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Commentary

Vectorscopic and Secamoscopic Decoding For Electrophoresis Data and Illumina BeadArray Technology (ASPBB Workshop Presentations)

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Abstract

The purpose of this work is to demonstrate the applicability of vector diagrams and vectoroscopic techniques in visualizing sequencing results in a spectrozonal or multispectral mode using phase-sensitive data transmission standards. It is also proposed in this context to consider the data produced by the circuits as descriptors of "analog bioinformatics". At the moment, testing has been carried out only for register grams (in real time) of various types of electrophoresis and separation methods in gel and partially ordered media, both in analog and digital formats, so it is possible to talk exclusively about the prospects of such developments, based on the mechanisms and criteria similarities between these methods and outdated classical sequencing techniques.

Keywords: Vectorscopic; Secamoscopic; Electrophoresis; Illumina BeadArray Technology

Introduction

The bulk of modern sequencing methods using fluorescent tags can be qualified as spectrozonal / multispectral, based on the principles of visualization and differentiation of the analytical signal. This also applies to on-chip sequencing technologies. Illumina BeadArray technology uses the principle of color/spectrocolorimetric encoding of microspheres (3 μm) with a 23 oligonucleotide targeting and a 50 oligonucleotide probe length. Formation of a code array response (after preliminary background leveling [1,2]) - the so-called. "calling" [3,4] - represents the analytical signal of microspheres (the so-called "raw data" [5]), tied to ROI (Region-Of-Interest). There are 1520 correct used addresses in the system, 272 correct but not used addresses and 4769 incorrect addresses. Their decoding hybridization, by definition, must be carried out in a position-sensitive mode, since the task is to determine the location of the target oligonucleotides in the given ROI coordinates, taking into account the initial location of the microspheres at a distance of 5.7 μm. The fluorescence of the label on the bead during color coding is obviously spectrozonal, that is, multispectral, but is measured in the target fluorescence regions.

Many specialized molecular biology research protocols are based on the use of multispectral labeling. Multispectral labeling of antibodies with DNA-based polyfluorophores is a common technique in cytometry and cell imaging [6] and protein mapping [7]. The multispectral measurement technique underlies protocols for studying DNA binding to xenobiotics and their cytotoxic and antioxidant activities [8]. DNA-peptide conjugates are used as specific enzymatic chemosensors [9]. Methods using rare-earth fluorescent probes (the so-called "rare-earth biochemistry" and "toxicogenetics of rare-earth elements") with multispectral recording of the response serve not only to directly study the toxic (or modifying) effect of these probes on the object of study, but also to analyze the mediation by the probe or intermediates formed during its assimilation, exposure of xenobiotics to tissue or nuclear material and cytoplasmons of the area of influence [10].

Multispectral data collection can be carried out not only in the visible, but also in the infrared region. The first works in this direction are descriptions of techniques for peptide sequencing implemented on short sequences, di- and tripeptides [11]. The authors are not aware of any work on infrared multispectral mapping techniques for sequencing nucleic acids, although laser scanning in the infrared range has been used in practice in DNA sequencing on the principles of electrophoresis since the early 1990s [12], PCR products have been measured by direct IR sequencing methods since the second mid-1990s [13], amplification of long repeated palindromic sequences of 16s rDNA is consistently compatible with Fourier transform infrared spectroscopy (FTIR / FT-NIRS) as a characterization technology [14], identification of lifetimes in near-infrared (NIR) areas in sequencing techniques based on capillary gel electrophoresis is becoming a classical approach [15], as well as DNA sequencing with time resolution (discrimination) when tagging with heavy atoms and dyes modified by them [16]. There is no doubt that the positioning of individual methods as multispectral or spectrozonal would be possible in the IR region (with the exception of MALDI-MS methods with multiphoton dissociation in the IR range, where the use of lasers with given wavelengths for desorption-ionization does not allow talk about multispectral data collection when sequencing high-molecular semantids and episemantids [17-19]), however, probably due to the lack of spectrozonal visualization of the signal in the infrared zone and, as a consequence, the use of either additive thermal detection of a single mark, or spectral registration of a complex of marks or the semantids themselves (a special case of the principle of "genetically encoded multispectral tagging" [20] is the method of encoding derivatives of xenonucleic acids, which are both DNA tags / polyfluorophores and the semantids themselves), this terminology is not used, although already well-known sequencers IR2 used a two-channel scheme with semiconductor solid-state lasers (at X=680 nm and X=780 nm) and an infrared detection range when recording the fluorescence signal of dyes that labeled dideoxynucleotides when separating the products of terminating reactions in gel electrophoresis.

The formation of the theoretical basis for the concept of multispectral sequencing and spectral multiplexing dates back to the late 1980s [21], when the principles of fluorophore photochemistry, multispectral recording, direct blotting or gel electrophoresis, electronic obtaining images (imaging) in digital format and multiplex sequencing with a specific set of probes and primers. Laser detection methods implied the presence of specific dyes with responses in different subranges; in particular, ABI 373 already used 4 visible dyes. The presence of dyes with different spectrocolorimetric response, adequate to the channels of the additive color model or the channels of the component video signal, makes it possible to use differentiation and discrimination along these channels for direct assessment of the content of coding agents bound to a given dye in a position-sensitive mode.

The problem of fast (real time) position-sensitive assessment of the content of dyes associated with the code carrier can be solved provided that measuring tools are used that differentiate and discriminate these color components of the signal in real time, which corresponds to modern trends in RTS [22] (real time se- quencing - including single molecule real time sequencing, SMRTS) with optical detection [23], including for modified (for example, methylated[24]) code carriers. More precisely, from a biological point of view that requires adequate mapping of the sequence code in situ, one could characterize the need for demultiplexing of color channels in the process of multispectral sequencing as the need to register the coding sequence under arbitrary influences (such as the above methylation) across all channels simultaneously with time resolution for each channel separately in situ. This formulation of the problem, appealing to the principles of in situ sequencing - ISS [25] (implemented both on specially prepared tissues and in gel electrophoretic separation techniques [26,27]; both in DNA sequencing and RNA sequencing), including with fluorescent detection [28,29], allows, in the case of optical registration, to implement multiplexing - demultiplexing with analogue division by color / wavelength (WDM - wavelength division multiplexing). The implementation of a system that performs the listed functions, including in the contact mode of registration on a chip [30], and testing on model drugs is the subject of this article.

Options for Implementation of Technology

Presentation of input data

When recording a signal with CCD and CMOS detectors in analog format (both using the simplest camera readers of early generations, and by using contact chips with built-in CCD and CMOS detectors), it is usually possible to extract primary data in the format of a component video signal, in in which the low-frequency color subcarrier is transmitted independently (without mixing the luminance and chrominance signals), or in the format of a composite video signal, in which the full color signal is transmitted, containing signals of luminance, color subcarrier, blanking, all types of synchronization (abbr. PTsTS or CVBS), one at a time channel, a single cable (according to GOST 21879-88, which stipulates the content of the synchronization signal in CVS). Registration using BNC cables (or RCA with an RCA-BNC adapter) can be carried out using not a monitor as the final receiver, but a set of measuring equipment that comprehensively measures and visualizes the characteristics of signals, and based on them - the properties of the object under study. We also tested the broadcast of signals produced by a chip with a primary RCA connector over a cable with a terminal interface under the SCART standard (Syndicat des Constructeurs d'Appareils, Radiorecepteurs et Televiseurs), which simultaneously broadcast a spectrum signal (green / red) and Y - brightness channel (both input and output) of the composite video signal; technically/physically excluded in this case was only the broadcast via SCART of the equipment control protocol in the CEC standard and via the corresponding bidirectional serial bus in the standard HDMI-compatible interfaces with copy protection (HDCP), since an analog signal was examined and required analysis, which should not have been protected from copying during experiments with parallelization of signal analysis to multiple output devices and copying information, which is regulated as a special case of application in the CENELEC EN 50157-1 standard (Comite Europeen de Normalization Electrotechnique), in addition to the CEC Implementation Guidelines regulations that are also redundant in this case (implemented by HDMI). When transmitting spectrum channels separately to visualization and radio measuring instruments in the form of a component signal, the principles of signal modulation that are different for each channel are not used, as well as converting the signal of these channels into brightness and color signals with subsequent reverse decoding, and therefore the metrological quality of this type of analog transmission significantly higher. This, in particular, is facilitated by the possibility of using an extended frequency band, much larger than that characteristic of a standard monochromatic luminance signal. Using a recording device of any format that stores color directly according to color coordinates in the RGB color space as a recording medium for analog data of multispectral registration (with color separation).

Synchronous/phase-sensitive detection (lock-in) in decoding synthetic cistrons

According to well-known data, the broadcast PAL standard, by definition, is distinguished by a line-by-line phase change during scanning, regardless of the principles of operation of the projection device (PAL stands for “Phase Alternating Line”). The so-called quarter-line shift used in this system is, in essence, a shift (“shifting” in engineering jargon) of the subcarrier from line to line by 1/4 of a period, as a result of which the complete cycle of alternating subcarrier phases and compensation phase is 4 frames of analog visualization data, that is - 4 serial cards/frames. This means that it is possible, consistent with theory, to use scanning the signal of fluorescently colored samples of synthetic code carriers in a multispectral / spectrozonal mode with separation by color or color-difference channels (RGB and its analogs or RY, BY and their analogs) with colocalization of fragments colored with different spectrally encoding dyes. Changing the polarity of the structure (subcarrier, but not only it) between frames should not shift the cyclicity / periodicity of the phases. Cycles encoded by a frequency corresponding to the difference in frequencies of spectrum harmonics can carry independent analytical information about the rate of reaction-diffusion and fluorescent processes in the sample/micropreparation under study. By using subcarriers with frequencies that are multiples of the horizontal scan frequency, and phase switching (180°) between its periods (from line to line), it is possible to achieve ultra-high resolution, ranging from 3 to 1 line per encoding source (optical signal of a cistron microspecimen located on a single ladder level or a single read line or line cell of a chip). Frequency synchronization in this case is ensured in a trivial way, introducing a rigid connection between the frequencies of the subcarrier and scanning lines through a normatively regulated multiplicity. An alternative is phase switching (regulated Sequentiel couleur avec memoire / Sequentiel couleur a memoire) with two subcarriers, and implemented interlaced - on the third lines, which provides high phase sampling (0°, 0°, 180°, 0°, 0°, 180°) when using two subcarrier frequencies, periodically alternating through the scanning line (the case of switching from field to field is not considered in this case, although it is regulated within the framework of the same standard). Despite the quite expected non-linear distortions of color signals in this case - since information about color difference signals is transmitted by frequency modulation of color subcarriers, due to which, if there are differential phase distortions in the path, at the vertical boundaries ("colorimetric transitions" of the phase), a sharp change in the phase of the color subcarrier signal occurs - this technology can also be used (with certain reservations) in practice in broadcast sequencing installations with display on vectorscopes and devices connected with them, subject to use in some links (see above) phase-sensitive/lock-in detectors and phase-sensitive amplifiers and analyzers. This requirement is due to the fact that, since frequency is a derivative of phase with respect to time, these phase changes are accompanied by short-term changes in the subcarrier frequency, leading to distortions in the spectrocolorimetric characteristics of code maps, which is unacceptable in correct sequencing and barcoding. Since the level of these distortions depends on the steepness of the difference in the brightness signal and the delta in the signal levels (before and after), and in fluorescent and dark-field measurements, this level and this delta, by definition, are very large, from a metrological point of view, it is necessary to take into account non-critical ones, if talk about "non-metrological" applications, phase characteristics. The amplitude detector selects signals approximately proportional to the deformed envelope with a 7-8 MHz band of the radio frequency channel, formed in the process of quadrature addition of the in-phase and quadrature components (with distortion).

With phase-sensitive (or, as this method is more generally called, synchronous) detection, quadrature modulation may not be an obstacle and a source of artifacts, but a means of correcting asymmetric phase distortions. Quadrature distortions are caused by a phase shift of the carrier, therefore, their compensation is possible by its reverse bias, which, in the case of the detector conductivity depending on time, can be implemented here (in a synchronous detector, in contrast to an amplitude detector with conductivity depending on the voltage applied to diode). Since the principles of operation of a synchronous detector are, in essence, equivalent to the principles of operation of a key modulator with a key that operates synchronously with the frequency of the modulating signal, closing the half-cycle of the input signal, perhaps using a narrow-band filter with an amplitude modulation limiter and a phase regulator (instead of the first, you can use a local oscillator, synchronized input signal), provide a phase shift by an angle equivalent to the angle of the parasitic phase, however, in sign, inverted relative to it. By avoiding in visualization the artifacts of coloring color ("spectrozonal") transitions, which usually arise when the resting frequencies of chromaticity subcarriers and subcarrier phase switching do not match, it is possible to improve the qualimetric qualities of the resulting measurement by three times or more (which cannot be applied in the case of a conventional amplitude detector and the presence of a discriminator in chromaticity decoder) Since in practice the characteristic of the group delay time is estimated by the frequency response (amplitude-frequency characteristic) of the receiver, and the flat part of this characteristic corresponds to the linear section of the phase-frequency characteristic (phase-frequency characteristic), and the phase-frequency response of the "slopes" is characterized by non-linear sections, it is possible to hybridize the measurement phase response on the signal transmission line from a selective spectrozonal fluorescence detection sequencer with telemetric signal transmission with measurement of nonlinear distortions and nonlinear analysis of the signal as a whole. The criticality of the phase shift of the frequency components of the signal and the group delay time adequate to it lies, in the case of cistron affimetrics according to fluorescent criteria, in the fact that the difference in the group delay time leads to a distortion of the brightness signal, and when measuring transient characteristics, and, consequently, the frequency-contrast function, manifests itself in a change in the front of the pulse. The only exception in which the operation of a phase detector in an experimental sequencer circuit may be useless is the case of a phase change by 90 or 270°, since detection is not possible in the case when the current in the circuit does not have a constant component. Synchronous or phase-sensitive detectors operate with a phase shift of 0° / 180°, and a phase deviation of several degrees is manifested by distortion of the test sinus-square pulses (and we are also talking about the baseline and original polarity, since with a phase change of 180°, by definition, the polarity of the rectified voltage changes). It should be noted that the nonlinearity of the end-to-end phase characteristic of the path, which increases in parallel with the increase in the steepness of the frequency response in the high-frequency region, can lead to “contouring” of the monochrome displayed signal, which can be used as a means of morphometry instead of processing according to Kirsch, Sobel, Prewite pits or laddering. identifiable elements in the signal scanogram. Oscillatory processes on the flat part of the code pulses of the general signal in the middle part of the signal spectrum with an oscillation frequency adequately corresponding to the frequency cutoff of the frequency response can also serve as a reference value, but only for methods such as FRAP and other comparative fluorescent kinetic measurements (meaning, of course, the number of periods of a damped transient process, which also depends on the path, therefore requiring it to be taken into account in the calibration of the analog sequence system as a whole). On the time scale, the deviation of the delay time, therefore - indirectly, the measurement accuracy parameter should not exceed 5 nanoseconds (in the case of line-by-line phase changes during scanning) or 30 nanoseconds (in the case of less metrologically optimal frequency modulation), which can be recorded with a stroboscopic oscilloscope of a fairly old model range (we used a two-channel type C7-16 with a modified computing attachment).

Transmission of visualized signal / information

Transmission can be carried out via two (or more) channels corresponding to two (or more) cables that provide broadcasting of signals with different colorimetric components; Accordingly, we are talking about broadcasting not a composite, but a component signal. At the same time, when using the simplest and chronologically earliest interface systems, the subcarrier of the colorimetric (color) signal can be switched separately to the basic colorimetric components - in order to reduce crosstalk or, correlatively, crosstalk.

It must be said that the use of this technology makes it possible to record signals of the colorimetric type of descriptors and the luxmetric / photometric type of descriptors (brightness and color) differentially, which makes it possible to analyze both quantitative and qualitative differences in the analytical signal of sequencing products by recording them in an array (magnetic tape) non-identical groups of heads on precisely differentiated and identifiable (based on signal characteristics) tracks. We, in particular, introduced a method in which a BETACAM type tape recorder was a recorder of qualitative and quantitative changes in the analytical signal on an active CCD or CMOS chip. It is possible to build such a multi-oscillographic / multi-vectoroscopy system in which two differential colorimetric channels for differently colored sequenced “analytes” and one luminometric / luxmetric quantitative assessment channel, which also transmits phase synchronization pulses, will simultaneously be recorded. It is obvious to a specialist that the difference channels (in blue and red, as a rule) in this scheme are the BY and RY channels, where Y is the channel of brightness measurements and broadcast of synchronization pulses.

For the purpose of statistical analysis of distributions, it is rational to use multichannel colorimetric methods, corresponding in the number of channels to the number of possible spectral sub-ranges of the label glow. Naturally, the BY and RY 9 nomenclature does not exhaust the real needs of molecular biology, but for the described variations of sequencing techniques it is only a successful demonstration of the principles/technologies of data conversion and transmission.

Problems of converting visualized/analyzed data

In principle, several variations of signal conversion are possible, on which the coordinates on the vectorgram and the commutation of the vectorscope to the telecine or video sequencing installation depend. In the case of using phase switching with two subcarriers (implemented interlaced - on third lines to ensure sufficient phase sampling (0°, 0°, 180°, 0°, 0°, 180°) when using a pair of subcarrier frequencies periodically interleaved through the line scanning), it is rational to use (and, in fact, uses) signal conversion into a “pseudo-phase” signal, detected separately or differentially into three colorimetric and one brightness. It is obvious and natural that data conversion (analog) is carried out not by changing the commutation, but by a signal converter, the input of which is the input from a phase-switched system with two subcarriers (see above), and the output is the output of a component / multi-channel spectrozonal signal of an arbitrary format. TDA 3300/3030, 356 .../359 ..., as well as previously produced in the USSR MTs41 (on KR1021H3, kr1021kh4) or Tauras 61CT402D (type 174 HA 33 {TDA3505, XA992} (K) K174HA32) allow you to implement format transcoding specified in the text. The corresponding class of devices is called, adequately for their functions, transcoders, although equivalent functions are inversely performed by the TDA3505 video processor (with inputs for luminometric / luxometric and colorimetric delta signal) and the TDA4555 bidecoder system, as well as “hybrid” single-chip processor-decoders (for example - 1021XA4 / TDA3562). The methods of real-time “analog bioinformatics” implemented with their help avoid the need for digital data converters and codecs that ensure compatibility of the installation’s computer with the corresponding file formats, which are products of preliminary digitization that, by definition, is not carried out in real time. Unfortunately, many such devices, specially adapted for video sequencing, are currently a patent-intensive subject of intellectual property law and, excluding references to proprietary documents that are not in the public domain, cannot be discussed here.

Problems of spectral-phase identification of individual horizontal scanning channels of a video sequencer

One of the common dyes for genetic purposes or one of the sources of excitation (depending on the use of positive or negative in the analysis of kinetic video sequences) are red dyes. The first systems were “tailored” for helium-neon lasers in the red range. The nonspecific dyes in the colorimetric calibration cassette were the most elementary dyes that, according to spectral criteria, fit into the red channel - such as rhodamine. Inversion and “negativization” of the color image made it possible to work with both spectrozonal or isolated and gradient-binarized patterns of the absorption mode of operation (absorption), and with direct patterns of emission analysis (usually fluorescence). Below we will consider phase difference analytics on the red channel. When a chroma signal is applied, the R-chroma signal is repeated in the following rows with a phase rotation of 180 degrees. To eliminate the phase error, the decoder adds the current line and the previous one (from memory 10), thereby eliminating phase errors typical of a number of video sequencer implementations on NTSC systems. When two signals are added, the R-color-difference components cancel out due to the change in signs. When subtracting two signals, the B-components are mutually destroyed (video engineering jargon: “annihilated”), as a result of which, at the outputs of the adder-subtractor, separated signals U and V are obtained, which are essentially scaled-up RY and BY. It should be emphasized that NTSC-type systems that are “erroneous” and require such recoding are predecessors to phase-type systems (PAL), which allows us to talk about partial compatibility of formats in the case of video sequencing of both types.

Essentially, modulation of the subcarrier amplitude by two difference signals when using this approach is performed by quadrature modulation, in which the subcarrier is divided into two components, shifted relative to each other in phase by 90°. One component is modulated by an R-difference signal, the other component by a B-difference signal, and the carrier frequency of signals modulated in this way is completely suppressed, leaving only the sidebands. In the case of phase switching with two subcarriers (interlaced - on third lines to provide phase sampling (0°, 0°, 180°, 0°, 0°, 180°) on a pair of subcarrier frequencies periodically interleaved across the scan line), phase the analysis is technically related to the colorimetric one, since during the period/phase when the R-spectrozonal channel signal is transmitted or read in line A1, the B-spectrozonal channel is transmitted or read in line A2, and the G-spectrozonal channel is transmitted or read in line A3, respectively. spectrozonal channel and then in a cycle over the entire sweep area. As a synchronizer pulse in phase systems, 8-10 pulses/oscillation periods of the reference oscillator are used on the back plateau of the horizontal damping pulse, which can be considered as a disturbance (burst) of the color signal subcarrier (“colorburst”), which has certain phase properties. In contrast to NTSC, where the phase of pulses of this type is invariant (constant) 11 , in PAL it varies from line to line by 90°, carrying information about the phase of the R-component of the subcarrier. In order to remove parts of the spectrum of signals in subcarrier frequency ranges, band-stop filters are used in most works.

It should be noted that during cable transmission / broadcasting of a signal, radio frequencies in the range of meter and decimeter waves are detected, as a rule, using transponders / multiplexes, in comparison with the absolute frequency ranges of the receive transmission or radio frequency channel as a whole, the frequency band of which is negligibly small (range, in in principle, may not have standardized values for radio frequency limits). In this case, it is advisable to talk about frequency modulation.

Strictly speaking, subcarrier modulation with difference signals uses, as a standard, frequency modulation, and the subcarrier frequencies are within the range of the frequency spectrum of the luminance signal, therefore, when receiving a color image on the screen, interference from the subcarriers becomes noticeable (in analog systems with cross-distortion along the color channels and along the subcarriers , in particular), which can be used as a metric for the spatial calibration of the monitored pattern. In the case of phase switching with two subcarriers (on third lines to provide phase sampling (0°, 0°, 180°, 0°, 0°, 180°) on a pair of subcarrier frequencies periodically interleaved through the scan line), additional interference occurs due to beats of subcarrier frequencies of color signals. In this regard, NTSC does not use color difference signals, but their linear combinations, since undistorted independent transmission of two video signals transmitted by quadrature modulation is possible only if the quadrature is preserved, i.e. - shift between signals equal to 90°. Thus, in a sense, we are talking about both quadrature and frequency-phase modulation. In this case, based on the spectroscopy data of the sequence signal, it is possible to determine the characteristics of the analyte through the characteristics of its detection signal (the nominal bandwidth of the luminometric or luxometric signal is 4.2 MHz, the colorimetric subcarrier is modulated by two color difference signals, one of which is transmitted in the frequency band 0 ...600 kHz, occupying two sidebands, and the second is broadcast in the frequency band 0..1.4 MHz with the upper sideband partially suppressed). Knowing the spectrum of the brightness signal, it is possible to obtain information in a mono channel by summing / superimposing the spectrum of the colorimetric signal at a subcarrier frequency on the spectrum of the brightness signal and subsequent differential analysis (the colorimetric signal can be obtained from RGB color signals by adding in a certain proportion: R - 30%, G - 59% and B - 11%; at the same time, the luxmetry / luminometry signal corresponds to a monochrome signal (arbitrary or so-called “black and white”, for which spectral conjugate colorimetric properties do not matter, although registration is carried out, for some tasks , using color or at least spectrozonal filters, in the simplest case - Bayer type).

Essentially, the problem of identifying biopolymer sequences using “analog bioinformatics” data comes down to the problem of detecting and identifying a signal corresponding to a particular modulation correlated with colorimetric reference data. In this case, the identification results must be invariant to polarity, since the polarity of the modulation of the video signal determines which level corresponds to the “black background” and which level corresponds to the “white” light source; in this case, the polarity of the modulation can be “negative” and “positive” (with a negative polarity, the maximum brightness / “white level” corresponds to the minimum modulation amplitude of the carrier waves, and with a positive maximum modulation amplitude. To decode the positive modulation of an analog recorder, it is possible to use devices with a circuit- an inverter of pulsed noise and, accordingly, signals that are pulsed in nature, in which the so-called “inversion threshold" was varied by a regulator made in the format of an agometer / potentiometer / rheostat. On the pattern displayed by the monitor (at a low luxmetric threshold) the corresponding zones or the pattern as a whole could be visualized in negative format.In these works, we did not use devices made in Great Britain and France from the 1960s, or even their analogues in terms of their basic design, but autonomous units on modern microcircuits with power supplied via the universal serial bus, USB (developed by F A. Nasirov).

Secamoscopic analog bioinformatics based on phase cross-connection with two subcarriers

A significant share of the available equipment fleet, which is optimal in terms of technical characteristics for the implementation of “analog bioinformatics” approaches, as practice shows, consists of phase-switched devices with two subcarriers within the framework of the “sequential colorization with memory” standard, differing in modulation polarity (SECAM-L - positive , SECAM-K1, SECAM B/G, SECAM D/K - negative), as well as technical details in the recording (for example, the use of an additional, as in PAL systems, local oscillator to transfer the signal spectrum to the low-frequency region of the MESECAM standard, while retaining redundant for the standard, phase data, while for the implementation of SECAM, the positive point is precisely the absence of redundant data for pattern transmission and, accordingly, a simplified configuration, when used in analog decoders, the use of a quartz resonator is not required, and the delay line operating in the ultrasonic range can have non-critical deviations of delay times up to 30 nanoseconds, and not up to 5 nanoseconds as in the case of phase registration in the PAL system, literally deciphered as a line-by-line phase change). As a consequence of this, it is advisable to use secamoscopes rather than standard vectorscopes as an analytical signal recorder (in cases where the original signal meets the physical parameters of the requirements of the SECAM standard).

Standard work with a secamoscope (and only a few such devices have survived), according to video engineering regulations of the 1970s - 1990s. (and until the early 2000s inclusive) has the following positions as the final result and intermediate goals: determination and decoding (by visualization on a cathode ray tube) of the amplitude characteristics of the tract; construction of amplitude characteristics at different positions of the gamma corrector switches (based on the brightness gradation signal corresponding to on-chip ladder methods when analyzed using the methods of “analog bioinformatics of secamoscopic sequence”), during which the device is also calibrated; control of monochrome signals (if we are talking about contrast-metric threshold detection, in particular) with encoding devices and synchroset blocks (with special generators, but with the button to disable frequency modulation in the encoding device pressed); control of signals according to metrological and qualimetric criteria when distributing the signal from amplifiers - distributors from the output of the installation complex to the switch, and at the output of the encoder the signal is distributed to the secamoscope, from one of the switches the signal is supplied to the monitor and parameter control unit, and camera signals are supplied to the inputs of the second registrations that are fed to the editing complex (eliminated from the system in cases of elementary experiments on video sequencing / telesequencing); encoding into the MPEG standard when digitizing registergrams (the signal is fed to a secamoscope, an analog head-end modulator and an MPEG-2 encoder, the output of which is a transport single-program stream via the ASI interface, and the secamoscope is intended in systems of this kind to monitor the output signal).

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Author's roles:

Orekhov F.K. – spectroscopic measurements; colorimetric measurements.

Nasirov F.A. – electronic design; metrology; design of CCD and CMOS PCBs.

Gradov O.V. – conceptualization; introduction / review; lens-less microscopy and lens-less imaging.

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