measurements (corresponding, to 0,1s dwell time, to 100s, 200s, or 15 min time periods).

2. Autocorrelation diagrams of FSs (in terms of spectral densities) covering, in most cases, the range of 0,2 – 60 s oscillations periods. The aim of such a representation was to look whether there is any kind of order (regular periodicity) in the arrangement of most pronounced spectral lines (maxima of a spectral density) creating a given FS. We define the obtained autocorrelations diagrams as AC-FS patterns.

3. Cross-correlation diagrams of FSs measured during several successive partially overlapped time periods. In most cases, the diagrams covered up to 20 periods of 1000 measurements each (= 100s) which were overlapped to 10%. This was done tor compare stability of a given FS pattern within a given time period for the biological and control samples.

Results

Normal development of fish embryos

As seen from Fig. 6, FS and AC-FS of the fish embryos at different stages differed significantly from each other. Also, they differed sharply from the control samples (*i.e.* a cuvette with water, but without eggs/embryos) (Beloussov *et al.*, 2003, Beloussov, 2003, 2006). These differences can be most clearly seen AC-FS diagrams (middle column): an almost non-correlated pattern typical for non-fertilized eggs (upper row) is replaced, several dozen minutes after fertilization, by a highly correlated one consisting of a series of extended and equidistant narrow peaks (row second from above). At the later stages, the correlation decreases again (rows 3rd and 4th from above), but, after hatching, a peculiar wave-like pattern appears (lower row). These data can be interpreted as follows. Immediately after fertilization, a single powerful oscillator with many harmonics (like a string with many overtones) comes into action. After the end of cleavage, this single oscillator is replaced by a large set of smaller ones that are at first non-correlated with each other. Later on, however, these oscillators become coupled together again around a few dominating frequencies; these entangle the neighboring ones in a more or less loose, but holistic, way. These latter events are associated with the start of active behavior, and hence the functioning of a central nervous system. We can see that a proper analysis of UWPE rhythmical patterns can tell us much about the holistic state of an entire organism, and even of a large

embryonic population (note that all the measurements were performed on the populations consisting of about 50 samples).

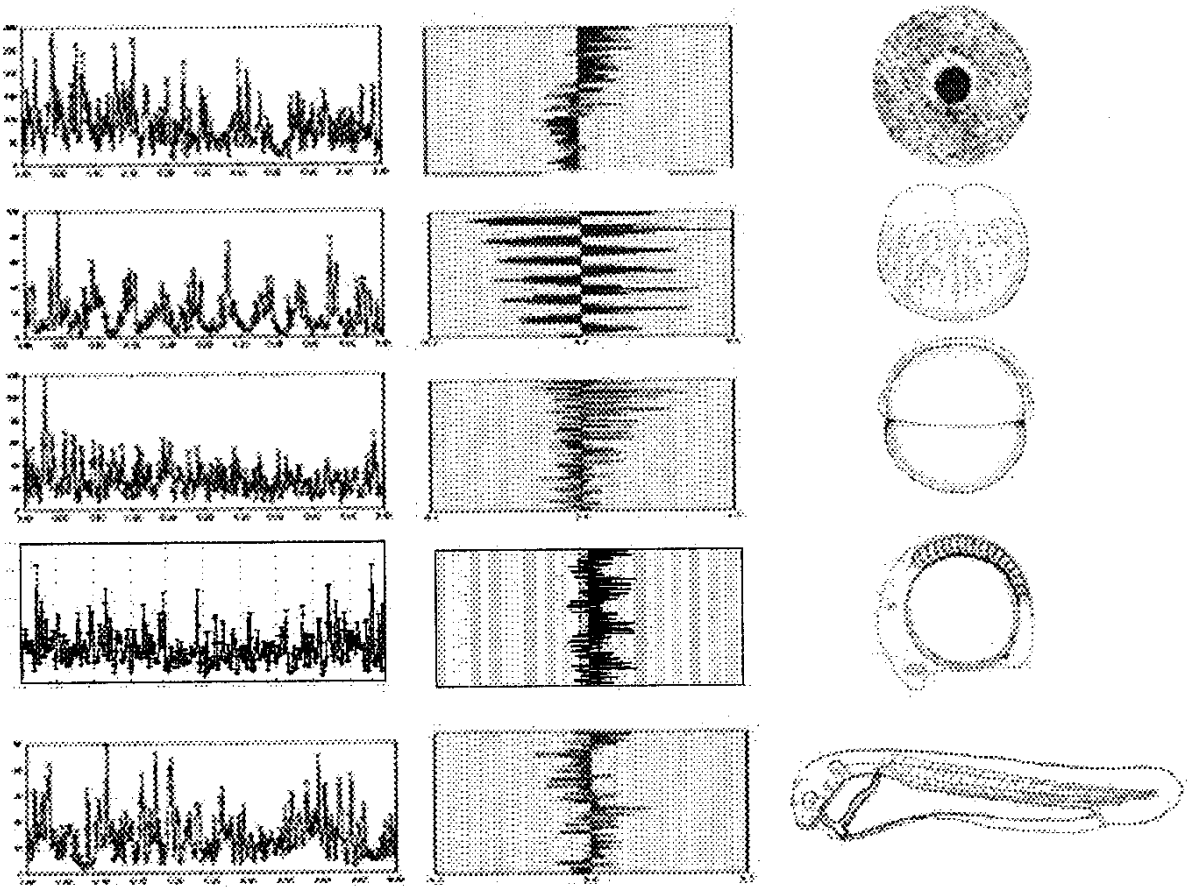

Figure 6. Fourier spectra (in terms of spectral densities) of loach eggs and embryos at successive developmental stages (left column), spectra autocorellograms of these spectra (middle column), and the pictures of the corresponding developmental stages (right column). From top to bottom: non-fertilized eggs, start of cleavage divisions (1 h after fertilization), end of epiboly, formation of somites and freely swimming larvae. Full horizontal scale of Fourier spectra is 1 Hz.

Mechanical perturbations of developing fish eggs

Several dozens of loach eggs at blastula-gastrula stages, maintained in a quartz cuvette, have been slightly compressed by a vertically arranged coverglass. UWPE has been recorded before, during and after the end of compression. In 15 experiments, either a brief impulse or a prolonged increase of photon emission during pressure application has been observed (Fig. 7A).

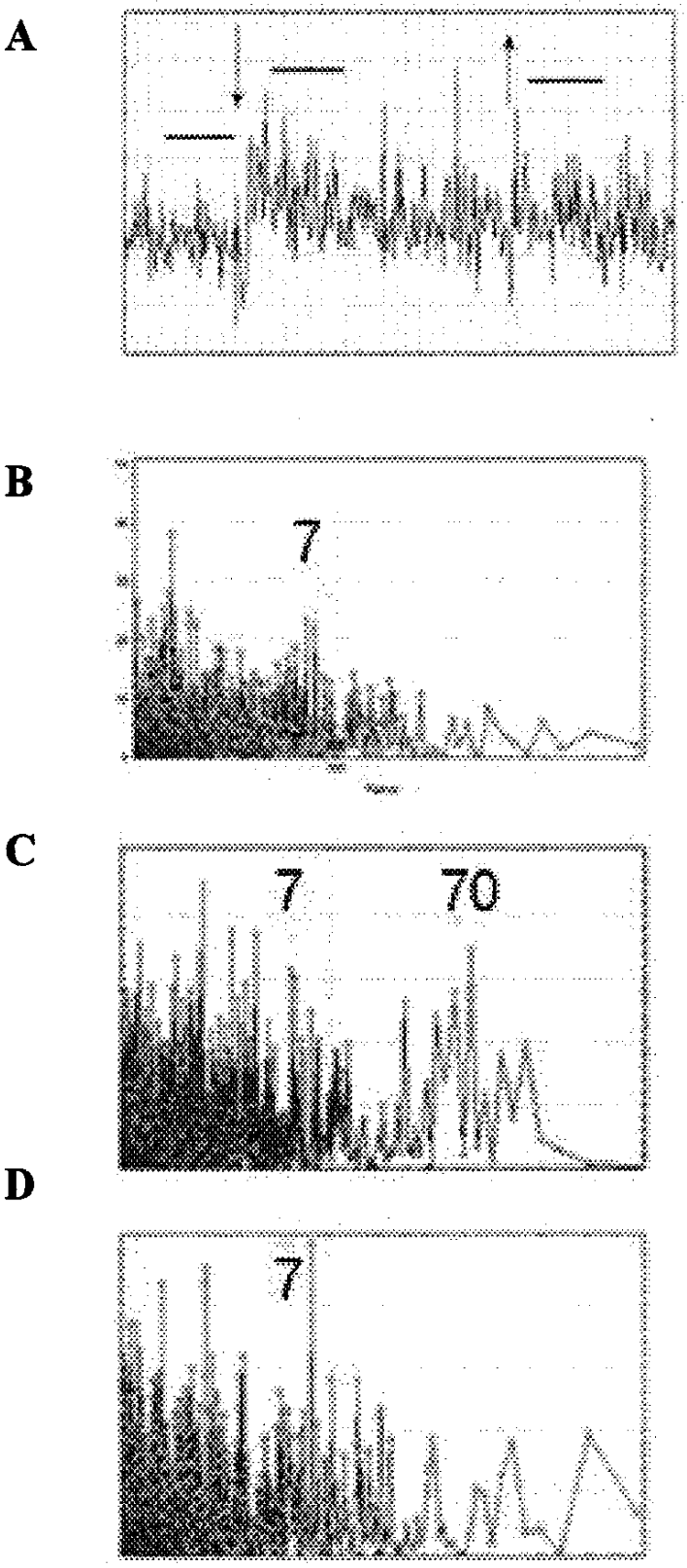

Figure 7. Mechanoemission of loach eggs. A: a record of biophoton emission. The moment force is applied is shown by a downward directed arrow and the moment of its release by the upward directed one. B-D: Fourier spectra (in terms of periodograms) before

(B), immediately after the application of force (C), and after its release (D). In this and other pictures, horizontal lines indicate the periods of FS measurements, and the numbers inside spectrograms indicate the periods of mostly expressed spectral peaks in seconds.

As shown by Fourier analysis, immediately after start of the pressure, an initial 7s period spectral peak has been supplemented by an extensive 70 peak (Fig. 7, cf B and C). After release of pressure, the latter peak disappeared, and only the initial one remained (Fig. 7D).

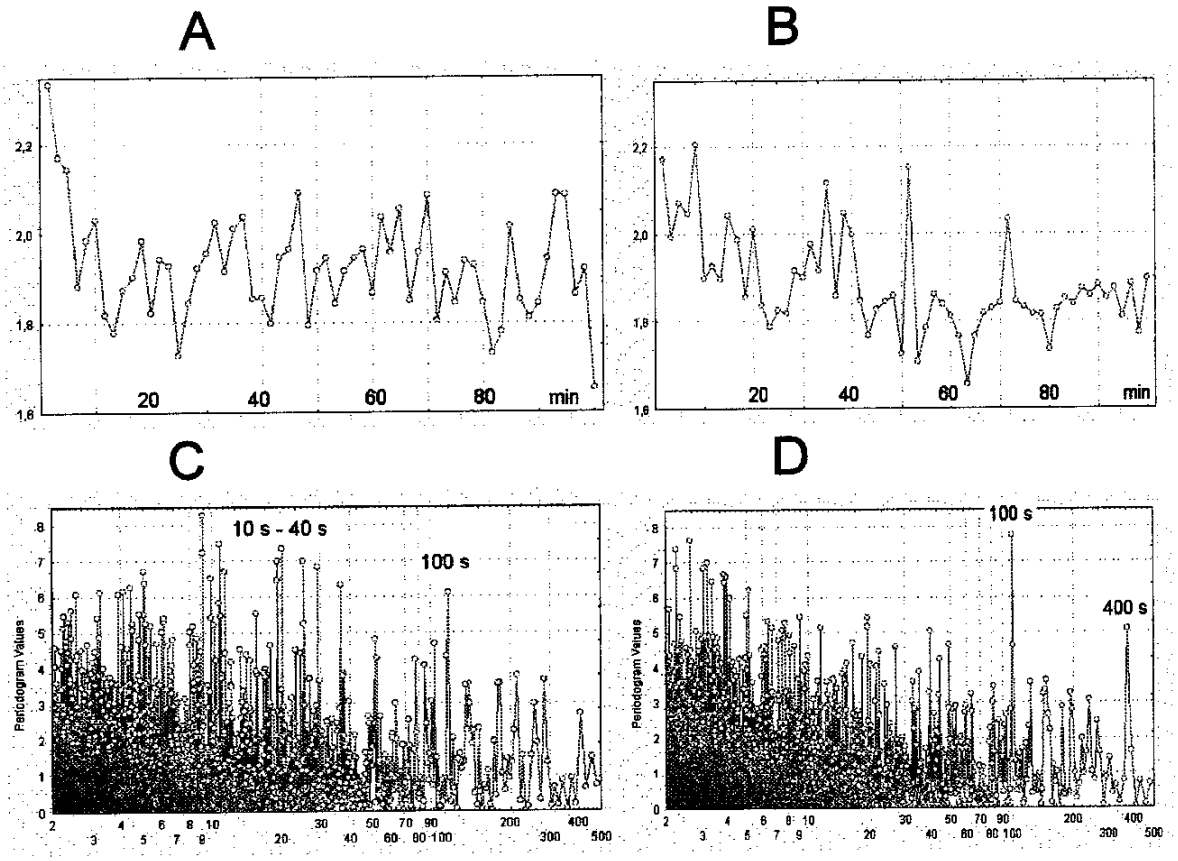

Figure 8. Records of biophoton emission (A, B) and Fourier periodograms (C, D) for a cell-free (A, C) and a cell-containing (B, D) sample. The periodograms cover 100 min periods (60000 successive measurements). Marked are the periods (in seconds) of the most outstanding peaks, which are much more distinct in cell-containing samples.

Fourier patterns of the intact and FGF-treated fibroblast cultures

Already by viewing the records *per se*, a certain periodicity in the emission pattern of cell cultures, as compared with that of a cell-free medium, can be seen (Fig. 8, cf A and B). This conclusion is confirmed by

Fourier analysis: while cell-free medium FS (Fig. 8C) reveals nothing more than smooth spectral bands corresponding to 10-40s and ≈ 100s periodicity, two very distinct peaks under 100 and 400s are seen in cell cultures FS.

Just after the addition of FGF, new low frequency spectral peaks appeared, which were harmonics of the pre-existing ones (Fig. 9, *c.f.* B and C). Such a tendency was retained after 7 h of incubation in FGF solution (Fig. 9, cf D and F).

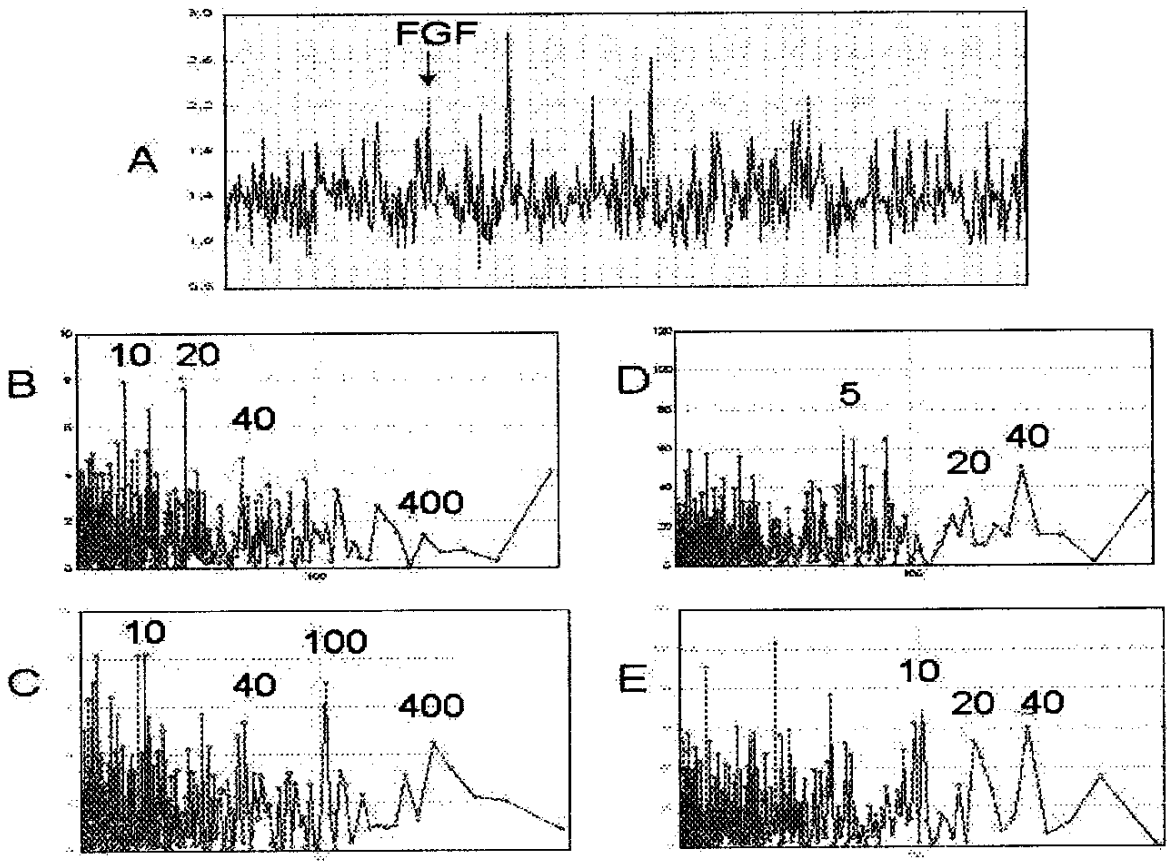

Figure 9. Effects of FGF-1 addition upon UWPE dynamics in fibroblast cultures. A: an experimental record showing the appearance of several UWPE peaks soon after FGF addition (full horizontal scale length corresponds to 66 min). B, C: periodograms covering 30 min periods before (B) and immediately after FGF addition (C) to the fibroblast culture, 1 h after seeding. Note in C the appearance of a new peak of 100 s period, and the increase of a 400 s one. D, E: similar periodograms covering about 3 min measurements periods recorded 7 h later. D: intact 7 h culture, E: that incubated for 7 h in FGF solution. Note in E, the increase of the 10, 20 and 40 s peaks. All the peaks belong to the same set of harmonics.

Effects of cytoskeletal drugs upon Fourier spectra (FS)

As seen from Fig. 10, very soon after the addition of cytoskeletal drugs considerable changes in FS took place. The main effect was, as in the previous cases, an enhancement of some spectral maxims either coinciding with or being multiple to pre-existed ones (Fig. 10).

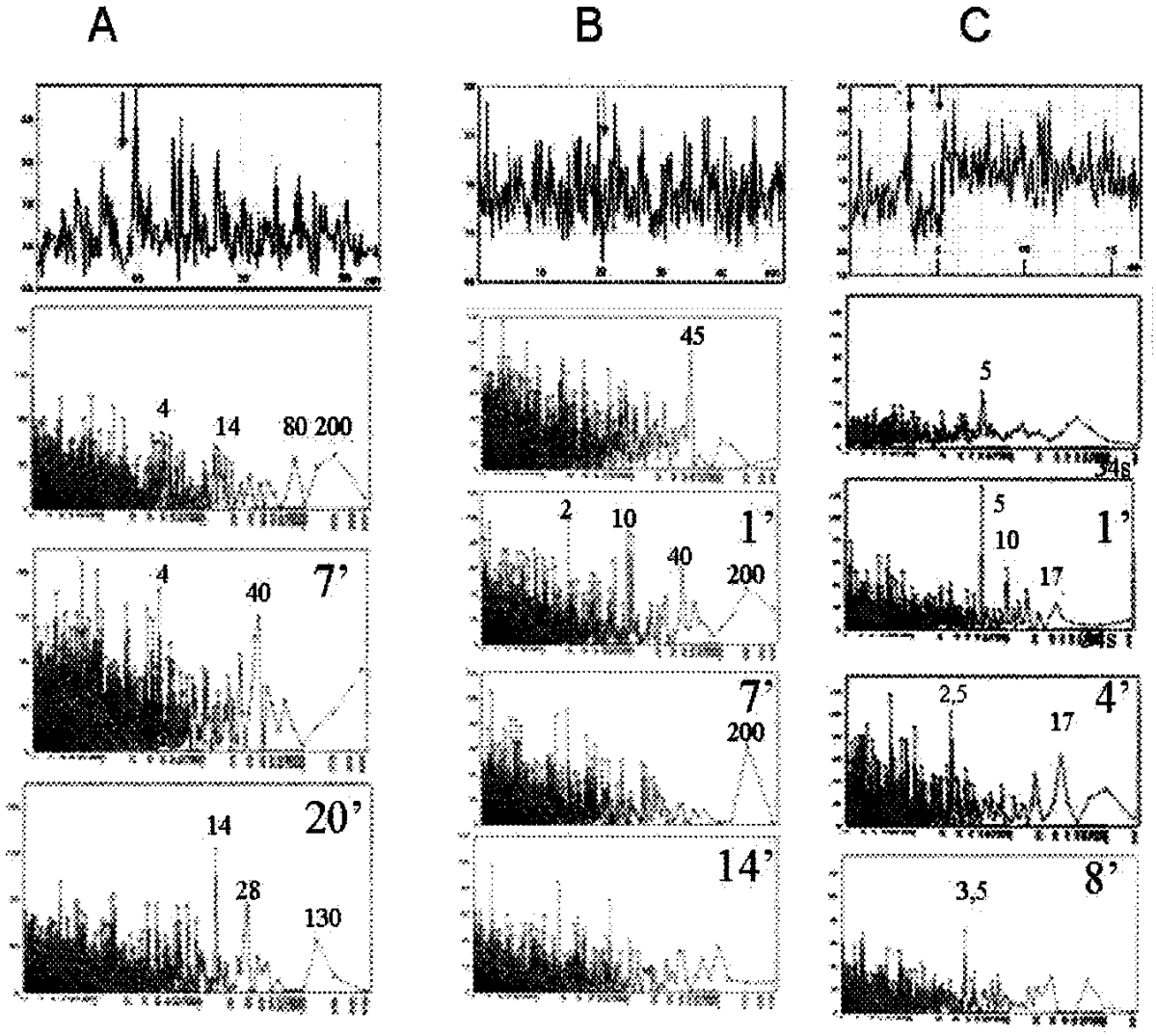

Figure 10. Changes in UWPE patterns of fibroblast cultures after addition of cytochalasine (A), colchicine (B) and trypsine (C). Upper row: records, single vertical arrows in A and B and a right vertical arrow in C indicate the time of drugs application. Second row from the top depicts periodograms of intact samples and the next rows to below those measured in successive times after drugs application (upper right the starting times of the given sets of measurements are given in minutes). Each periodogram corresponds to a 3,5 min time period. Note an extensive increase of several spectral peaks, which are mostly the harmonics of each other and the pre-existed peaks of the intact samples.

AC-FS representation of cell reactions to various agents.

Similarly to the developing fish embryos, the construction of autocorrelation patterns within Fourier spectra became useful in revealing the internal structure of spectra (presence and width of harmonics). As a rule, a few dozens minutes after affecting cell cultures by various stressful factors (FGF, trypsin, cytochalasine, optical interactions with the same culture in another quartz cuvette, transfer to a hunger medium, *etc.*) the AC-FS patterns became more pronounced and the correlation maxims wider (Figs 11-15).

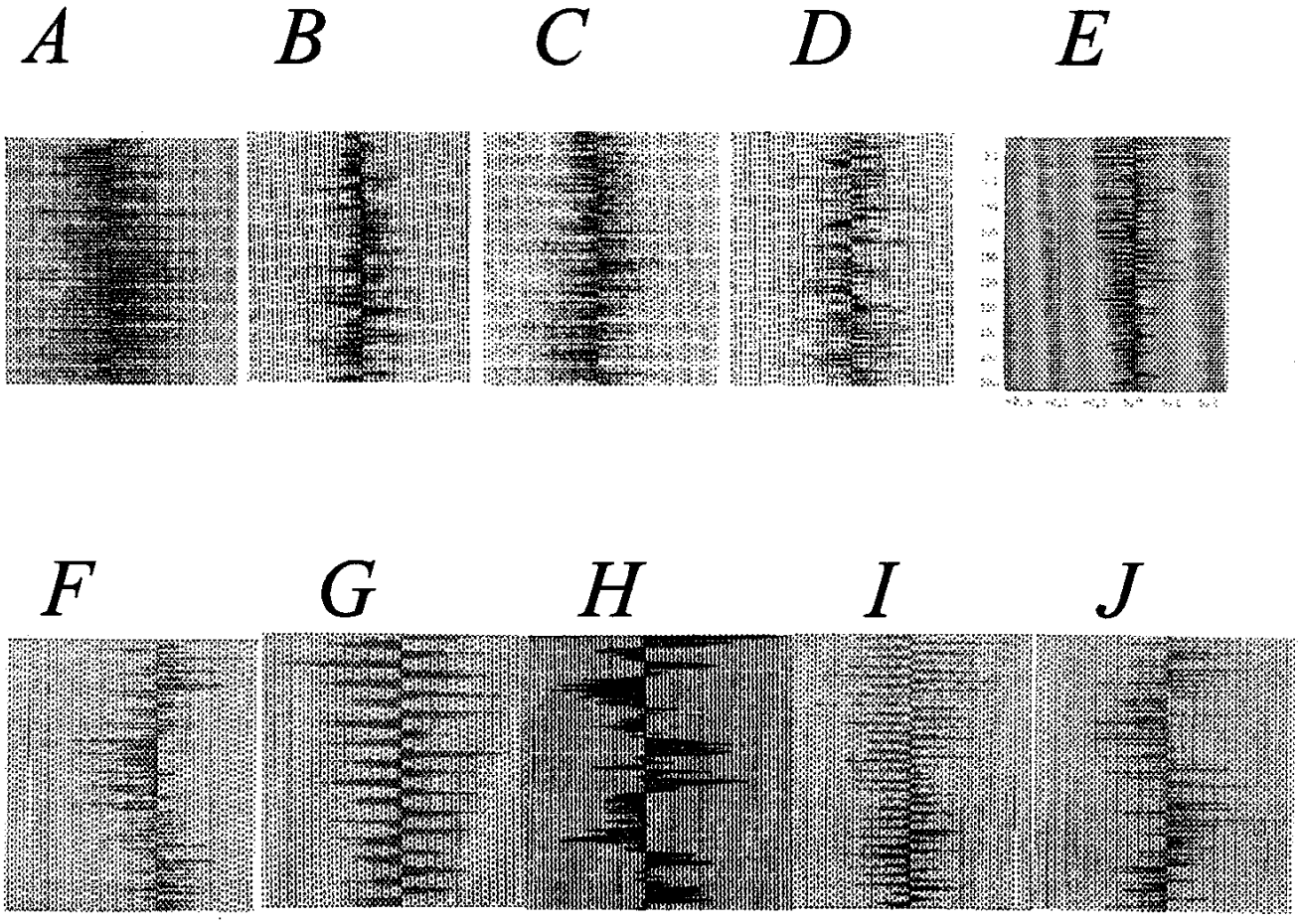

Figure 11. AC-FS patterns of the intact fibroblasts (A-E), and those measured immediately, 10, 20, 30 and 40 min after FGF treatment (F-J successfully). Frame E gives the diagram measured immediately before F on the same sample. Periods of measurements were 3,5 min.

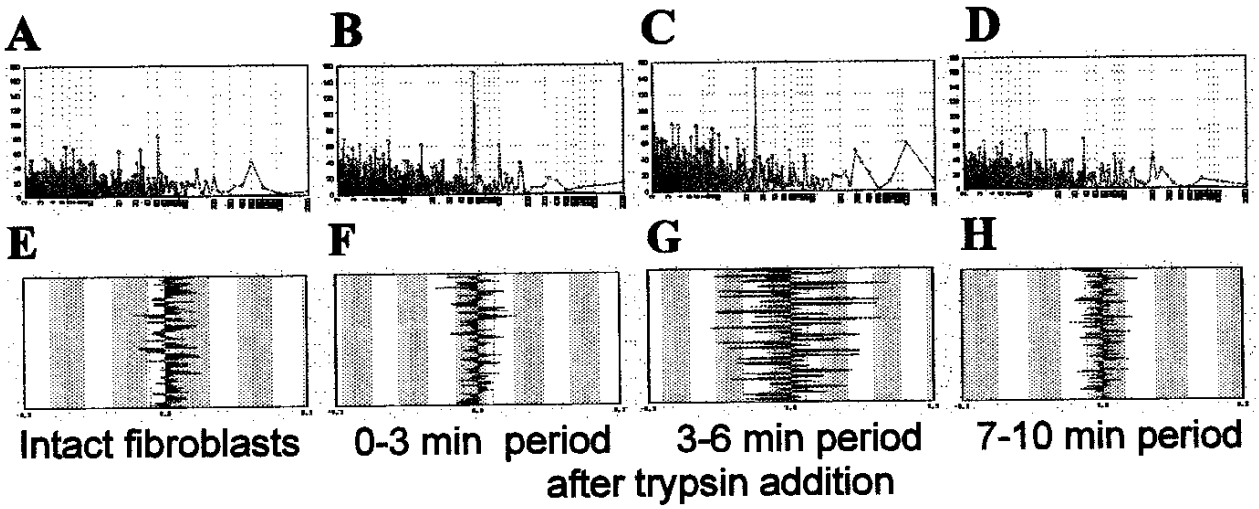

Figure 12. Effects of trypsin addition upon AC-FS patterns of fibroblast cultures. A-B: periodograms, E-F: corresponding AC patterns of Fourier spectra during successive 3 min periods. Although immediately after start of trypsin treatment (F), an outstanding 5s spectral peak appeared, a pronounced autocorrelation pattern have been established only 3 min later (G). Later on all the spectral characteristics have been deteriorated due to destruction of cell monolayer.

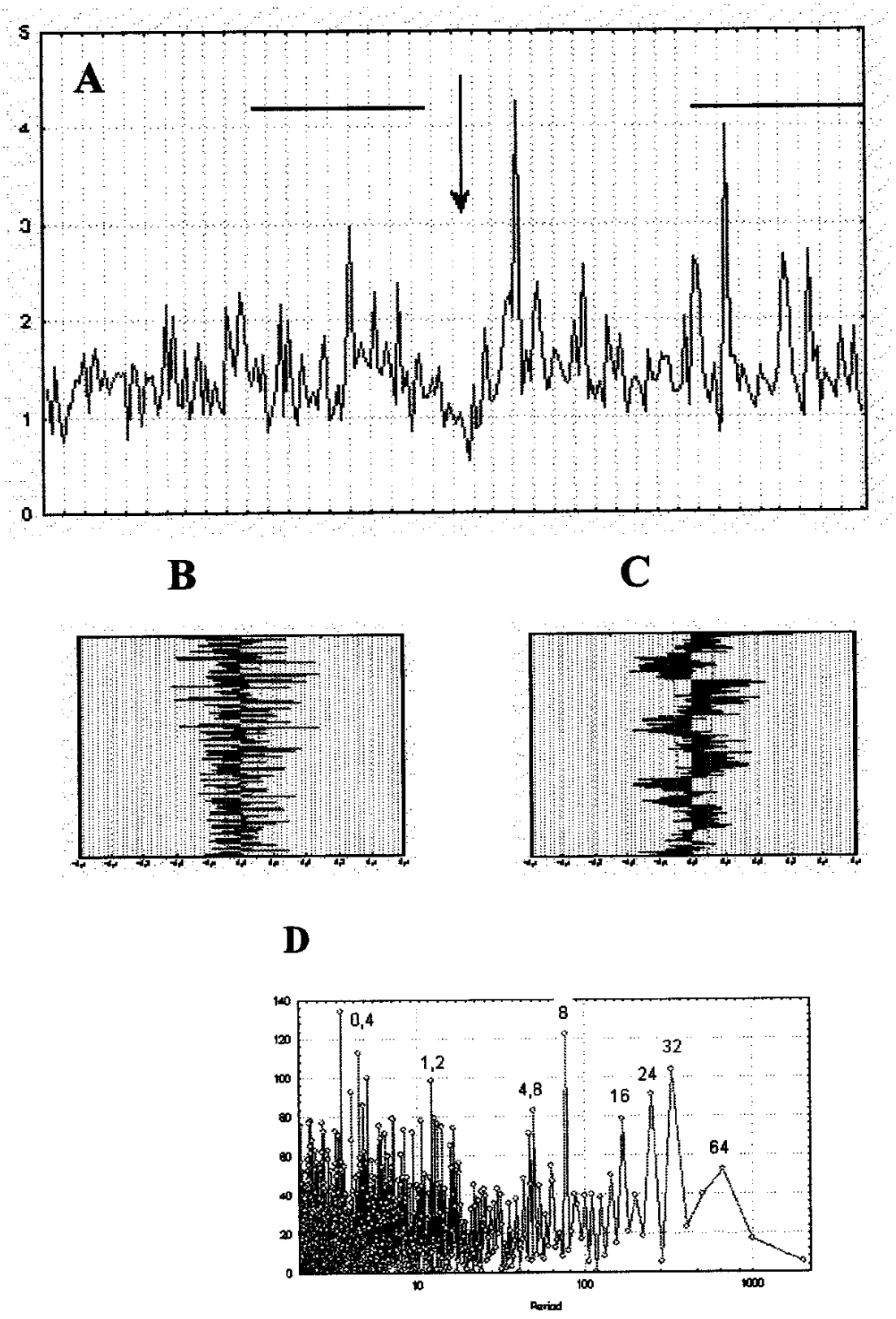

Figure 13. Changes in AC-FS patterns of fibroblast cultures after cytochalasine D treatment. A: record of biophoton emission. Arrow indicates addition of cytochalasine. B, C autocorrelation diagrams of Fourier spectra within 3,5 min periods before and 3min after application of cytochalasine (measurements periods indicated by lines in A). D:

periodogram corresponding to C period. Note a large number of harmonics (figures show periods in sec).

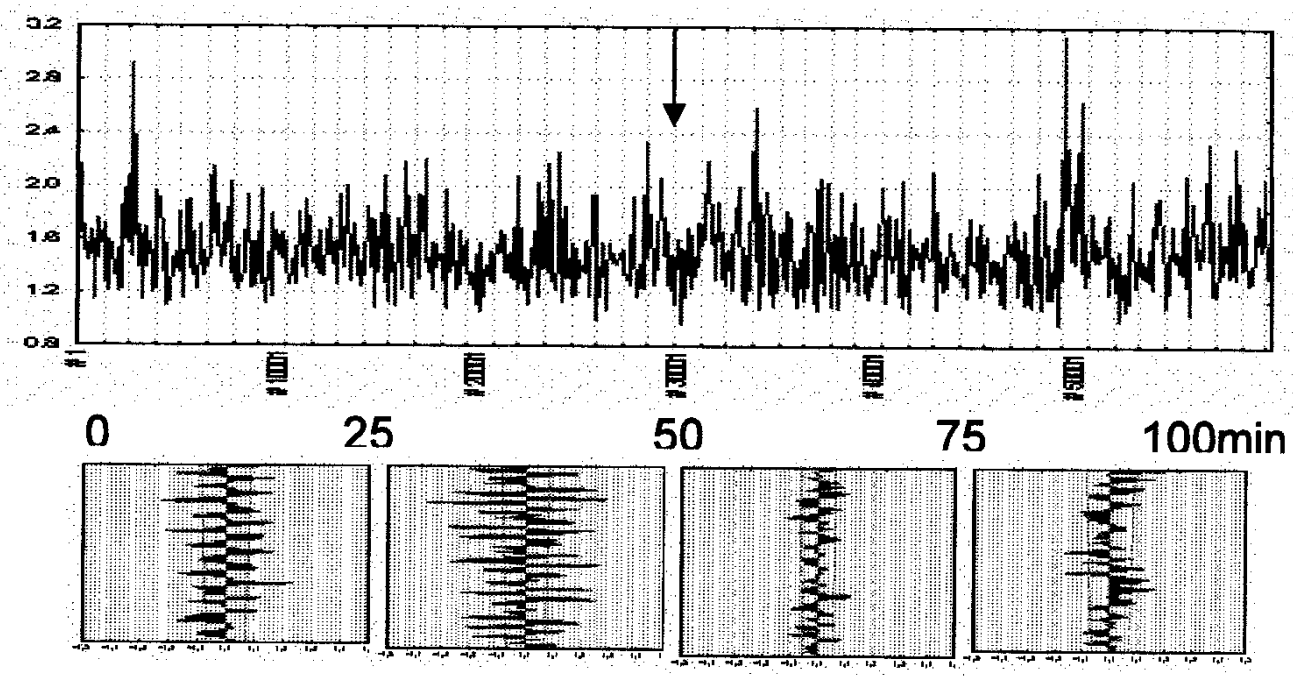

Figure 14. Changes in AC-FS patterns associated with optical interactions of two fibroblast cultures. Above is a 100 min record of biophoton emission. The vertical arrow shows the cessation of optical interactions, which lasted from the beginning of the record. Below are AC-FS patterns for successive 25 min periods. Note the increase of AC values in the second half of optical interactions (25-50 min), their decrease after cessation of interactions (50-75 min) and the appearance of residual wavy pattern in 75-100 min. Such a retarded effect is reproducible.

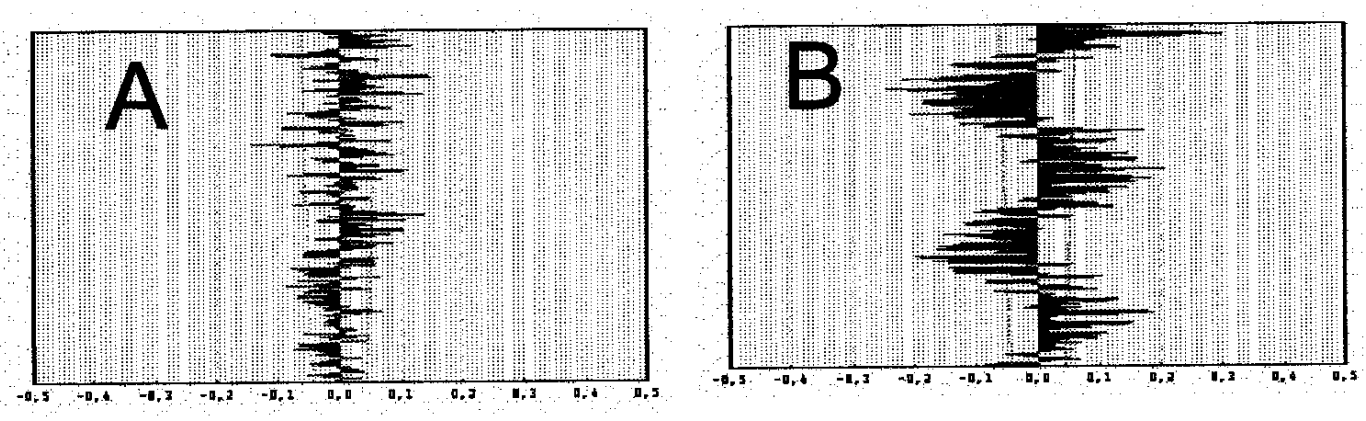

Figure 15. AC-FS patterns of cardyomyocytes cultures before (A), and 20 min after their transfer to a hunger medium (phosphate buffer solution) (B). Measurements periods are 3,5 min.

8.7. Discussion

In this last part of the paper we report two main interrelated (but not identical) findings. First, we can see that photon emission of different biological objects demonstrates a pronounced periodicity, at least in the range $10^{-1} - 10^{-3}$ Hz, which is lacking in non-biological samples (such as a nutritive medium for cell cultures), in spite of the same average intensity. As shown by studies of fish eggs/embryos emission, these periodic (Fourier) patterns show clear stage-dependence. Also, the periodicity is greatly and rapidly enhanced under stress conditions (mechanical pressure, destruction of the cytoskeleton and cell contacts structures, application to growth factors, cessation of optical interactions, cooling, transfer to hunger medium).

Next, as shown by autocorrelation analysis, Fourier spectra possess an obvious internal order, in the sense that the main frequencies are themselves arranged periodically. In the other words, they are multiples of each other, so creating a set of harmonics. Such a structure is also stage- and stress-dependent. In several cases, the corresponding AC-FS patterns differ from each other by a width and smoothness of the spectral maxima. Sometimes (Fig.6, line 2 from above; Fig. 9D) the maxima are narrow, while in other cases (Fig.6, lowest line; Fig. 9B), they are not so pronounced, but more extended. One may conclude that, in the latter cases, the spectral maxima are acting like dominating centres that entrain the oscillators to similar frequencies. As a result of such an entrainment,

each individual oscillator's amplitude becomes inversely proportional to its spectral distance from a dominating centre. In a rough approximation, this may be illustrated by a simple physical example. Suppose that we have a set of strings of the different flexibility, which are and bound to each other (by some kind of friction) to different degrees. If a given string has a great flexibility, and one can neglect its bounding with other strings, it will produce, after being excited, a series of pure overtones (harmonics).

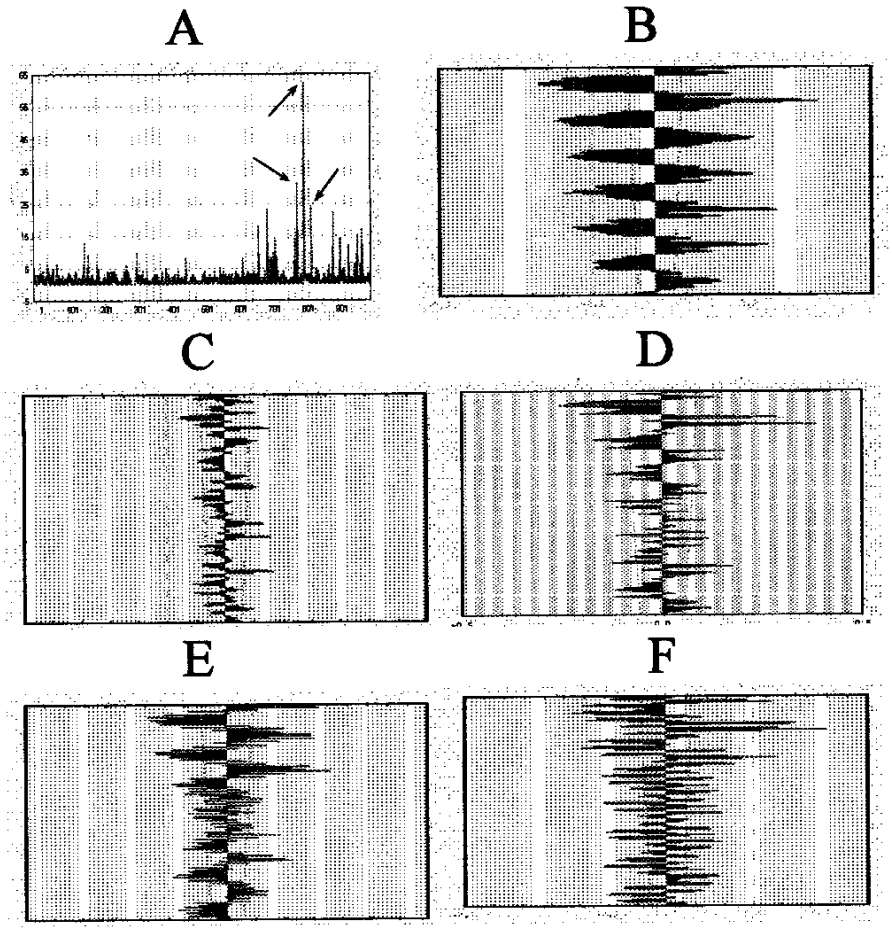

Figure 16. Analysis of AC-FS patterns by means of 'computer surgery'. A: a typical record for a postfertilization period of *M. fossilis* eggs (cf Fig. 6, row second from above). Arrows point to three main emission bursts. B: AC-FS pattern for this record. C: same, after erasing the main peak. D: same, after shifting a left peak to 3 measurements' units out from the main peak. E,F: same, after 'transplanting' all the 3 peaks to other positions removed from their natural positions to 100 and 200 units correspondingly. The mutual distances between all the peaks are preserved.

This corresponds to a series of narrow peaks, which are best of all seen in the autocorrelograms. On the other hand, a string that is less flexible, but which is bound more firmly with other oscillators, will produce smoother spectral patterns. As development of the fish embryos proceeds, the first kind of 'strings' seem to be exchanged by the second one.

An objection can be made that the AC-FS patterns are more or less an artifact of Fourier analysis, in the sense that they reflect nothing more than a presence of single solitary peaks of biophoton emission. To test this point, we took a typical emission record from *M. fossilis* eggs at the postfertilization stage (which demonstrates a perfect periodicity of autocorrelation maxims, see Fig. 6, row 2nd from above), and made some 'computer surgery' procedures by erasing, or transplanting in other positions, some of the most outstanding peaks (Fig. 16A, arrows). Erasing of the main peak indeed led to an almost complete smoothing out of AC pattern (Fig.16, cf B and C). However, an extensive deterioration of the AC pattern took also place when the main peak was left intact, but a smaller one (left arrow) shifted no more than three units to the left of the main one (Fig, 16D), or even when an entire group of three outstanding peaks is shifted out of its 'natural' position without changing the distances between these three (Fig.16E, F). Therefore, the presence of a single outstanding photon emission peak anywhere within a recorded set is itself a necessary, but not sufficient, condition for obtaining a pronounced AC-FS periodicity. To obtain the latter, the positions of all peaks must be finely adjusted, not only in relation to each other, but also, according to a set of the minor emission bursts. One may conclude, that AC-FS patterns indicate a real holistic temporal structure of biophoton emission.

In this paper we showed that Fröhlich's basic idea of coherent excitations in biological systems becomes more and more substantiated as soon as one takes Fröhlich's example as a paradigm, and takes into account other spectral ranges of oscillations in the living state. The inclusion of Glauber's coherent states, the extension to the optical range, the concentration on the coupling of Fröhlich modes, and the rather efficient transfer of non-thermal energy over the extremely broad band of biological oscillations all provide a completely new understanding of biological regulation. We showed that there is already surprising experimental evidence in support of the correctness of Fröhlich's great and

deep picture of the living state as a harmonic concert rather than as a stupid sequence of random chemical events, but under one provision: One has to dare to look seriously through this fascinating window.

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