

## HGMF INTERPRETER Vers 2.0 Microbial Colony Counter

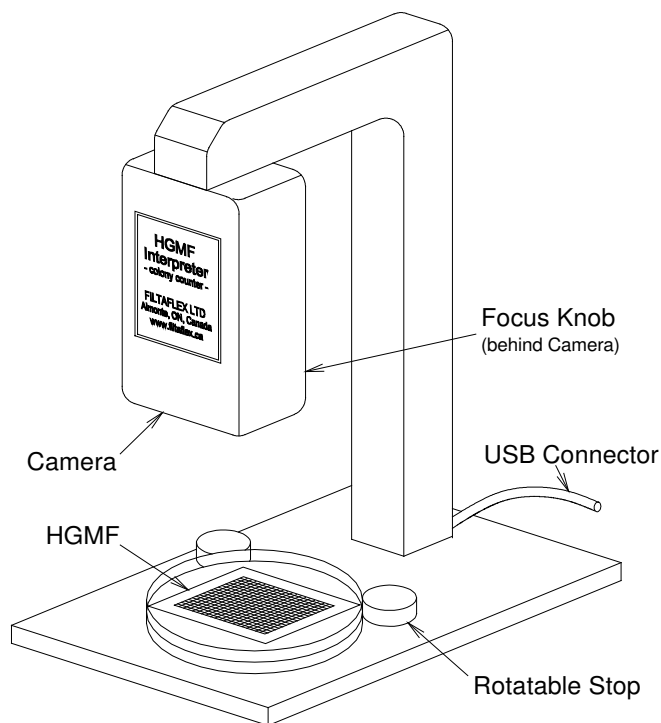
This version of the HGMF Interpreter will enable you to do preliminary simple profiling, based on whether all grid cells in the same coordinates across a set of HGMFs were positive. The data file it builds for this can also be examined in more detail by more sophisticated database software.

### **PLEASE NOTE THAT:**

This Vers 2.0 HGMF Interpreter software is relatively untested. Its profiling capability is limited to showing which grid cell coordinates were positive across *all* HGMFs out of a chosen profiling set of up to *eight* HGMFs. To make more detailed profile examinations (for example, whether a particular grid cell coordinate was sometimes "0" and sometimes "1"), or on profile sets of more than eight HGMFs, you will need to use a more sophisticated database program.

Frankly, we do not know what profiling tests users will eventually find they need, nor how they would like to organize their work...if we receive useful feedback on this we will be happy to rewrite the software accordingly. For example, currently, all operations are carried out on just one computer monitor, with the **Grid cell biochemical profile** screen overlaying and hiding buttons and textboxes of the main Interpreter screen -

if users were prepared to use two monitors, then one could be used for counting and the other for profiling (with much more information and options being made available).



The HGMF Interpreter

### **IMPORTANT:**

- **MAKE FREQUENT BACKUPS OF FOLDERS - "..\Data", "..\Images", and "..\Officework"...**
- **NEVER DELETE ANY OF THE IMAGES IN THE "\Images" FOLDER**
- **NEVER EDIT THE FILES "..\Data\HGMFData.txt" or "..\Data\ProfileData.txt".**

(One more thing):

**... PLEASE READ THE MANUAL AT LEAST ONCE BEFORE STARTING...**

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## UNPACKING YOUR HGMF INTERPRETER

The shipping package contains:

- The HGMF Interpreter Camera unit; An Installation CD; This Manual

## SYSTEM REQUIREMENTS

- CPU speed 250 MHz or faster
- USB 1.0 or 2.0 port
- 128 MB RAM minimum
- Windows 98 or later
- 8 MB disc space for program files - extra required for image and database storage
- (Note: each record of the HGMFData.txt file occupies 2,304 bytes of disc storage space, each record of the ProfileData.txt file occupies 13,600 bytes, each filed HGMF .jpg image occupies approximately 45-60 KB, depending on their complexity)

**Optimum monitor/screen size:** A monitor with aspect ratio 5:4 should be used, with a resolution of 1280 x 1024 or greater. Other screen sizes or formats may give unpredictable results.

## INSTALLING THE CAMERA

- Place the Camera where it will receive diffuse daylight or normal lab illumination. If fluorescent lights cause "strobing" patterns on the image an incandescent lamp may help. Avoid shadows from other equipment.
- Plug the Camera's USB connector into your computer's USB port. Windows should detect new hardware and install the correct driver. If you are requested to obtain a driver from an external disk insert the CD, navigate to the folder "Camera Video Drivers" and follow the instructions.

## INSTALLING THE SOFTWARE

- Please first read the CD's "README.TXT" file for up-to-date instructions.
- Read the Section below, regarding software piracy.
- Insert the CD. Navigate to and run SETUP.EXE.
- *It is strongly advised that you use the default folders suggested by the installation program.*

## GETTING STARTED

- When you press "**Start | Programs**" you will see "HGMF Interpreter" in the list of programs (it has an "HGMF" icon). To access the program easily in future use the right mouse button to create a desktop shortcut.
- Connect the camera and double click the HGMF Interpreter icon. The program will load.
- Use a pen to make a mark in one corner of an HGMF and place the HGMF in a half-filled Petri dish under the camera. Note the rotatable Stops - set them so that the HGMF aligns centrally under the Camera, as indicated by the onscreen image.

The following adjustments will require several seconds to appear onscreen:

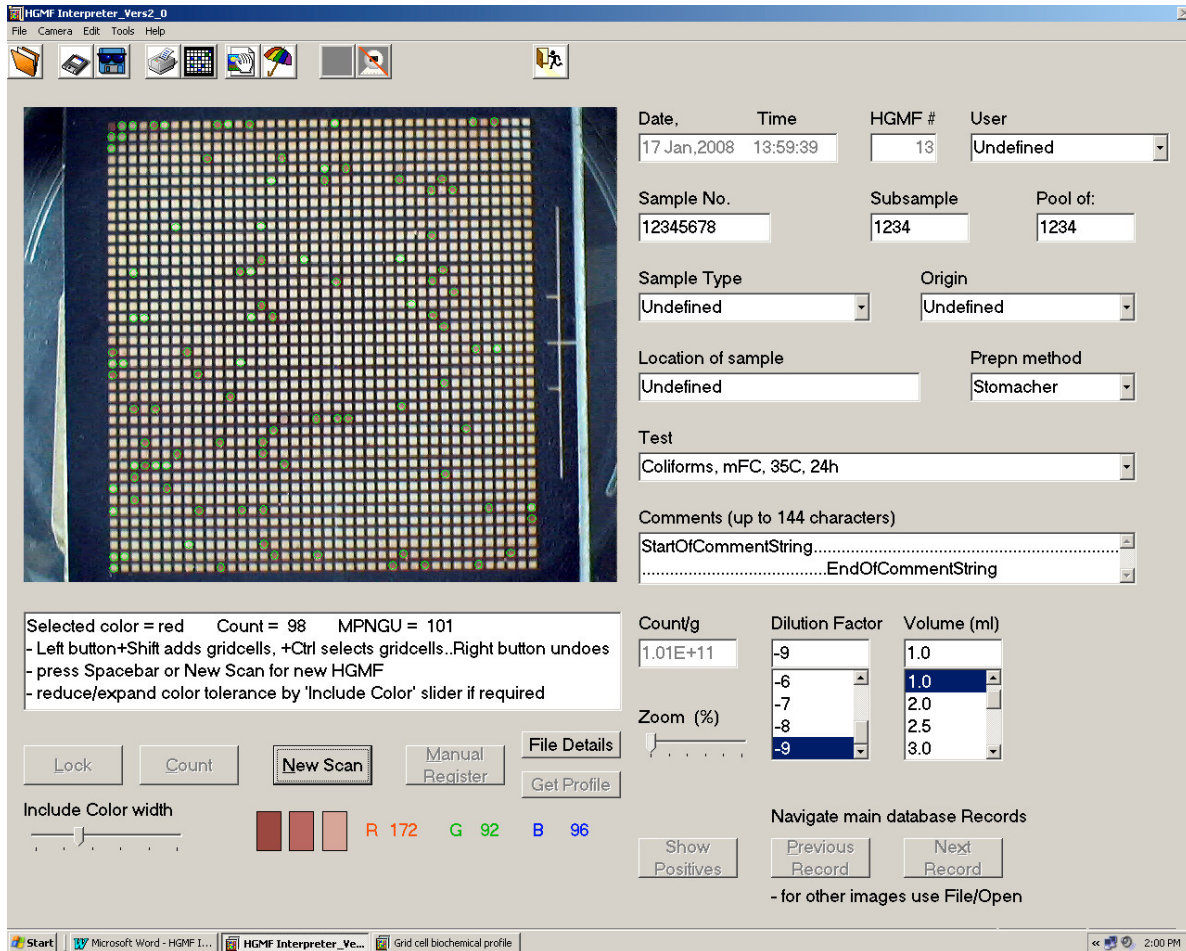
- *Resolution:* From the Interpreter menu select "Tools-Video Format". Check that "Resolution" is set at 640 x 480 pixels.
- *Video Quality:* From the Interpreter menu select "Tools-Video Source" and the tab "Main". Make sure the radio button "Optimized for best image quality" is clicked.
- Observe the HGMF - the mark you made should appear the right way around - if not, from the Interpreter menu select "Tools-Video Source" and the tab "Image". You have options to "Mirror Image" or "Flip Image"
- *Focus:* The Camera was shipped prefocussed. If the image is unfocussed, slowly rotate the knob behind the lens to reach maximum sharpness.

That's it! Your HGMF Interpreter should be ready to use. If you still experience problems, contact the Vendor via:  
Tel: 613 256 3066; Fax: 613 256 8681; Email: [tsharpe@filtaflex.ca](mailto:tsharpe@filtaflex.ca)

## Copying this Software

Your HGMF Interpreter was purchased as a combined software and camera hardware unit. *The software was developed to work properly only with the camera unit that was purchased with it and may give unpredictable results with other camera hardware.* You or your organization are authorized to copy or install this software on as many computers as you wish within your Organization and operate any one of these as an HGMF Interpreter by plugging the camera into one of its USB ports before starting up the software. *If you are not authorized to use or copy this software you are advised to read the Section **Protection Against Piracy.***

## The HGMF INTERPRETER OVERVIEW



**Typical HGMF Interpreter Vers 2.0 screen just before filing data**

The HGMF Interpreter was developed by Filtaflex Ltd. to count microbial colonies on ISO-GRID HGMF™ hydrophobic grid membrane filters. It comprises a stand-mounted camera connected to a computer via a USB port, and dedicated image analysis software operating under Microsoft Windows. The camera used in the current version of the HGMF Interpreter produces images of 640x480 pixels. The Interpreter operates in three modes.

In "**Real time**" mode it will recognize an HGMF placed under the camera and calculate ("register") the positions of its 1,600 grid cells. When you indicate (with the mouse) the desired color of target colonies, the Interpreter will flag grid cells containing colors close to the chosen color and display the Most Probable Number of Growth Units (MPNGU). You can then indicate *dilution factors* and *inoculum volumes* and read the sample's *Count/gram*. You then have the option of:

- immediately counting another HGMF;
- adding the sample data to a database file;

In cases of "difficult" HGMFs (e.g., badly obscured grids) you can use the mouse to help the computer accurately "register" the HGMF. And whenever an HGMF is being counted you can control the range of colonies included in the count by using the Shift and Ctrl keys in combination with the left and right mouse buttons, to ensure that the count completely reflects the target distribution.

In "**File**" mode you can reexamine file images produced either by from the Interpreter's camera. The various text boxes will also display the text data filed when the image was saved.

In "**Profile**" mode you can construct a database of growth/no growth results at all grid cell coordinates across a selected set of HGMFs.

## **EXITING THE PROGRAM**

Always exit the program using the File | Exit menu. This will allow the program to disconnect the camera and end smoothly. If you try to exit in other ways (for example, by pressing Alt+F4) the camera may not be disconnected and you may have to reboot your computer before continuing other applications or restarting the Interpreter.

## **LIGHT LEVELS**

The HGMF Interpreter will operate satisfactorily in normal laboratory ambient light levels. Minor intensity variations across the HGMF will not affect performance. Avoid excessive light levels (e.g, direct sunlight) on the HGMF. If the light level is too low small horizontal lines may appear inside the HGMF grid cell image - these can cause grid cells to be counted by mistake - avoid this condition by increasing the illumination level. A "Moiré pattern" may appear on the image when the only illumination is from certain types of fluorescent lamps - if this occurs try to arrange a contribution from an incandescent lamp or daylight.

The camera has an automatic exposure control. After removing your hand from the Petri dish the camera may require a few seconds to accommodate to the changed light level - the image on the monitor may brighten or darken. It is not necessary to wait until a steady-state is reached - you may press Lock (or Spacebar, or Enter) whenever the image colors seem sufficiently saturated for the comfort of your own eyes.

## **IMAGE JERKINESS**

The frequency at which the image strobos or jerks is governed partly by the inherent frame refresh rate of the camera and by the CPU speed - it will be less noticeable at higher CPU speeds. Push the Petri dish against the HGMF Interpreter's stops and rotate the HGMF *slowly* until it is suitably aligned - by moving too quickly you may overstep the position.

## **CAMERA HEIGHT**

The HGMF Interpreter is shipped with the camera height set to provide optimum image size for HGMFs in Petri dishes approximately 2/3 full of agar. At this agar depth the HGMF image occupies approximately 90% of the vertical height of the picture box. For thinner agar layers the image will appear somewhat smaller because the HGMF is further from the camera. It is not essential for the HGMF image to be the optimum size; however, if desired it may be increased digitally using the slider labelled "Zoom %" on the screen. The Zoom control is set at zero when the program starts and can provide magnifications up to about 10%.

*(Note that after adjusting Zoom:*

- you will have to use the "Lock" button rather than Spacebar or Enter to lock and register the image for counting;
- you may have to adjust the position of the Petri dish using the Interpreter's stops, in order to center the HGMF image correctly;
- if the image is too large the Interpreter may not be able to register it automatically and you may have to use Manual Registration).

## **NON-FLAT, CURVED OR DAMAGED HGMFS**

The HGMF Interpreter will be able to cope with HGMFs on sloping agar surfaces, and with minor deviations from flatness (planarity). However, results with HGMFs that are curled, corrugated or otherwise damaged will be unpredictable.

## **YELLOW CORNER-TARGET CIRCLE**

In real time mode a yellow circle in the upper left area of the image box indicates the area in which the HGMF Interpreter will "look" for the HGMF's top left hand corner as soon as you press "Lock" (or Spacebar or Enter). As long as the top left hand corner is somewhere within this circle the Interpreter should be able to find the corner and complete the Registration.

## **ALIGNMENT ACCURACY**

In general, as long as all the HGMF grid cells are visible in the image box the HGMF Interpreter should be able to register and count it. If AutoRegistration fails you might find it easier to press "New Scan" and readjust the HGMF, rather than do a Manual Registration.

## **AUTOREGISTRATION**

In real time mode, the HGMF Interpreter will default to AutoRegistration mode - that is, on pressing Lock (or Spacebar, or Enter) it will attempt to detect the top-left-hand grid cell, then "step around" the border intersections to register the HGMF automatically. The majority of HGMFs will register satisfactorily. Whenever the HGMF Interpreter is ready to AutoRegister an HGMF the text box below the HGMF image will display the message:

*Place HGMF TopLeft grid-cell inside yellow circle, with all grid-cells visible.  
Press Spacebar or click 'Lock' when HGMF is suitably aligned...*

First position the HGMF so that its top-left-hand grid cell is within the yellow circle (or just below it), and ensure that all of the HGMF grid cells are visible in the image. Then press "Lock" (or Spacebar, or Enter) - in quick succession you will see a solid yellow bullet blink just outside the top-left-hand grid cell, then the Interpreter will beep, the four corner grid cells will be "flagged" by green circles, and the text box below the HGMF image will display the message:

*Registered for counting ..... use mouse to indicate typical colony  
- Left button+Shift adds gridcells, +Ctrl selects gridcells..Right button undoes  
- press Spacebar or New Scan for new HGMF  
- reduce/expand color tolerance by 'Include Color' slider if required.*

If for some reason the AutoRegistration fails you will hear a warning tone and the information box will display the message:

*can't AutoRegister ... press Count if corner flags seem acceptable  
-press Spacebar to retry, or Manual Register to register manually ...*

If the four corner flags (green circles) look very close to being in their correct positions the Interpreter is probably still capable of giving a good count, and you can try counting by pressing the "Count" button - in this case you will see the same message as above:

*Registered for counting ..... use mouse to indicate typical colony.....*

If the four corner flags are not close to their proper positions you have two options:- either try to AutoRegister again by pressing "New Scan" then "Lock" (or twice on Spacebar or Enter), or; press the "Manual Register" button.

### **MANUAL REGISTRATION**

Manual Registration is normally only needed when the definition between an HGMF's corner grid lines and grid cells are badly obscured by dark colonies, causing AutoRegistration to repeatedly fail. The HGMF Interpreter indicates its need for the assistance of human eyesight by asking you to press the "Manual Registration" button - the text box displays the message:

*can't AutoRegister ... press Count if corner flags seem acceptable  
-press Spacebar to retry, or Manual Register to register manually ...*

After pressing "Manual Registration" the text box displays the message:

*Align HGMF properly, then press 'Lock'*

Align the HGMF so that all grid cells are visible, then press the "Lock" button (or Spacebar, or Enter). The text box will now display the message:

*Now click mouse pointer exactly in centres of corner grid-cells. Click in any order*

Follow the instruction, positioning the cursor carefully *at the centre* of each corner grid cell and clicking once. The text box will then display the message:

*Registered for counting ..... use mouse to indicate typical colony  
- Left button+Shift adds gridcells, +Ctrl selects gridcells..Right button undoes  
- press Spacebar or New Scan for new HGMF  
- reduce/expand color tolerance by 'Include Color' slider if required*

### **USING SPACEBAR AND ENTER KEYS**

To make life easier, the HGMF Interpreter will often respond to pressing the keyboard's Spacebar or Enter key as if you had "pressed" the next appropriate button by using the mouse. For example, when you are inserting the next Petri dish you may find it easier to simply have one hand on the dish and the other hand touching the Spacebar to "lock" the image, rather than also having to control the mouse to press "Lock" - the HGMF Interpreter will respond to pressing Spacebar (or Enter) by locking the image. Similarly, unless you have started to file data, pressing Spacebar or Enter will have the same effect as pressing "New Scan" with the mouse. At times, however, the Interpreter will not be sure what next action you intended and these keys will have no effect - in these cases simply press the desired button by using the mouse.

### **"NEW SCAN" BUTTON**

Pressing this button (or Spacebar or Enter) puts the HGMF Interpreter into "real time" mode, allowing you to adjust or replace the Petri dish and HGMF.

## "LOCK" BUTTON

Pressing this button (or Spacebar or Enter) causes the HGMF Interpreter to "grab" the current image frame and remain locked on it. If you had not previously pressed "Manual Register" the Interpreter will attempt to "register" the HGMF automatically. If you had previously pressed "Manual Register" the Interpreter will instruct you to accurately indicate the HGMF corners (see above).

## "MANUAL REGISTRATION" BUTTON

This button is disabled unless the HGMF Interpreter finds it cannot register the HGMF automatically.

## COUNTING POSITIVE GRID CELLS

As soon as the HGMF is registered you can count grid cells containing growths of a particular color. To make the HGMF Interpreter count the the grid cells of interest, simply position the mouse pointer on a typical growth (color target) and click with the *left* mouse button.

Experiment by dabbing an HGMF with colored markers and clicking different colony colors. Each time you click the HGMF Interpreter will flag grid cells containing that color (plus or minus a certain color tolerance value) and display the count as MPNGU, both in the text box and in the Count/g box. For example, if you clicked on a yellow growth and the HGMF Interpreter counted 197 positive (similar) grid cells the text box will display the message:

*Chosen color = yellow    Count = 197    MPNGU = 210*  
*Hold 'Shift' to add to count...use Spacebar or 'New Scan' for new HGMF.*  
*- reduce/expand color tolerance by 'Include Color' slider as required.*

and the Count/g box will display the value  $2.10E+11$  (i.e.,  $2.10 \times 10^{11}$ ). At the same time, the centre box of the three color boxes below the text box will fill with the color you chose, and the boxes to its left and right will fill slightly darker and slightly lighter, respectively, showing the limits of color tolerance to the count.

If the flagged grid cells appear to adequately represent the grid cells you would like to have counted you can proceed to correct the Dilution Factor and Volume (ml) and thus the Count/g by clicking the appropriate list item or typing in values - you will see the value of Count/g change accordingly.

If the grid cell count is *less than* the number of grid cells you would prefer to see you have several options:-

- left click on another colony of slightly different shade to the first
- hold down Shift and left click an uncounted grid cell - a new set of grid cells (based on the extra color indication) will be added to the count
- hold down Ctrl and left click uncounted grid cells to add them individually to the count
- move the Include Color slider left or right to decrease or increase the color range included in the count, then again click a colony. The Include Color default value of 30 will usually encompass a suitable range - by reducing it to 10 you may have to Shift/Click many times to count - by increasing it above 50 you may include too many "false-positives" in the count.

*(Note that the HGMF Interpreter calculates colony colors as a function of their RGB (red, green, blue) contents - black has a value of (0,0,0), white is (255,255,255) - for any clicked colony color and Include Color value of 'C' the counted colors will be all those in the range (red  $\pm C$ , green  $\pm C$ , blue  $\pm C$ ).*

If the grid cell count is *greater than* the number of grid cells you would prefer to see you have several options:-

- left click on another colony of slightly different shade to the first
- *if you already used Shift* to add grid cells you can hold down Shift and *right* click a *counted* grid cell - the added set of grid cells (based on the extra color indication) will be *subtracted from* the count
- hold down Ctrl and *right* click *counted* grid cells to *subtract* them individually from the count
- move the Include Color slider left or right to decrease or increase the color range included in the count, then again click a colony, as described above.

You can recount the HGMF as many times as you like before you press the "File Data" or "New Scan" buttons.

*(Note that "Dilution Factor" defaults to -9, i.e., a dilution factor of  $10^{-9}$ . This ensures that the HGMF Interpreter returns a "worst case scenario" in case you forget to correct it before using the data. "Volume (ml)" defaults to 1.0 ml).*

## MPNGU AND HGMF STATISTICS

The Most Probable Number of Growth Units (MPNGU) is derived from a statistical treatment of the HGMF by treating it as though it were a set of 1,600 most probable number tubes all inoculated at the same dilution factor. Unlike the conventional 3x3 or 3x5 tube-MPN method, which has a very low precision, the large number of "tubes" in the HGMF allows it to yield a precision as high as or much higher than a conventional Petri plate count, depending on the number of positive grid cells. At low inoculum levels the MPNGU equals the number of positive grid cells (N). The value of N does not increase as fast as the MPNGU. The relation:

$$\text{MPNGU} = 2.303 \log_e(1,600/(1,600 - N))$$

applies, as long as the HGMF was inoculated uniformly across its surface. This mathematical relation allows the HGMF to yield a linear recovery (MPNGU) against inoculum CFU, until  $N = 1,599$ , for which the  $\text{MPNGU} = 1.2 \times 10^4 (1.2E04)$ . In practice, counts will have low precision when N is less than 100 or greater than 1,500 and further dilutions should be examined. Precision will be highest when 50% of the grid cells are positive (800 grid cells).

## ADDING NEW USERS, SAMPLE TYPES, ORIGINS, TEST TYPES, AND PREPARATION METHODS

When you file HGMF data you must enter data in the combo boxes labelled: User, Sample Type, Preparation Method, and Test. For each of these, if the entry you require does not appear in the drop-down list you may add a new entry.

### *Temporary additions*

If you want to use an entry that is not in the list but do not wish to make a permanent addition to the list, simply click on the existing text to highlight it, then type the entry you wish to use. The entry can be filed with the HGMF data and will stay in the box for succeeding HGMF data entries until you make a further change, but it will not appear the next time you use the HGMF Interpreter.

### *Permanent additions*

You can make permanent additions to the listings in these combo boxes whilst the HGMF Interpreter is running in "real time" mode. Depending on which combo box you wish to update, select (click on) the appropriate list item:

- \*ADD USER\*
- \*ADD ORIGIN\*
- \*ADD PREPARATION METHOD\*
- \*ADD SAMPLE TYPE\*
- \*ADD TEST\*

A new box will appear requesting that you type the new entry you wish to add to the combo box. When you click "OK" the item will be added permanently to the box's drop-down list.

You can also edit these files from outside of the HGMF Interpreter program. They are in the folder "..\Officework\" within the directory in which your HGMF Interpreter program is located. The five files are named:

- ..\Officework\Users.txt
- ..\Officework\Origin.txt
- ..\Officework\PreparationMethods.txt
- ..\Officework\SampleTypes.txt
- ..\Officework\Tests.txt

## EDITING USERS.TXT AND OTHER FILES

You can add to or edit, any of these files with a wordprocessor program such as Notepad. Terminate each list with a single Enter (carriage return). If you use Word, WordPerfect or other formattable wordprocessor *be sure to save the file again as ".txt"*. **Do not delete the various items: "\*ADD USER\*", "\*ADD PREPARATION METHOD\*", etc, or you will not be able to access the real-time updating facility...**

## COUNTING WITHOUT FILING DATA

If you do not want to make a permanent file record of the count you just made, simply press "New Scan" - the HGMF Interpreter will return to AutoRegister mode to allow you to insert a new HGMF sample.

## FILING THE TEXT DATA

All alphanumeric information about an HGMF is filed in the file "HGMFData.txt", in the folder \...\HGMF Interpreter\Data\.. As soon as you click the mouse on a typical colony (after the HGMF Interpreter indicates that the HGMF is registered and ready to count), the following happens on your computer screen:-

- the box "HGMF No." displays the next record number in the file
- text in the boxes on the right of the screen changes from grey to black, indicating that they are now accessible for entering sample identification details.

Note that if you file HGMF data you may leave some of the boxes blank, but you must enter data in certain other combo boxes - these are: User, Sample Type, Preparation Method, and Test. For each of these you may:

- enter an existing item from the drop-down list by clicking on that item;
- add a new entry if the item you want does not appear in the drop-down list (for details of how to do this see the sections **Adding New Users, Sample Types, Origins, and Test**

### FILE DETAILS BUTTON

After counting an HGMF to your satisfaction and recording your "office work" in the various boxes, press this button. The Interpreter will:

- save an image of the HGMF in the file "\Images", in the form "00.xxx.jpg" (where "xxx" was the HGMF number shown in the box "HGMF #");
- and append a record of this HGMF to the "\Data\HGMFdata.txt" (see **HGMFData.txt file Record Structure** for details of the structure).
- return to the real time mode (yellow circle at top left) state ready to register and read another HGMF.

### HGMFData.txt FILE RECORD STRUCTURE

All fields contain only "strings" (numbers are recorded as strings also)

<i>Field contents</i>	<i>Characters</i>	
Time & Date	20	Format (Now,"dd mm,yyyy hh:mm:ss"
tab		
HGMFNumber	10	
tab		
SampleNumber	8	
tab		
Subsample	4	
tab		
Pool	4	
tab		
SampleType	30	
tab		
Origin	30	
tab		
Location	30	
tab		
Preparation	20	
tab		
Test	50	
tab		
Volume	6	
tab		
Dilution	6	
tab		
HighColor	12	- as "R,G,B" - separated by tabs
tab		
LowColor	12	- as "R,G,B" - separated by tabs
tab		
MPNGU	8	
tab		
CountPerGram	10	
tab		
Comments	144	
tab		

User	20	
tab		
xTL	5	- xcoords of HGMF corner
tab		
yTL	5	- ycoords of HGMF corner
tab		
xTR	5	- xcoords of HGMF corner
tab		
yTR	5	- ycoords of HGMF corner
tab		
xBL	5	- xcoords of HGMF corner
tab		
yBL	5	- ycoords of HGMF corner
tab		
xBR	5	- xcoords of HGMF corner
tab		
yBR	5	- ycoords of HGMF corner
tab		
Zoom	3	- Zoom factor for images
tab		
ScreenWidth	5	
tab		
ScreenHeight	5	
tab		
BlankField	195	- define extra fields as user requires
tab		
GridCell	1600	- one byte for every gridcell
CR/LF		
<b>Total</b>	<b>2304</b>	

### TOOLBAR OPERATIONS

Ten Toolbar buttons are variously enabled at various stages of counting an HGMF. These enable you to open file images instead of real-time ones, carry out various manual saves or printouts of images and data, refresh an onscreen image, turn the camera ON or OFF, or exit the program.

#### Open Image

Press this button to bring a stored file image onto the screen. In real-time mode there will be several seconds delay while the Interpreter closes down the camera. Use the standard directory dialogue to bring up the required file.

#### Save Raw Image

When you press the **Save Raw Image** button on the Toolbar you will see a **Save As** box. You can save the current image (in .bmp form only) under a discrete filename. This may be useful if you wish to transmit the data elsewhere.

### MANUAL FILING AND PRINTING

When you press the **File Data** button the Interpreter automatically saves the screen textual data as a numbered record in the file HGMFData.txt. You can also store the screen data manually in a separately named, discrete file - this may be useful if you intend to transmit the data elsewhere. Press the **Print Record** Toolbar button to print out the data list.

#### PRINT RECORD

If you wish you can print (make a hardcopy of) the screen data before proceeding to count another HGMF. Press the **Print Record** Toolbar button to see a Print Preview of the screen data, including the image. From the menu buttons you can print simply the text data or the image also.

#### COPY RAW IMAGE

You can copy the raw image (i.e., the image minus the green circles showing counted grid-cells) to a separately named, discrete file - this may be useful if you intend to transmit the data elsewhere. Press the **Copy Raw Image** Toolbar button. You will see the message:

The image has been copied to the Clipboard. Press OK to open PAINT.EXE then use Paste or Ctrl+V to paste the captured image.

Once the image has been transferred to the Paint.exe screen you can manipulate it as you wish and file it using **Save As**.

### **COPY COUNTED IMAGE**

You can copy the counted image (i.e., the image plus the green circles showing counted grid-cells) to a separately named, discrete file - this may be useful if you intend to transmit the data elsewhere. Press the **Copy Raw Image** Toolbar button. You will see the message:

The image has been copied to the Clipboard. Press OK to open PAINT.EXE then use Paste or Ctrl+V to paste the captured image.

Once the image has been transferred to the Paint.exe screen you can manipulate as you wish and file it using **Save As**.

### **REFRESH LAST IMAGE**

At times, you may find the residue of a menu or message box has "left a hole" in the image. To restore the complete image press the **Refresh Last Image** button on the Toolbar.

### **TURN CAMERA ON/OFF**

You should normally only need to turn the camera ON - for example, to return to real-time scanning mode after examining a file image. For most operations requiring the camera to be OFF (e.g., after using the **File | Open** menu or **Get Profile** button, the Interpreter will turn the camera off automatically.

## **BIOCHEMICAL, ANTIBIOTIC, OR OTHER TYPES OF PROFILING**

### **IDENTIFYING HGMFS FOR A PROFILE SET**

As all operations currently are carried out on one computer monitor, you will note that the **Grid cell biochemical profile** screen overlays and hides buttons and textboxes of the main Interpreter screen. When you come to choosing HGMFs for profiling, you may wish that identifying information were more easily seen. For the time being we suggest two possible methods of identifying HGMFs. Whichever method you use - *after you have counted each HGMF that you will use in a particular profiling experiment, be sure to record details of it in the "office work" side of the Interpreter screen, in such a way that you can identify it easily again.*

In profiling experiments you may find it easier to record simple identifying information in the textboxes **Sample No.**, **Subsample**, and **Pool of** - these can have text in them instead of numbers...

#### ***1. Manual recording***

Every time you count an HGMF in a profiling experiment, write down its number (the number in the "**HGMF #**" textbox) in your lab notebook.

#### ***2. Reopening HGMF records***

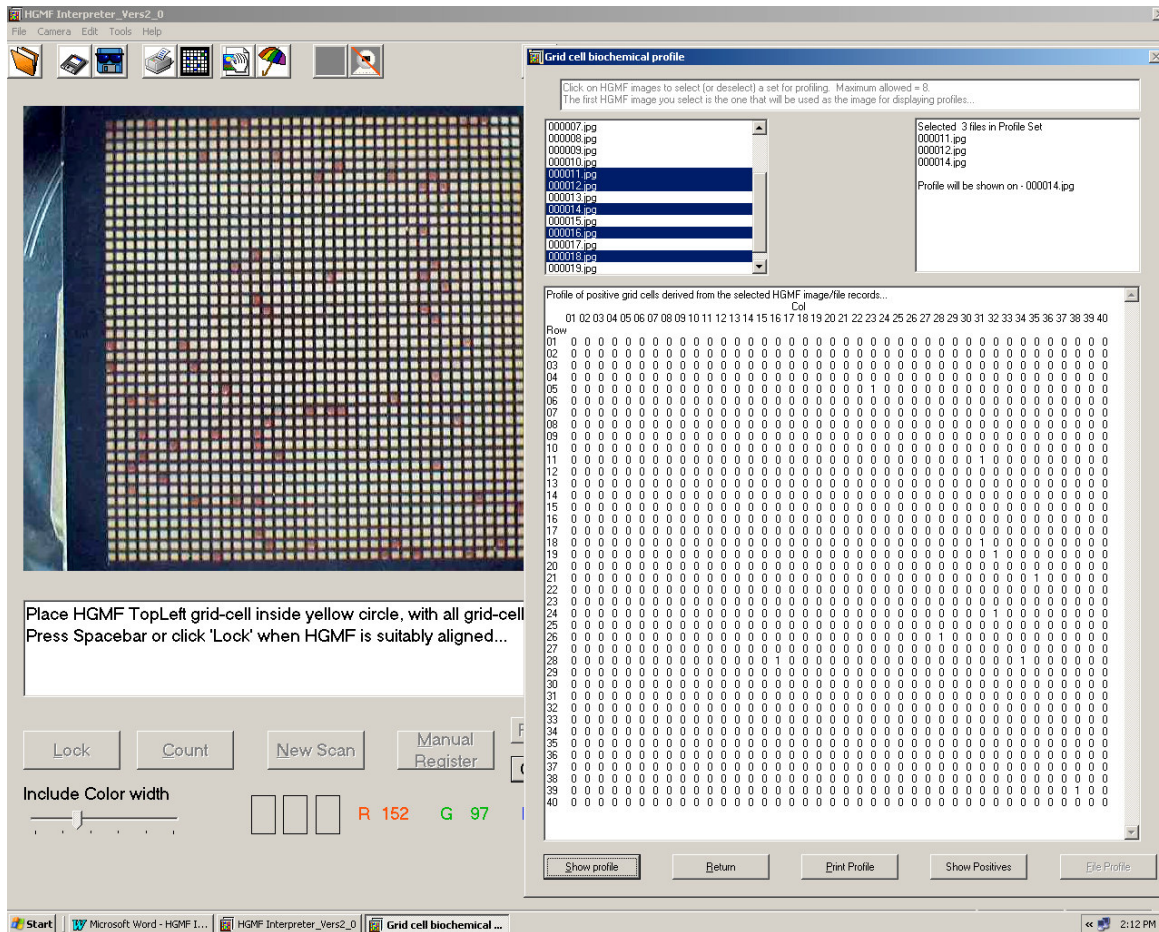
Before opening the **Grid cell biochemical profile** screen, use the **File | Open** menu to call filed images back into the Interpreter - (unlike Vers 1.1, Vers 2.0 will only let you see images it has stored itself). The various boxes will show the original information filed with that image, including the Date, HGMF number, Test, etc. If the HGMF is one you want, record its number. To look at other HGMFs you can either use **File | Open** again, or navigate backwards and forwards one at a time using the two buttons **Next Record** and **Previous Record**.

### **GET PROFILE button**

This button becomes enabled after you have pressed the **File Details** button, and starts the process by which you will be able to see which grid cells were positive on every one of a set of HGMFs that you will select from the list of those you have already counted. Only press this button if you want to do some profiling.

When you press **Get Profile** the Interpreter will change to Profile Mode and a new screen captioned "**Grid cell biochemical profile**" will appear, overlaying the right ("office work") side of the Interpreter screen. On the left you will see the camera image of an HGMF.

***NEVER DELETE ANY OF THE IMAGES IN THE "\Images" FOLDER AND NEVER TRY TO EDIT THE INFORMATION IN THE "\Data\HGMFData.txt" FILE. Each image corresponds to a record in the file - if you make any changes the records will not correspond to images, and vice versa....***



**Typical HGMF Interpreter Vers 2.0 screen after pressing "Show Profile"**

## PROFILE MODE

In "Profile" mode (i.e., the Grid Cell Biochemical Profile screen is visible) you will see several text and listboxes, also the buttons:

**Show Profile**

**Return**

**Show (or Hide) Positives**

**File Profile**

which will become enabled or disabled at various times. The textbox at the top contains the instructions:

*Click on HGMF images to select (or deselect) a set for profiling. Maximum allowed = 8.*

*The first HGMF image you select is the one that will be used as the image for displaying profiles...*

The listbox on the left lists the filenames of the images of all the counted HGMFs in the Interpreter's database.

The large textbox initially will simply say:

*xxx files/images available to select from...* (where xxx is the total number of HGMFs in the Interpreter's database).

The textbox on the right will initially simply say:

*No HGMFs selected*

Decide which image you would like to use to display the profile of all grid cells that are "1"s in the profile set you are going to choose, and click on its filename. The HGMF image in the image box of the Interpreter's screen will be replaced by the stored image of your selected HGMF. This is called the "*Template Image*". Initially it will not have any grid cells highlighted.

After your first selection (say, 000023.jpg) the textbox on the right will say:

*Profile will be shown on - 000023.jpg*

Click once on other image filenames to select the other HGMFs that you want in your profile set (clicking on an already selected filename will deselect it). As you select HGMFs the textbox on the right will be updated, for example, as:

**Selected 5 files in Profile Set**

000003.jpg

000009.jpg

000011.jpg

000023.jpg

000031.jpg

Profile will be shown on - 000023.jpg

**Do not try to deselect the Template image.** If you want to change the Template image you must press **Return** to return to the real-time Interpreter, then go back to the **Profile** screen.

**Do not select more than eight images.** Normally the Interpreter will be able to correct your error; however, there may be circumstances where it will "hang up" and you will need to press Ctrl+Alt+Del to end the program.

**SHOW PROFILE button**

After you have selected the HGMFs you wish to profile, press this button. The large textbox will now fill. Under the heading:

**Profile of positive grid cells derived from the selected HGMF image/file records...**

will appear 40 numbered Rows and 40 Columns corresponding to the Rows and Columns of an HGMF. If all grid cells at a particular coordinate in your selected set of HGMFs were positive, that coordinate will display as "1". If any grid cell out of your selected set was negative at that coordinate that coordinate will display as "0".

**GRID CELL COORDINATES**

**NOTE THAT:** grid cell coordinates of an HGMF are defined by Row and Column number of the 40 x 40 Rows and Columns, starting at the top left hand corner of the HGMF, proceeding across the Columns of the first Row, then proceeding down in the same way, Row by Row. For example, the coordinates of the grid cell that is 7 Rows from the top and 23 Columns from the left are (7,23).

**SHOW POSITIVES button**

This button becomes enable after you have pressed **Show Profile** to see the text display of the profile. If you press it you will see green circles around all (profile positive) grid cells on the Template image in the Interpreter's image box. The **Show Positives** button will change to **Hide Positives**. Repeatedly pressing this button will toggle the display on and off - this may help you examine a particular grid cell

**PRINT PROFILE**

This button becomes enabled after you have pressed the **Show Positives** button. Currently it simply prints the complete **Grid cell biochemical profile** screen, with its information about selected files, the 40 x 40 text display, etc.

**FILE PROFILE button**

This will append the details of your profile experiment to the file \Data\ProfileData.txt.

The file record structure is as follows:

<b>Field contents</b>	<b>Characters</b>
"Nonsense" string	2
tab	1
Expt #	10
tab	1
Date/time of profile test	20
tab	1
No. of HGMFs used	10
tab	1
HGMF#	10
tab	1
Tests for this HGMF#	50
tab	1
Data for this HGMF#	1600
tab	1
HGMF#	10
tab	1
Tests for this HGMF#	50

tab	1
Data for this HGMF#	1600
tab	1
HGMF#	10
tab	1
Tests for this HGMF#	50
tab	1
Data for this HGMF#	1600
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HGMF#	10
tab	1
Tests for this HGMF#	50
tab	1
Data for this HGMF#	1600
tab	1
HGMF#	10
tab	1
Tests for this HGMF#	50
tab	1
Data for this HGMF#	1600
tab	1
Unused	248
CR/LF	2
<b>Total</b>	<b>13600</b>

Note that, for all HGMFs you chose for profiling, the HGMF#, Test, and Data fields will contain the data for those HGMFs. If you did not select eight HGMFs for profiling, the remaining fields corresponding to those unselected HGMFs will be filled with "H"s, "T"s, and "D"s, respectively. The "Unused" field will be filled with "U"s. You can make a simple inspection of the ProfileData.txt file using Notepad or Excel, but **NEVER ATTEMPT TO EDIT THIS FILE**.

Also note that the ProfileData.txt file **does not record** the simple profile you reached using the Interpreter. To make a more detailed examination (for example, whether a particular grid cell coordinate was sometimes "0" and sometimes "1"), you will need to use a more sophisticated database program.

#### **RETURN button**

Press this button at any time to exit the **Grid cell biochemical profile** screen and return to the normal real-time Interpreter.

#### **EXITING THE PROGRAM**

Always exit the program using the **File | Exit** menu. This will allow the program to disconnect the camera and end smoothly. If you try to exit in other ways (for example, by pressing Alt+F4) the camera may not be disconnected and you may have to reboot your computer before continuing other applications or restarting the Interpreter.

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