cal thioflavine S, and the intensity of the specific fluorescence could be preserved for several months if sections were kept at 4 °C. The pretreatment with $KMnO_4$ and NaOH totally removes lipid autofluorescence, resulting in an improved definition of the pathological lesions[11,12]. Results were comparable for short or long fixation periods.

Reactive astrocytes are occasionally stained with MTST and are predominantly located in the superficial part of layer I and in the white matter. Diffuse amyloid deposits are also evidenced in the hippocampal cortex, as well as amyloid accumulation in the walls of several small blood vessels (fig. 4). The intense staining of neuropil threads (fig. 3) may be another advantage of this new method. The modified thioflavine S technique (MTST) presented here may be very convenient for routine neuropathological diagnoses and, on floating deparaffinized or frozen sections, could be used as a rapid standard protocol for developing reliable criteria for AD diagnosis.

Moreover, the fact that this improved thioflavine S technique can be used to visualize neuropil threads[13], amyloid deposits, and vascular lesions, indicates that new criteria may be added to those currently used for AD diagnosis[14–17].

In conclusion, the use of our modification of the thioflavine S method permits rapid and reliable quantitative assessments of histopathological changes resulting from AD and could therefore be useful for further detailed neuropathological investigations.

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Photon emission from normal and tumor human tissues

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Abstract. Photon emission in the visible and near ultraviolet range by samples of human tissue removed during surgery has been measured by means of a low noise photomultiplier coupled to a data acquisition system. The results show that among the 25 analyzed samples the 9 from normal tissues had an emission rate of the order of some tens of photons/cm^2 min, while most of the 16 tumor tissue samples had a very much higher rate.
Key words. Ultraweak radiation; cancer; low level luminescence; biophoton.

The emission of radiation from living systems was suggested at the beginning of this century by G. Gurwitsch[1], to explain some experimental observations on duplicating cells. The presence of this radiation was revealed in Italy forty years ago by Facchini et al.[2,3], who discovered that living systems emit light at a very low level. Although a number of scientists in the Soviet Union[4] and in Eastern countries[5,6] continued to work on photon emission from biological systems, it is only in the last few years that some interest in the problem has been growing in Western countries[7–10].

The original Gurwitsch hypotheses suggested that living systems transmit and receive information by means of electromagnetic waves. This statement is at present controversial and neither the experiments nor their interpretation are conclusive[11]. It is widely accepted, however, that the intensity of the emitted radiation is closely connected with the biochemical processes occurring in a sys-

tem, as well as its physiological state. This suggested the possibility of using the emitted radiation as a non-destructive tool to investigate the 'status' of a living system[9,12,13].

The development of this method is at present in a quite preliminary stage and a large effort seems to be needed to

a) improve the experimental technique, and bring it up to date;
b) widen the experimental basis, looking for statistical significance of results, and using strictly controlled experimental conditions; and
c) gain a better insight into the phenomena involved and thereafter design new and more specific experiments capable of discriminating between different hypotheses.

Attempts have already been made to use the measurement of ultraweak radiation in agriculture[13], biology and medicine[12] as a qualitative diagnostic method for different pathologies including cancer. Some preliminary results have been reported in this field[14,15], however the correlation with the pathological characterization of the examined samples was quite incomplete.

The present paper is concerned with the intensity of radiation emitted by 25 samples of human tissues coming from surgical operations, of which 9 were from normal tissues and 16 from tumors.

Materials and methods

To measure the photon emission from biological systems a special experimental set up has been designed and assembled, as illustrated in figure 1. It consists of a steel dark chamber, where the sample to be analyzed can be maintained at a constant temperature by means of a thermostatic sample holder, the temperature of which can be set between 5 °C and 80 °C and maintained within ± 0.1 °C. The sample is put into a pyrex or plastic cup, which is covered by a Suprasil quartz window, transparent to UV light up to 190 nm; the window prevents the evaporation of liquids contained in the sample. To avoid condensation of the window, this is heated to a temperature 1–2 °C higher than that of the sample.

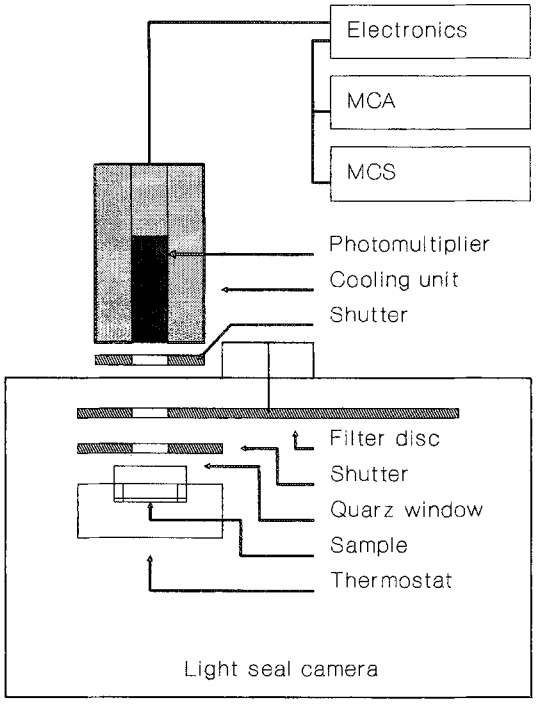


Figure 1. Schematic block diagram of the experimental set-up.

A light shutter is placed just above the window. The shutter can be closed by an electromagnetic actuator to measure the chamber emission background; in the absence of sample, the background has been shown to be the same whether the shutter is open or closed. Above the shutter lies an aluminium disk which acts as filter-holder for 15 different filters. The disk can be rotated electrically so that different filters are interposed between the sample and the photomultiplier; this allows the spectral distribution of the emission from the sample to be measured in the range between 200 nm and 700 nm. The dark chamber is closed by a second light shutter above which the photomultiplier is fastened.

The photomultiplier (Thorn Emi type 9635 QA or 9558 QA) is outside the main chamber, and is cooled to − 30 °C by means of a Peltier cooling system. This temperature-reduction makes it possible to decrease the dark current by three orders of magnitude.

Pulses coming from the photomultiplier are amplified, shaped, discriminated by an appropriate electronic chain, and sent to a multiscaler which counts the number of pulses arriving during each preset time interval; up to 4096 equal time intervals can be fixed, in the range from 2 μs to 30 min each.

Under operating conditions, the background counting rate is only about 8 pulses/s as is necessary if the low rate emission from samples is to be detected.

The intensity of the emitted light depends on several factors. These include the physiological condition of the sample itself and its preparation and handling, and also the physiological and pathological conditions of the living system from which the sample has been taken, i.e. of the patient in the present case.

To reduce masking effects due to sample handling, a standard procedure for sample preparation and treatment was followed. Samples were human tissues coming from surgical operations. As soon as they had been taken from the living body, the samples were put into a dark container thermostatically maintained at 37 °C, and immersed in Ringer's solution to allow the metabolic processes to be maintained. Samples were put into the measuring chamber 30–100 min since removal. After the photon emission measurements the size was measured and it was of the order of one or a few cm^3.

Results and discussion

Of the 25 samples examined, 16 were from tumors and 9 were normal (non-tumor) tissues. The characteristics of

Measured photon emission from tissues.

Sample #	Patient code	Description of pathologies	Sample size (mm)	Sample weight (g)	Emission photons (cm^2/min)	Standard deviation (cm^2/min)	Elapsed time (min)
1	sa46	Metastasis from infiltrating ductal carcinoma	30 × 15 × 5	1.64	266	3	30
2a	fm56	Thymoma with preminent lymphocytic component	5 × 3 × 2	.02	66	50	100
2b	fm56	Perithymic normal lymph node	4 × 2 × 2	.02	0*	12	110
3	xx	Gastric adenocarcinoma	–	–	348	12	45
4	pg60	Mild differentiated adenocarcinoma	5 × 3 × 2	.03	1440	80	120
5	lr69	Light cell kidney carcinoma	–	–	228	12	70
6	va58	Lymphnodal metastasis from infiltrating ductal carcinoma	9 × 6 × 2	.18	510	24	90
7	c174	Omentum metastasis from liver carcinoma	20 × 16 × 8	2.92	819	15	55
8	ma57	Infiltrating ductal carcinoma	40 × 30 × 10	9.75	24	1	30
9	g	Chronic appendicitis	25 × 20 × 3	1.29	34*	2	30
10	fr	Lithiasic chronic cholecystitis	35 × 25 × 4	2.68	13*	1	45
11	na	Poorly differentiated adenocarcinoma	10 × 20 × 5	1.23	84	6	50
12	mr70	Diffuse malignant lymphoma w/ small and great cells	12 × 15 × 3	.30	84	6	350
13	rs67	Epidermoid well-differentiated carcinoma	30 × 20 × 8	3.66	24	3	35
14	dc	Pleomorphic sarcoma	10 × 15 × 5	1.14	138	8	40
15	s	Chronic appendicitis	45 × 12 × 4	2.92	34*	2	30
16	fr	Mild differentiated adenocarcinoma	30 × 15 × 6	2.67	108	12	30
17a	cr68	Ductal carcinoma with peritumoral lymphocytic infiltrate	10 × 10 × 1	.24	24	24	95
17b	cr68	Normal breast tissue	20 × 10 × 4	.95	12*	12	85
18	at	Melanoma	10 × 6 × 4	.24	320	24	20
19a	tn61	Lobular infiltrating carcinoma	13 × 8 × 4	:35	306	36	55
19b	tn61	Normal breast tissue	30 × 20 × 7	4.31	40*	6	85
20a	vm39	Chronic appendicitis	17 × 8 × 4	.41	40*	9	90
20b	vm39	Lithiasic chronic cholecystitis	14 × 10 × 4	.52	0*	9	100
21	m66	Normal thyroid tissue	20 × 20 × 6	2.15	26*	3	130

The symbol * indicates non-tumor tissues.

the samples, including their size and weight, and the pathological description, are shown in the table. The column 4 'sample size' reports the average dimension of the sample in mm; the first and second figure are the dimensions of the side facing the photomultiplier, while the third is the sample thickness. The table also shows the intensity of light emitted (number of photons/cm^2 min after subtraction of background), the estimated error in the counting rate, and the time that elapsed between the surgical removal of the sample and the emission measurement.

From the table it is evident that all the normal (non-tumor) samples emitted light with negligible intensity, whereas the tumor samples emitted light with an intensity which could be as high as 1400 photons/cm^2 min. All of the 9 samples belonging to the sub-set of non-tumor tissues have an emission rate lower than 40 photons/cm^2 min, with an average value of 22 ± 6 photons/cm^2 min. On the other hand, the 16 samples belonging to the sub-set of tumor tissues showed a much wider distribution of emission rates. The average value was 300 ± 90 photons/cm^2 min.

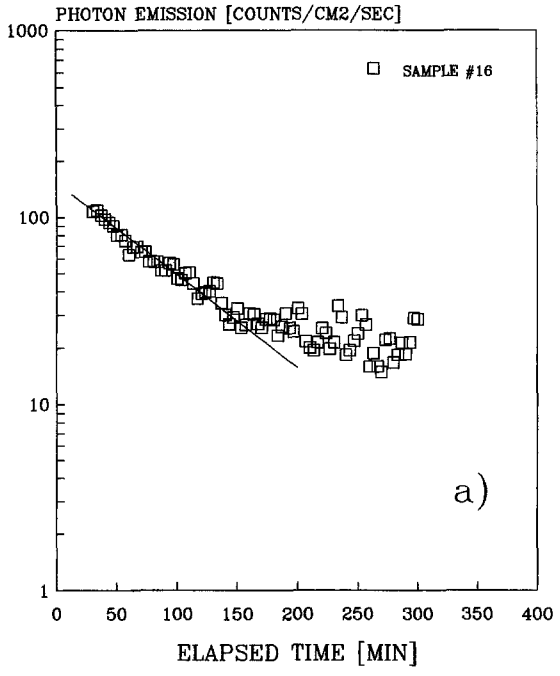
The results indicate that the two sub-sets have different populations. If they actually belong to the same distribution, the measured intensities of emission by the tumor samples should be distributed with equal probability below and above the average value for the non-tumor subset (22 photons/cm^2 min). However, in fact the rates for only 3 samples out of 16 fell below the average, whereas the rates for 13 samples were above. This difference remained even when the most unfavorable condition was considered, i.e. when the statistical error for the photon counting is subtracted from the measured value.

One problem was that the time intervals between surgical removal of the samples and measurement of emission ranged from a few minutes to a few hours. The time course of the change in emission was determined for a few samples. The emission decay of sample # 16 is shown in figure 2a, and it was found that the emission intensity decayed exponentially, with a measured half-life of 65 ± 10 min. Figure 2b shows, however, that the time-dispersion of the measurements affects both sets of samples in a similar way, and the differences cannot be due solely to differences in the time before measurements.

The experimental data show that some tumor samples (e.g. # 8, # 13 and # 17a) have a low emission rate which cannot be distinguished, within the limits of statistical error, from that of normal tissues. At present there is no evidence to show whether or not this is due to anomalous behavior. The present data are not sufficient to show whether there is a significant correlation between the degree of malignancy and the emission intensity. However, some tumor samples that emitted at a very low intensity (e.g. # 13) are definitely characterized by a low degree of malignancy, whereas some of those that emitted at high intensity are from highly malignant tumor tissues.

The histogram of the obtained results for normal and tumor samples is reported in figure 3.

The results demonstrate that many samples from tumors have a photon-emission rate higher than that of normal tissues, which allows them to be distinguished from nor-



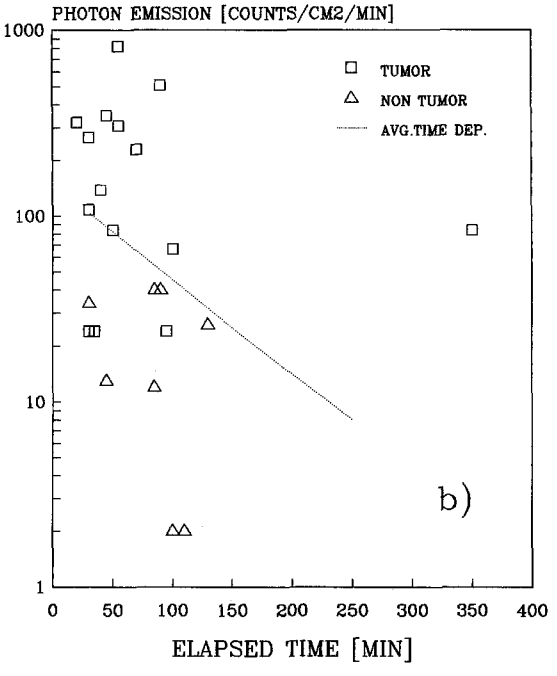


Figure 2. *a* Intensity of light emitted by a tumor sample vs time elapsed from the surgical removal; *b* Intensity of light emitted by normal (△) and tumor (□) samples vs time elapsed from surgical removal (see text). The dashed line represents the average measured decay.

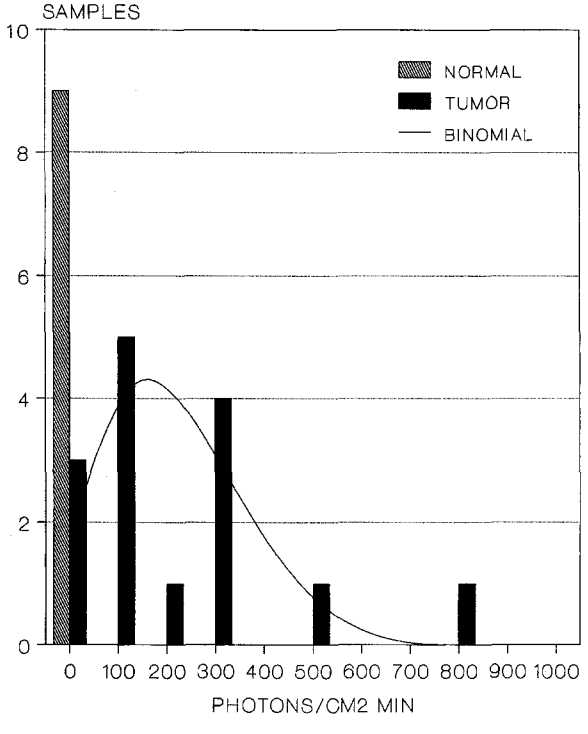


Figure 3. Histogram of the measured intensities for normal and tumor tissues; the line shows the binomial distribution, which has an average value of 220 photons/cm^2 min.

mal tissues. Clearly, a wider experimental basis is required to gain more insight into this phenomenon, but it is possible that photon emission could represent, in addition to the standard techniques, a simple and non-destructive analytical tool for tumor diagnosis.

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