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# MEDICAL AND BIOLOGICAL MEASUREMENTS

## ANALYSIS OF THE ACCURACY OF CLINICAL LASER FLUORESCENCE DIAGNOSIS

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*We consider a systematic theoretical and experimental approach to statistical analysis of the accuracy of clinical laser fluorescence diagnosis. We propose an improved procedure for treatment of the diagnostic findings which decreases the average relative error of the measurements. This pushes to the forefront the problem of uniform metrological certification and standardization of the apparatus and procedures presently used for laser fluorescence diagnosis.*

Recently reports have begun to appear in major scientific journals connected with the development of optical (including laser) diagnosis in medicine [1-3]. The leading edge in this research direction is laser fluorescence diagnosis (LFD), based on detection of stimulated luminescence from endogeneous and exogeneous fluorochromes [1]. But the questions of the metrological accuracy and confidence level of the findings of these investigations has been practically undiscussed in publications, even though these questions should be of critical importance for actual use of LFD in clinics.

In fact, existing LFD procedures provide very little protection from the action of various external random noise factors such as external artificial and natural illumination, contamination of the examined surface and the working end of the fiber-optic device, instability of the power of the probing laser radiation, instability of the parameters of the CCD-based photodetectors, changes in the optical properties of living tissue when exposed to laser radiation, etc., which all together determine the total error in the diagnosis. Furthermore, LFD includes such imprecise technical operations as manually bringing the end of the fiber-optic device into light contact with a section of tissue to be examined. In our opinion, this operation includes some element of subjectivity associated with the uncertainty in the angular position of the fiber-optic device and the very concept of "light contact" (even slight compression of the tissues may alter their optical characteristics [2]), and also with the subjective choice of the contact point between the fiber-optic device and the tissue, which even by itself may be a source of error in repeated measurements.

The goal of our research was evaluation of the actual errors in LFD for the LESA-4 apparatus (Biospek, Russia) in multiply repeated measurements; statistical analysis of such errors; and working up suggestions for using mathematical statistics methods in actual LFD clinical practice to rationally decide if the required parameters can be monitored under established conditions with sufficient accuracy and confidence.

According to the LFD procedure described in [1], information about the presence and degree of severity of a disease is embedded in the intensity of the fluorescence signal $I_F$ from endogenous fluorochromes. For healthy (intact) tissues, the intensity of fluorescence is relatively low and increases when various destructive inflammatory and cancerous processes are present in tissues, as a result of (according to the current hypothesis) the ability of damaged cells to accumulate an elevated number of fluorochromes. Even though this hypothesis concerning the reasons for the elevated fluorescence is quite controversial, we can use it to carry out differential diagnosis by measuring only the intensity of the fluorescent "response" of the tissue. However, the value of the fluorescence also depends on the power of the original laser radiation penetrating the tissue, which we also must monitor in order to obtain results which can be systematically compared. This information may be indirectly obtained by analyzing the light reflected (scattered) by the tissue in the backward direction, as allowed by the LESA-4. So the

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TABLE 1. Results of Multiple Measurements of Laser Peak Intensity on the LESA-4 Apparatus for Different Diagnostic Objects

| Number of measurement, parameter | Reference | Intact tissue | Tumor |
|---|---|---|---|
| 1 | 1748 | 1100 | 346 |
| 2 | 1548 | 1032 | 210 |
| 3 | 1742 | 984 | 293 |
| 4 | 1740 | 1148 | 881 |
| 5 | 1807 | 1084 | 410 |
| 6 | 1804 | 1180 | 506 |
| 7 | 1540 | 1048 | 354 |
| 8 | 1796 | 1168 | 546 |
| 9 | 1780 | 1144 | 368 |
| 10 | 1736 | 1028 | 330 |
| 11 | 1884 | 1154 | 342 |
| 12 | 1780 | 928 | 515 |
| 13 | 1764 | 924 | 768 |
| 14 | 1832 | 988 | 482 |
| 15 | 1816 | 1012 | 335 |
| 16 | 1550 | 1062 | 514 |
| 17 | 1764 | 1004 | 362 |
| 18 | 1716 | 901 | 314 |
| 19 | 1716 | 1090 | 322 |
| 20 | 1724 | 864 | 494 |
| 21 | 1712 | 956 | 373 |
| 22 | 1524 | 1020 | 493 |
| 23 | 1732 | 1056 | 433 |
| 24 | 1660 | 1056 | 361 |
| 25 | 1700 | 1009 | 518 |
| 26 | 1707 | 1122 | 370 |
| 27 | 1668 | 1064 | 338 |
| 28 | 1716 | 876 | 301 |
| 29 | 1730 | 956 | 741 |
| 30 | 1650 | 1030 | 454 |
| M | 1720 | 1033 | 436 |
| σ | 87,8 | 85,8 | 149 |
| δ | 0,05 | 0,08 | 0,34 |
| $\chi^2$ | 3,08 | 3,78 | 10,3 |

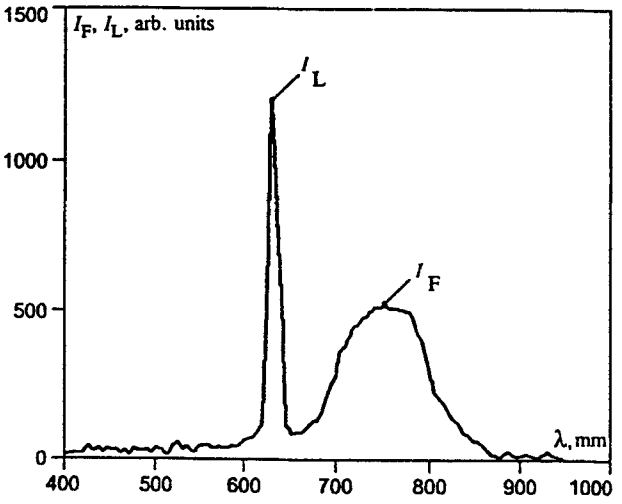


Fig. 1. Shape of spectrum on the monitor screen: λ is the wavelength.

authors of [1] for rather sound reasons used as one of the diagnostic criteria the fluorescence coefficient (*FC*), which in their terminology is equal to the "ratio of the intensity of fluorescence at the wavelength where the fluorescence peak is observed to the intensity of the laser radiation diffusely reflected from the tissues." Moreover, we should not forget that the intensity of the reflected laser radiation ($I_L$) also can be changed when pathological tissue processes develop [2] and accordingly it can

TABLE 2. Values of the Significance Test Variables for the Experimental Results

| Test variable | Reference – intact | Reference – tumor | Intact – tumor |
|---|---|---|---|
| $t$ (1,67) | 30,7 | 40,7 | 19,0 |
| $F$ (1,84) | 1,05 | 2,88 | 3,02 |

TABLE 3. Comparative Results of Patient Examinations

| Patient | Intact region | | | | Center of tumor | | | |
|---|---|---|---|---|---|---|---|---|
| | $I_L$ | $I_F$ | $K_F$ | $\kappa$ | $I_L$ | $I_F$ | $K_F$ | $\kappa$ |
| 1 | 1437 | 368 | 0,26 | 0,41 | 678 | 406 | 0,60 | 0,75 |
| | 1537 | 332 | 0,22 | 0,36 | 654 | 516 | 0,79 | 0,88 |
| | 1418 | 400 | 0,28 | 0,44 | 624 | 505 | 0,80 | 0,89 |
| | 976 | 234 | 0,24 | 0,39 | 607 | 570 | 0,94 | 0,97 |
| | 1241 | 352 | 0,28 | 0,44 | 616 | 588 | 0,95 | 0,98 |
| | 1108 | 320 | 0,29 | 0,45 | 958 | 786 | 0,82 | 0,90 |
| | 1061 | 338 | 0,32 | 0,48 | 928 | 734 | 0,79 | 0,88 |
| | 1465 | 426 | 0,29 | 0,45 | 917 | 730 | 0,80 | 0,89 |
| | 1344 | 386 | 0,28 | 0,44 | 881 | 728 | 0,83 | 0,90 |
| | 1017 | 310 | 0,30 | 0,57 | 893 | 750 | 0,84 | 0,91 |
| M | 1264 | 347 | 0,28 | 0,43 | 776 | 631 | 0,82 | 0,90 |
| σ | 210 | 54 | 0,03 | 0,04 | 150 | 131 | 0,10 | 0,06 |
| δ | 0,17 | 0,16 | 0,11 | 0,09 | 0,19 | 0,21 | 0,12 | 0,07 |
| 2 | 775 | 281 | 0,36 | 0,53 | 1409 | 553 | 0,39 | 0,56 |
| | 769 | 277 | 0,36 | 0,53 | 1337 | 603 | 0,45 | 0,62 |
| | 849 | 320 | 0,38 | 0,55 | 1231 | 559 | 0,45 | 0,62 |
| | 687 | 252 | 0,37 | 0,54 | 1311 | 528 | 0,40 | 0,57 |
| | 849 | 326 | 0,38 | 0,55 | 1317 | 564 | 0,43 | 0,60 |
| | 673 | 264 | 0,39 | 0,56 | 1247 | 544 | 0,44 | 0,61 |
| | 793 | 288 | 0,36 | 0,53 | 1279 | 552 | 0,43 | 0,60 |
| | 921 | 342 | 0,37 | 0,54 | 1385 | 602 | 0,43 | 0,60 |
| | 657 | 262 | 0,40 | 0,57 | 1295 | 544 | 0,42 | 0,59 |
| | 668 | 292 | 0,44 | 0,61 | 1369 | 624 | 0,46 | 0,63 |
| M | 764 | 290 | 0,38 | 0,55 | 1318 | 567 | 0,43 | 0,60 |
| σ | 91,3 | 29,9 | 0,02 | 0,02 | 58,2 | 31,3 | 0,02 | 0,02 |
| δ | 0,12 | 0,10 | 0,05 | 0,04 | 0,04 | 0,06 | 0,05 | 0,03 |
| 3 | 1100 | 305 | 0,28 | 0,43 | 346 | 311 | 0,90 | 0,95 |
| | 1032 | 312 | 0,30 | 0,46 | 210 | 222 | 1,06 | 1,03 |
| | 984 | 208 | 0,21 | 0,35 | 293 | 242 | 0,83 | 0,90 |
| | 1148 | 254 | 0,22 | 0,36 | 881 | 524 | 0,59 | 0,75 |
| | 1084 | 267 | 0,25 | 0,40 | 410 | 357 | 0,87 | 0,93 |
| | 1180 | 311 | 0,26 | 0,42 | 506 | 530 | 1,05 | 1,02 |
| | 1048 | 265 | 0,25 | 0,40 | 354 | 273 | 0,77 | 0,87 |
| | 1168 | 271 | 0,23 | 0,38 | 546 | 469 | 0,86 | 0,92 |
| | 1144 | 275 | 0,24 | 0,39 | 368 | 398 | 1,08 | 1,04 |
| | 1028 | 300 | 0,29 | 0,45 | 330 | 265 | 0,80 | 0,89 |
| M | 1092 | 277 | 0,25 | 0,40 | 424 | 359 | 0,88 | 0,93 |
| σ | 67,3 | 32,1 | 0,03 | 0,04 | 188 | 116 | 0,15 | 0,08 |
| δ | 0,06 | 0,12 | 0,12 | 0,09 | 0,44 | 0,32 | 0,17 | 0,09 |

carry certain diagnostic information. In this case, the method of artificial smoothing (normalization) of the laser lines leads to loss of some of the information, and we did not use this method in our work.

In the first stage of the work, we dealt with the problem of statistical analysis of the errors in determination of the intensity of reflected laser radiation $I_L$ when measurements are multiply repeated (30 times) from the same point, in the physician's opinion (we remove and replace the light guide many times for each measurement). As the diagnostic object, we chose a reference (nonbiological) fluoroplastic diffuser, intact tissue from the wrist, and the central region of a malignant tumor of the auricular concha. The amplitude $I_L$ was measured in arbitrary units (arb. units) lit up on the monitor screen of the LESA-4 apparatus (see Fig. 1). The results of 1 of the 10 series of experiments (30 measurements each) we did for each object are presented in Table 1. The data file obtained was analyzed to estimate the empirical mathematical expectation $M$ of the measurement results, their empirical deviation $\sigma$, and the mean relative error $\delta$ by the Bernoulli method [4]:

$$\delta = \sigma / M.$$

From the experimental population obtained, using the $\chi^2$ test for statistical agreement we additionally tested the hypothesis of a normal error distribution when dividing the population into six classes. These data are also reflected in Table 1. As we see from the results obtained, the relative error in the measurements increases from 5% to 34% as we go to biological intact and tumor tissues, while the error distribution is practically no longer a normal distribution (the critical value for three degrees of freedom and significance level $\alpha = 0.05$ is equal to 7.8 [5]). Obviously such an increase in error on going from a nonbiological to a biological object is connected with the structural and functional characteristics of the tumor process in biological tissue.

The confidence for the differences obtained in $M$ and $\sigma$ was estimated using the Student and Fisher tests for statistical significance ($t$ and $F$). The calculated values of the test variables are presented in Table 2. In the first column of Table 2, in parentheses we indicate the critical values of the test variables for significance level 0.05 [5]. Thus in the experiment we observe a statistical difference between the means at confidence level 95% for the different diagnostic objects, and a statistically significant difference between the deviations on going to tumor tissues. Combined with the estimate of the test variable for compatibility with the hypothesis of a normal error distribution, in our opinion the values of $t$ and $F$ are additional quantitative indicators of the presence or absence of pathological changes in the tissue structure. All this can be a basis for developing methods for treatment of LFD findings, taking into account and minimizing the random errors arising by using mathematical statistics methods.

In fact, according to the law of propagation of errors [5], for a function of independent arguments $f(x,y)$ the mean value of the deviation of the function $\sigma_f$ will be equal to

$$\sigma_f = \sqrt{\left(\frac{\partial f}{\partial x}\Delta x\right)^2 + \left(\frac{\partial f}{\partial y}\Delta y\right)^2}.$$

where $\Delta x$, $\Delta y$ are the mean errors in determination of each argument. When determining $FC$ according to [1], we determine the mean value of the deviation of $FC$ from the expression

$$\sigma_{FC} = \frac{1}{I_L^2}\sqrt{\left(I_L^2 \Delta I_F^2 + I_F^2 \Delta I_L^2\right)}, \tag{1}$$

and we estimate the relative error in the measurements $\delta_{FC}$ using the formula

$$\delta_{FC} = \frac{1}{I_L I_F}\sqrt{\left(I_L^2 \Delta I_F^2 + I_F^2 \Delta I_L^2\right)}, \tag{2}$$

from which we see that it decreases in inverse proportion to the product of the intensities. But of course the value of $FC$ is not the only possible method for representing the diagnostic findings. In optics, for example, a widely used method for evaluating the quality of an image uses the concepts of intensity contrast or visibility of an image element [6], when we calculate the value equal to the ratio of the difference and the sum of two intensities. In this case, the intensity contrast may vary from $-1$ to $+1$ with maximum derivative (sensitivity of the method) in the region about zero. In LFD practice, the appearance of negative values may confuse the physician too much, so it is advisable to consider an approach to calculating the fluorescent contrast ($K_f$) of biological tissue using the formula:

$$K_f = \frac{I_F - I_L}{I_F + I_L} + 1. \tag{3}$$

In this case, we write the mean value of the deviation for $K_f$, as in (1), in the form:

$$\sigma_{Kf} = \frac{2}{\left(I_F + I_L\right)^2}\sqrt{\left(I_L^2 \Delta I_F^2 + I_F^2 \Delta I_L^2\right)}, \tag{4}$$

and the relative measurement error will be equal to

$$\delta_{Kf} = \frac{1}{I_F(I_F + I_L)} \sqrt{\left(I_L^2 \Delta I_F^2 + I_F^2 \Delta I_L^2\right)}. \quad (5)$$

A simple analytical comparison of (1), (2), (4), and (5) shows that using relation (3) for treatment of LFD findings is preferred: without any special extra efforts, we can always reduce the relative error of the diagnosis, and it is inversely proportional to the square of the fluorescence intensity. And the deviation will also decrease, starting when the fluorescence intensity reaches a value of about 0.5.

As a graphic test of the analytical constructions we have proposed, we performed statistical measurements on the intensity of fluorescence and the laser response for a number of cancers and treated the results by both methods discussed above. As an example, in Table 3 we present the results of examinations of three patients: 10 measurements each in the intact region and in the center of the section damaged by the malignant tumor for each patient. These results completely support the theoretical prediction concerning the reduction in the measurement error. Furthermore, in many cases we noted a reduction in the $\chi^2$ variable for the fluorescent contrast $K_f$ at the center of the tumor. This may indirectly suggest improvement of the approximation to a normal error distribution when using this method for treatment of diagnostic findings.

And recently in [1], two parameters were indicated as the results of the diagnosis: *FC* and the contrast coefficient. The latter, although similar in spirit to the quantity $K_f$ which we have proposed, is qualitatively different. It is equal to the ratio of the fluorescence coefficients for intact and tumor points. Based on its value, the authors of [1] have proposed assuming an upper limit for the normal value of the contrast coefficient of 1.25. That is, for our second patient from Table 3, it would be automatically concluded that he is healthy (the contrast coefficient calculated from the average is equal to 1.13), even though a tumor has been confirmed histologically and the value of $K_f$ is clearly elevated. This may be a consequence of the elevated background fluorescence we noted for most of the observed cancer patients, even from intact tissues. So the use of such mixed (combination) parameters as a diagnostic criterion requires caution and more thorough study. Since such a discussion would go beyond the scope of this paper and is more appropriate to the area of medicine and biophysics, here we will not consider the contrast coefficient or its limiting and accuracy characteristics.

Thus a more rigorous approach to the problem of analysis and treatment of the findings of laser fluorescence diagnosis pushes to the forefront the problem of metrological certification and standardization of the equipment and procedures used. In our opinion, work in this direction on standardization of existing procedures for treatment and representation of LFD findings to make measurements done in different clinics and on different apparatus metrologically comparable is both necessary and already feasible today. For example, simply by an appropriate choice of the procedure for representation of the results, without making any changes in the apparatus it is already possible to significantly decrease the measurement error. And even after this, using comparable combined results from different clinics, we will be able to competently work out the numerical criteria for differential cancer diagnosis.

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