

Towards Ultraviolet Microbeam Scanning and Lens-Less UV Microbeam Microscopy with Mirror Galvanometric Scanners: From the History of Research Instrumentation to Engineering of Modern Mechatronic Optical Systems

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Abstract. This article aims to ensure continuity between classical methods of ultraviolet microscopy or/and micromanipulation using ultraviolet microbeam and lens-less ultraviolet microscopy and microbeam exposure of cells and tissues. Considering the history of the development of the method and the possibility of working with different methods of mechanical scanning, the authors propose to use mirror galvanometers and an electromechanical scanning system in the mechanical engineering of lensless microbeam installations. These installations make it possible to provide both scanning with an ultraviolet microbeam to obtain a line scan image, and precision micromanipulation at the level of individual cells or individual organelles (in the case of large cells). We propose to extrapolate mathematical models previously developed for galvanic mirrors of light-beam oscilloscopes for microbeam scanning systems, position-sensitive micromanipulation, and real-time microphotometric cell analysis.

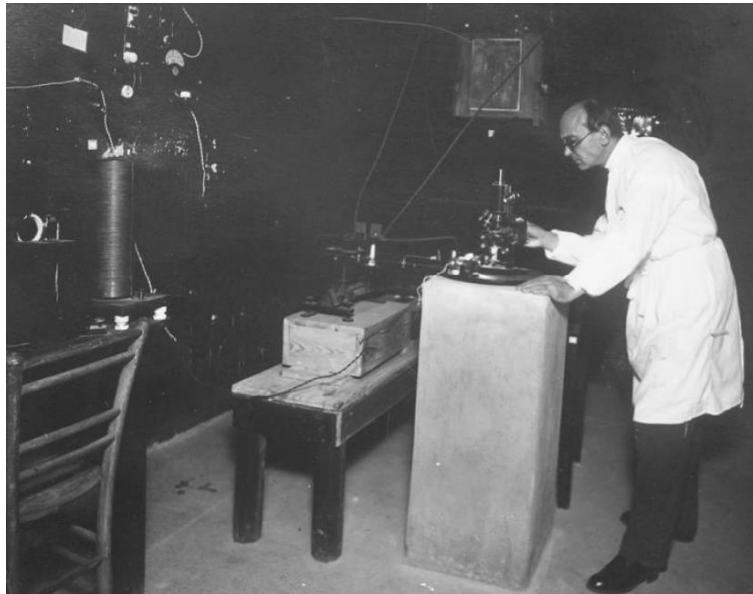
Keywords: ultraviolet microbeam, mirror galvanometer, galvanometric scanners, radiopuncture microscopy, lens-less FRAP, lens-less FLIP.

1 Introduction.

1.1 The History and Emergence of Chakhotin UV Beam Method

As Chakhotin's pioneering works about exposing cells [1-3], organelles [4,5] and cytoplasmic colloids providing phase physiochemical cell morphology adaptability [6, 7] to UV emission showed, the ultraviolet microbeam is an efficient and accurate tool for solving experimental cytology and morphology problems [8]. The author originally called it "UV radiopuncture methods" [2,9]. However, in one of his papers (in *Compt. Rend. Soc. Biol.*, 1920) he calls it "radiopuncture microscopie". Perhaps he meant that the UV irradiation effect and the microscopic observation of cell changes in UV microbeams are simultaneous. Chakhotin's "radiopuncture" should not be confused with the radium radiopuncture method that was common in the same period. Subsequently, other researchers working with UV irradiation in experimental morphology called it the same, including Anne Dürken [10]. She researched UV

irradiation effects on newt sensory system morphogenesis. These studies were initiated by Bernhard Dürken [11], the German experimental zoomorphology founder, in the first half of the 20th century. It turned out that the UV irradiation applications are much more extensive than Chakhotin believed in his first works.



a



b

Fig. 2: S. Chakhotin with his microbeam setups: a) in the laboratory of the Max Planck Institute in Germany, 1931; b) in the Laboratory of Prof. Pietro Di Mattei at the Pharmacological Institute in Rome, 1957 (from the Posudin's book).

In the 1930s he demonstrated that a UV beam could induce parthenogenesis in invertebrate (and the morphogenesis, as a result) [12-14]. About at the same time, his studies found the correlation between the biomorphology changes induced by the beam (as much as UV beams were applicable to ultramicroscopy of colloid particles or cytoplasm organoids [6,7] and ultramorphology) and the functional or physiological changes. This enabled the microbeam to be applied to morphophysiological and functional morphology research with great accuracy for that period (Fig. 1 - Fig. 3).

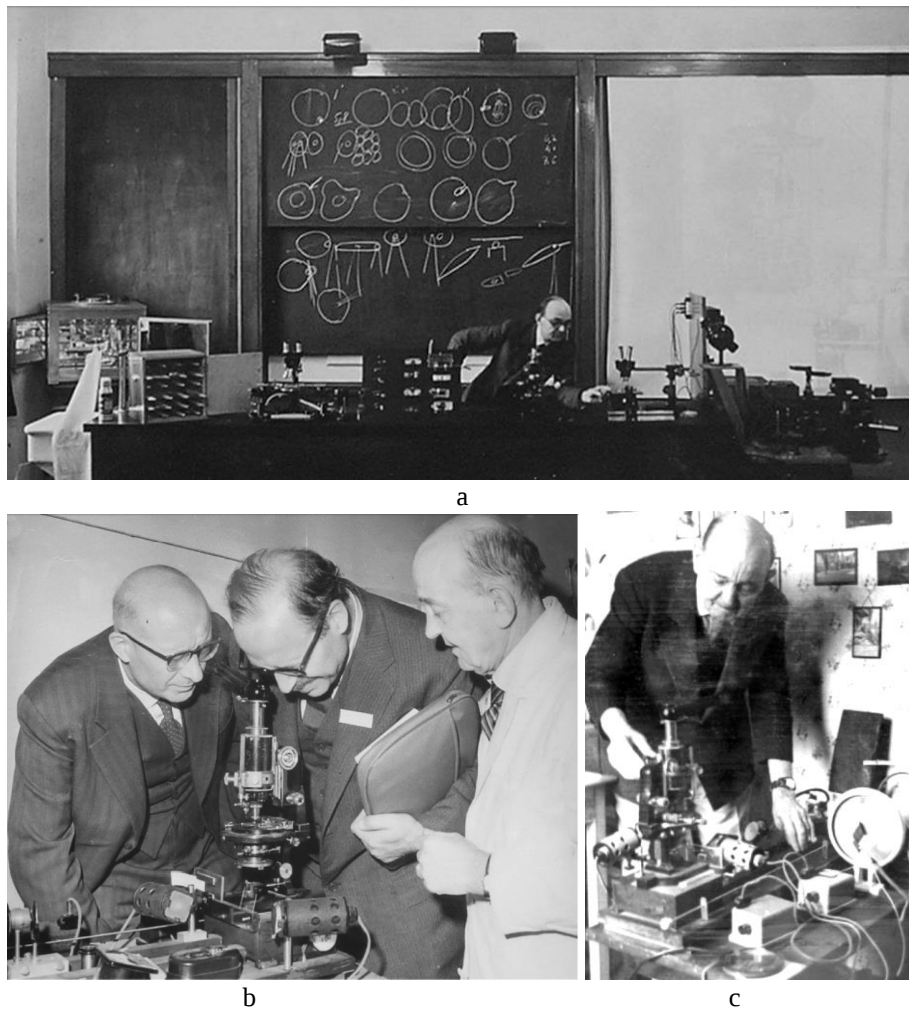


Fig. 2. S. Chakhotin demonstrating his microbeam experiments: a) in Heidelberg, 1929; b,c) in Moscow, 1960th.

These works include daphnia heart rate suppression by the UV beam [15], the UV microbeam effects on myonemes (myonemes are similar to myofibrils, and they also have a smooth or striated thready muscle structure) of *Campanularia* [17] in

subcellular level, and other UV beam micro operations on protozoans [18]. Chakhotin's experiment skills were unparalleled because even nowadays the daphnia heart rate suppression problem cannot be solved without interferometric autodyne detection tools [16]. Micro manipulations with individual cell populations were also introduced [19].

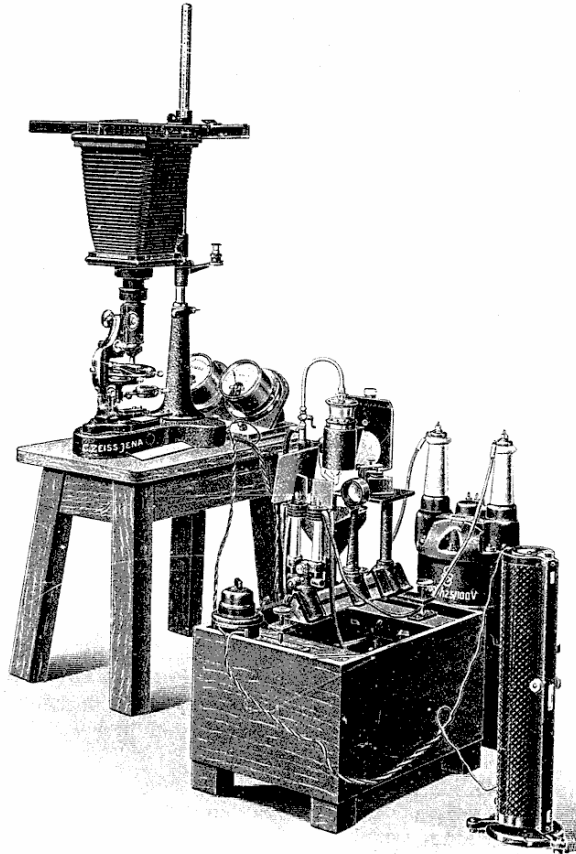


Fig. 3. S. Chakhotin experimental system for cell micro-irradiation.

Chakhotin was working at Wilhelm Röntgen's labs for a long time. He managed to handle the methodology of physical interpretation of experimental results, that is why his physiological-morphological results were always accompanied by physical-chemical and biophysical explanations. In that way, he was ahead of the research conducted in the first third of the 20th century, because biophysical morphology as a separate field emerged only in the last quarter of the century. Internationally, the emergence is commonly attributed to the *Techniques of Biochemical and Biophysical Morphology* monograph by David M. Glick, Robert M. Rosenbaum (Editors) published in the 1970s. The cytoplasm reaction in Chakhotin's experiments to UV irradiation was associated with pH and membrane permeability [20] following the physical reductionism principles. The cell hydrostatic reaction was described in

terms of the imbalance between the cytoplasm and the vacuolar system of the cell [21].

The method proposed by S. Chakhotin subsequently was adopted in various biology areas and turned into a multi-discipline tool. A UV micro-beam scanning TV system developed in 1962 [22] enabled digital processing and storing of acquired morphological data. Later, as semiconductors replaced vacuum tubes in 1970s, such systems became simpler and more affordable [23], which contributed to their extensive adoption. While the method was mesoscopic in terms of the exposure magnitude and beam detection, mostly tissue and histochemical effects were studied. Particularly, in 1960s experimental exposures of muscles [24] and tissue cultures [25] to the microbeam were common. For the same reason, mesoscopic experiments with relatively large *Ciliata Euplotes* species continued. For instance, the UV beam effect on cell morphogenesis was studied [26].



Fig. 4. Contemporary computerized version of the UV microbeam setup based on ZEISS OPTON photomicroscope with optical waveguide spectrometer, UV microspectrometer, ZEISS OPTON micromanipulator and computer-assisted data acquisition system. Semenov Institute of Chemical Physics, Gradov's multiparametric microscopy infrastructure, 2018.

Still, as early as at the end of 1950s, the research began to be more and more focused on multi-beam applications for cytogenetic, karyomorphological, and mitotic studies, and for the associated experimental morphology studies. The effects and contribution of nucleoli on the mitosis process were studied with an UV micro-beam in 1958 [27]; in the 1960s the research was continued and verified by another team [28]; later they switched to cell membrane studies not related to genetics [29]. The UV micro-beam effects on nucleoli were studied through 1970s, and 1980s [30, 31] (see Fig. 4, Fig. 5).

Since the 1960s spindle apparatus microbeam effect studies were undertaken but in the first works the additive physical methods allowed to register only optical changes, for example, the spindle apparatus birefringent effects [32]. Subsequently, the studies in this field became more extensive. In 1970, cytomorphological effects were considered and interpreted as “false anaphase”, true anaphase delay, and the spindle apparatus destruction / disorganization [33]. Then, in the 1980s the spindle apparatus extension under the UV beam was observed [34]. After introducing the novel methods for working with single microtubules *in vitro*, the microtubule asymmetric behavior and destruction under UV microbeam was registered [35]. The action spectrum of the beam for this case [36] was studied, and then the forces emerging from the spindle mitotic dynamics were assessed [37]. Some research teams continued to study protozoa [34,38,39] in the 1970-1980s, but more of them focused on histological test objects (tissue sections, bacterial cultures, grafts in isolated chambers, etc.) However, it was not related to the additive effects on these objects, but due to the equipment that enabled working with the relatively small cells.



Fig. 5. Ultraviolet (micro)beam setup prototype based on nitrogen laser ILGI-503 and scanning microspectrophotometer MSPhP (Inst. En. Prob. Chem. Phys., 2016).

At the end of the 1980s, technically feasible UV microbeam (almost DIY) equipment based on commercially available components [22] became common. Cheap UV lasers with different wavelengths, for example, nitrogen or helium–cadmium ones, enabled to study chromatin photodegradation and genetic material photoreactivation [40] in a single experiment in a static object using the same experimental facility. The first laser UV microbeams entered the microscopic practice in the early 1970s [41]. The above prerequisites made it possible to operate with extremely narrow, highly coherent beams and resulted in UV subcellular microdissection introduced by S. Chakhotin. This method was expected to boost in the 1990s.

However, the non-coherent UV equipment was still used during the massive change-over to lasers (even in 1991, the technical support documentation for lamp-based microbeam experiments using monochromators and filters was still published). This is due to the fact that lamp-based microbeam sources with monochromators possess a wider wavelength range than the laser ones, and hence, lead to the various biological effects under different optical parameters of the microbeam [42]. At the beginning of the 21st century, more compact designs based on laser diodes and DPSSL, as well as compact gas lasers were implemented (O.V. Gradov, GEOKHI RAS, 2010 – Fig. 6a; Andrew Skrynnik, 2017, based on inverted phase-contrast microscope by the Gradov's idea, INEPCP RAS, Gradov's group – Fig. 6b).



Fig. 6. Contemporary Russian compact microbeam setups for field and (auto-)mobile lab conditions: a) combined laser diode / DPSSL compact microscope for hydrobiochemical / geobiochemical and hydrobiological microbeam investigations (GEOKHI RAS, Oleg Gradov, 2010); b) HeNe laser microbeam setup based on inverted phase-contrast microscope (INEPCP RAS, Andrew Skrynnik, 2017).

The research trends of the 1990s resulting from the emergence of the above UV lasers and laser systems should be briefly reviewed. First of all, the studies of microbeam effects on the microtubules and genetic material were continued. In particular, Soviet researcher Uzbekov's team explored the postmitotic nucleolus reconstruction in cell culture during centrosome microbeam photoinactivation [43]. The centrosome defect in mitosis induced by the microbeam [44] was considered as a special case of the mitotic system dysfunction under UV microbeam irradiation of the spindle apparatus [45]. The same studies were carried out outside of the USSR. For

example, Forer's team studied epithelium cells and spermatocytes, but they were focused on UV effects on kinetochore considering it as a protein structure that fastened the spindle apparatus fibers to the chromosome during the mitosis [46,47,48,49].



Fig. 7. Computerized multi-module multi-wavelength UV microbeam setup with two possible cooling pathways (O.V. Gradov, Research Center of Neurology, Brain Research Department, Laboratory of Neural Brain Structure, Moscow, 2012-2013).

These studies continue the cytogenetic works in the UV microbeam method which were successfully performed by the Soviet scientists in it in the 1970s [50]. They also developed the interphasic control methods with UV microbeams. These methods started to be developed in the 1960s through Amenta's team classic studies on the UV microbeam irradiation on *Triturus viridescens* granule and dysgranule cells [51,52]. Such works are beyond the molecular-genetic trend in the UV microbeam method development that was presented mostly by the immunological and virological papers [53], and rather belong to experimental ultramorphology. The possibility to detect irradiated areas after the cell treatment with uranyl acetate enables to map the irradiated sites and cellular compartments depending on their sensitivity or resistance. This method was common in the 1990s [54]. In our group, proto-

types of systems for synchronous manipulations on cells and microphotometry or luminometry were created (see Fig. 7, Fig. 8). In particular, similar experiments were planned in gas microchambers (Fig. 9), which, however, did not happen due to the budget deficit and staff leaving.



Fig. 8. Prototype of the ultraviolet microscope and ultraviolet microbeam setup in the external fields in the Faraday cage rag (based on mobile luminescence / fluorescence microscope MLD, multi-wavelength laser photometer FM-01 and photomultiplier-assisted quantometer, which also was used in the prototype UV setup on the Fig. 5). Project initiator: F. Orekhov (Gradov's Biophysical instrumentation group, 2017).

UV microbeam irradiation according to the Chakhotin's experimental methodology is a modern high-tech experimental approach based on the effectively focused laser systems. It is compatible with the probe and atomic-force microscopy [55], lab-on-a-chip techniques [56], optical tweezers and manipulators [57], systems for synchronous chemical and morphological control of the free radical species [58], immunofluorescence methods and tools for radiobiological / photobiological comparative studies [59]. The microbeam applications to the experimental morphological studies have been expanding. In the 1970-1980s the microbeam effects were macroscopic or mesoscopic with the morphological results achieved by the direct effects on the organism or its embryo [60-62], while nowadays the same results are achieved with a "molecular resolution" by genetic switches or changes in the membrane ion channel operation. In neuromorphology, where UV microbeams were used for control and inhibition of the cell and tissue differentiation since the 1980s [63], an optogenetic trend has emerged which is based on the optical stimulation of the light-gated ion channels embedded into the cell membrane (e.g., opsins) with the genetic engineering methods used to express such channels. There are known several methods of pulse near-ultraviolet (UV-A) irradiation for optogenetic research [64,65] and the related areas [66] (consider the reference list in the review paper [67] for more in-

formation on this). This novel tool for genetic research drastically differs from the laser UV beam induced DNA introduction into plant cells popular in the 1980s [68], as well as from chromosome / kariogenetic experiments with UV microbeams in animal cells conducted at the same period [69].



Fig. 9. Project of UV experimental setup with gaseous chamber for experimental investigations in coacervate morphogenesis with microspectral and electrophysical monitoring with Fourier transform based signal analysis and spectral morphometry (Gradov's Biophysical instrumentation group, 2016; F. Orekhov, 2015-2016; this project was cancelled by the mass spectrometric lab management in 2016).

2 UV Microbeam Positioning and Focusing Devices Similar to Galvanometric Scanners

Considering the capabilities described above, the UV microbeam method should be introduced in many areas of biology where its application seems to be rational. However, despite the fact that UV light sources, including lamps, are affordable, it does not happen due to the inaccessibility of the other components of the installation required. Devices for accurate UV microbeam positioning required for a wide cytological, histological, embryological, and experimental-morphological application of UV microbeam technique possess high cost and low commercial availability. A modern microbeam facility is not only the optics, but also the accurate micro-electromechanical and computer-based adaptive control systems that manipulate the beam with a high speed and micron / sub-micron accuracy. Nowadays, galvanometric mirrors / scanners [70-72] are used in experimental biology research for these purposes. Their scanning rate is up to a kilohertz, and they allow to operate with fine micro-

structures, and even perform laser transfection [73,74] or laser cloning [75] by membrane fusion [76].

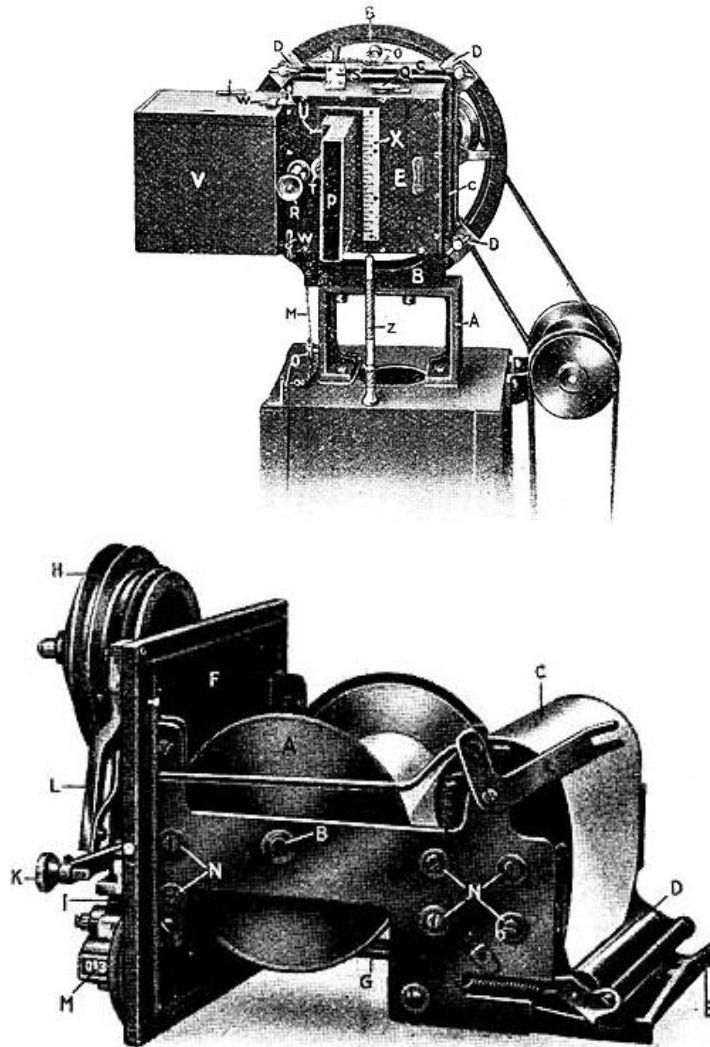


Fig. 10: A typical Photo-Kymograph. The first half of the 20th century: designed by Prof. Broemser (Photo-Kymographion nach Prof. Broemser), Image courtesy: Jaquet J. Jaquet Apparatus: Catalogue. No. 503: a: p. 51, fig. 1022; b: p. 0052, fig. 1022a.

The galvanometric scanner operates as follows: the mirror head rotates about two axes and changes the beam incident angle on the sample controlled by electric signal with the the computer-generated waveform. The higher the control signal frequency, the higher the scanning rate. However, the higher the frequency, the more expensive the galvanometric reflector is. For this reason, in developing countries

lower scanning rates are often used. For many cytophysiological researchers, this is an ultimate obstacle preventing them from experiments with the UV microbeam. Nevertheless, bifilar / light-beam oscillographs with movable galvanometers integrated into the recorder cassette are commonly used. These are galvanometric scanners that are controlled not by a computer over a dedicated controller but by any signal supplied to the analog input of the light-beam oscillograph. When the sequences of signals / pulses generated by the DAC are used as a “control program” to generate the required light beam trajectories, the galvanometer modules of the old light-beam oscillographs can be used to scan microscopic slides / tissue cultures with a UV beam created by a Hg UV lamp integrated into the oscillograph housing (see below).

It should be noted that the application of light-beam (or bifilar) oscillographs to biomedical research and the development of dedicated measurement devices for practical medicine were drastically accelerated as early as at the beginning of the 20th century as the vacuum tube technology was improved, and further intensified by the 1930s. In Germany, this approach was developed in the most comprehensive way. As early as in 1930 the nerve electric currents (“nervenaktionsströme”) were studied with bifilar (“schleifenszillograph”) oscillographs [77], and in 1931 the vacuum tube and light-beam registration technology were used for physiological acoustics studies [78] and acoustic analysis of breath sounds [79]. In 1932 for the first time heart tones were recorded by a light-beam oscillograph on photosensitive paper [80]. In 1935 for the first time, a light-beam oscillograph was applied to study an acoustic stimulation of the vestibular system [81], while in 1938 the same device was used to register the oscillations of vibrating vocal cords with a setup based on a sensitive photoelement [82]. In the same year, Richter published a paper that presented the advantages of Brown tube-based oscillographs over other bioelectric potential registration methods [83]. The paper was welcomed by the medical community. After that (post hoc non est propter hoc) as early as in 1939 the first study of electrophysiological brain activity measurement errors was published. It noted that due to the physical principles of signal registration, light-beam oscillographs were not free of electrophysiological signal recording errors [84].

A detailed study of the available references in chronological order showed that the first international papers about the possible errors of light-beam recording and bifilar oscillograph registration techniques as applied to electrophysiology and neurophysiology were published more than 30 years earlier than the first comprehensive works by the Soviet researchers who analyzed such errors in a general case [85-87]. However, through the first half of the 20th century, the bifilar oscillograph remained a high precision electrophysiological activity analyzer. For a long time, they were used for electrophysiological research abroad. Furthermore, at least till the end of WWII Germany was a leader in the number of research papers where this method was used. In 1940 in Germany they registered muscle tissue electrophysiological activity [88]. Afterward, except for several insignificant papers that mentioned this type of oscillographs, till the second half of the 1940s, the research with this method slowed down, and the number of such papers in German drastically reduced. Only in 1948, the first large post-war research that used a bifilar oscillograph to register induced nystagmus was published [89] and several works on biomechanical dynamometry have also been published [90]. An electroscritograph based on a bifilar oscillo-

graph for psychophysiology [91], a registration system for determining the actual intrathoracic pressure [92] and observing tetanus caused by damaging of a frog heart [93] using a bifilar oscillograph were developed in 1950s. Then the bifilar oscillograph as it had existed since the 1920s ceased to be used for lab research and was replaced with the new light-beam oscillographs.

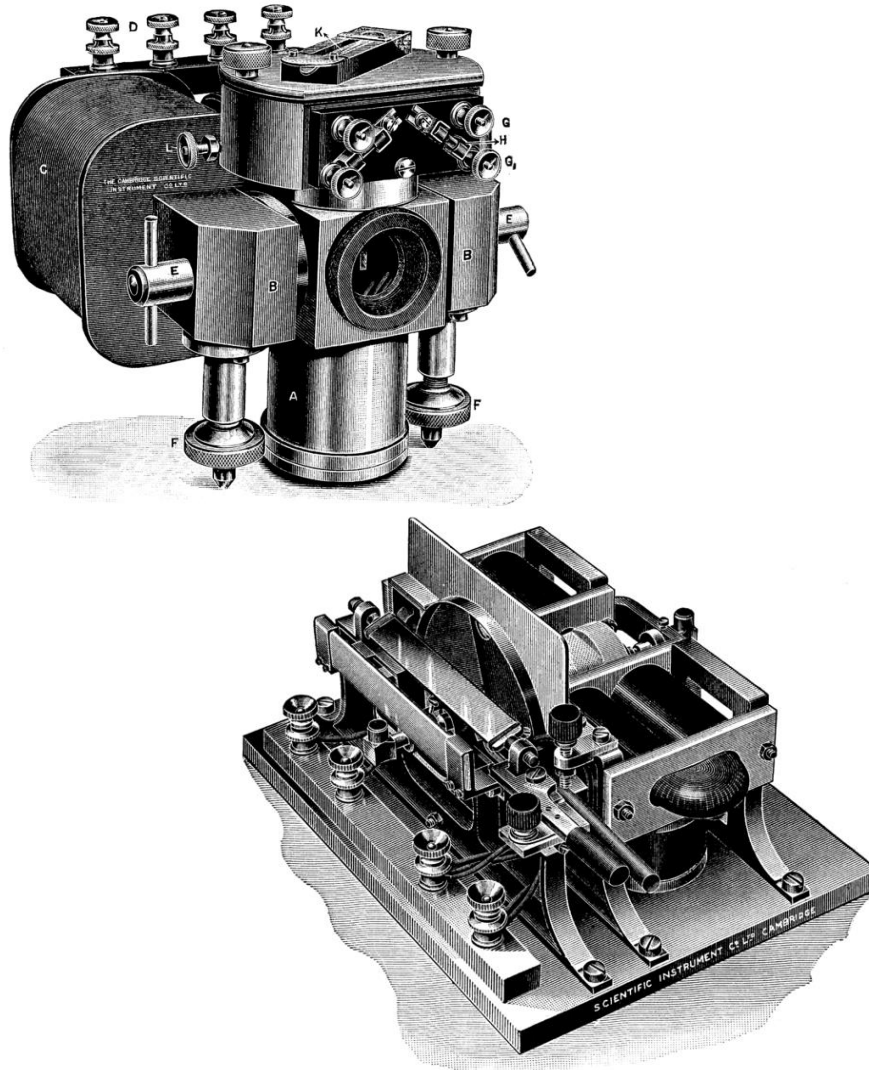


Fig. 11. Duddell moving-coil oscillograph components (1917). Image courtesy: Hawkins Electrical Guide, Vol. 6, Chapter 63: Wave Form Measurement.

It should be noted that the term “light-beam oscillograph” (“lichtstrahloszillograph”) first appeared in German literature not before 1952 in an international journal review [94]. As to commonly available lab literature, it is first found in the Registri-

erinstrumente handbook, 1959, and Meßzeuge und Meßanordnungen, 1965. No wonder the term entered medical and biological practice only in the early 1970s. Initially, light-beam oscillographs were introduced in West Germany as a histochemical instrument for the fast decay fluorescence spectroscopy [95]. It was probably the first reference to a light-beam oscillograph application in biomedical chemistry since the reference to a bifilar oscillograph application to studying blood alkali reserve in 1931 [96]. Subsequently, many works in biomechanics [97, 98], bioacoustics, and ENT [99] also used the instrument that strengthened its positions in the lab practice.

One of the “most biological” devices based on the light-beam principles and components was the photo-kymograph (refer to Fig. 10). The photo-kymograph was quite popular in Soviet research at its time (1950-1960s). It is confirmed by an abundance of photo-kymography experimental research papers published just in *Bulletin of Experimental Biology and Medicine* over that period (e.g., [100-106]). In 1963 in the USSR they developed a photographic accessory for cathode-tube oscilloscopes to register the visualized signals similar to the conventional photo-kymograph [107]. However, it did not undermine the popularity of photo-kymography. Globally, photo-kymographs continued to be in service until the 1970s [108] and beyond. Although, the first biomedical studies with basically similar equipment were published as early as in the 1940s [109], and the optical oscilloscope as a direct ancestor of the photo-kymograph was invented in the 1920s.

In the USSR, light-beam oscillographs were also used and refined until the end of the 1970s [110, 111] as a substitute for photo-kymographs. Concurrently, similar RFT instruments were imported from Germany [112, 113], and the units with the US pre-war designs such as Duddell or the moving-coil oscillograph were also in service [114]. Fig. 11 shows some mechanical and optical components of such an instrument. Numerous modifications made to the US instrument design in the USSR can be attributed to the lack of equipment suitable for specific measurement techniques and experimental physiology procedures.

This study can probably be categorized as a part of the same trend since while declaring the galvanometer scanner functionality fundamental to the light-beam oscillograph operations we restrict ourselves to the available instrument manufacturing technologies, not to the optimal design solutions. It should be noted that any modification shall not render the instrument non-compliant to the GOST 9829-81 national standard in terms of thermostatic control, ramp-up time, beam length (galvanometer mirror to the sensor) range since the optimal electromechanical and metrological properties of bifilar oscillographs are interrelated.

As a follow-up, we should mention here another simplified controlled scanning method that we tried to some extent. The method is suitable, among others, for research teams that unfortunately do not have light-beam oscillographs, but do have mirror galvanometers as separate modules of light-beam oscillographs. It is commonly known that the first mirror galvanometer was invented in 1826 by Johann Christian Poggendorff. The simple mechanical design was in use as a separate instrument until the last quarter of the 20th century. Consequently, it is very easy to find such an instrument for retrofitting. Galvanometer cassettes (from multichannel oscillographs) can be used for studies with many beams at the same time. We have achieved a record experiment: 5 different beams at the same time (see Fig. 12). For example, it can

be rational for multi-angle experiments and SPIM-like geometries of experimental setups.

Galvanometers with photoelectric feedback became available in Eastern Europe at the end of the 1940s [115]. The principles of photoelectric amplification of beam deflection had been presented by many European researchers. These principles were expanded in the more modern East European instruments in terms of better dumping and a wider oscillation period range by feeding back the current signals proportional to the first and second derivatives of the beam deflection. This solution resulted in a 20-fold sensitivity increase. The highly sensitive instruments at that time had their best accuracy in the low-frequency range [116]. Oscillographic galvanometers for registering fast processes were invented in the 1970s [117].

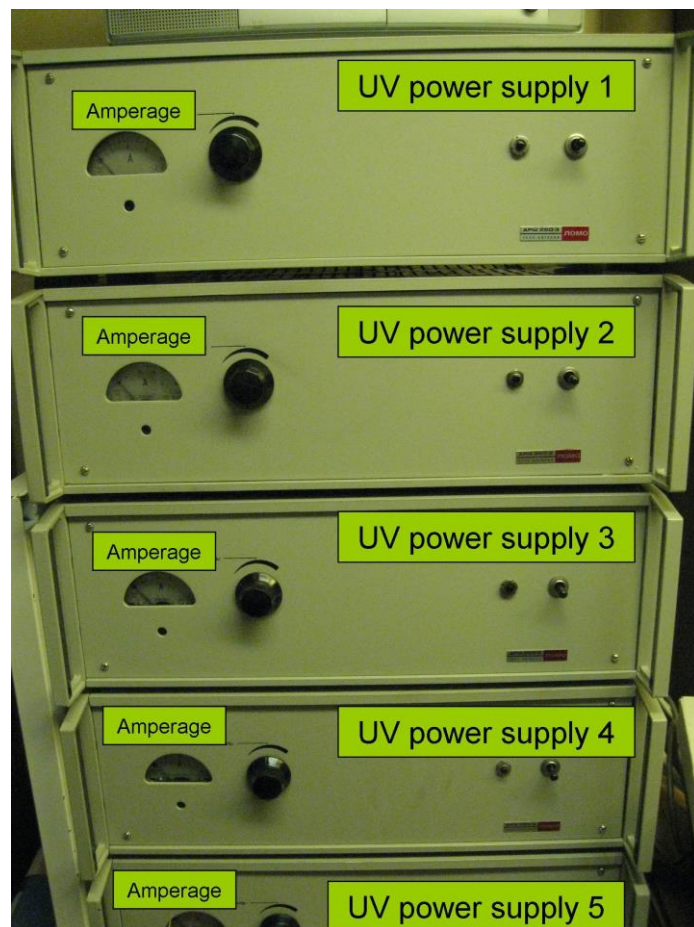


Fig. 12. Computerized multi-module multi-beam UV microbeam setup power cluster (O.V. Gradov, Research Center of Neurology, Brain Research Department, Laboratory of Neural Brain Structure, Moscow, 2012-2013).

The USSR conducted large-scale R&D activities to improve the performance of such galvanometers by:

1. Reducing the fluctuative dispersion [118]
2. Registering low-amplitude, “weak” signals [199] up to 10 Hz
3. Frequency response optimization [120]
4. Developing a contactless linear photoamplifier used with a high voltage gain potentiometer [121]
5. Developing procedures for measurement and monitoring of the mirror galvanometer properties [122, 123].

The most relevant Soviet light-beam oscillograph reference published in 1982 [124] notes that “prototypes of multichannel UV oscillographs made with standard components and arrangements including the optical path and the galvanometer modules were introduced almost simultaneously and independently in the USSR and the USA about 20 years ago” (that is in the early 1960s). But what is missing here is the fact that by the time the book was written the USA and Europe had already initiated a large-scale switch to new oscillographic instruments that ultimately ended in digital registration systems while the Soviet engineers refined designs that were considered obsolete in the relevant Western literature (refer to Figs. 5, 6 for some examples of such technology and design improvements).

Hence, it is possible to make well-shielded Chakhotin instruments in the original enclosures with its optical path integrated into the oscillograph light beam path, since all the Soviet mirror galvanometer designs after the 1970s were so optimized that could be adapted to high-precision microbeam exposure experiments (however, since they lack a UV source unlike most light-beam oscillographs, such a source is to be integrated).

Using finely adjusted galvanometric mirror devices or mirror galvanometers controlled by a virtual or physical waveform generator is not obsolete (as of today.) On the contrary, as the microscopic morphology methods such as the optical coherent tomography [125] (including redox-metric ones – see Fig. 13, Fig. 14) advanced, there is a need to select the optimal scanning functions (saw-tooth, triangular, sine, squarewave, etc.), to optimize the frequency and phase responses [125].

Since the end of the 1990s, we have coherent mapping and imaging methods that require the use of galvanometer scanners [126] (e.g., for *in vivo* scanning in ophthalmology and retinal biometry.) It is also needed for scanning fluorescent capillary arrays containing biochemical analytes or labeled cells [127], ultrasound range mapping [128] and terahertz range research [129]. There are slightly different approaches to solving confocal fluorescent microendoscopy problems [130] (so-called hypodermic needles / probes). However, until now multiphoton confocal microscopy and related to it micromanipulations (laser microsurgery, trapping, tweezers, localized photolysis, photobleaching / burning out of compartments and ultrastructural networks) also require the use of galvanometer scanners [131]. As early as in the 1970s, three basic types of optical “beam deflectors” existed: acousto-optic, analog electro-optic, and (the most conservative design) galvanometric [132]. Today, the latter type (controlled galvanometric reflectors) is most commonly used in biological research. Such reflectors can rotate for scanning [133] so they are suitable not only for 3D morphological scanning but also for time-based and space-based event scanning, or slow-motion scanning with rotating mirror sectors. It is not surprising that

galvanometric mirrors are used to manipulate individual cells and cell microstructure for precise positioning with optical tweezers [134] and experimental measurements of evoked potentials [135]. Hence, the mirror galvanometer is a general-purpose device. Restoration and modification of obsolete precise mirror galvanometers and their direct connection to scanning sweepers is a way to obtain an advanced instrument for low-budget research teams without huge expenses associated with purchasing new equipment.

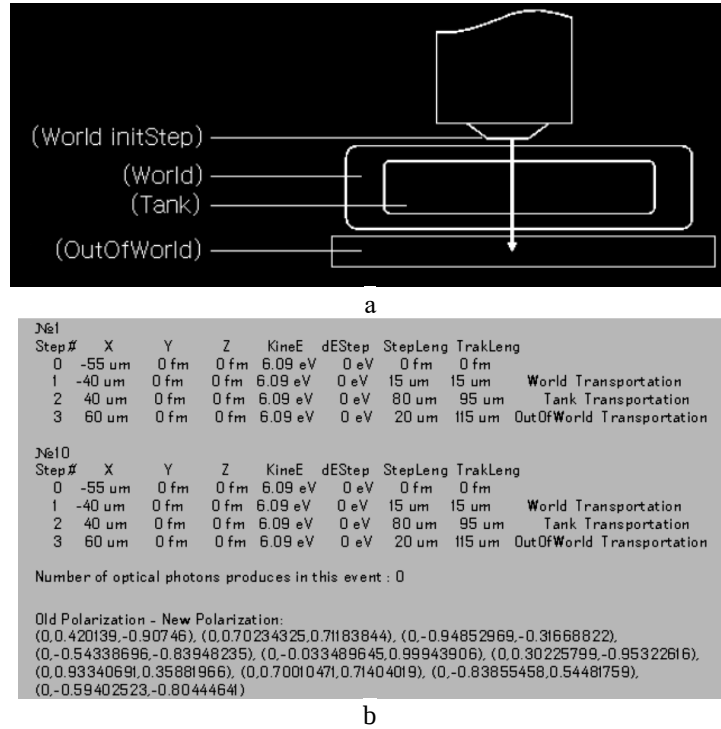


Fig. 13. An example of Monte Carlo modeling of the UV-beam propagation through the algae cell (World) with a vacuole (Tank) on the mineral surface (Out of world) under the ozonometric microscope with the UV light source (World InitStep). This result shows that one should track not only spectrum changes, but also polarization changes in microbeam experiments, integrated with UV microbeam microanalysis.

To turn mirror galvanometer modules of oscillographs into optical path components for microbeam instruments the mirror galvanometer dynamics models are to be extrapolated to cover the new design. To select the domestically manufactured instruments suitable for the upgrade we analyzed the monographs on light-beam oscillographs [137, 138], optical devices for oscillation recorders, and magneto-electric oscillographs [139-141]. The references are well-known to optical recording experts. The mathematical model used for our feasibility study is an adapted version of the models presented in [137] and related papers.

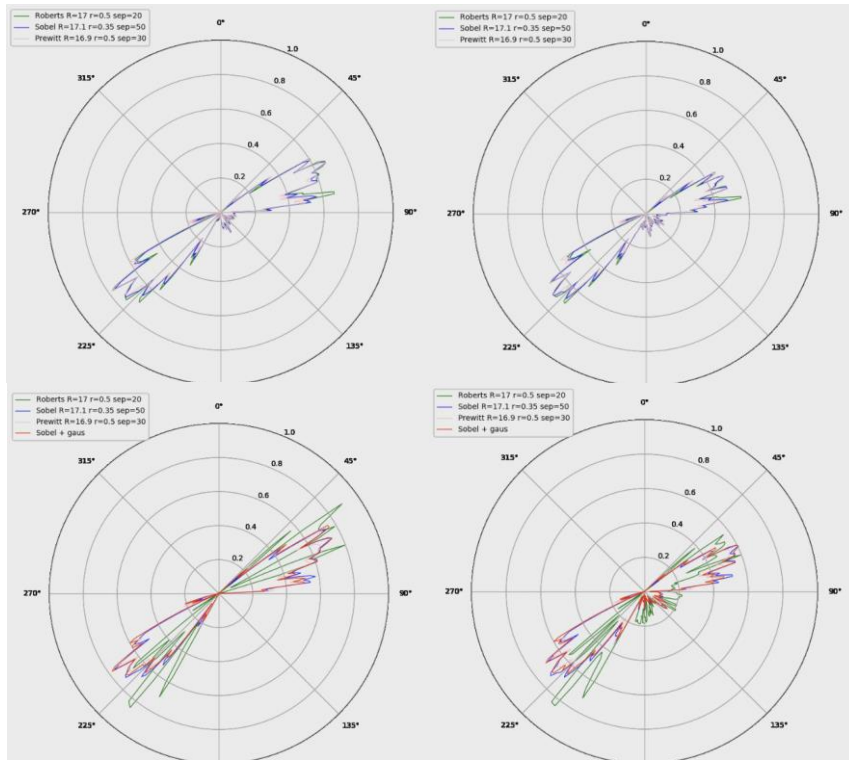


Fig. 14. “Radiation patterns” of different vector fields of diffusion in FRAP (From the IEEE Dataport-deposited Supplement to the preprint: Notchenko A., Endaltsev N., Gradov O.: Novel FRAP techniques...) [DOI: 10.21227/kfdk-eq33]. Software for these measurements has been developed by A. Notchenko and N. Endaltsev before 2017-2018.

It is commonly known that the light sources in light-beam oscillographs are EHP vapor lamps (Hg vapor pressure up to 5...6 MPa). They mostly radiate short waves in the UV range. Xenon lamps were also used in US and European light-beam oscillographs manufactured in the late 1970s but such lamps are not sufficiently efficient for microbeam methods since their spectrum is different. Since the vapor pressure in a cold EHP lamp does not exceed 1000 Pa, the discharge is unstable for the first minutes after ignition, and the spectrum is distorted. As the pressure rises to 15...30 MPa, the share of the continuous radiation component increases, and the relative amplitudes of the individual spectral lines are decreased. So an optimal effect in terms of providing standard metrological properties can be achieved only in a narrow range that we used for micro manipulations. The optimal range can be estimated from the source luminous element brightness that generates the UV illumination in the affected area. All prerequisites for the possibility or impossibility of using a microbeam with specific characteristics can be calculated and simulated using software (for example for GEANT4, see Fig. 13).

By using filters, individual spectral lines can be filtered out to adjust the effect of light on cells in Chakhotin microscopy and microdissection (if enabled by the galvanometer mirror.) Usually, the spectral lines of interest are two lines in the C band (184.949 nm and 253.651 nm), one line in the A band (365.015 nm) and one line in the near-UV visible band (404.656 nm). As a rule, these lines are sufficient for simple photobiological operations. In the steady mode, the luminous element is a sphere with the brightness at the middle of $1,500 \text{ Mcd/m}^2$, while the focused light spot illumination is close to a million lux.

The cylindrical condenser lens, the aperture, the galvanometer mirror and lens, the adjustable aperture, and the cylindrical lens create the light beam in the illumination channels. There may be more components in the optical system such as intermediate mirrors between the Hg lamp and the galvanometer, but we ignore them here for clarity.

The characteristics of the microbeam projection spot determine the reaction-diffusion processes in the cell in the irradiation zone, as (for example, see Fig. 14) in the FRAP (Fluorescence Recovery After Photobleaching) and FLIP (Fluorescence Loss In Photobleaching) methods.

Focusing by the condenser is a way to optimize the light beam affecting the sample. Another way to adjust the exposure is by controlling the illumination period by changing the light spot speed and the object manipulation speed. Of special interest is the implementation of a UV microbeams in lensless microscopes (Fig. 15, 16).

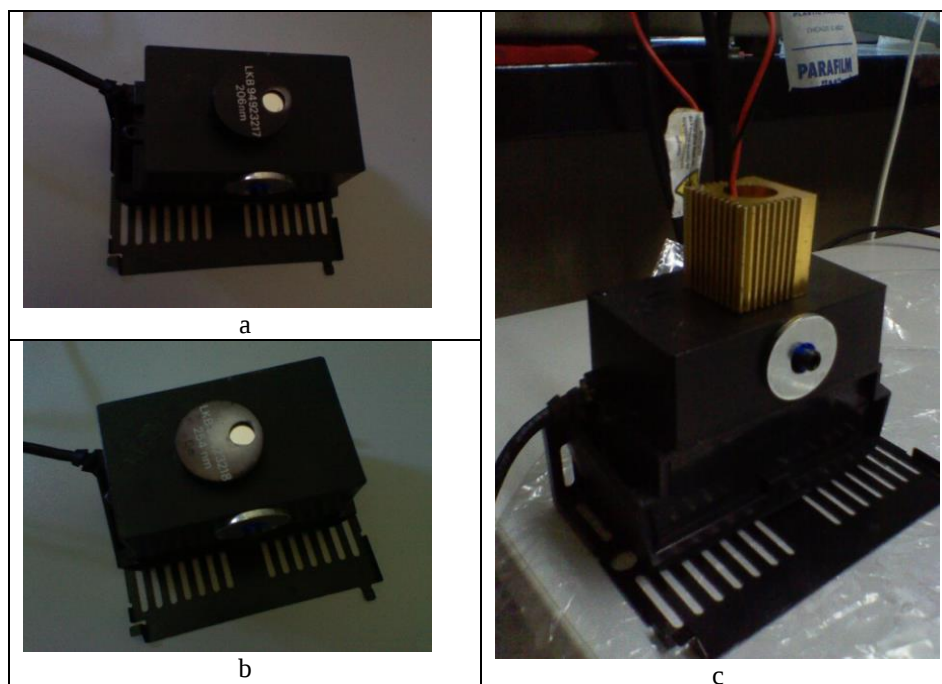


Fig. 15: Lens-less microscopes with 206 nm (a) and 254 nm (b) filters and with UV laser (c) source (design: O. Gradov, F. Orehov).

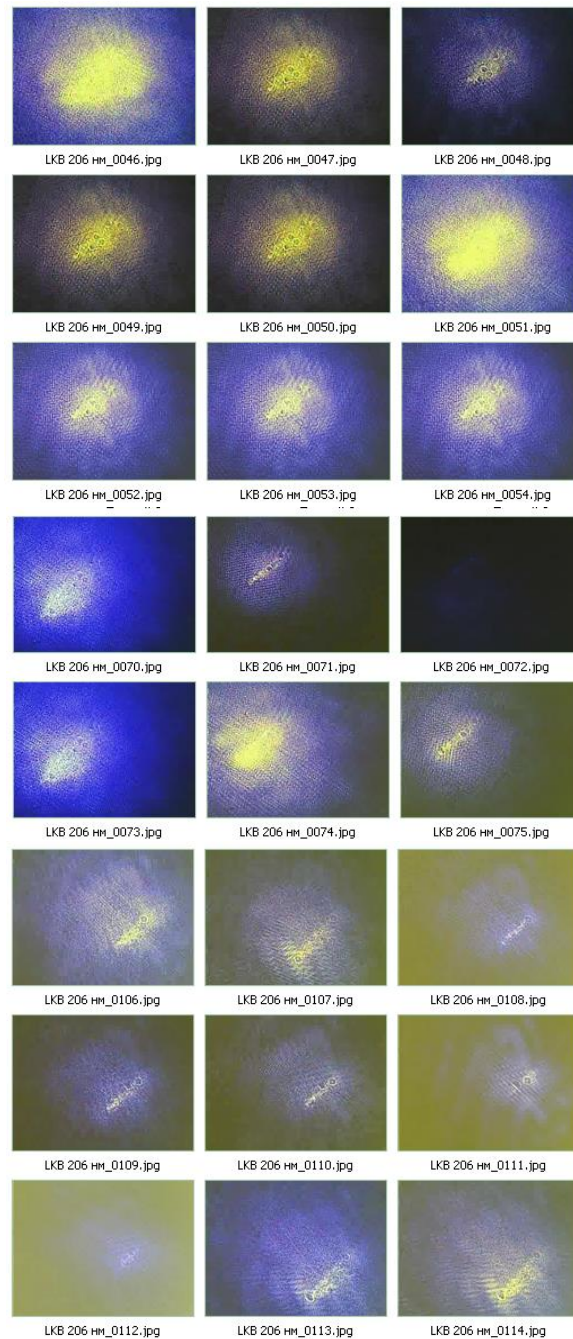


Fig. 16: Focusing of the UV microbeam (with diffraction slit) at the surface of the sensor array (CMOS array) of the lens-less microscope (from the author's works).

The object manipulation speed depends on the light spot speed which is controlled by the input signal. In early light-beam oscillographs, the light spot speed was about hundreds of meters per second. In the 1960s the speed reached 1000 m/sec, and in the 1970s, up to 2000 m/sec. However, the speed reading also includes the relative drum motion. The galvanometer head itself moves at up to 10 m/sec in the frequency range up to 10...15 kHz, so the speed can be monitored by analyzing the head movement frequency spectrum. In our experiments, we used the same low scan rates and lower scan rates (of the order of units - tens - hundreds Hz). To control the scanning, as well as to control the signal of the lensless microscope, a SCADA GUI.

The galvanometric component comprises of the replaceable galvanometer insert and the magnetic block with a permanent magnet and the magnetic conductor parts. The replaceable galvanometer insert is installed in the receptacle on the magnetic block. It consists of a movable frame with power supply tension wires, a mirror attached to the tension wires, and a lens embedded into the insert housing. Another design option is a loop mechanism without a magnetic system common for each galvanometer insert. Instead, each frame has an independent magnetic system as a separate measuring instrument. However, in the case of Chakhotin methods, it does not make much difference. The frame consists of a wire coil between two supports. The coil plane orientation is the same as the magnetic field direction in the gap. Electromagnetic forces produce elastic deformation, and the coil plane orientation is changed. As a result, the mirror attached to both halves of the coil, is rotated.

Correspondingly, the beam scanning can be software-controlled by an analog signal at the DAC output. To program various cell exposure modes, it is sufficient to develop signal sequences resulting in various responses of the electromechanical components. It is possible to generate rather complex and multistage exposure processes, and perform multi-coordinate cell / tissue exposure using multichannel DACs with software-controlled signal routing from the DAC output to various galvanometers located at the respective optical planes along the light spot path.

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