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ADAPTER FOR REGISTERING MICROSCOPIC IMAGES TECHNICAL DOCUMENTATION





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Introduction

Adapter registering microscopic photos consists of electronic stage (which differs depending on the model and type of a given microscope) and software part (VID.EXE program). Adapter is used for acquisition and analysis of collected photos (scanned photos). Thanks to this very adapter it is possible to upgrade almost every microscope type. Elbit Company developed and introduced a method of collecting photos from various types of microscopes:

- optical microscopes
- scanning microscopes
- transmission microscopes.

Optical microscopes.



Upgrade of a microscope is possible thanks to the use of photo converter (as in the case of high definition video camera or photo camera converter). We use only Canon photo cameras in our project. Joining optical track is possible with prism or eye-piece.

Data acquisition is in other words preliminary preparation of a signal containing a photo information (electronic stage), followed by conversion and pre-processing (in the software stage) and its display on PC screen.

Depending on Client's needs and requirements we use various types of photo converters:

- 640x480 pixels;
- 800x600 pixels;
- 1000x750;
- 200x1500 pixels and more.

Other important parameters:

- number of colours: 24 million ;
- save formats: BMP, JPG, TIF, AVI.

The program offers an updated preview of a microscopic photo allowing to set sharpness precisely.

It also allows to make ordinary photos and time lapse photo.

Scanning microscopes.



ELBIT Company developed an electronic adapter converting electric signal from scanning microscope converter.

The signal conveying pictorial information is digitalized and sent to a computer in digital form and then, after pre-processing (in software stage) displayed on a PC screen.

In some microscope types it is possible to join the drive steering track of a table in such a way that sample's movements as well as reading and position are made by means of the program.

Depending on Client's needs we use various types of photo converters:

- 640x480 pixels;
- 720x576 pixels;
- 1024x768 pixels;
- any (for photo mode adapter).

Other important parameters:

- shadows of grey: 64 thousand;
- save formats: BMP, JPG, TIF, AVI.

The program offers an updated preview of a microscopic photo allowing to set sharpness precisely.

Transmission microscopes.



ELBIT Company developed a special converter for transmission microscope. It is a device similar in size to a film cassette. It is to be placed right under the electron gun. The main element of a converter is a luminophore plate. Sample's image is displayed on it, digitalized and next sent to the computer in a digital form. Then after pre-processing (in software stage) it is displayed on PC screen.

Special correction element allow to decrease geometrical deformations of a photo in such a way that the photo is as close to the original as possible.

The Company also developed a special vacuum camera mounted to a microscope column which records the image from the original luminophore through a special prism in a microscope.

Together with a software there is an element for diffractive analysis. The program automatically selects prism's nodes and next calculates basic information about each node.

Depending on Client's needs we use various types of photo converters:

- 720 x 576;
- 1024 x 768;
- 1400 x 1050;
- 2000 x 1500 pixels.

Other important parameters:

- shadows of grey: 64 thousand;
- save formats: BMP, JPG, TIF, AVI.

The program offers an updated preview of a microscopic photo allowing to set sharpness precisely.



Equipment and software requirements

Recorder program ought to be run on a computer with the following parameters:

- USB port 2.0;
- CPU Core 2 duo 2x2.6GHz;
- 4 MB Ram for video memory.

The second USB port is required to steer the table drive.

Operating system:

- Windows XP (with Service Pack 3) or
- Windows 2000 (with Service Pack 4).

In case of other systems, i.e. Windows 98 the program works in limited resolution.

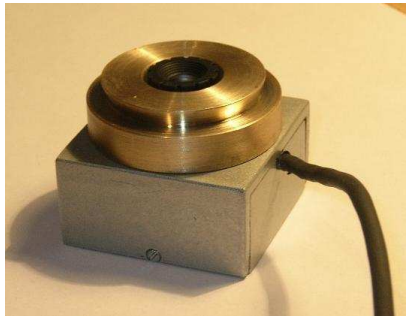
It should be noted that in scanning microscopes electronic part joining detector's track is galvanically connected by means of an adapter with a computer unit. Therefore, it has to be remembered that power feeding of a microscope and feeding of a computer need to join the same phase.

Adapter's feeding operated by means of USB port. In order to avoid network hums which may deform scanned photos (constant or multi-variant horizontal stripes) computer set used for scanning needs to use good-quality feeding distributors with filters.

Electronic part

This very part is different for different types of microscopes and in each case it can be modified according to individual Client's needs (resolution, additional functions, etc.). However, there are several parts which are the same for each adapter.

Optical microscopes.



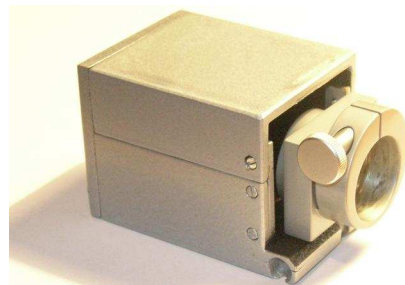
There is a CCD converter mounted inside each adapter, either as a high resolution video camera or stand-alone converter, or as a photo camera converter.

All those adapters communicate with a computer (send the images) by means of a USB cable.

All optical microscopes' adapters have axes control system.

Such an adapter is to be mounted to a microscope both ways: either using parasymphetic system of a prism or instead eye-piece.

In each case, the way adapter is mounted to a microscope is selected and discussed with a Client.



Scanning microscopes.



Electric signal converter is mounted in a special aluminum casing.

What is more, table drive steering systems, as well others, can be mounted there, too.

Converter is powered by means of a USB port, and feeding of any possible extra systems – from the network - 230VAC.

There is also one BNC cable coming out of a microscope.

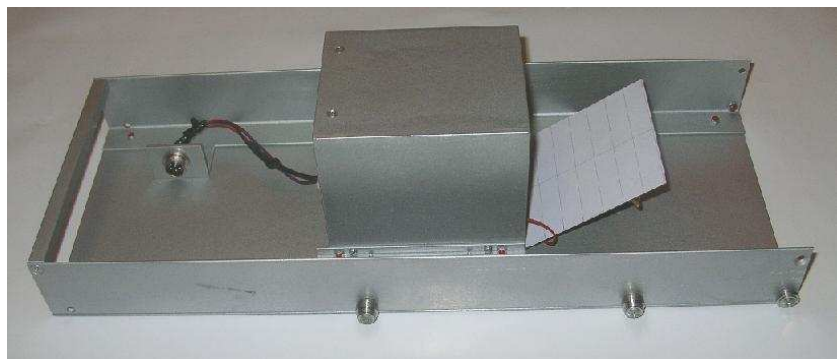


Transmission microscopes.

Transmission microscope's adapter is a device similar in size to a film cassette. It is to be placed under the electron gun.

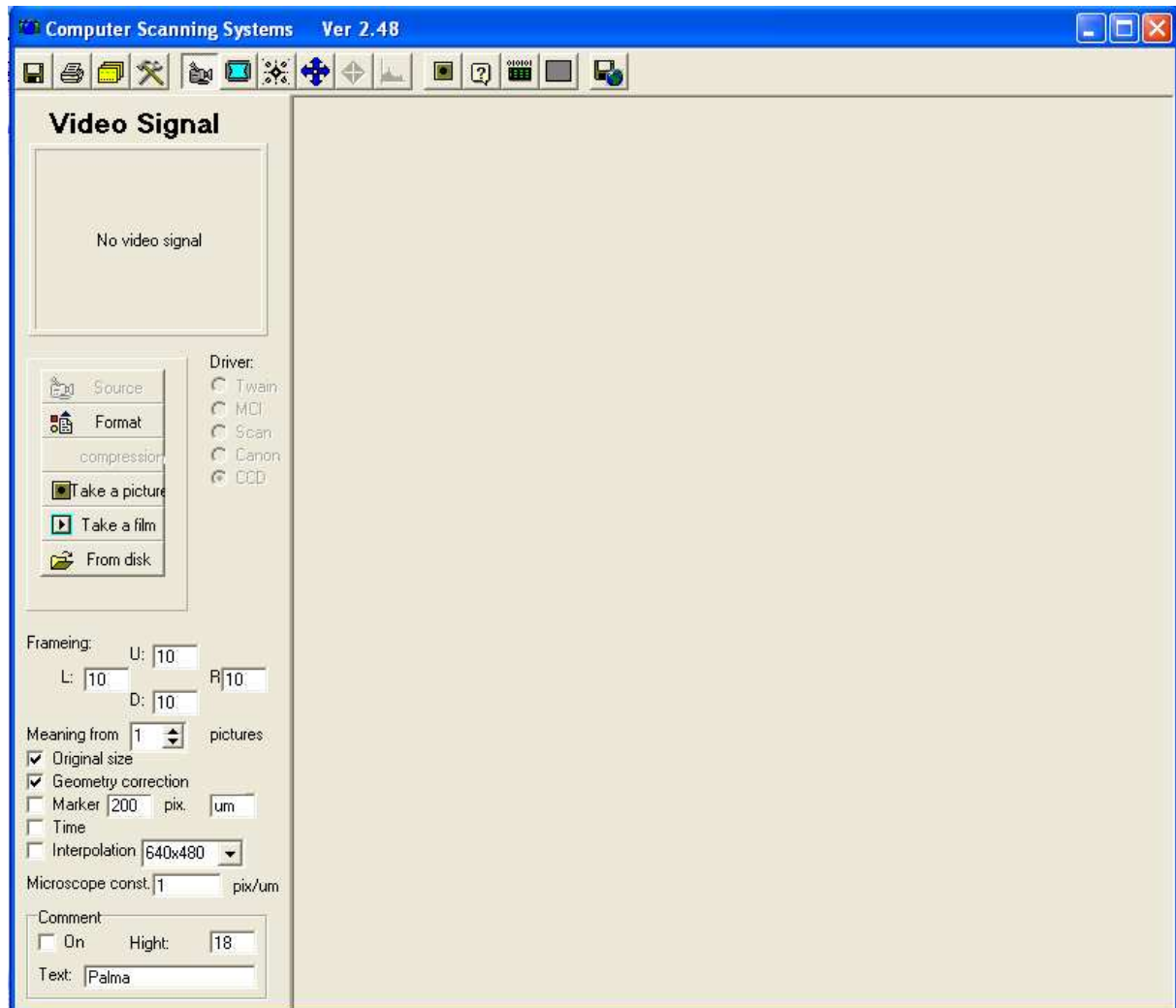
The main part of a converter is a luminophore plate. Image of a sample displayed on it is digitalized and sent to a computer in digital form. The whole device is inside a microscope in high vacuum.

Electric signals from the inside of a microscope go through a special cap.



Software part - VID.EXE program





When run, the window of a tool program looks as presented below:









The program has an icon bar, working panels activated when needed and proper working area.

Buttons on an icon bar, divided into three sub-groups have the following meaning:






Ordering functions

-  Automatic save of all the scanned images to the disc.
-  Print of a chosen photo.
-  Ordering scanned photos on the desktop.
-  Program parameters settings.

Working panels

-  Video preview panel, panel where scanning parameters are to be changed.
-  Image geometrical correction panel.
-  Diffraction analysis panel.
-  Servo control panel.
-  Microindenter function panel.
-  root analyser

Other functions

-  Start scanning of an image.
-  Information about the program and the producer.
-  Program registration in a system.
-  Screen saver.
-  Internet transfer.

Automatic save of all the scanned images to the disc.

Using this function saves all the open scanned photos in a chosen format (bmp, jpg or tif) and using currently set resolution. They are saved in a catalogue in which VID.EXE program is located. If a program finds already existing photo with a given name, a dialogue window pops out allowing to save the photo with its current name, change the name of a photo or discarding save option.

File name where each scanned photo is to be saved can be found in the top tool bar of each photo. It is to be set in the program's parameter window.

Print of a chosen photo.

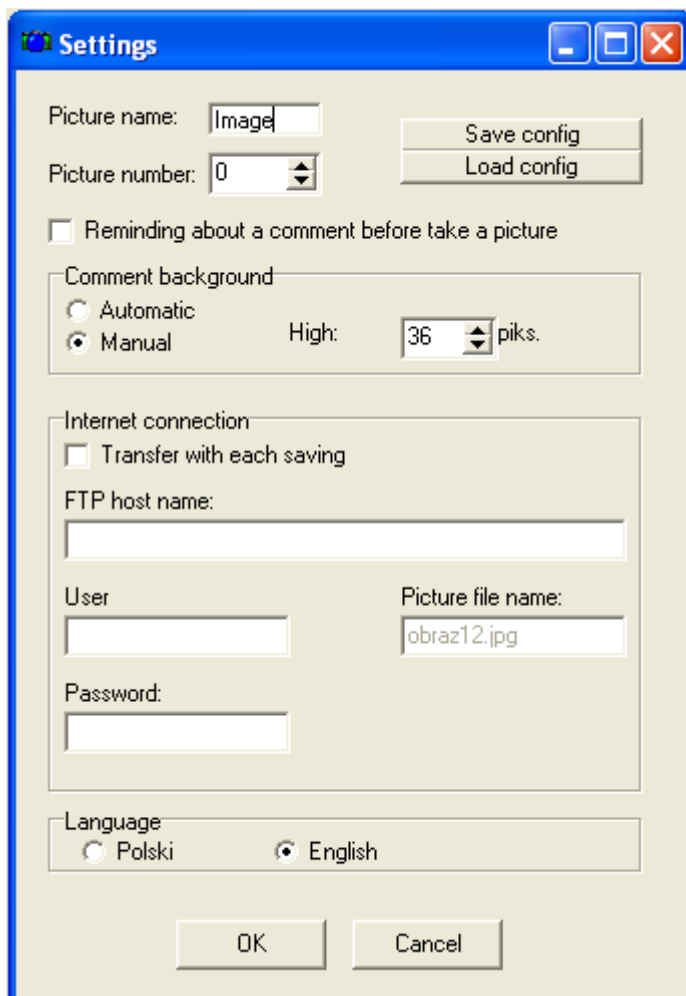
When selecting this function, printing setting window appears followed by printing of that very photo.

Ordering scanned photos on the desktop.

Running this function results in cascading windows (one after another) with scanned photos on the screen.

Program parameters settings.

If you run this function, a dialogue window opens – example presented below:



The program gives to each new photo a name under which each scanned photo is to be saved.

In the settings window it is possible to provide the name for a group of scanned photos and its ordinal number. Photos' extension depends on the format in which a given photo was saved. It is to be chosen in the window from among the following: BMP, JPG or TIFF.

If we take the picture on the left as an example, after selecting BMP as an extension, the first scanned Photo receives the name: Image1.bmp, and the next one - Image2.bmp, etc.

Running remember function will result in a situation in which before every single scanning, the program will ask for a comment to it.

The program enables saving and reading of the whole set of parameters. Configuration files require „abk” extension. It makes it easy to work on the program in multi-user mode (each of them can define his own set of settings).

Transfer of scanned images to the Internet is an interesting extra option. It activates each time a photo is saved to disc. It can also be initiated by choosing a transfer icon.

FTP file transfer is used in the program. Required parameters comprise the name of FTP server (host), user's login and access password.

The files are always saved under the same name. As a standard, when installing the program, Elbit FTP server is a default one.

Video preview panel, panel where scanning parameters are to be changed.

When choosing a camera icon, input preview panel appears on the screen. There are also some buttons allowing to change the parameters of video conversion.

The way such a panel looks is presented on the right.

Image appearing in the preview window does not go through any filters and is displayed in a real time (depending on computer's speed it is from 1 to 30 frames/second).

Source button opens a dialogue window allowing to change basic parameters of a scanning device (brightness, contrast, saturation, diaphragm, white balance and others).

Format button opens a dialogue window of a stream associated with a capture device allowing to change resolution and the number of colours in scanned photos.

Compression button allows to set video compression (it is recommended not to compress the video stream).

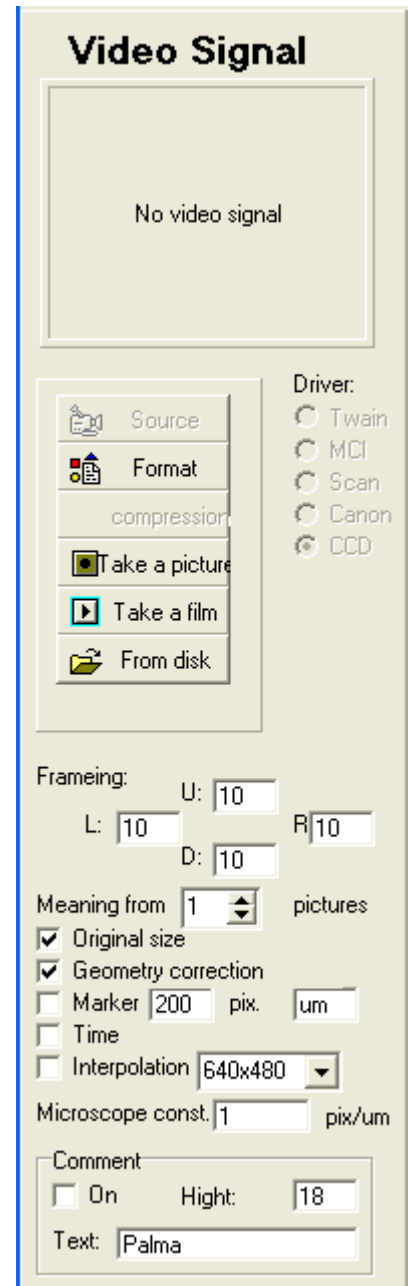
There are two separate button to scan photos and download the videos.

Scan photo button starts the process of signal scanning from the adapter using all previously set parameters. What we get is a photo generated by the program.

Download video opens a daughter window where downloading parameters can be configured. Current preview is automatically displayed as a large image.

The program also provides an option of automatic cropping of a given photo (done while scanning). The size of a cut is to be set in cropping parameters. Unit used is pixel. In the attached picture, scanned image is cut by 90 pixels from the left and 200 pixels from the right. Top and bottom edges remain unchanged.

In case of photos with a large number of image noises it is recommended (and advantageous) to scan several photos which later are averaged with the use of special function (i.e.g. photos from scanning microscope's converter or photos taken in conditions of low illumination). The function is **averaging** which gives an opportunity to scan up to 32 photos. The bigger the number of averaged photos, the longer time of processing photos. However, the advantage is that noises (white noise) are eliminated.



Original size parameter influences only the display size of the scanned photo on computer screen. It is especially recommended in case of high resolution photos. It makes it possible to display the whole scanned photo in full-screen mode (photo is scaled down respectively).

In optical systems with small focal distances (FD) and in transmission microscopes adapters, where maximum faithfulness in scanned image mapping is required, geometrical correction is used. Parameter to be used is called **Photo geometrical correction**. It allows to stretch or squeeze a photo on axes X and Y and to correct the spherical aspect of an optical system ('fish-eye' effect reduction). Particular correction parameters are to be set in correction panel.

The program allows to enlarge registered scanned photo to requested resolution. It is to be achieved when choosing **Interpolation** function. Averaging functions (**Averaging**) make such an interpolation to higher resolution not only and ordinary zoom but also increase the number of registered details.

It is not recommended to do interpolation bigger than 1:1.5 (ratio of original image to interpolated image). For instance, in case of an image with horizontal resolution 720 pixels, the highest interpolated resolution, at which the number of details increases even more, is $720 \times 1,5 = 1080$. Therefore, the first (closest) to be chosen in the program is 1024x768.

Scanned photo can be accompanied by information on time of photo development and on the zoom. Parameters to be used are **Time**, **Tag** and **Comment**.

Choosing **Tag** function adds to the scanned photo (at the top, in the middle) lines (of length defined by the number of pixels) with a comment such as „200 um”. The function is especially useful in microscope systems with permanent zoom option (i. eg. Optical microscopes with measured zoom). In order to measure zoom, zoom patterns are to be used (glass plate with digested nonius).

Choosing **Time** parameter adds an information about day and hour when scanning took place (left bottom corner).

Choosing **Comment** parameter adds in left bottom corner a comment of the size defined in pixels. Maximum length of a comment is 200 letters. It is not possible to add date and time and a comment as well automatically at the same time.

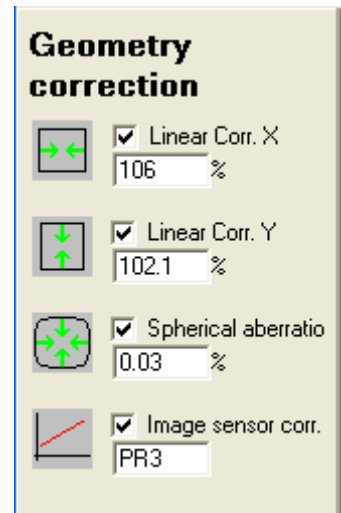
Photo geometrical correction panel.

Choosing a proper icon opens correction panel. Example to be seen in the attached picture (on the right).

Linear correction, horizontal (X) and vertical (Y) is about squeezing or stretching the final image compared to the original. I.e.g. transmission microscope converter, in which luminophore plate is set at a given angle against electron gun, creates image deformations. Value of the change/deformation is given in percentages.

In case of optical microscopes it is necessary to correct the 'fish-eye' effect (being the result of spherical aspect of optical system).

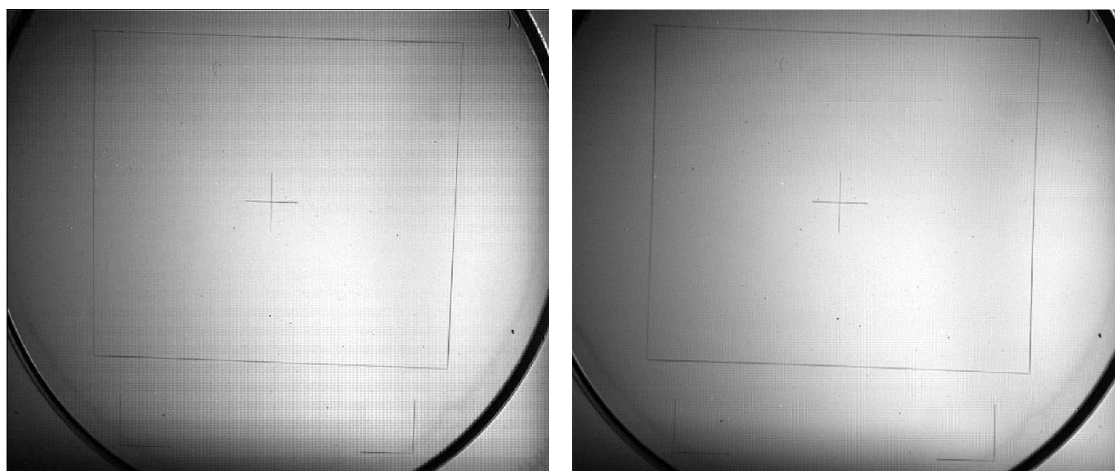
Image converters used (CCD) do not have the same amplification of each pixel. In some microscope systems (i.e.g. transmission ones) in which the value of each pixel is an input data for further analysis, correction may just be inevitable. The weight of particular pixel in the image is provided for completely blacked-out converter and saved in correction file. When working normally, information on each pixel is multiplied by already established weight of that very pixel.



Linear correction as well as correction of spherical aspect of lenses is appointed and corrected in optical microscopes. When it comes to transmission microscopes, sensitivity of converter is appointed as well. Scanning microscopes do not need geometrical correction of an image.

Particular correction parameters are chosen each time individually by ELBIT Company, when adapter is installed.

Image before and after correction are presented below:



Diffraction analysis panel.

When choosing a proper icon, diffraction analysis panel appears on the screen. Example presented on the right.

Only transmission microscopes are equipped with software element responsible for diffraction analysis.

In the analysis nodes in crystal lattice are automatically appointed and the results are given in the form of coordinates and angles.

Diffraction analysis

dynamic lev.:

background lev

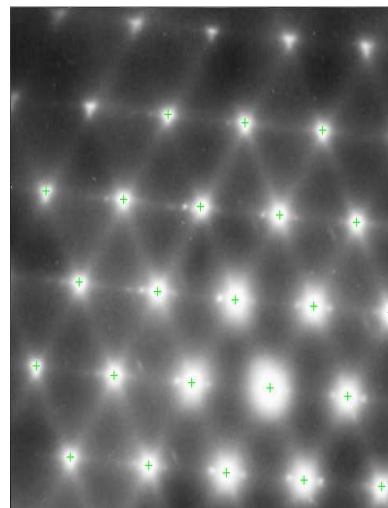
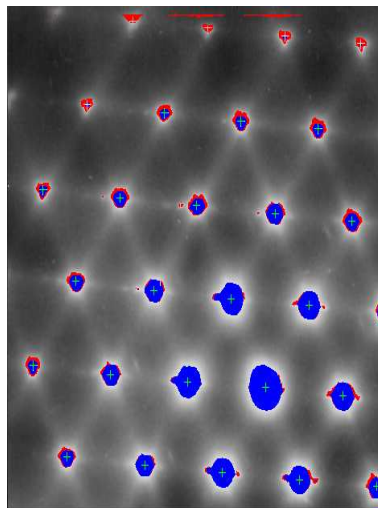
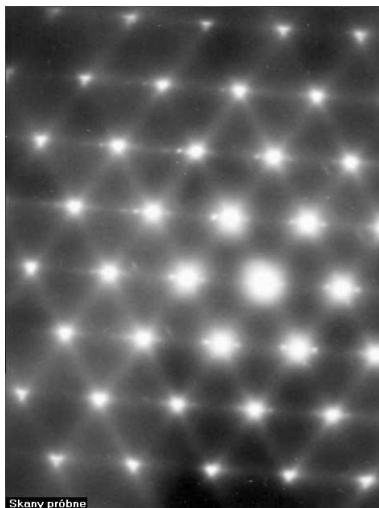
☐ Monitoring

Nodes:

<input checked="" type="checkbox"/>	1	104	169	100
<input checked="" type="checkbox"/>	2	144	256	100
<input checked="" type="checkbox"/>	3	185	340	100
<input checked="" type="checkbox"/>	4	91	334	100
<input checked="" type="checkbox"/>	5	144	256	100
<input checked="" type="checkbox"/>	6	196	175	100

First line:
Nodes: 3
Distans:
D1 =95.75490 um
D2 =93.47192 um
Second line:
Nodes: 3
Distans:
D4 =94.30270 um
D5 =96.25487 um
Angle:121.23 st.

Exemplary effects of automatic lattice appointment are presented below.



In manual mode nodes, for which calculations are to be made, need to be appointed.

Appointing a node is in other words 'catching' it in a rectangular window on a scanned image, as presented on the picture.

The program automatically allocates a point from within a selected area and places a marker on that very spot (cross). That point is a mean value of the brightest points.

At the same time, coordinates of the point are put on the list of nodes, on diffraction panel.

Each node can be deleted anytime by selecting it (highlighting it) on the list and pressing DEL key.

This functions works only when the diffraction analysis panel is open.

Note: all the calculations are to be conducted on the original size (real-size) of the scanned photo.

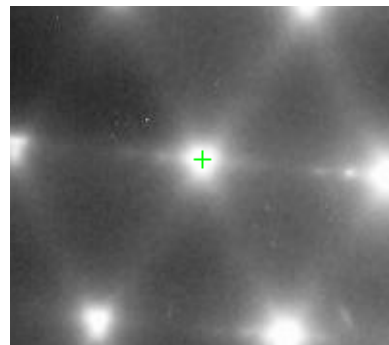
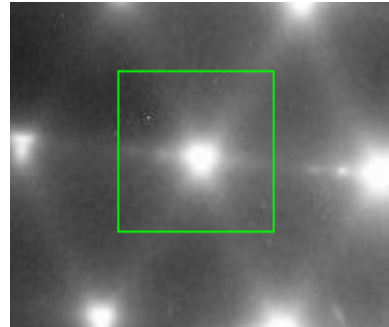
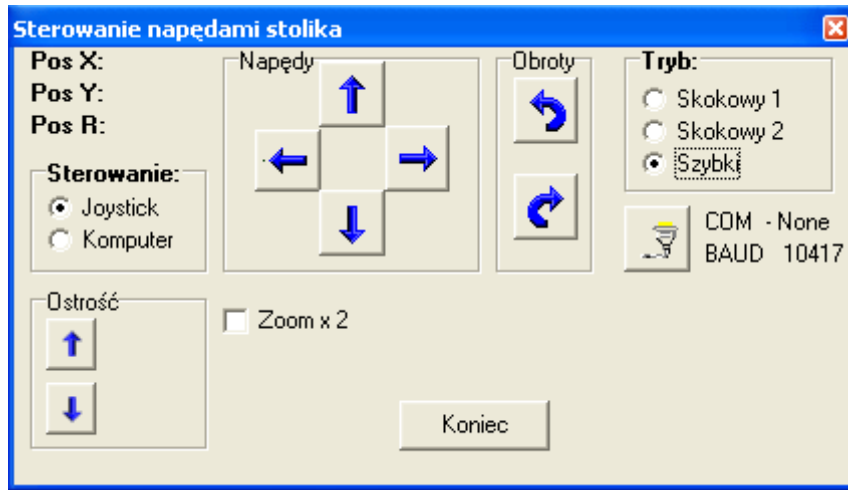


Table drive control panel.

Choosing a proper icon opens a window as the one presented below.



Depending on a solution chosen following options are to be chosen:

- table movements on the axes (X i Y) in three work modes;
- right/left turns;
- readings of a current location of the table (in μm);
- focus movements (sharpness);
- doubled zoom made by a program

Microindenter panel.

Selecting that option opens a window as in the picture below:

**Mikro-
twardościomierz**

Obciążenie:

Stała mikroskopu:

Węzły:

<input checked="" type="checkbox"/>	1	39	132	0
<input checked="" type="checkbox"/>	2	252	116	0
<input checked="" type="checkbox"/>	3	144	17	0
<input checked="" type="checkbox"/>	4	159	241	0

Długość 1: 0.17um
Długość 2: 0.18um
Średnia: 0.18um

Twardość: **11.78**

Before the measurements are made, weight which was used for the test is to be chosen (in grams) and constant values for a microscope and measurement system are to be provided (in pixels/millimeter).

Next, when scanning is complete, diagonals of the track (in case of rectangular tracks) or diameters (in case of round tracks) are to be appointed manually. When the second diagonal or diameter is already appointed, the program automatically draws appointed sectors/segments and makes calculations:

1. calculating the length of the sectors/segments from pixels to μm . (result to be found on the panel);
2. calculating arithmetic mean value of two sectors/segments (result to be found on the panel)
3. calculating hardness according to the formula below:

$$HV=0.1891 \cdot F/d^2$$

where:

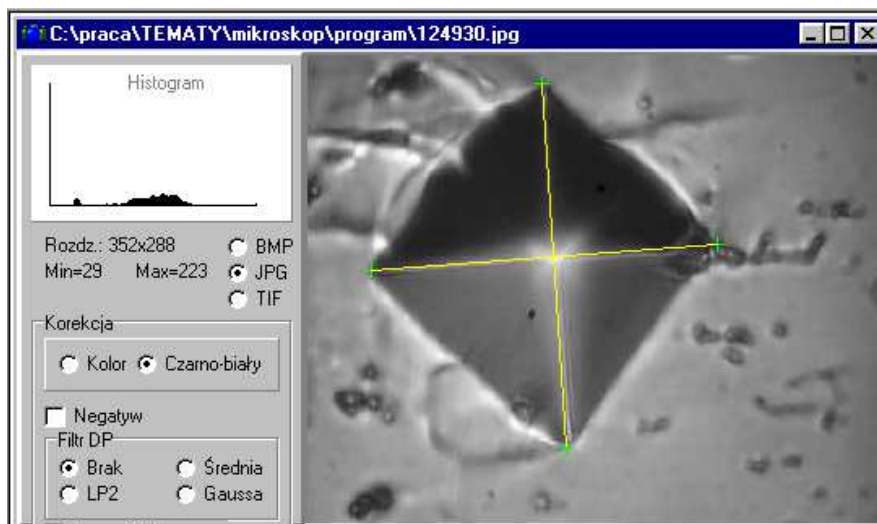
HV – hardness;

F – force in N;

d – arithmetic mean of measured diagonals in mm

Note: all the calculations are to be conducted on the original size (real-size) of the scanned photo.

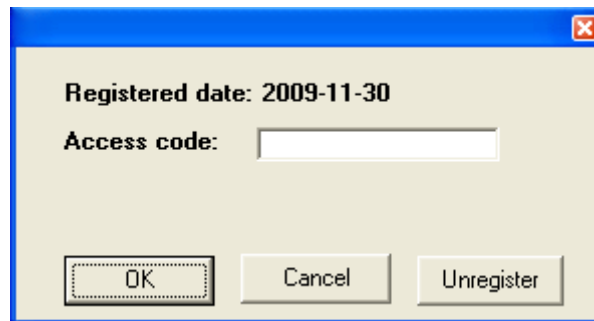
Example of a scanned photo with properly appointed diagonal to be found below:



Program registration in a system.

Due to the fact that some of the solutions that are to be found in the adapter registering microscopic photos are quite novel and VID program is entirely in-house ELBIT's program, some of its elements are secured with passwords.

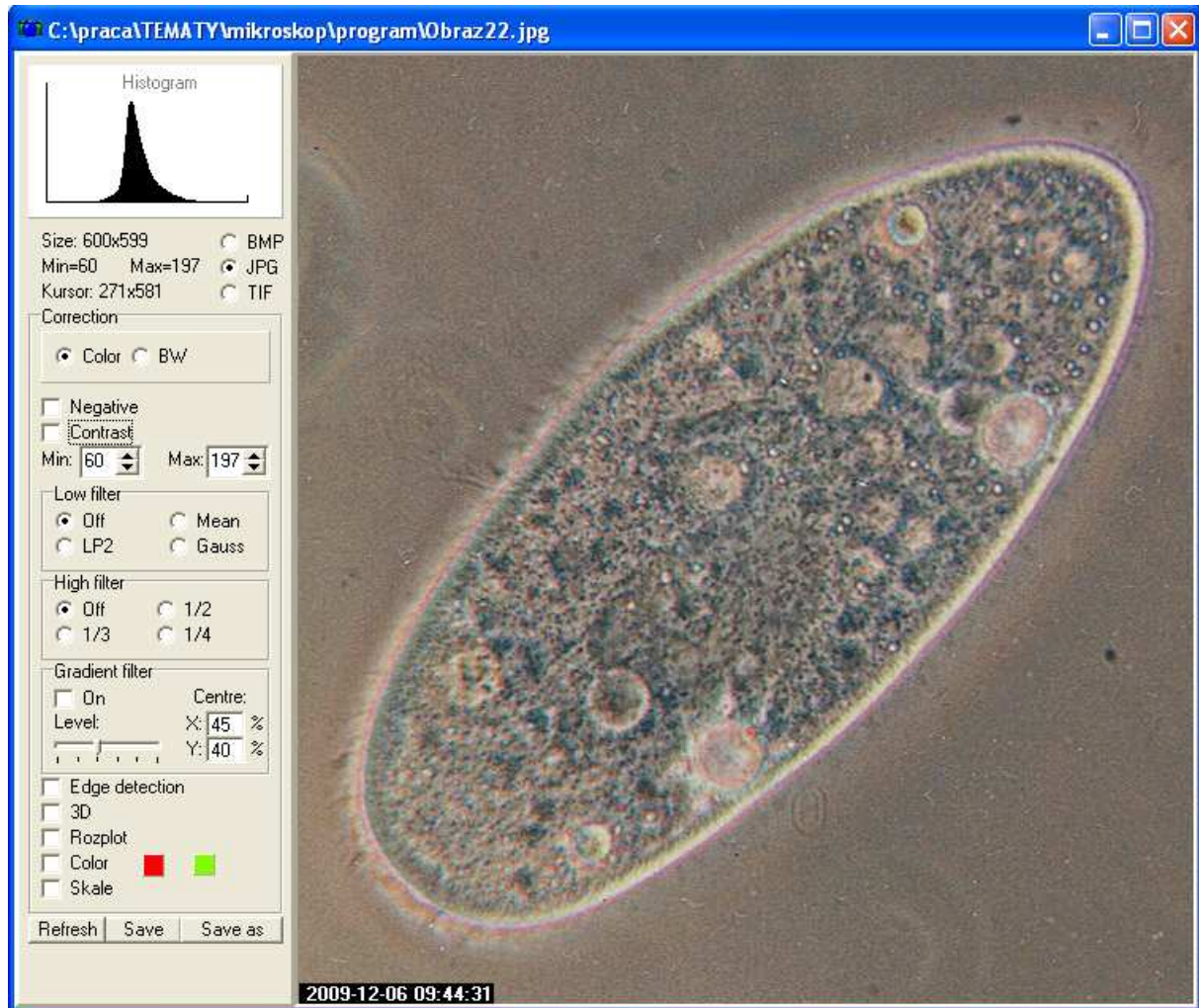
While registering the program in the system, a single-use access password provided by ELBIT Company (when purchasing the program) is to be put in the registration window.



Properly written password registers a copy of a program in the system. It needs to be remembered that the program distinguishes different font sizes.

Options available in the window containing scanned photo

Example of a scanned image looks just like the one in the picture below:



On the left of the panel, there is an image processing control panel to be found. The remaining space is filled with a scanned photo. At the bottom of the window, there is a marker with the time and date of scanning. At the top (title bar) – full access path to a file containing a photo accompanied by its name and extension.

A panel is divided into the following:

- histogram, that is weight of an image and the number of particular pixels presented in the form of a diagram;
- background information on a scanned image (the size given in pixels, min and max weight of a pixel on a scanned image as well as the format when saved);
- correction part of a panel containing:
 - colour and black&white scanning options;
 - negative-option;
 - low-pass filters;
 - brightness and contrast adjustment with edge parameters to be set;
 - high-pass filters;
 - edge detector function;

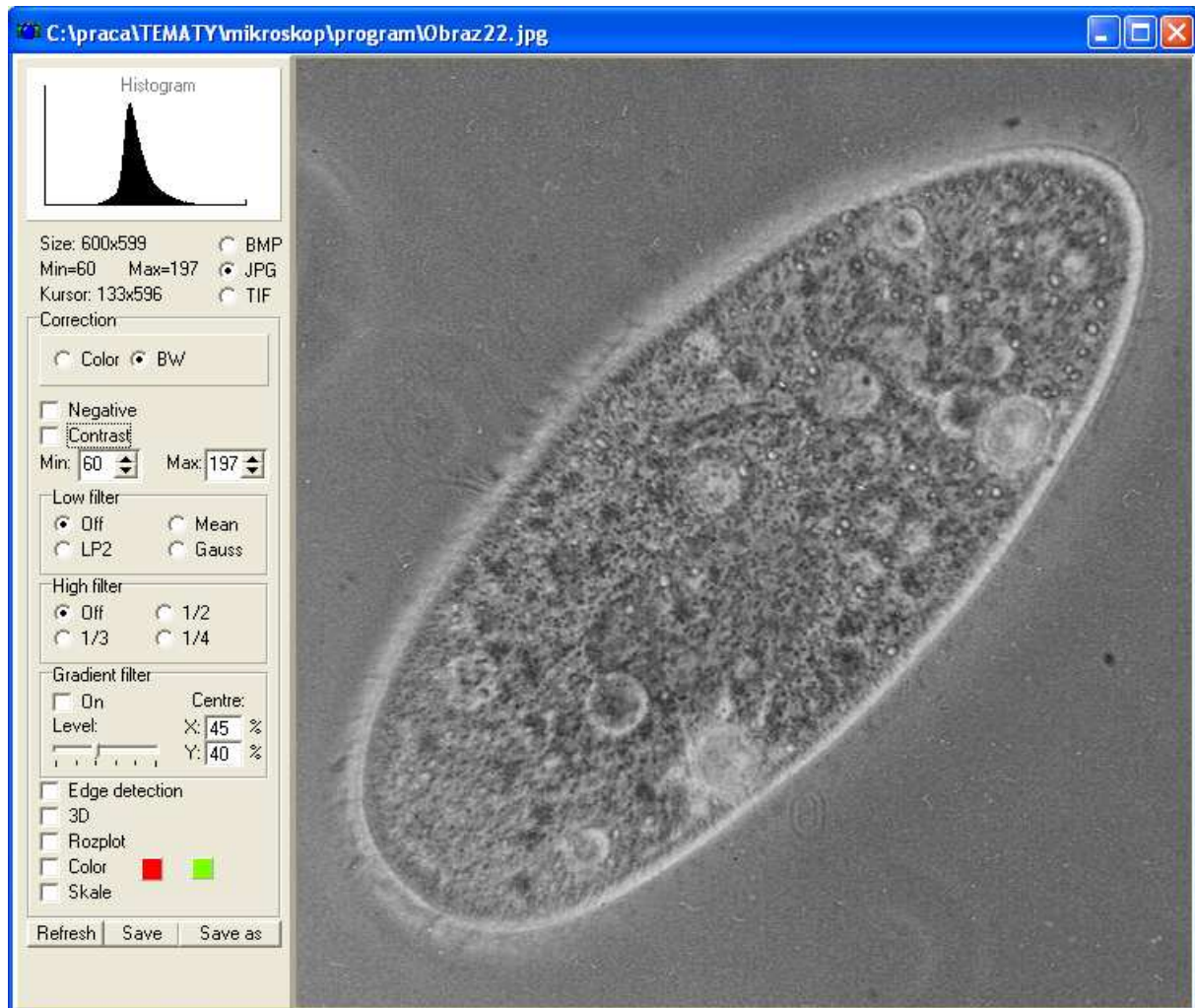


- special function (rozpl);
- function adding colours artificially;
- function adding multiple colours;
- **Refresh** and **Save** buttons allowing respectively refreshing of the scanned image and saving it under the name that is to be found on a title bar at the top of the window.

Meaning and description of the following pre-processing functions

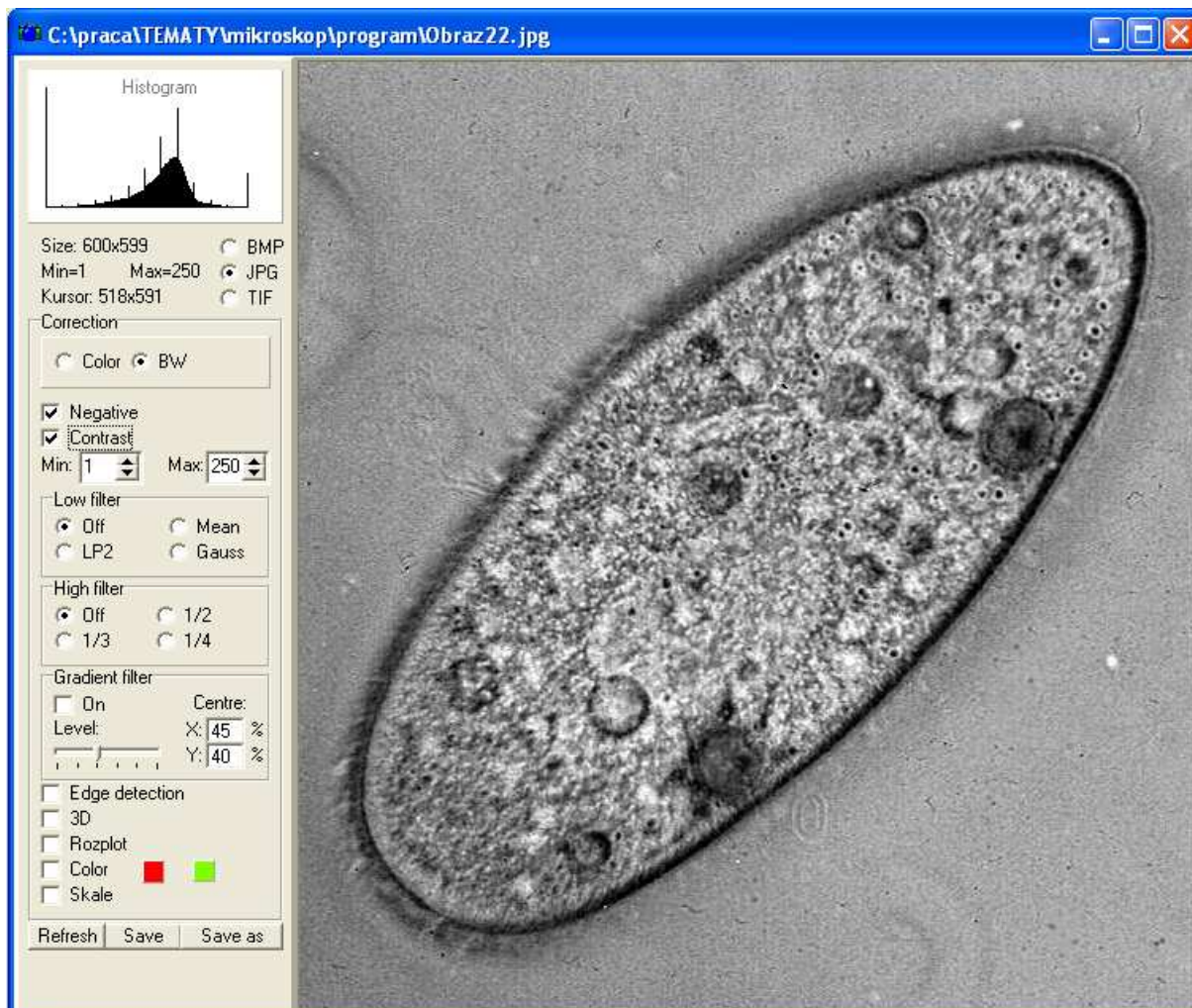
Image from a scanning microscope (does not concern optical microscopes) is basically black & white, therefore there is no point in scanning it in colour version. However, the program was written in a universal way allowing (with some modifications) scanning images from ordinary video cameras. Selecting black&white scanning mode accelerates the process of data processing to a large extent.

Exemplary scanned photo in black& white is presented below:



In some cases, negative-option allows to obtain clearer (however inconsistent with the real image) image.

Example to be found below:

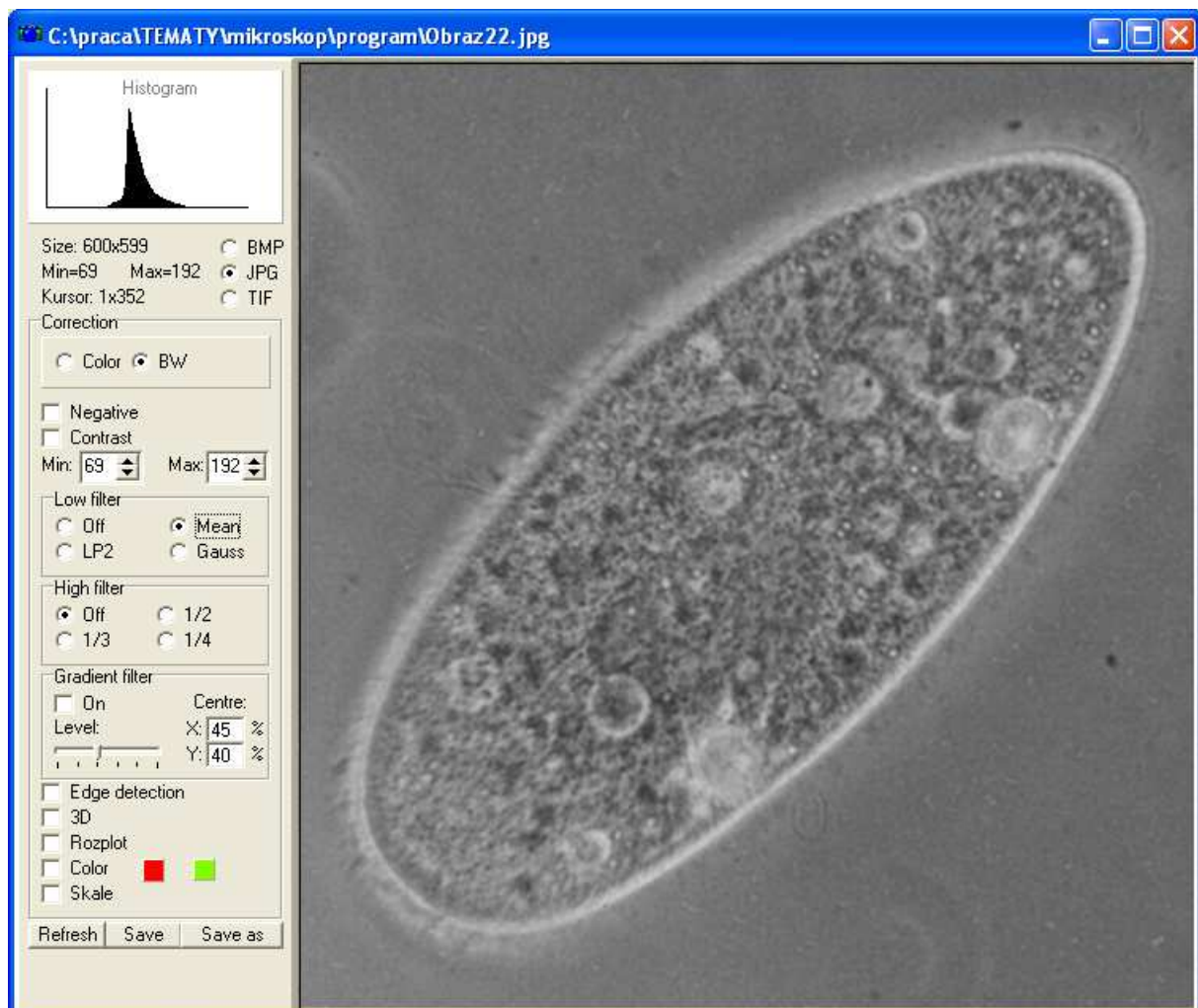


Low-pass filters are used in order to 'smoothen' the image and reduce the pixel-sized noises. It makes sharp transitions softer and those which are soft - leaves almost unchanged. An image becomes somewhat 'blurry' (fuzzy). Low-pass filters can paradoxically be used to make the image sharper. Fuzzy image is deducted from the original one. In that way, details can be emphasized.

Below, implemented low-pass filters to be found (below - a standardization factor, by which the result is to be divided):

averaging	LP2	Gauss
1 1 1	1 1 1	1 2 1
1 1 1	1 4 1	2 4 2
1 1 1	1 1 1	1 2 1
9	12	16

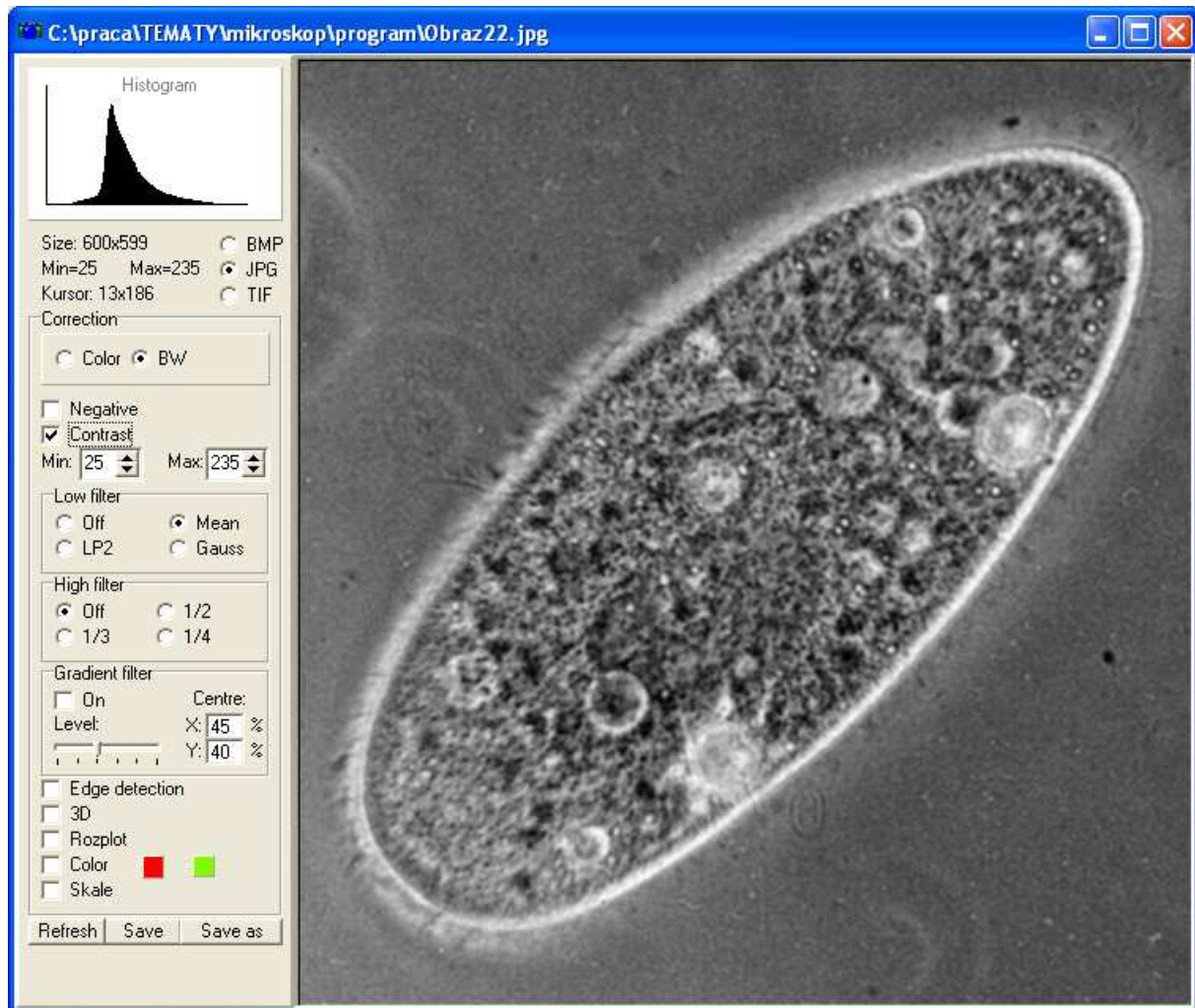
Scanned image with the use of averaging filter to be found below:



As can be noticed on histograms attached to particular scanned photos, as a rule pixels have weights (brightness) similar to one another, thanks to which an image has similar colours. 'Stretching' curve on a histogram artificially, we obtain improved brightness and contrast of an image.

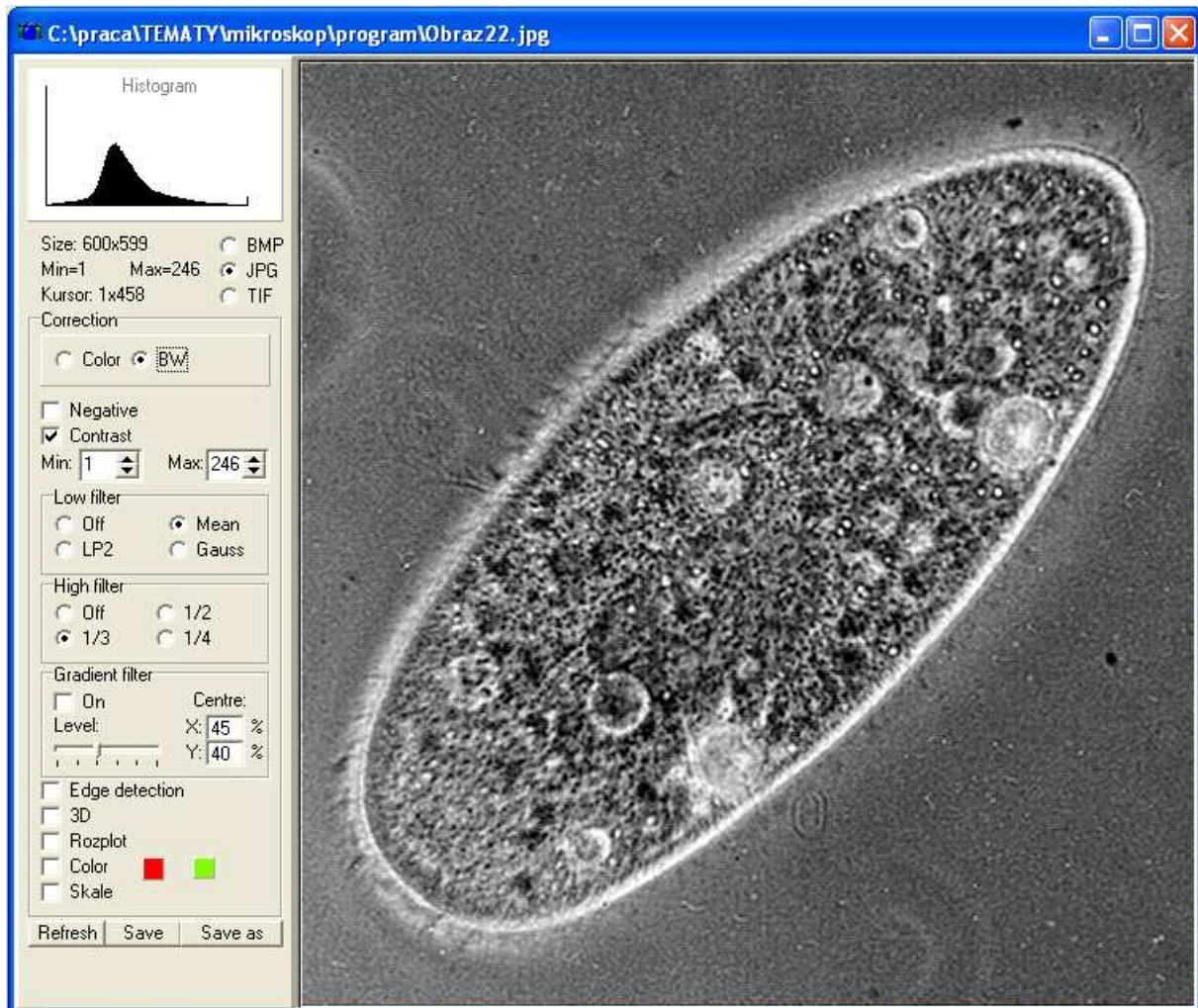
Edge parameters of the function (Min and Max) define the weight starting from which changes will appear. The program suggests the most optimal edge points, which however can be changed.

Example of a scanned image after choosing contrast-improving function:



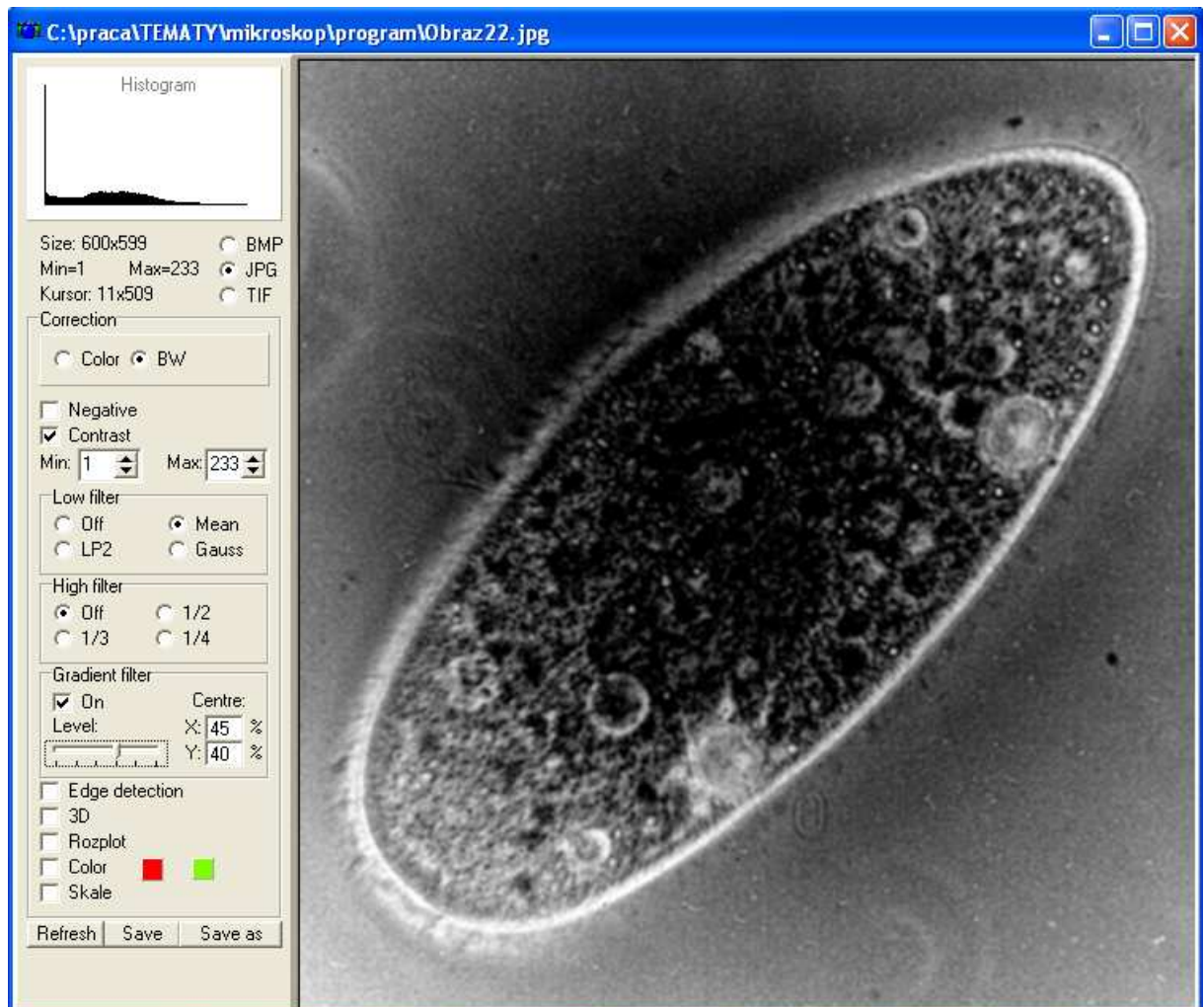
High-pass filters are used when we want to intensify details in the photo – enhance the edges, change brightness. After using such a filter a photo becomes sharper and increases its ‘sharpness’. However, that filter enhances noises as well – that is accidental imperfections of the value of pixels in the photo.

Exemplary use of filter nr 9 is presented below:



In some cases (i.e. for diffraction images) is need to decrease brightness in a part of image. In this program we can use a gradient filter.

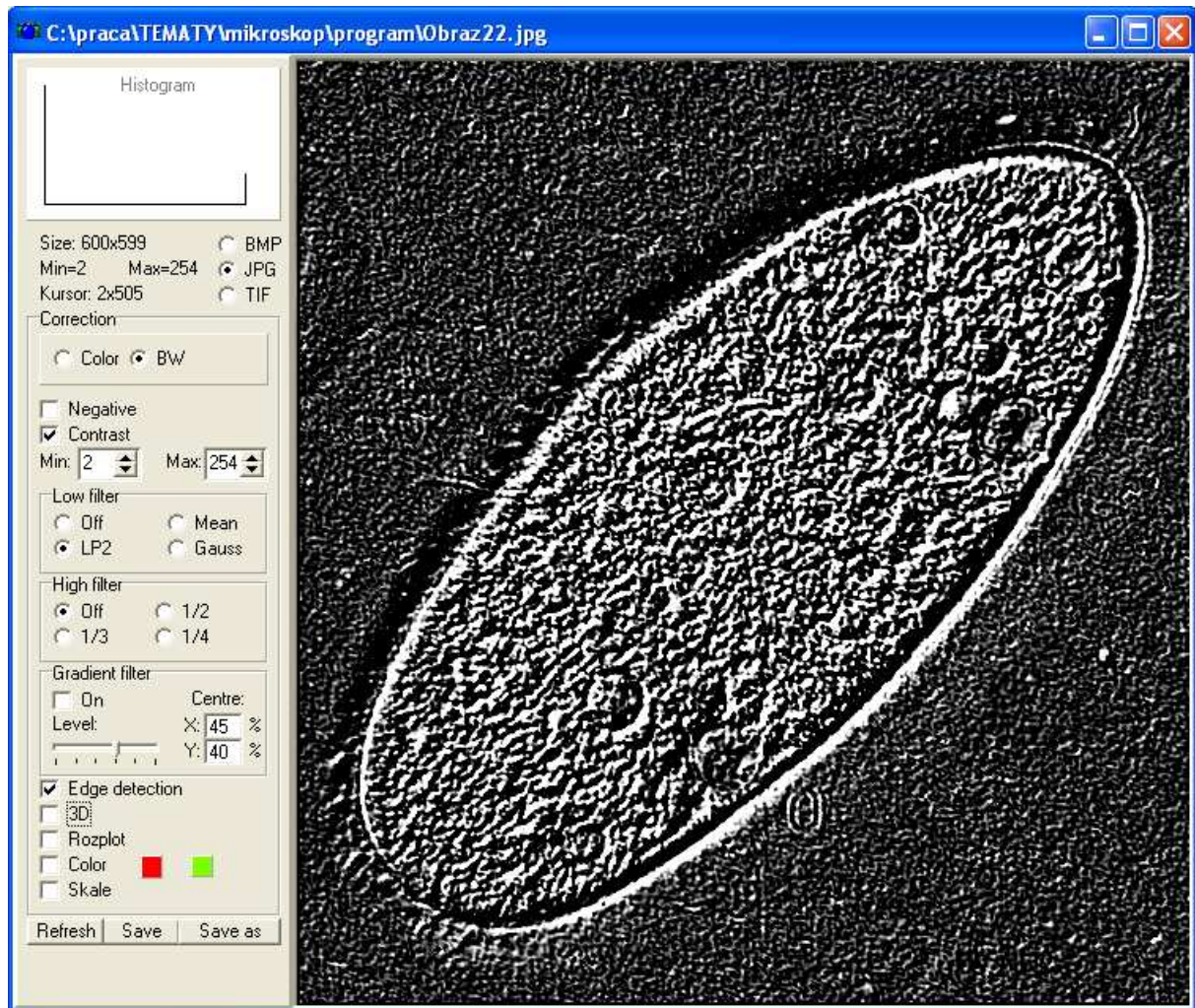
Scanned photo with the use of filter described above:



When we are interested in the edges in the photo, edge filters can be used. The whole photo is black then, and only the edges and -dark transitions are visible.

This filter is similar to the next named 3D.

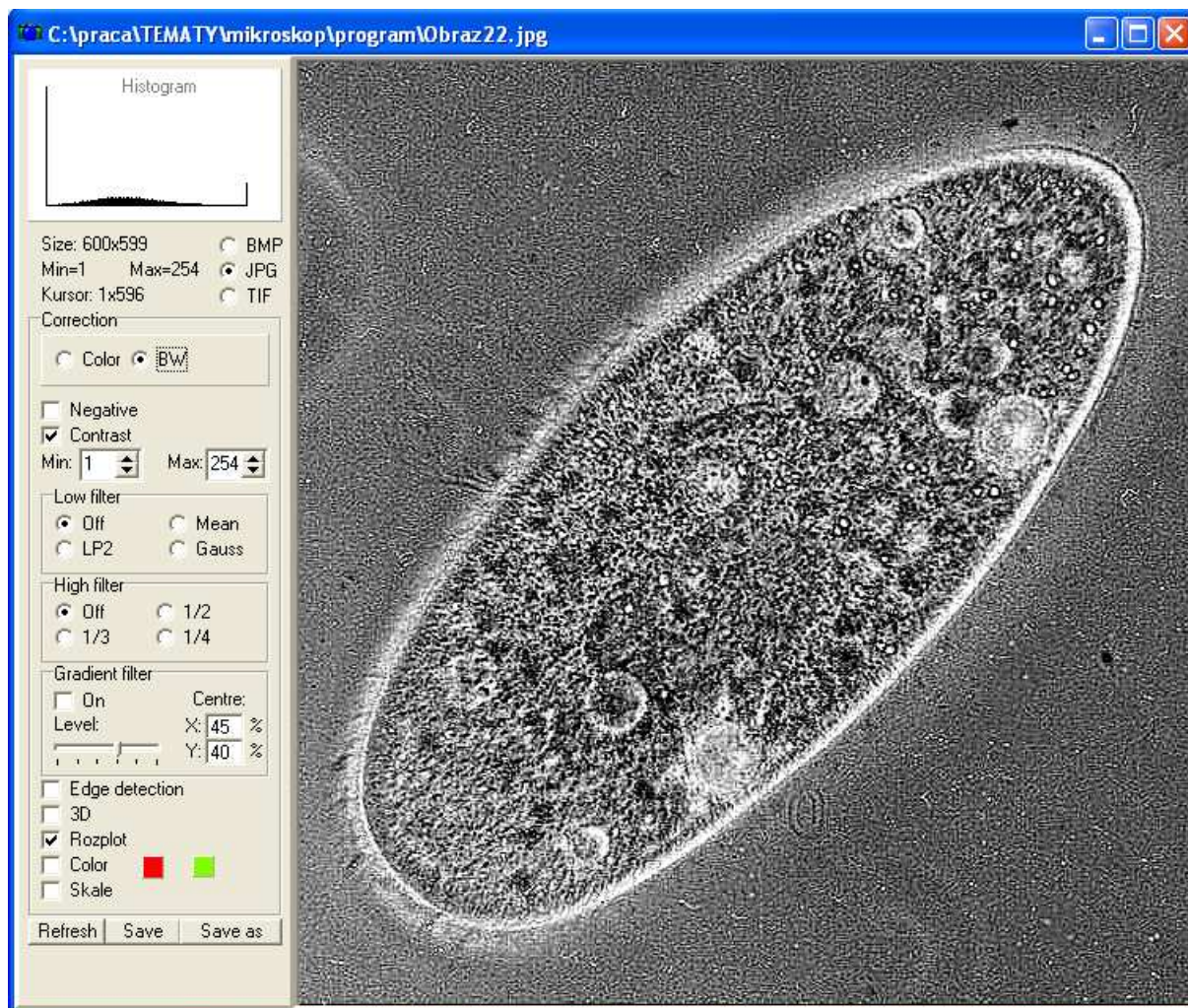
Scanned photo with the use of filter described above:



It happens quite often that images due to many various reasons are of poor quality. For instance imperfect optics or wrongly set sharpness, etc. Since these noises have similar arrangement to that of Gauss filter, they become blurry. If there was a method reversing the effect of combination with that filter, we would obtain a better quality of a photo.

Simple function disabling such a combination with Gauss filter was implemented into the program.

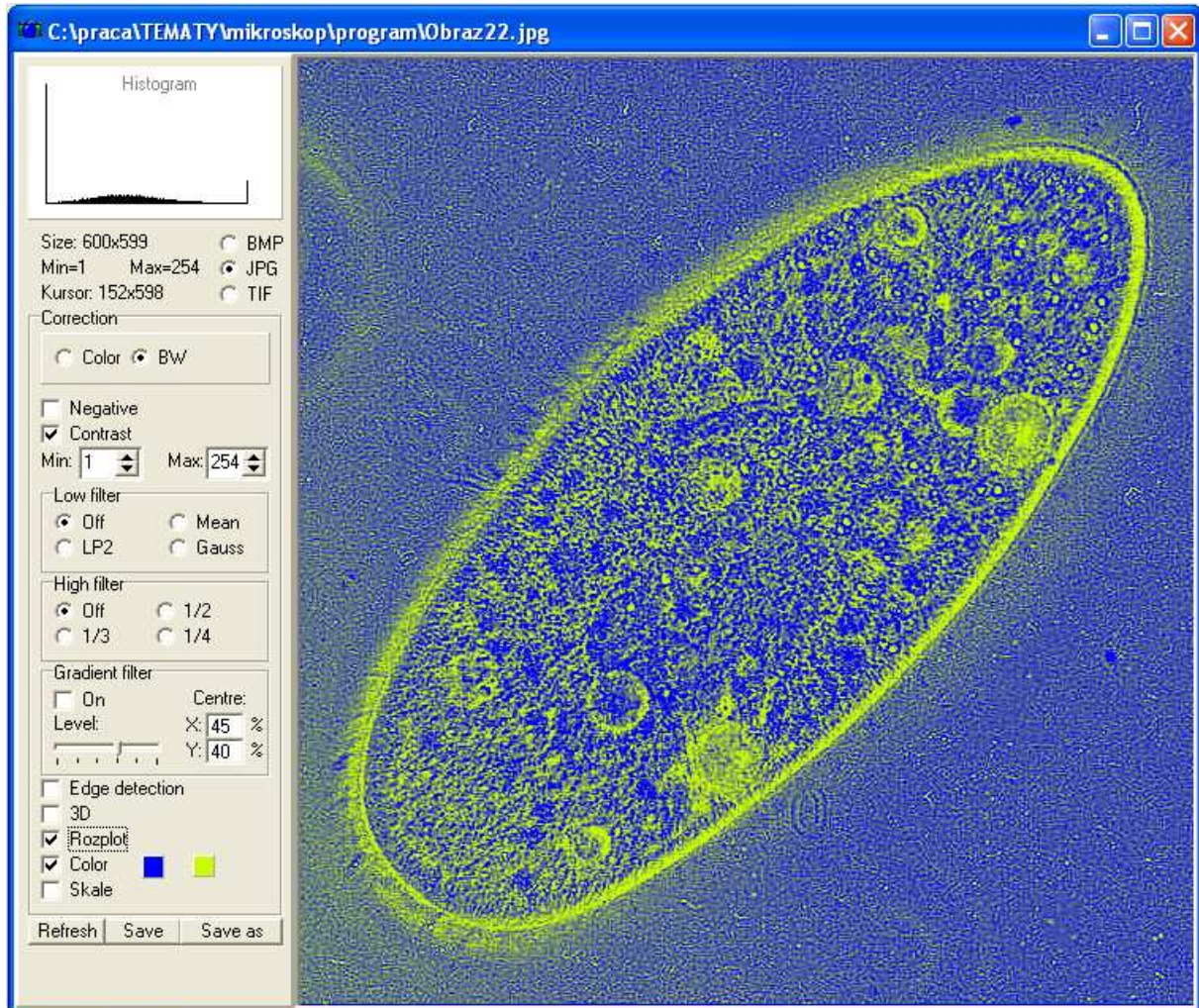
Example of how it works to be seen below:



In some cases in black & white photography artificial colour adding is done on purpose. It does not increase the number or the quality of the information that the image contain. It, however, allows to create images of more natural looks.

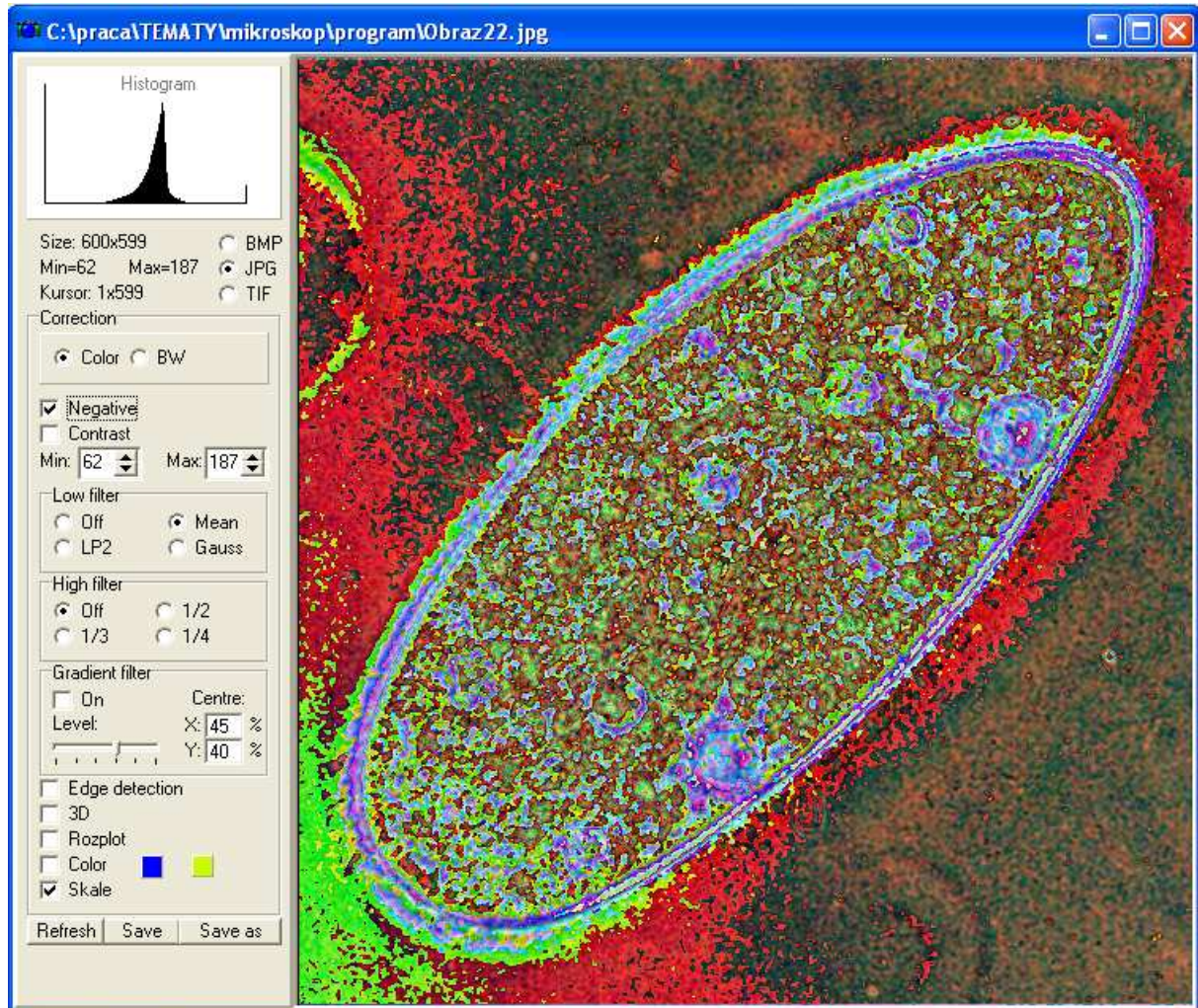
VID.EXE allows such a treatment with additional parameters: colour corresponding to black and the colour corresponding to white.

Example of 'coloured' image to be found below:



Additional function of the program is adding colours in the spectrum similar to the one that is found in maps (cartography). It is a sort of a specific filter allowing to enhance details in very low contrast (requires choosing colorful scanning option).

Example to be found below:



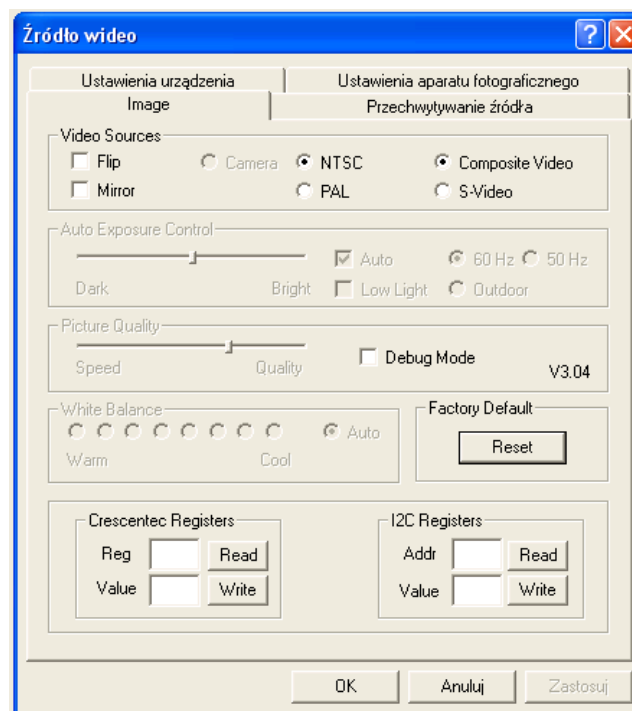
Drivers

Particular adapters are made using various integrated circuits. Produces usually provide ready-to-use drivers so that they work properly. Some of those used by ELBIT Company can be found below.

Converter Crescentec

Choosing **Source** button opens a dialogue window in which parameters of converter's work can be changed.

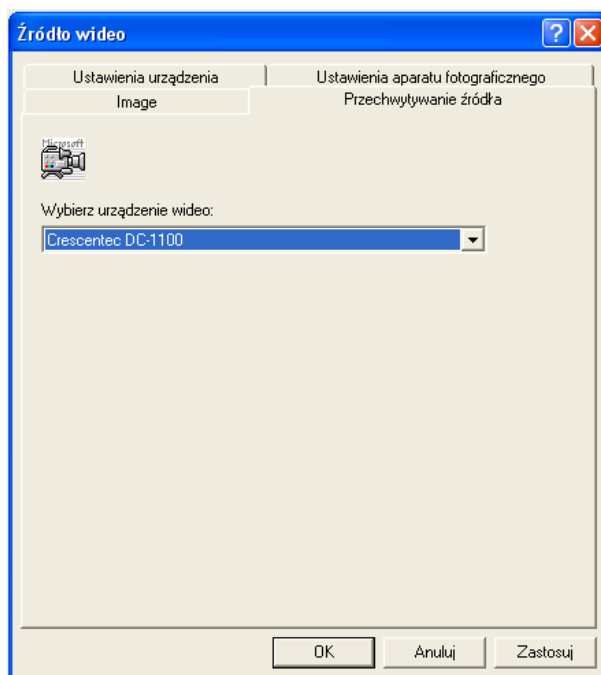
Example of a window is presented below:



Only upper panel was left for a user to change (Video sources) with functions):

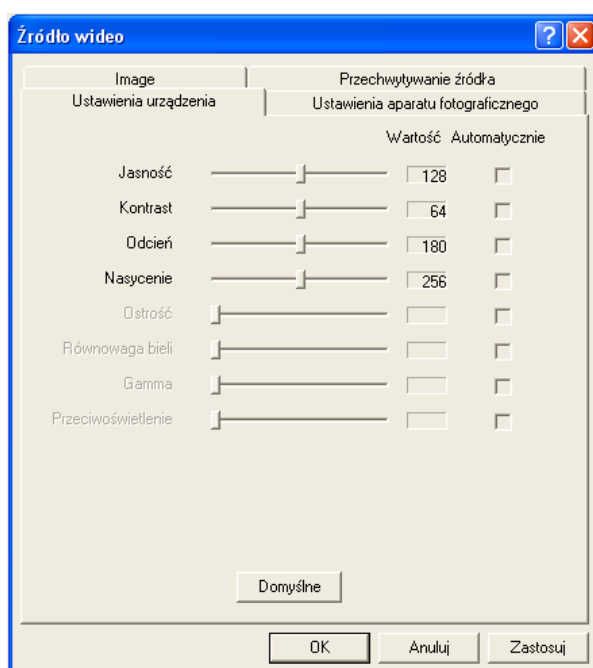
- **flip** – flips a scanned image;
- **mirror** – mirror image of a scanned photo;
- **NTSC/PAL** – signal type selection;
- **Composite Video/S-Video** – input type selection.

The next tab (**Source selection**) allows to choose the type of an input device. In this case it is always a device called *Crescentec DC-1100*.



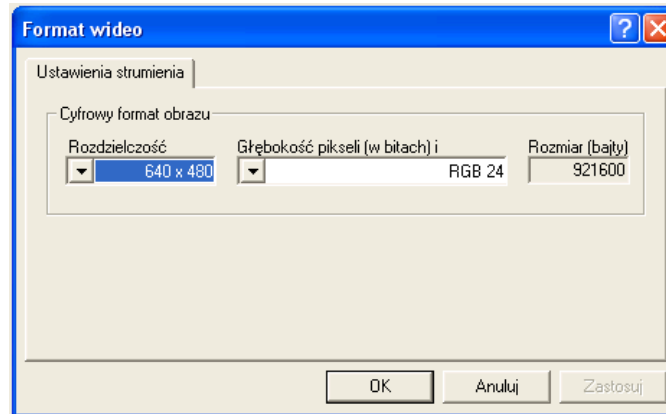
The next tab (**Device settings**) allows to change basic parameters of the scanned photo, such as:

- brightness;
- contrast;
- shades;
- saturation;
- sharpness.



Choosing **Format** option in video input control panel opens a dialogue window where scanning parameters can be changed.

Example of such a window to be found below:



Faster computers may scan photos in formats listed below:

- 160 x 120;
- 176 x 144;
- 320 x 240;
- 352 x 288;
- 640 x 480;
- 720 x 480;
- 720 x 576.

Pixel depth can be set using three different standards:

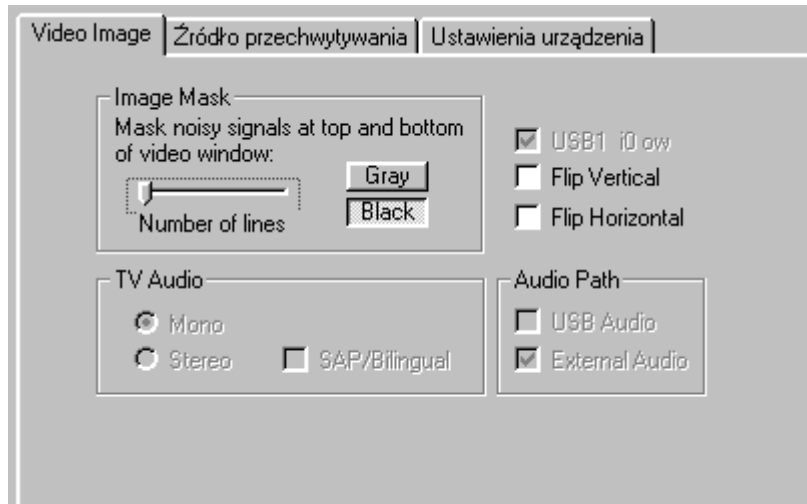
- YUY2;
- I420;
- RGB 24.

Suggested coding standard RGB 24-bit.

Converter 2820

Choosing **Source** button opens a dialogue window in which parameters of converter's work can be changed.

Example of a window is presented below:

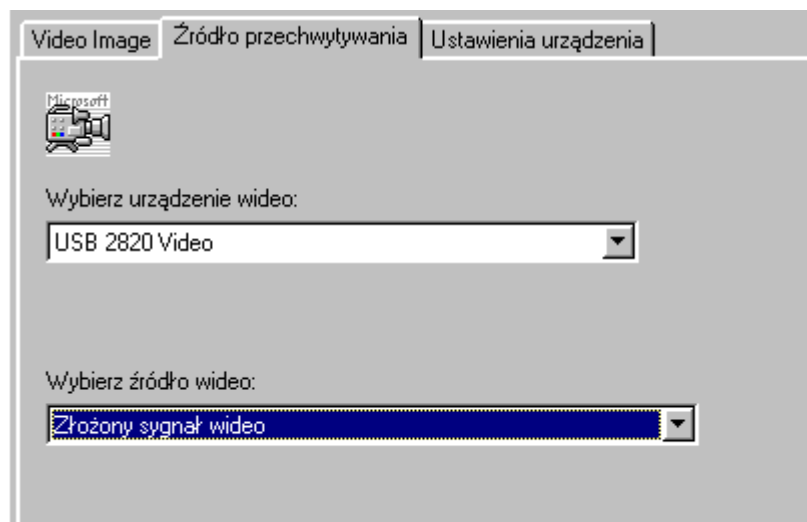


User can change the number of horizontal lines in the photo that are to be masked as well as mirror image of a photo:

- **Flip Vertical** – vertical flip of a scanned photo;
- **Flip Horizontal** – horizontal flip of a scanned photo;

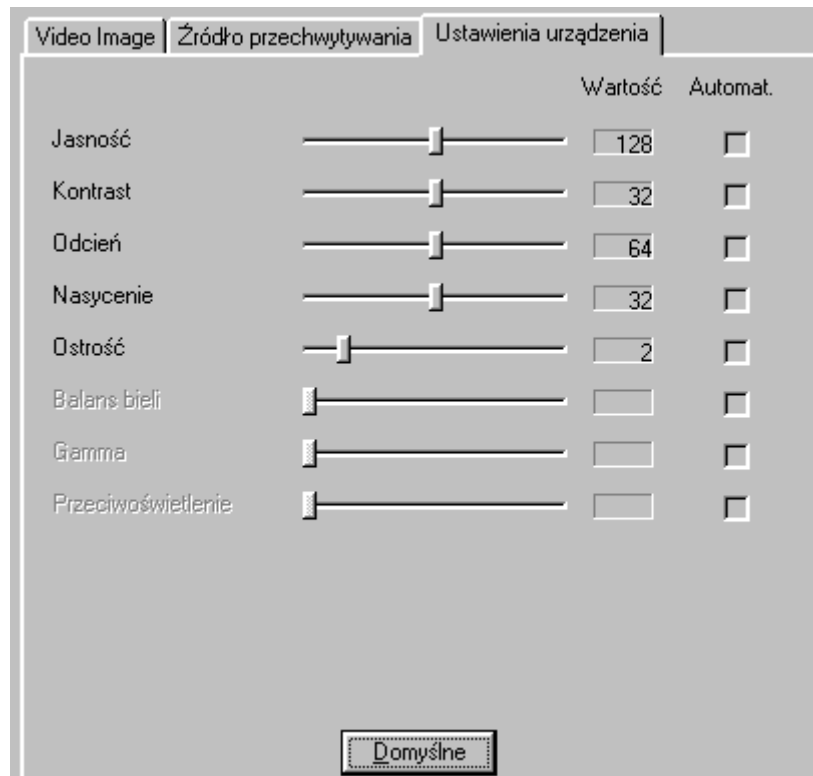
The next tab (**Source selection**) allows to choose the type of an input device. In this case it is always a device called **USB 2820 Video**.

Complex video signal is used as an input signal.



The next tab (**Device settings**) allows to change basic parameters of the scanned photo, such as:

- brightness;
- contrast;
- shades;
- saturation;
- sharpness.

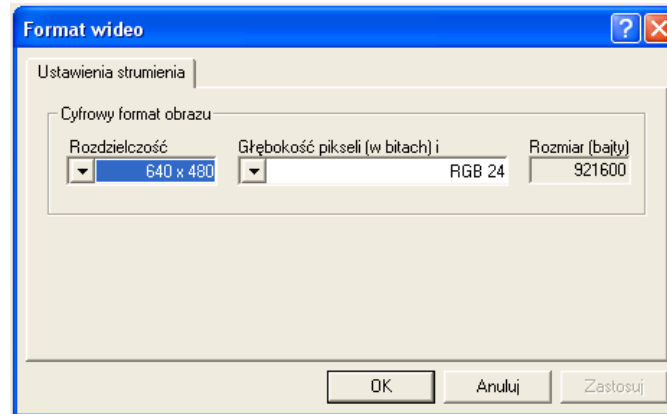


		Wartość	Automat.
Jasność		128	<input type="checkbox"/>
Kontrast		32	<input type="checkbox"/>
Odcień		64	<input type="checkbox"/>
Nasycenie		32	<input type="checkbox"/>
Ostrość		2	<input type="checkbox"/>
Balans bieli			<input type="checkbox"/>
Gamma			<input type="checkbox"/>
Przeciwoświecenie			<input type="checkbox"/>

Domyślne

Choosing **Format** option in video input control panel opens a dialogue window where scanning parameters can be changed.

Example of such a window presents the picture below:



Faster computers may scan photos in formats listed below:

- 160 x 120;
- 176 x 144;
- 320 x 240;
- 352 x 288;
- 640 x 480;
- 720 x 480;
- 720 x 576.

Pixel depth can be set using three different standards:

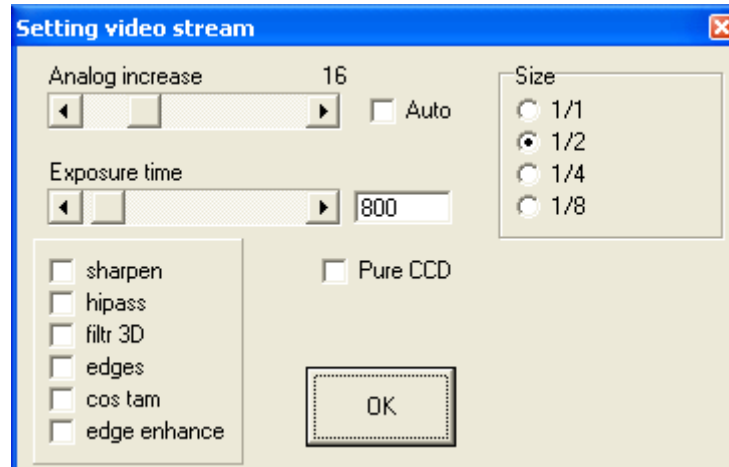
- YUY2;
- I420;
- RGB 24.

Suggested coding standard RGB 24-bit.

Converter Arco

Choosing **Format** option in video input control panel opens a dialogue window where scanning parameters can be changed.

Example of such a window to be found below:



The Analog increase and Exposure time allows to change a brightness of the scanned photo. A parameter Auto enable an automatic adjustment of analog increase. Always is better to change the brightness with analog increase.

A resolution can be chosen from listed below (for Arco2 converter):

- 1/1 (2000 x 1500);
- 1/2 (1000 x 750);
- 1/4 (500 x 375);
- 1/8 (250 x 187);