

Hemoglobin electrophoresis film analysis

Scan Films

HellabioScan analyzes images that are stored at any picture format (*.jpg, *.pif, *.tif, *.bmp etc) in your computer. The user can use any peripheral device in order to obtain these images (such as Scanner, Digital Cameras etc.) and store them to his computer.

Recommended analysis for a typical Electrophoresis film is **100 dpi** by 256 gray level color depth (it combines a very good final result with a small image size in computer's hard disk). At this point we must refer that the basic scanning parameters such as dpi analysis or color depth have small effect to the final results of HellabioScan software.

Saving Images

After scanning completion HellabioScan program asks the user to save the image. Recommended storage file \rightarrow Follow the path C: \rightarrow program files (X86) \rightarrow evresis \rightarrow HellabioScan(SQL) \rightarrow Images. Alternatively the user can save the archives to a folder of his choice.

After saving completion HellabioScan captures automatically the scanned image and presents it on the Application's Desktop (default position is on the right side of desktop). **Figure 1** shows HellabioScan desktop after a typical electrophoresis film scanning.





Additionally the user can load a saved image at any time, using the Open Image selection in File submenu (Ctrl + O) or by click on Toolbar's Open Image icon.

Importing patient data

After scanning and saving completion, the user should enter the patient's personal data. HellabioScan software uses a database for patient registration. Using the option **tables** (above the toolbar), select the **Patients** option (**Fig. 2**)





The database table will appear (Fig. 3).

| 🞽 Hellabio Scan | | | | | | |
|-----------------|----------------|-----|--------------------|----------|--------------------------------|----------|
| File Edit Ta | bles Windows H | | | | | |
| N | | | + - 🔀 🔁 | | $\mathbf{k} \times \mathbf{k}$ | 0 |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | Patients (0 Re | | - | 0 | | |
| | ID | SSN | PersonName Address | City Cou | Intry Phone | BirthDay |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| | • | | | | | Þ |
| | | | | | | |
| | | | | | | |
| | | | | | | |



Using the option Tables again, select the New Record option, from the Tables submenu (Fig. 4)



Fig. 4

The program displays a submission form. The user should fill in all details, for each patient. **Figure 5** shows the submission form. Click on the option **Save**, to register the new patient.

| Patients | |
|---------------|---------------|
| | |
| SSN | |
| PersonName | |
| Address | |
| City | |
| Country | Protocols |
| Phone | |
| Date of Birth | 1/1/1990 |
| Doctor's Name | |
| Laboratory | Δ |
| Comments | |
| | |
| | |
| | Save 🔀 Cancel |
| | |

Fig. 5

Selecting Lanes

In general occasion Electrophoresis films contains more than one lane. The user can select for analyzing one or more lanes by drawing a rectangle using the computer's mouse. Just click on upper left corner and drag the mouse to the bottom right corner, drawing a rectangle that contains the lanes you want to analyze (**Fig. 6, 7, 8**).



After the lane selection the user can analyze the selected lanes using the Analyze button on Toolbar or choosing **Analyze** in File submenu (**Fig. 9**).

The program asks about the type of Electrophoresis film. Select **Hemoglobin (in alkali or in acid)** Electrophoresis Type (**Fig. 10**).







After the type selection the program automatically appears as many Calculation Windows as the selected lanes of the film. Figure **11** shows the HellabioScan Desktop after a 4-lanes selection of **4 blood samples**.



Fig. 11

Every Calculation window is an independent object. User can minimize, move or close any of the windows. Every Calculation window is labeled by a number according to the number of the lane in the film. In a Calculation Window the user can input several data about the examined sample. The **Patient** field must contain information about the examined sample (*e.g. the patient's name*) because it will be the filename of the specific examination. This is the name that the user must search in the future, if he wants to retrieve data about this examination.

Using the button **1** the user can enter the patient's name (**Fig. 12**).





Select the patient by double-clicking on the arrow that appears on the left side of the table (**Fig. 13**).

| 10 | CCN | | 0.1 | | | D: 4 D |
|------------|-----|----------------------|------|---------|-------|-----------|
| ID | SSN | A PersonName Address | City | Country | Phone | BirthDay |
| 45a423b9- | | Sample 1 | | | | 16/2/1997 |
| 7e10e7af-9 |) | Sample 3 | | | | 1/1/1990 |
| 898de7d1- | | Sample 2 | | | | 1/1/1990 |
| | | | | | | |
| | | | | | | |

Fig. 13

Using the Calculate button on Toolbar the program executes an image processing algorithm that automatically defines the fractions of the image and calculates the density of each area. The results are presented at the down white part of the calculation window (**Fig. 14**).





The user can enter various data information's (e.g. hemoglobin value, comments concerning the diagnosis) (Fig. 15).



Fig. 15

After the electropherogram analysis, the user can **Save** and **Print** the protocol (**Fig. 16**).





| 1 | | | | | | |
|----------------|--------------------|-----------------------------|-------|----------|--------------|---------|
| Patient SSN | sample 1 012345 | | | 2 | Date | - |
| Туре | | n (in Alkali) | • | То | tal Quantity | 13,8 |
| (| Comments | | | | | |
| | | Sample | | | | ~ 1 |
| | | Result Image | | | | |
| | | | , | | | 🎯 Print |
| | | | | | | 💐 Save |
| | | | | | | |
| Fractions | Values(% |) g/dl | NV(%) | | | ^ |
| | | 2,69 96,7 - 9),31 1,5 - | | | | |

Fig. 16

Adding an image to the protocol

Before printing, the user has the ability to preview the protocol (**Fig. 17**). HellabioScan software is automatically inserting the image of the electropherogram into the protocol. The user can choose whether or not to display the image in the protocol (**Fig. 18, 19**).



Fig. 17

| 🖳 Labels & Normal Values | 2 Town Towney | |
|--------------------------|--------------------------------|------------|
| Band Label | Norm | nal Values |
| НЬА | 96,7 | 98,5 |
| HbA2 | 1,5 | 3,5 |
| | | |
| Header Image | | <u> </u> |
| Secondary Image | C:\Users\Desktop\IMAGE0002.JPG | |
| 0 | k 🛛 | Cancel |

Fig. 18 The scanned film will appear in the protocol.

| SN : 012345 | I | | | |
|----------------|-----------|-----------|-------|-------------|
| xamined Sample | Fractions | Values(%) | g/dL | NV(%) |
| i | НЬА | 97,60 | 13,47 | 96,7 - 98,5 |
| | HbA2 | 2,40 | 0,33 | 1,5 - 3,5 |
| | | | | |
| Comments: | | | | |

Hemoglobin electrophoresis protocol

| 🖳 Labels & Normal Values | | | |
|--------------------------|----------|---------------|----------|
| Band Label | | Normal Values | |
| HbA | 96,7 | 98,5 | |
| HbA2 | 1,5 | 3.5 | |
| | | | |
| Header Image | | | <u>7</u> |
| Secondary Image | <u>г</u> | | _ |
| Coolinally image | 1 | | |
| 0 |)k | 🔀 Cancel | |

Fig. 19 Blank window. The scanned image will not appear in the protocol.



Hemoglobin electrophoresis protocol

Hemoglobin variants

Hemoglobin variants are mutant forms of hemoglobin in a population (usually of humans), caused by variations in genetics. HellabioScan program performs an image processing algorithm that automatically defines the fractions of the image and calculates the density of each area. However, recognition of the hemoglobin variant is not supported by the software. User has to identify the type of hemoglobin variant and enter the data to create the protocol (see example below).

Example



Electropherogram of pathological sample. An extra band appears between HbA and HbA₂ (HbS, HbG, HbD, Lepore). After electrophoresis on acidic pH, the hemoglobin variant was identified as HbS.

Protocol creation

After analyzing the electropherogram, the following data appear in the table of results (**Fig. I**)

| Fractions | Valu | es(%) | g/dl | | NV(%) |
|-----------|-------|-------|------|---|-------|
| HbA | 59,60 | 0,00 | 96,7 | - | 98,5 |
| HbF/S | 38,11 | 0,00 | 0,0 | | 1,0 |
| HbA2 | 2,29 | 0,00 | 1,5 | | 3,5 |

Fig. I



By clicking on the **PrintPreview** option, the user can modify the data (**Fig. II**). After modification, the user can **Save** and **Print** the protocol.

Fig. II HbS Normal values NV (%): 0%

| Patient : sample SSN : 012345 Date : | 1 | | | |
|--|-----------|-----------|------|-------------|
| Examined Sample | Fractions | Values(%) | g/dL | NV(%) |
| 1 | НЬА | 59,64 | 0,00 | 96,7 - 98,5 |
| | HbS | 37,99 | 0,00 | 0,0 - 0.0 |
| | HbA2 | 2,37 | 0,00 | 1,5 - 3,5 |
| | | | | |
| Commenter | | | | |
| Comments: The Doctor | | | | |

Hemoglobin electrophoresis protocol

Manual correction

In some cases the software may not define the fractions properly. This may be due to technical errors (improper sample application, gel injury, insufficient staining-destaining). In this case the software allows the user to correct the errors manually.

<u>Toolbar</u>

In the toolbar, the user has the following options:





Example 1



Displaced position of separator.

For manually correction, the user must follow the following steps:

- I. Click on the option
- II. Move the mouse to the displaced separator. A cross will appear on the separator (**Fig. 1.1**).



Fig. 1.1

III. Move the separator (Fig. 1.2)



Fig. 1.2

Example 2

| Sample | |
|--------------|--------------|
| Result Image | PrintPreview |
| | 🎯 Print |
| | I Save |

More separator /separators

For manually correction, the user must follow the following steps:

- I. Click on the option
- II. Click on the option
- III. The following table will display on the desktop (Fig. 2.1).

| - Delete a Separator | |
|----------------------|--------|
| Separator Number | 1 🕂 |
| - Delete | Cancel |

Fig. 2.1

- IV. Select the separator you want to delete (**Separator Number**). Click on the option **Delete.**
- V. Alternatively move the mouse over the separator you want to delete. A cross will appear on the separator.
- I. Right-clicking will display the following table (Fig 2.2)

| Hellab | io | X |
|--------|-----------------------|----------|
| Dele | te this Fraction Sepa | arator ? |
| | Yes | No |

Fig. 2.2

II. Click on the option **Yes.** The separator has been deleted (**Fig. 2.3**).



If you need further assistance or have any questions related to the HellabioScan program, please do not hesitate to contact our team.

Email: hellabio@hellabio.com & hellabio@thermokoitida.gr