Complex test bench for optical diagnostics of biosystems

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Instrumental test bench for study of absorption, scattering, and fluorescence properties of vegetation structures as well as processes of exchange of minor gas components and aerosol with atmosphere and phytocoenotype is described. Some new results obtained with the test bench are presented.

Monitoring of Earth's vegetation cover is one of the topical problems for predicting the sustainable development of our civilization. The globality of the subject dictates the use of remote, first optical, facilities for gathering information on the phytosystem state.

Remote sensing of vegetation is mainly based on measuring spectral and integral parameters of the radiation reflected from plants. Though these measurements are relatively easy, they are low-informative due to insufficient knowledge on the nature of reflectance spectra. New remote sensing techniques are working out lately on the base of full accounting for plant-absorbed energy transformation processes, manifesting themselves in particularly in the broadband fluorescence of pigments of the photosynthetic plant apparatus in visible and UV spectral ranges. A characteristic example is the chlorophyll fluorescence in living leaves, observable in two narrow bands at $\lambda = 685$ and 740 nm. It is necessary to admit that the correlation between optical properties and state of vegetation, masked by its seasonal behavior, are insufficiently studied. In particular, there are no physical models for correlation of parameters of optical signals, resulted from the phytosystem–radiation interaction, with commonly accepted structure-functional parameters of phytosystems.

Thus, finding the correlating bonds between vital functions of phytosystems and their optical parameters is of primary importance. The principal problems there are:

– the study of spectral transmission coefficient α, spectral and integral scattering phase functions of leaves in reflected and transmitted radiation β, working out of methods for determination of true absorption spectra τ of a living leaf as the whole;

– experimental determination of Stokes parameters of the radiation interacting with a living leaf; as well as the transfer matrix, carrying the information on a leaf as the light-scattering medium;

– finding correlations between the state of photosynthetic plant apparatus and fluorescent properties (spectral content, intensity, times of radiation building-up and damping, and so on) of large molecules participating in photosynthesis;

– finding correlation bonds between the state of a plant with the character of the plant–atmosphere gas exchange, in particular, with the emission and discharge of minor gas components of the atmosphere, as well as the nature and intensity of generation of aerosol fields over phytocoenotypes.

A unique instrumental setup for studying absorption, scattering, and fluorescent properties of vegetation structures was designed at the Institute of Atmospheric Optics SB RAS, which allows studying the exchange processes of minor gas components and aerosol between the atmosphere and phytocoenotypes as well. The setup includes a lidar system for measuring integral parameters of optical signals formed by plants and the ground air layer above them. Objects of study are plants specially grown in controllable conditions, corresponding to the aims of the study, as well as plants grown in natural conditions. The available instrumentation allows the solution of many above problems with uniformly obtained biomaterial, optimization of algorithms for processing the recorded signals, and interpretation of the found regularities.

The complex test bench was implemented into operation in 1998 by researchers of the IAO SB RAS and the Department of Biology and Soil Science of the Tomsk State University. Many experiments, carried out at the test bench, were supported by Russian Foundation for Basic Research (Grants Nos. 96–04–49150, 98–04–03099, and 99–04–49085) and the SB RAS Program "Atmospheric and Environmental Physics."

The setup includes the following devices:

1) lidar system for *in-situ* measurements of integral-over-crown fluorescent parameters of needle and foliage seedlings, grown in artificial experiment-specified conditions; the system also allows the study of plant samples of natural phytosystems;

2) lidar system for *in-situ* study of both reference and anthropogenic molecular-aerosol formations (caps) over separated vegetation areas, including the study

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of changes of the cap composition under controllable physico-chemical impacts on plants;

3) the spectrometer with an integrating sphere for laboratory studies of absorption, scattering, and fluorescent properties of the living plant material;

4) laser gas-analyzers for studying the gas exchange between the atmosphere and phytosystems at the sub-background level;

5) test plant nursery (phytotron), where plants are grown following the preset programs in controllable conditions for their further testing by standard means. There are two forest polygons for *in-situ* experiments.

The test bench as the whole and its components are used now both for individual and cooperation programs. It is a part of the free-access sharing center of the Tomsk Scientific Center SB RAS.

Below are sited the most interesting, in authors' opinion, results of experiments performed in 2005–2006.

1. Based on diode and CO_2 lasers, photoacoustic (PA) analyzers of minor gas components of atmosphere were designed, unique in the sensitivity and selectivity.[1] Their high concentration sensitivity is due to the use of PA detectors with resonance cells, including the differential Helmholtz resonator (DHR).

The gas analyzers allow the real-time analysis of the plant respiration at all stages of their development; all measurements are computerized. Parameters of the gas analyzers are given in Tables 1 and 2.

Table 1. Concentrations of molecular gases detectable by PA gas analyzers with near-IR diode lasers

Gas	Wavelength, μm	Absorption cross section, 10^{-22} cm^2	$C^{(1)}_{min}$, ppmV	$C^{(2)}_{min}$, ppmV
HF	1.330	820	0.45	0.03
HBr	1.341	118	3.2	0.2
H_2O	1.365	710	0.53	0.03
HI	1.541	10	37.2	2.3
CO	1.579	0.525	710	43
CO_2	1.579	0.54	690	40
CH_4	1.651	44.3	8.4	0.5
HCl	1.741	395	0.94	0.06
NO	2.67	36.5	10	0.6

Note. 1) Absorption cross sections were calculated by HITRAN-04 data for Lorentz profile. 2) $C^{(1)}_{min}$ and $C^{(2)}_{min}$ are the minimally detectable gas concentrations with a Knowless microphone (type EK1024) at a laser power of 1 mW for a single-pass and multipass PA DHR cells, respectively.[1] 3) The use of a more sensitive microphone (of Bruel&Kjaer company of the BK4144 type) allows approximately 30-fold decrease of $C^{(1)}_{min}$ and $C^{(2)}_{min}$ (Ref. 1).

The list of gases detectable by gas analyzers of such type comprises several tens.[2] As an example, Tables 1 and 2 present main biogenic (CO_2, CH_4, NH_3) and most wide-spread contaminants. Data on minimal concentrations were obtained when fitting gas analyzers with PA detectors of the same type (in case of CO_2 laser – only a single-pass variant).

Table 2. Gas concentrations detectable by PA gas analyzers in the CO_2-laser lasing range

Gas	Wave number, cm^{-1}	Absorption cross section, 10^{-20} cm^2	C_{min}, ppbV·W
C_2H_4	949.48	170	0.02
CH_3OH	1033.48	104	0.04
C_2H_3OH	1057.30	31.8	0.12
C_6H_6	1037.43	11.1	0.34
NH_3	1084.63	319	0.01
C_2H_3Cl	942.38	27.1	0.14
C_6H_3Cl	1084.63	12.9	0.30
C_7H_8	1031.47	3.99	0.95
C_5H_8	929.97	3.1	1.20
C_8H_{10}	1031.47	2.25	1.70
C_3H_6O	920.83	1.26	3.00
C_2H_5Br	944.19	0.515	7.20
CH_3Cl	920.83	0.167	22.3

To illustrate the technical and analytical potentialities of the laser PA gas-analyzers, the data on dark respiration of Siberian stone pine needles, obtained from analysis of the absorption spectra of gas samples within the lasing range of a CO_2 laser (gas analyzer emitter), are shown in Fig. 1.

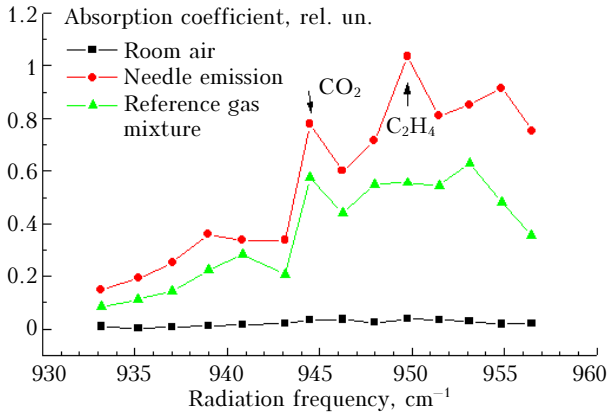

Fig. 1. Gas absorption spectra within the CO_2-laser lasing range (*P*-branch of the 10 μm band).

A biogenic gas sample was prepared from needles separated from branches. Needle samples (10 g) were placed into a shadowy pressurized vessel of 0.5 l in volume filled with air. After a 3-hour dark exposure, the gas was taken from the vessel and then the measuring cell of the gas-analyzer was blown through by this gas.

The absorption spectrum of the obtained gas sample is shown in Fig. 1, as well as prerecorded spectra of the reference gas mixture CO_2–N_2 (CO_2 concentration is 5000 ppm), and of the room air filling the vessel before placing there samples.

When considering the observed variations of the gas composition in the vessel as manifestations of dark respiration of needles under the experimental conditions, note, that it is accompanied by emission of not only carbon dioxide (10*P*(20) line; 944.194 cm^{-1}) but a significant amount of ethylene (its characteristic

absorption peak is at $10P(14)$ CO_2-lasing line; 949.479 cm^{-1}, Fig. 1).

2. The next experiment was aimed at studying of such stressor as the disturbance in the mineral nutrition of trees causing changes in their optical parameters. Soil, where five-year plants of the Siberian stone pine were growing, was polluted by oil; optical parameters of tree crowns and the chlorophyll concentration in needles as a function of such pollution were under study. Concentration of chlorophyll and carotenoids in a photosynthesizing cell is very important indicator of the plant response to changes in root nutrition and the degree of plant adaptation to new conditions.[3,4] Siberian stone pine samples were grown in special polyethylene vessels placed at a height of 3.5 m into a specially purposed box 70-m distant from a lidar. Such arrangement allowed sufficiently high averaging of laser-induced fluorescence (LIF) sources over the tree tops. The LIF was measured with the fluorescent lidar,[5] the ratio of Siberian stone pine needle fluorescence signals $f = E(685\ \text{nm})/E(740\ \text{nm})$ was determined in two bands of 2 nm in width centered at 685 and 740 nm.[6] The chlorophyll concentration in needles was measured by the spectrophotometric method. The Siberian stone pine was chosen because of high resistance of conifers to atmospheric and hydrological changes.

Instrumental measurements were carried out during 40 days (from the middle of July to the end of August), visual observation continued a month longer, to the end of September. Siberian stone pines, grown in the similar soil, were divided in two groups by four plants in each. The soil of the experimental group was periodically (10-day period) polluted. The LIF of needles in both plant groups was measured 3 days after polluting the soil of the experimental group. The chlorophyll concentration in experimental and reference needle samples was measured at the end of each time period.

No visual distinctions between plants of two groups were observed during the first observation period, while the fluorescence response of experimental samples increased more than 1.5 times to the end of the first period (f changed from 1.82 to 2.94). This difference is seemingly caused by primary disturbances in the electron-transport photosynthesis circuit of experimental samples: the increase of the fluorescence quantum efficiency in this case is caused by the decrease of the efficiency of initial photosynthesis processes, i.e., absorbed light energy is not used in photosynthesis, therefore fluorescence intensity increases. A sharp decrease of f occurred during the second period (Fig. 2) due to plants adaptation to the stressor. Visually observed changes, i.e., yellow needles in the understory were observed on trees of the experimental group during further oil pollution (to the end of the third period). The fluorescence response of the experimental samples decreased due to, on the one hand, partial destruction of chlorophyll and, on the other hand, corresponding adaptation of the plants to new root nutrition conditions.

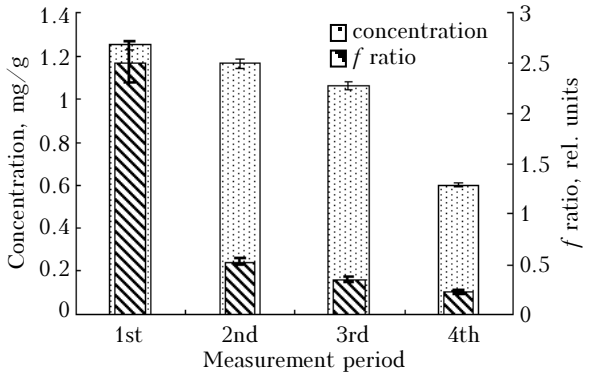

Fig. 2. Ratio of the fluorescence signals $f = E(685\ \text{nm})/E(740\ \text{nm})$ and chlorophyll concentration for plants of the experimental group during four measurement periods after first oil pollution of the soil.

By the end of the fourth measurement period, plants of the experimental group essentially differed visually from those of the reference group. Apparently, the level of the stressor (oil pollution) exceeded adaptive abilities of the Siberian stone pine, which resulted in its irreversible degradation.[7]

The variation range of the ratio of fluorescence signals in conditions of standard root nutrition corresponded to the standard vegetation behavior in July and August.

Since the remote LIF methods are meant in future to detect pre-visual stages of disturbances in vital functions of plants, the fluorescence spectra of Siberian stone pine crowns were recorded, related to the first period (Fig. 3).

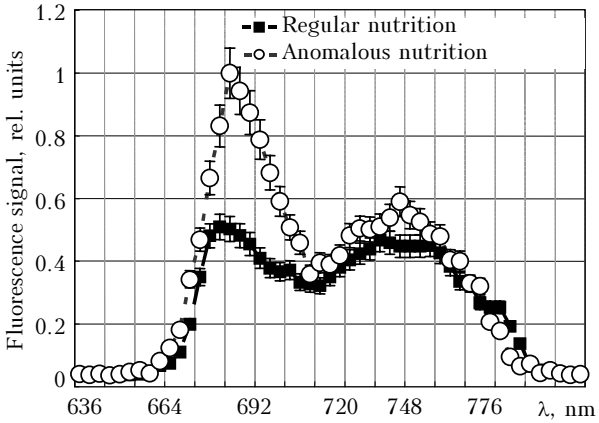

Fig. 3. Fluorescence spectra of the reference and experimental groups of plants.

The spectra were obtained through the wavelength-scanning of fluorescent lidar signals. The analysis of the obtained spectra shows significant variations of fluorescence values in the 685 nm band as compared to 740 nm. Such redistribution of signal intensity inside the spectrum is seemingly due to the decrease of efficiency of photosynthesis processes.

Experimental results of this work are in good agreement with the obtained earlier LIF measurement

results related to the marcescent conifers and foliage trees at Western Siberia.[8]

3. Along with model experiments, the *in-situ* experiments on pigment composition and the state of photosynthetic apparatus of plants were conducted. The evergreen plants were growing in natural mountain ecosystems of the Gorno-Altaisk region. Though the region is of low ecological tension, there are air-polluted areas: urbanized territories, highways with heavy traffic, regions impacted by launching rockets from Baikonur, and so on. As the plant-bioindicators, Siberian spruce, Common pine, and Siberian stone pine were used. Pigment composition was determined spectrophotometrically (Fig. 4), the state of photosynthetic apparatus — by the time of plant termination of their hibernation.

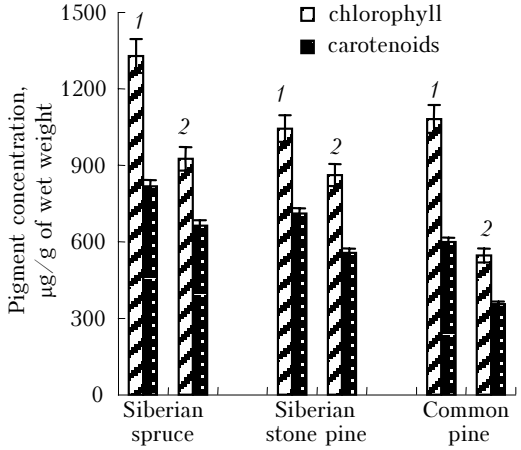

Fig. 4. The effect of air pollution on the concentration of photosynthetic pigments in needles of trees: grown in clear atmosphere (*1*) polluted (urban conditions) (*2*).

The concentration of chlorophyll *a* and *b* for all plant species under study was 25–100% higher in standard conditions then in polluted (see Fig. 4). The pigment ratio *a*/*b* also differed for reference and experimental plants (this is evident from fluorescence spectra as well, see item 2), which says about profound changes in chlorophyll–protein complexes of chloroplast membranes under the effect of air pollution. For trees growing in urban conditions, along with green pigments, the carotenoid pool was on the average 1.4 times lower. Besides, the reaction of the needle pigment complex to the chemical air pollution strongly depends on the species-specificity. The ratio of sum of green pigments to the sum of yellow ones decreased to 15–20% for Siberian spruce and the Common pine while it remained stable within the measurement error for Siberian stone pine. In general, Common pine turned out to be less pollution-resistant in comparison with Siberian spruce and Siberian stone pine. Therefore, it appears to be interesting to carry out the above-described experiments (item 2) with Common pine and Siberian spruce.

4. To increase the information content of remote methods for vegetation observation, the technique for measuring reflection spectra was worked out and tested on a series of living plant structures, since the detailed study of reflection and absorption spectra of phytostructures is an essential stage in working out optical remote methods for their study.

The integrating photometer technique (integrating sphere) was used for measuring spectra of absorption and diffusive reflection of phytostructures within 680–750 nm range. The integrating photometer, accounting for all the radiation scattered by a sample under study, allows physically correct measurements of the optical reflection coefficient K_{samp}, transmittance α, and absorptivity τ of complex natural structures, such as plant leaves and other light-scattering objects. Meters of such type are widely used in biophysical researches.[9–11]

The scheme of the integrating sphere setup for measuring absorption and diffusive-reflection spectra is shown in Fig. 5 (to measure an absorption spectrum, the sample is mounted at the inlet of the sphere). A sphere of 110 mm in diameter ("Carl Zeiss Jena") was used.

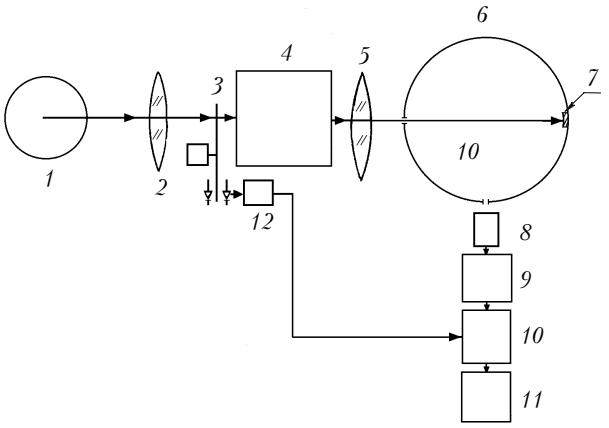

Fig. 5. A scheme of the setup for measuring diffusive reflection coefficient: radiation source (heat lamp) (*1*); consistent optical system (*2*); mechanical modulator (modulation frequency is 800 Hz) (*3*); MDR-12 monochromator (*4*); collimating lens (*5*); integrating sphere (*6*); sample (*7*); photoreceiver (*8*); ac amplifier (*9*); synchronous detector (*10*); automatic recorder (*11*); and timing sensor (*12*).

The integrating sphere was calibrated using two reference reflectors, which were the standards of white and black bodies. We used "white" model of a SF-4 device preliminary removing the blanket of the model's facing material (pressed MgF_2) by a diamond stylus. We assumed that the reflection coefficient of the thus-renewed model was close to maximal for this material, i.e., 95%. As a blackbody standard was taken the standard of "Carl Zeiss Jena" company (an inky velvet) with the reflection coefficient 5% — minimally known for "blackbodies".

As plant samples, we took leaves of birch, lilac, broad-leaf indoor plants, as well as conifers (Siberian stone pine, Siberian spruce, and Common pine), which were used in 5–10 min after separation from the plant.

The sequence of measurement procedures was used, worked out earlier, when measuring diffusive

reflection coefficients of natural and artificial materials in the UV-range. Photomultiplier signals U_w (of the white standard), U_b (of the blackbody one), and $U_{samp.}$ (of a sample under study) were recorded consequently at every wavelength from a chosen range. The reflection coefficient was obtained from the evident equation

$$K_{samp.} = K_b + (K_w - K_b)\frac{U_{samp.} - U_b}{U_w - U_b},$$

where K_b, K_w, $K_{samp.}$ are the reflection coefficients of the black and white standards, as well as a sample. U signals were counted from the zero signal level corresponding to the automated recorder indication when screening the lamp light.

Figure 6 shows the measured reflection coefficients for foliage trees and conifers in the 600–750 nm range.

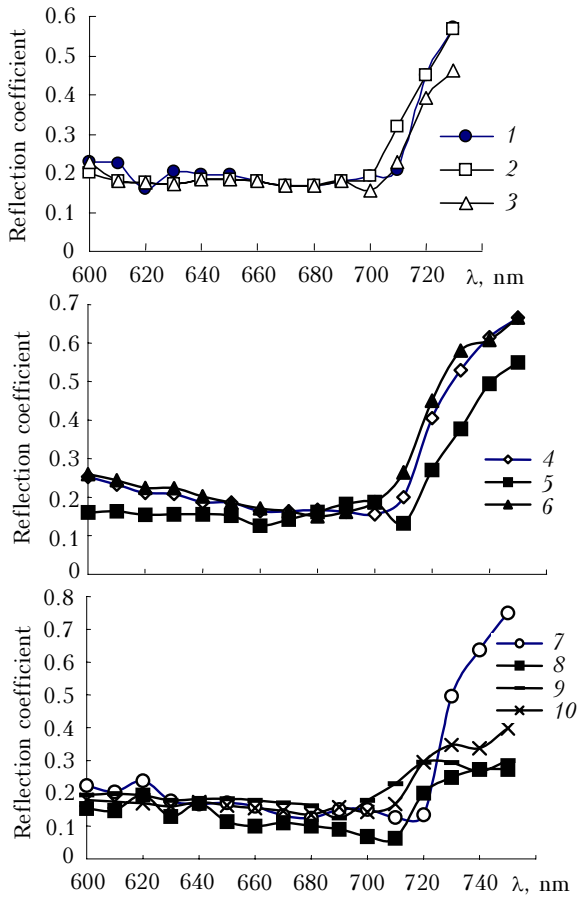

Fig. 6. Spectral behavior of the reflection coefficient $K_{samp.}$ of birch (*1–3*), lilac (*4*), indoor plant (*5*), larch (*6*, *7*), Siberian stone pine (*8*), Siberian spruce (*9*), and Common pine (*10*).

As it follows from the measurement results for diffusive reflection coefficients of the studied leaf materials, the shift of long-wave absorption limit of conifers is well pronounced as compared to foliage trees. This indicates the dependence of the chlorophyll absorption spectrum on molecular surroundings; hence, the position of the long-wave absorption limit carries information on the density and the state of molecular components, surrounding phytostructures. To justify this conclusion experimentally, it is necessary to study the long-wave limit as a function of the plant state.

The comparison of the reflection spectra with the observing LIF shows that LIF at 685 nm is absorbed and scattered while at 740 nm it is only scattered. Hence, LIF at 685 and 740 nm can be considered as the unique spectroscopic pair for determination of the pigment density in leaves by the differential method. By analogy with the differential absorption method of the laser sensing, this method can be called the method of differential absorption of fluorescence in laser sensing of vegetation covers.

Acknowledgements

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