

Elispot Reader Quick Start Guide

&

Manual

Elispot Reader Installation Date:

Getting Started

After Elispot plates are developed spots in each well need to be quantified to determine the frequency of cytokine-producing cells. Quantification can be done manually under the dissection microscope, but manual quantification is inaccurate, time-consuming and a very tedious procedure. That is why quantification of spots is done using computers – it is much faster, much more accurate and very easy to perform.

What is QHub Elispot Reader?

QHub is a semi-automated system Elispot reader allowing accurate quantification of spots in developed Elispot plates. QHub reader is a user-friendly system, which requires little training. The heart of QHub is an image acquisition and spot quantification software which has been designed using real Elispot images rather than artificial model drawings. The system has three sensitivity levels, which can be set by the operator depending on the density of spots and their staining intensity. QHub Elispot reader consists of a *PC workstation* linked to an *image acquisition system*.

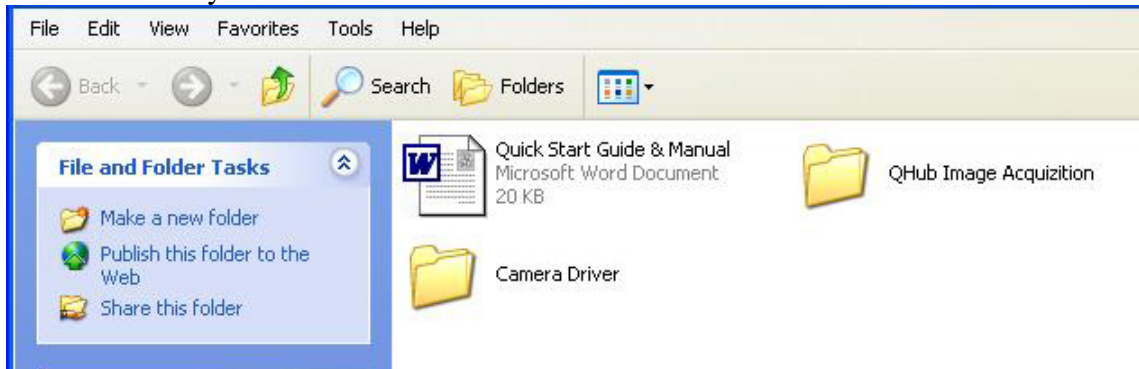
Installation of software

Don't plug Sony camera into your computer before installing software !

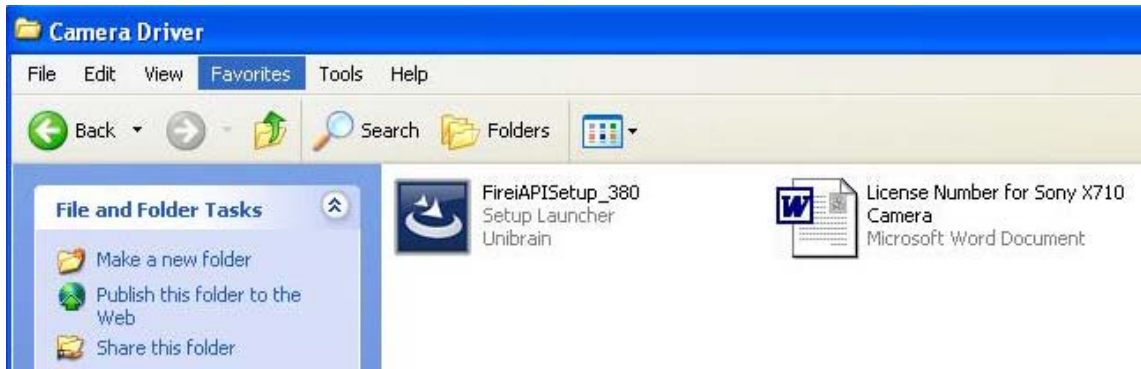
There are two major software modules that need to be installed:

1. Camera driver (to allow Sony camera transfer digital images to PC).
2. QHub Image Acquisition software (Image capture and quantification software for Elispot plates).

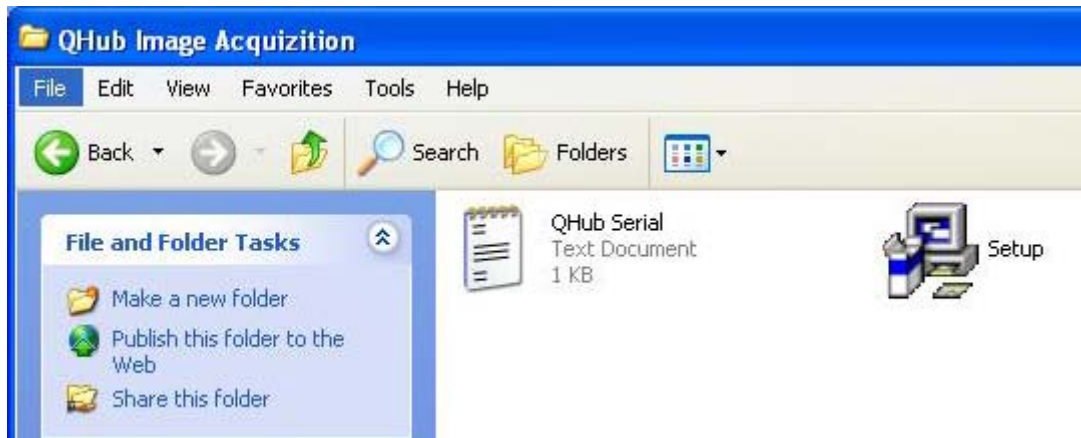
On the USB flash drive you will see files shown below:



First install camera driver: open **Camera Driver** folder which has two files:



Double-click on FireIAPISetup_380 and install it on your hard-drive (follow-up software prompts).
After that install QHub software: open **QHub Image Acquisition** folder where you see files shown below:



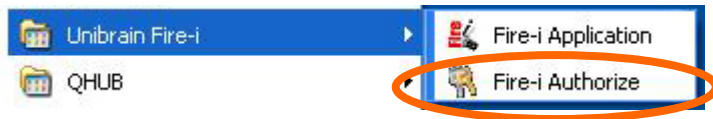
- Double-click on **QHub Serial** file to open it and copy a serial number (select it while pressing the left mouse button followed by then Ctrl-C). Close this file.
- Double-click on **Setup** file to install the software as prompted.
- When authorization window opens enter information about the user and a serial number (Ctrl-P).

If QHub starts, close it and proceed to entering authorization key for Sony Camera.

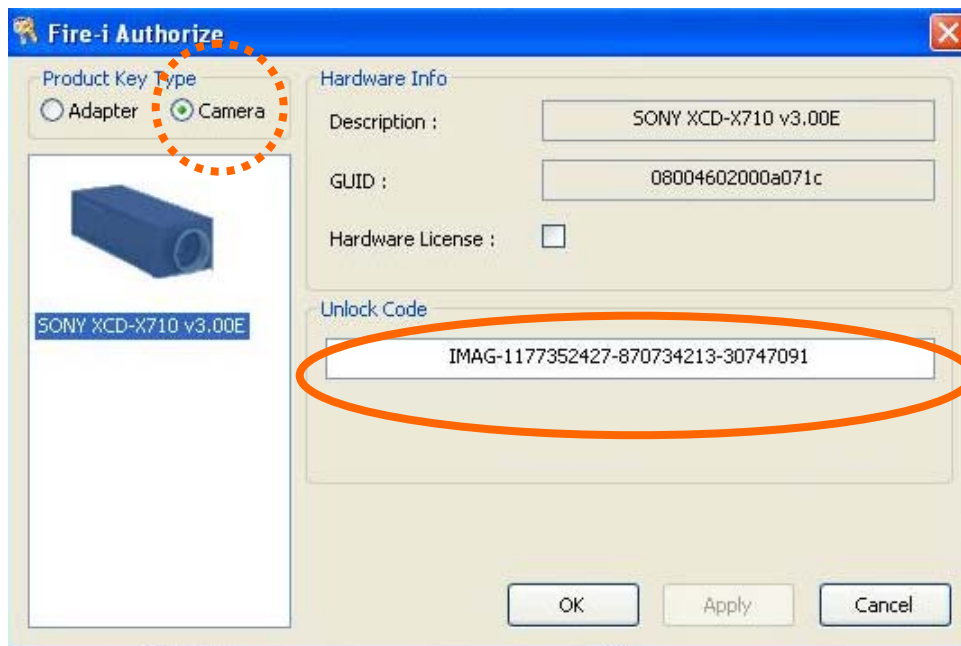
Now plug in your Sony Camera into FireWire port in your PC

In order camera driver recognizes your Sony camera you need to enter the license key number for this particular Sony camera as follows:

Start > All Programs > Unibrain Fire-I > Fire-I Authorize (see image below).



When this window opens (see below) first click with the left mouse button on the **Camera** button (outlined by dotted circle on the image) and then enter exactly the same code shown on this image into **Unlock Code** window. This number can be also found in the **License Number for Sony X710 camera** in the **Camera Driver** folder (see above). Click Apply and then OK.

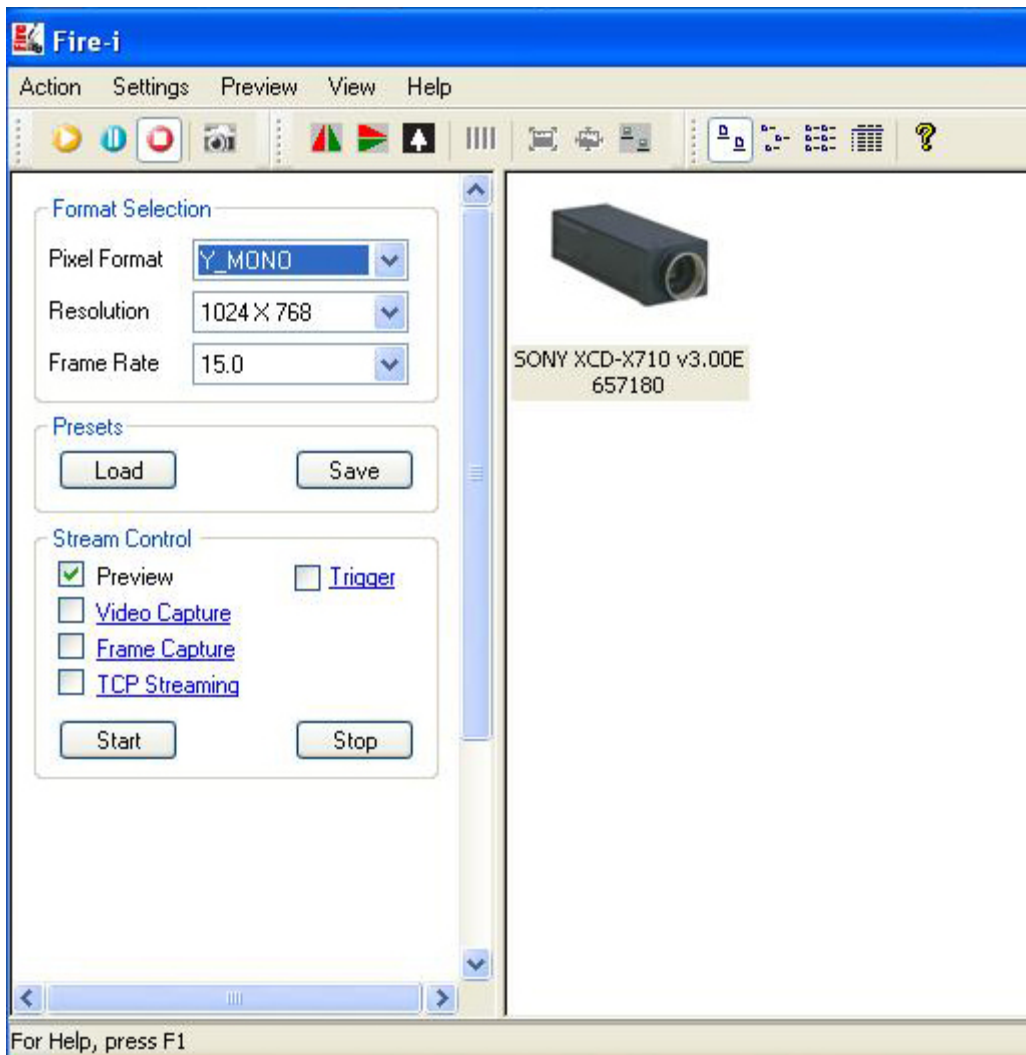


Your computer will now communicate without interruptions with the Sony camera.

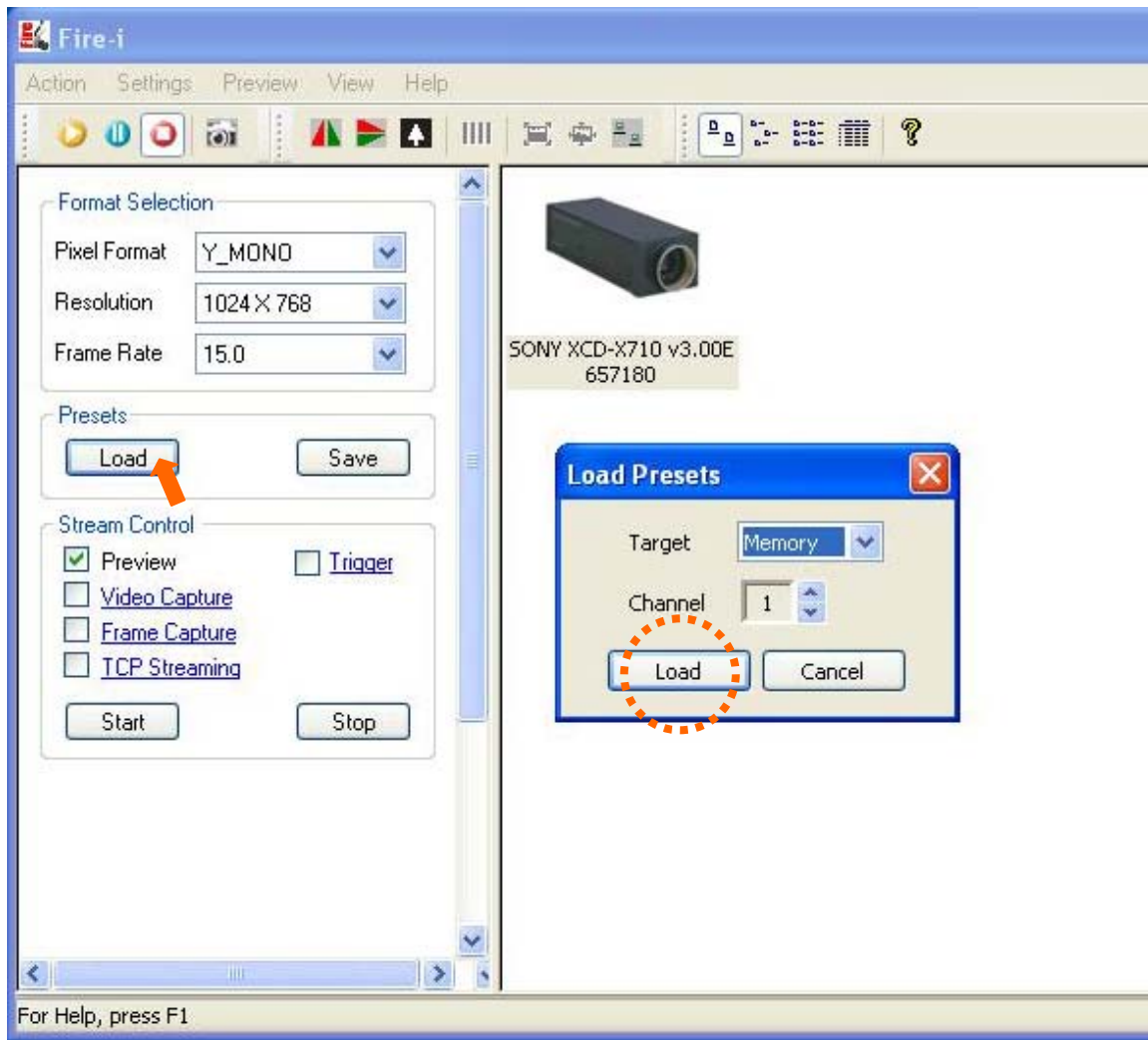
Reboot the computer with Sony camera attached and start **Fire-I Application**



When window opens (see below) enter parameters shown on the image



Now click on the **Load** button (left – arrow) and when this window opens (see below) make sure that **Channel 1** is selected and then click on **Load** button on the small window (outlined with dotted circle)




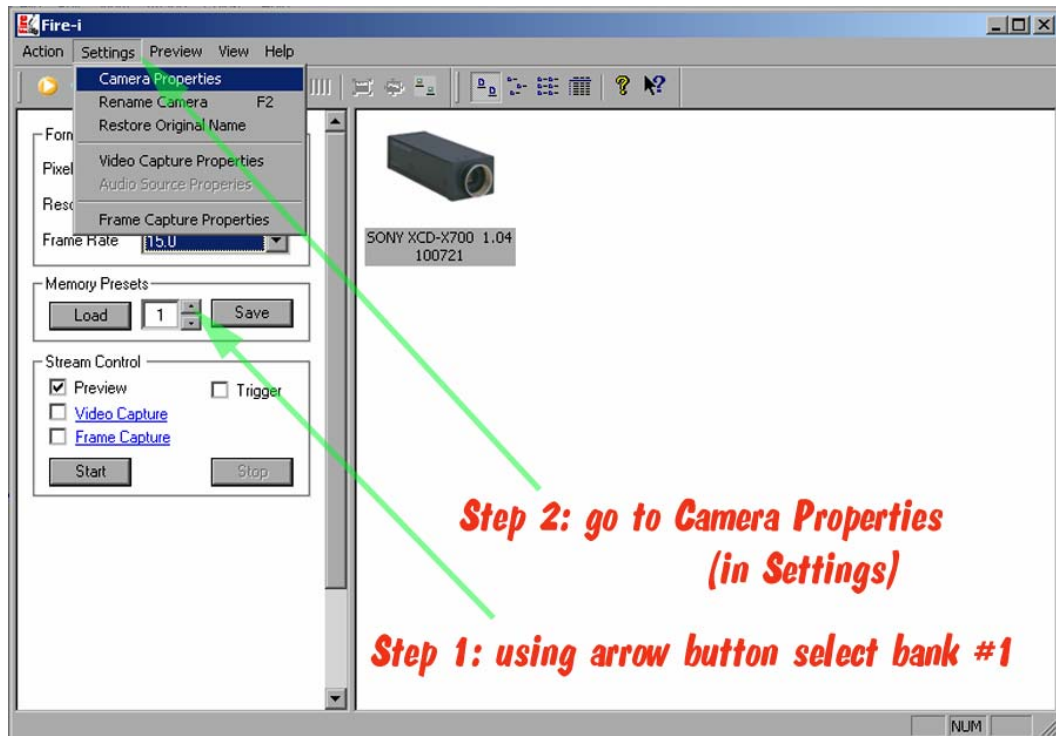
again

Configuring Sony High-Resolution FireWire Digital Camera

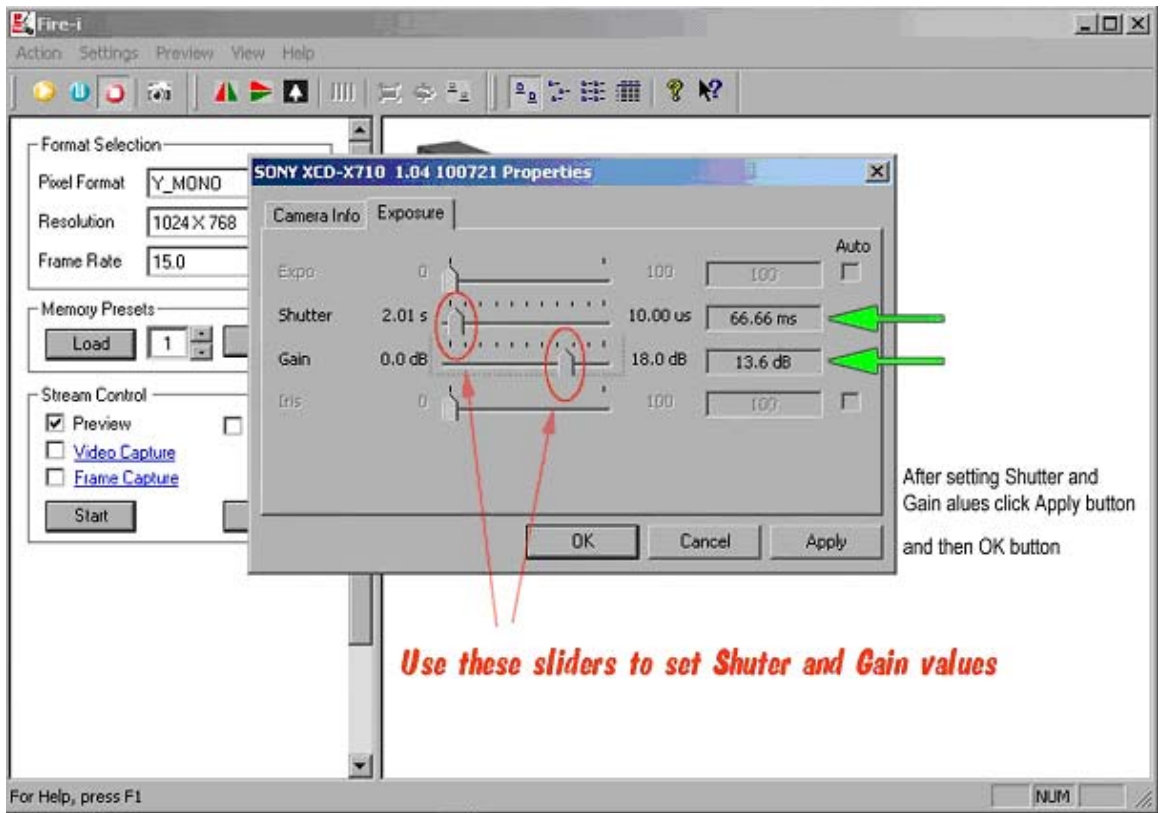
On some computers every time the computer is turned on, it is necessary to set Shutter and Gain parameters for Sony camera (on other PCs these parameters will remain as set after rebooting and don't need to be adjusted. Whether parameters changed or not can be checked out when PC is turned on again). Setting camera parameters can be done from either (1) within Fire-i application (contain only drivers operating the camera) or (2) within QHub application.

1. Camera Setup Using Fire-I application.

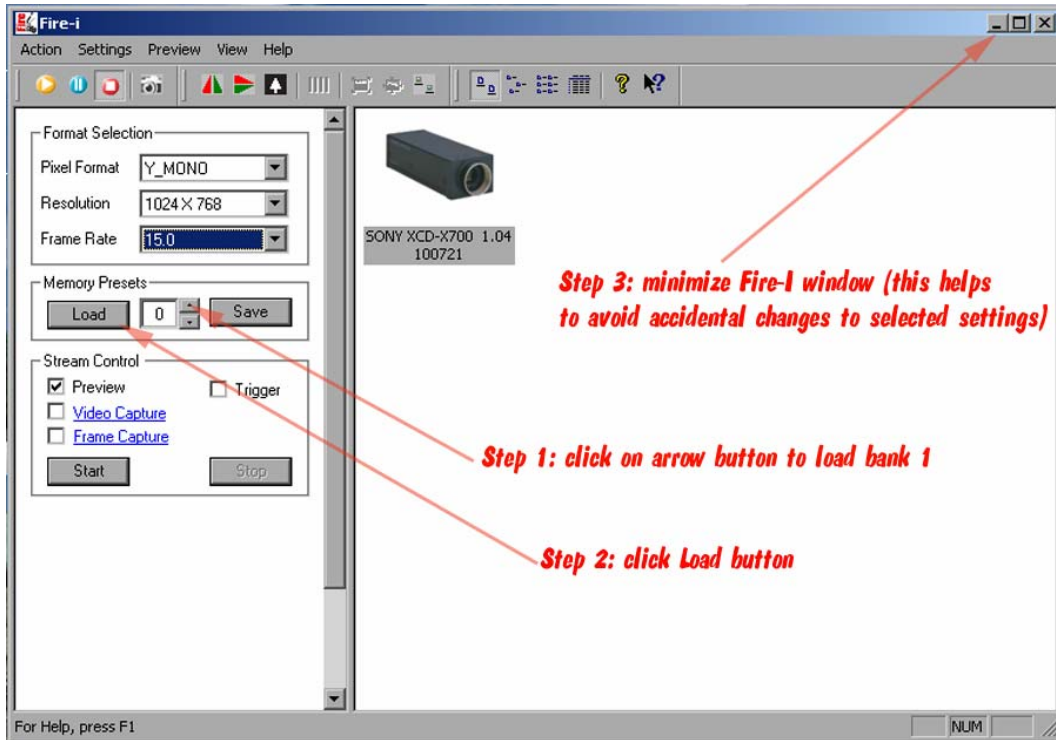
Open Fire-i program as explained above or make a shortcut  (keep on the desktop) and double-click on it with the **left** mouse button. With storage bank #1 selected open camera setting dialog box by clicking on **Settings > Camera Properties** (see below).




In **Camera Properties** box open click with the **left** mouse button on **Exposure** to open a drop-down camera setting box as shown below. Investigator can experiment with entering various combinations of Shutter and Gain settings, but we suggest to set Shutter at or around 66.66 ms and Gain at or around 13.6 dB. These settings are suitable for analyzing Elispot plates within a wide range of staining intensities. Shutter and Gain settings can be changed by moving horizontal sliders left or right (move mouse pointers over the slider, press and hold **left** mouse button to hold the slider to move it). With the **left** mouse button click **Apply**, **OK** and then **Save**.




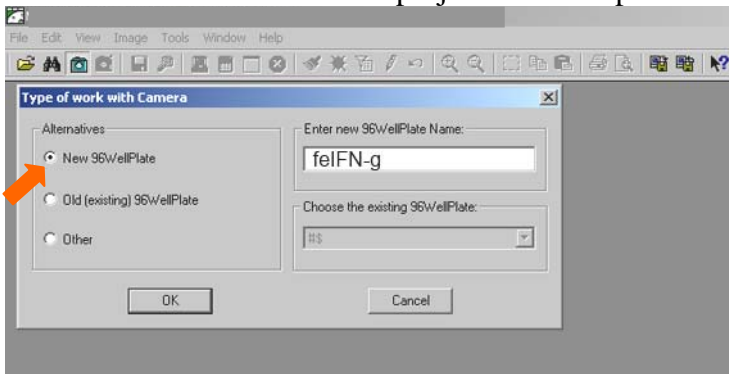
Next time when computer is turned on there is no need to enter Shutter and Gain settings again – just open Fire-i application as described above, with the **left** mouse button select **Load #1** (where settings were stored previously) and then click **Load** button. After that you can minimize Fire-i application. These steps are summarized on Figure below.



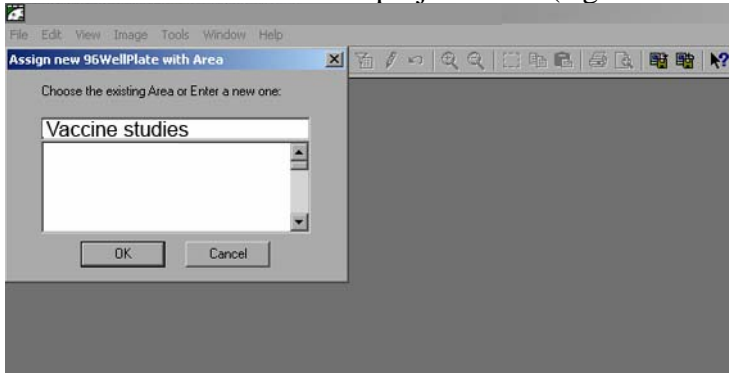
2. Camera Setup Using QHub application.

Start QHub application either from Start>All Programs or by making short-cut icon  on the desktop and double clicking it with the **left** mouse button.

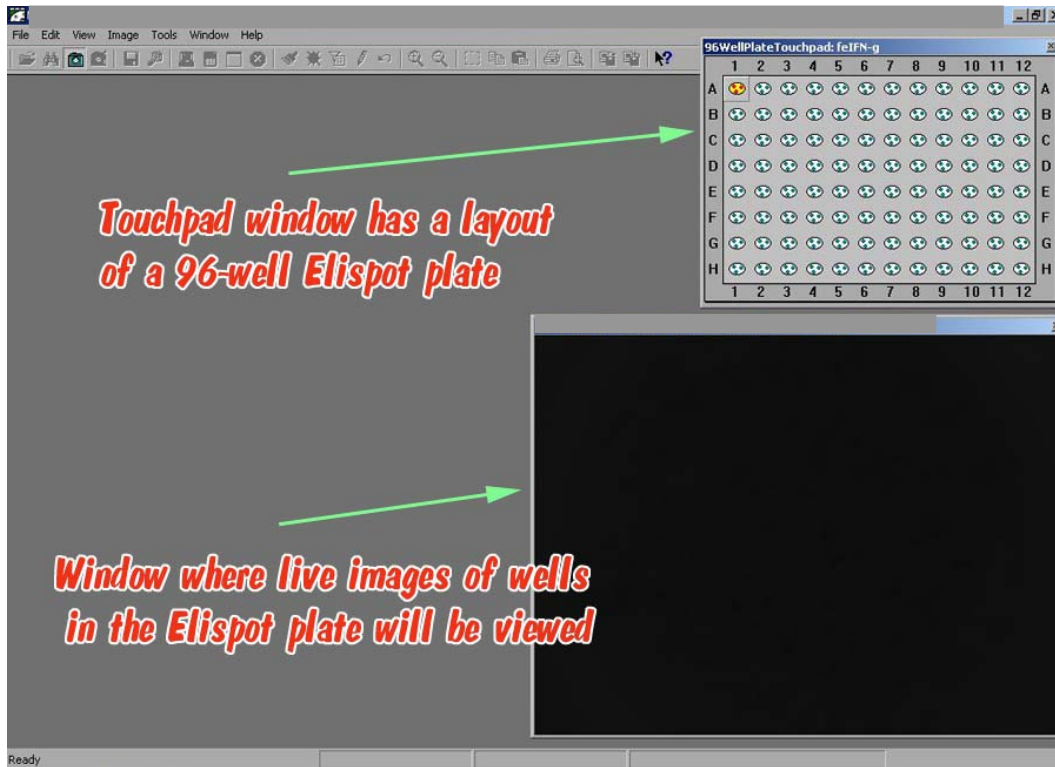
Camera settings can be entered only after to-be-analyzed Elispot plate is assigned a file name. To give a file name click with the **left** mouse button on the camera symbol  on the Tool Bar, select **New 96WellPlate** (arrow on the image below) and enter the file name for particular plate (e.g. “feIFN-g”) and then the name of research project in the drop-down window.



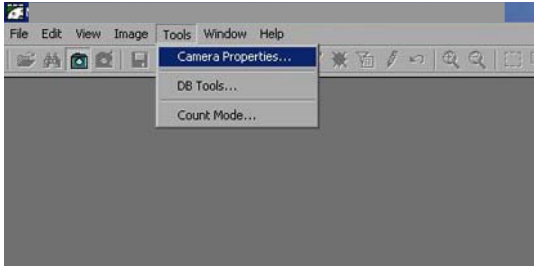
Click **OK** and then enter the project name (e.g. “Vaccine studies”) as shown below.



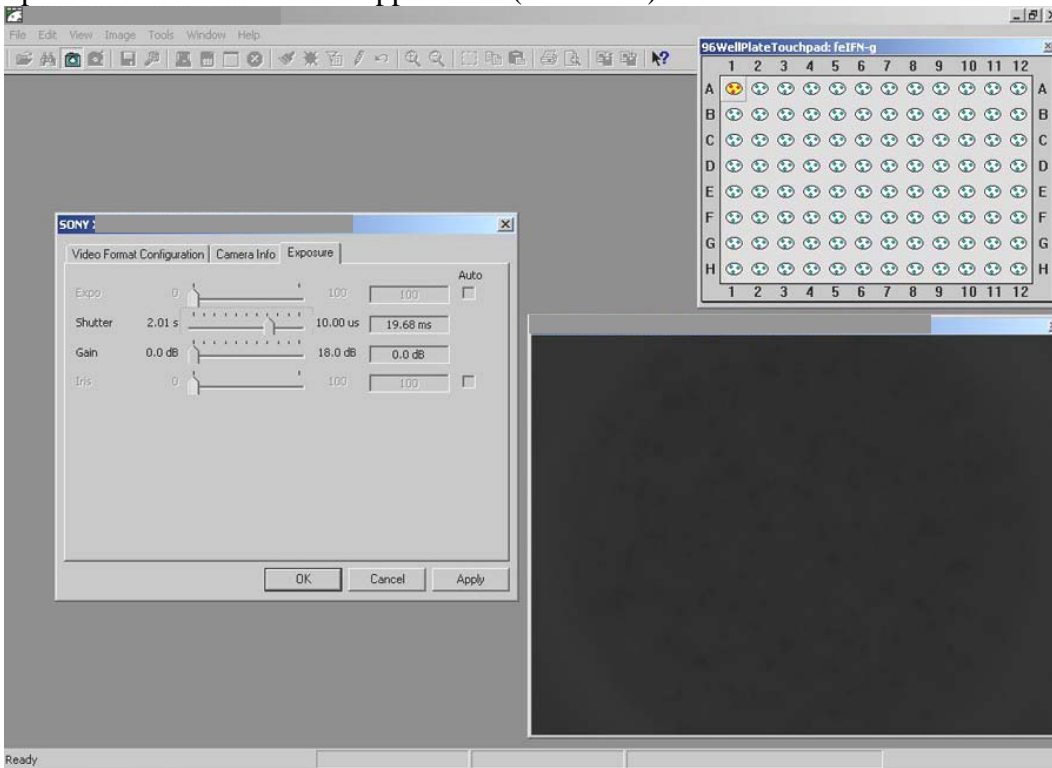
Click **OK** and QHub project window will open up as shown below.



With the **left** mouse button click on **Tools** and then on **Camera Properties**.



This opens the camera setting dialog box which looks exactly the same as the dialog box that can be open from within the Fire-i application (see above).



In **Camera Properties** box click with the **left** mouse button on **Exposure** to open a drop-down camera setting box. Investigator can experiment with entering various combinations of Shutter and Gain settings, but we suggest to set Shutter at or around 66.66 ms and Gain at or around 13.6 dB. These settings are suitable for analyzing Elispot plates within a wide range of staining intensities. Shutter and Gain settings can be changed by moving horizontal sliders left or right (move mouse pointers over the slider, press and hold **left** mouse button to hold the slider) and then click **Apply** and **OK**.

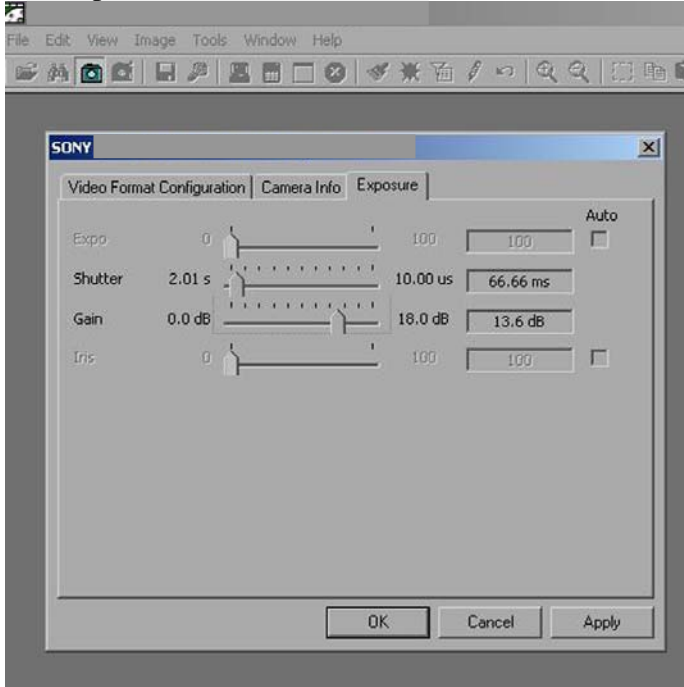
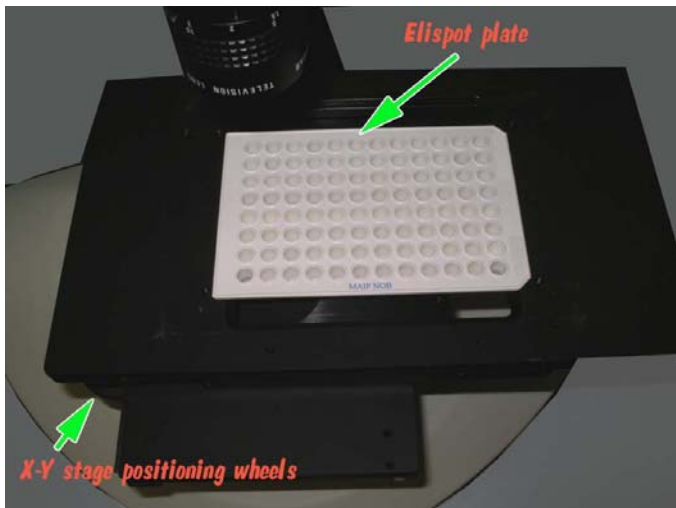


Image-Acquisition System (hardware may be slightly different)

Put developed Elispot plate (on Millipore plates it is necessary to remove plastic underdrain first) in the middle of X-Y stage holder. Elispot plate can be moved in the horizontal plane using X-Y stage positioning wheels located underneath the stage in its front left corner (Figure 10).



Lens Adjustments

Release Iris Knob and Iris Ring on the camera lens set the aperture to 4.0 (match it with the reference mark on the lens – orange arrow). Secure Iris Knob but don't over tighten it. To adjust the focus, release Focusing Knob and turn focusing ring until the image on the computer screen is in focus. Secure Focusing Knob but don't over tighten it

Iris will be set to 4.0 at the factory, but focus may need to be slightly adjusted when first setting the system.



Turn on the fiber optics illuminator and adjust the intensity of light using dial knobs on the front panel. It is recommended to dim the lights in the room to avoid their interference with Elispot reader illumination. Figures A, B and C below provide guidance how to set the correct illumination allowing for accurate quantification of spots in the well.

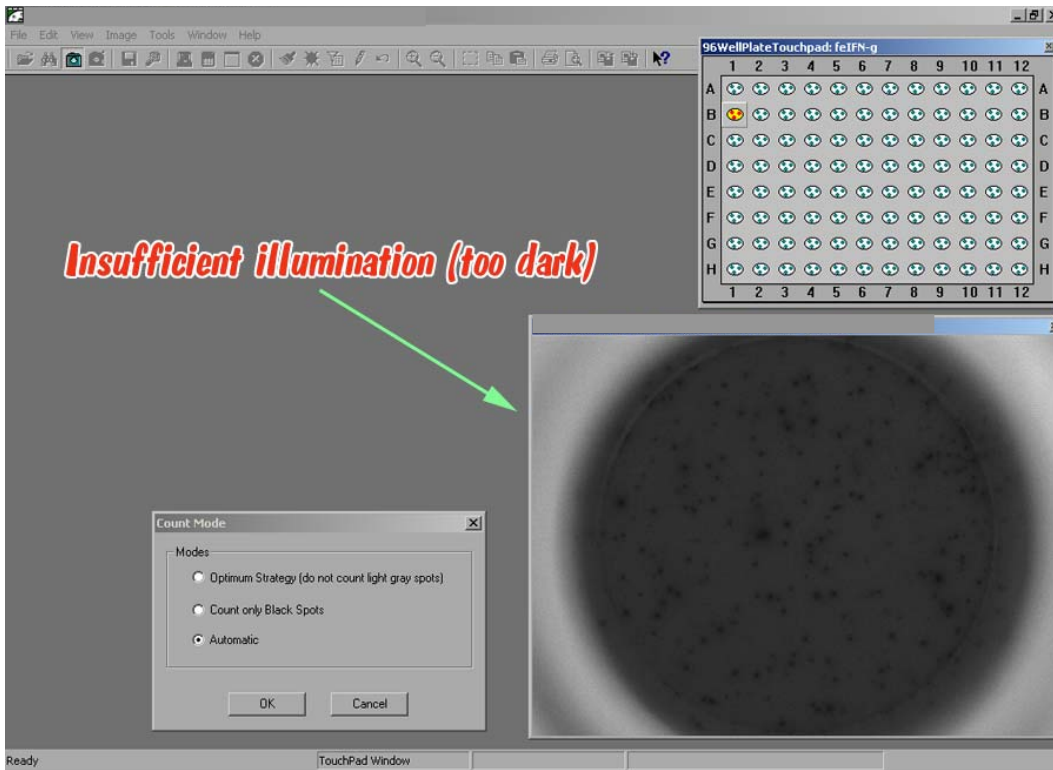


Figure A

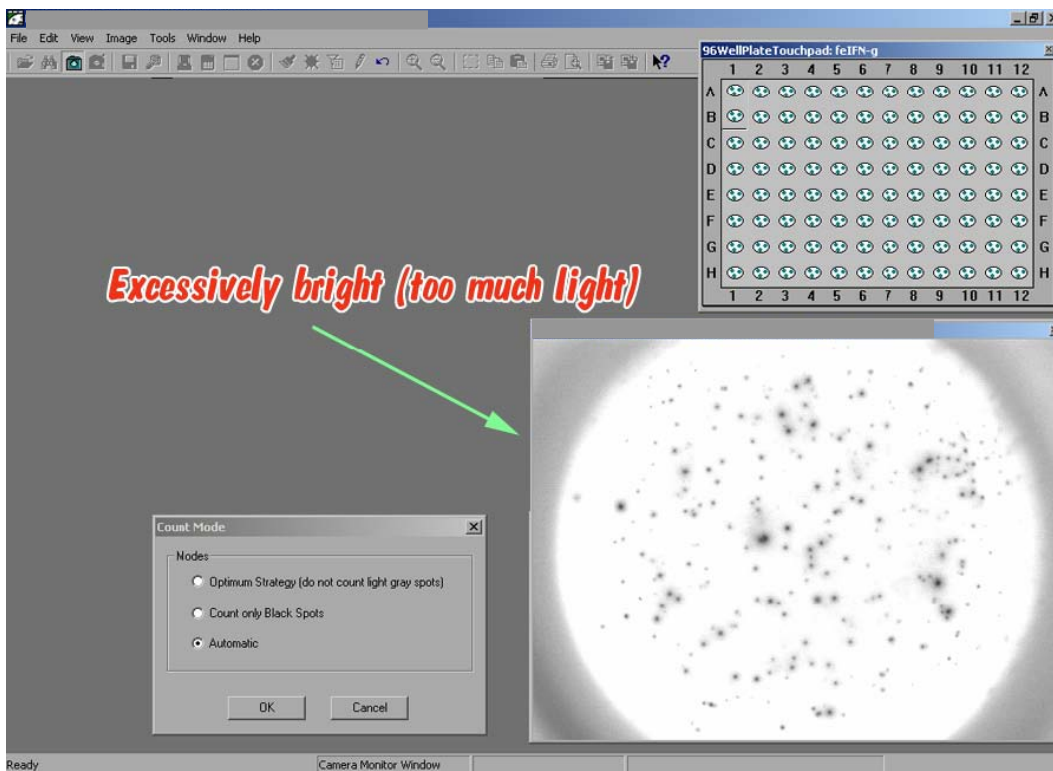


Figure B

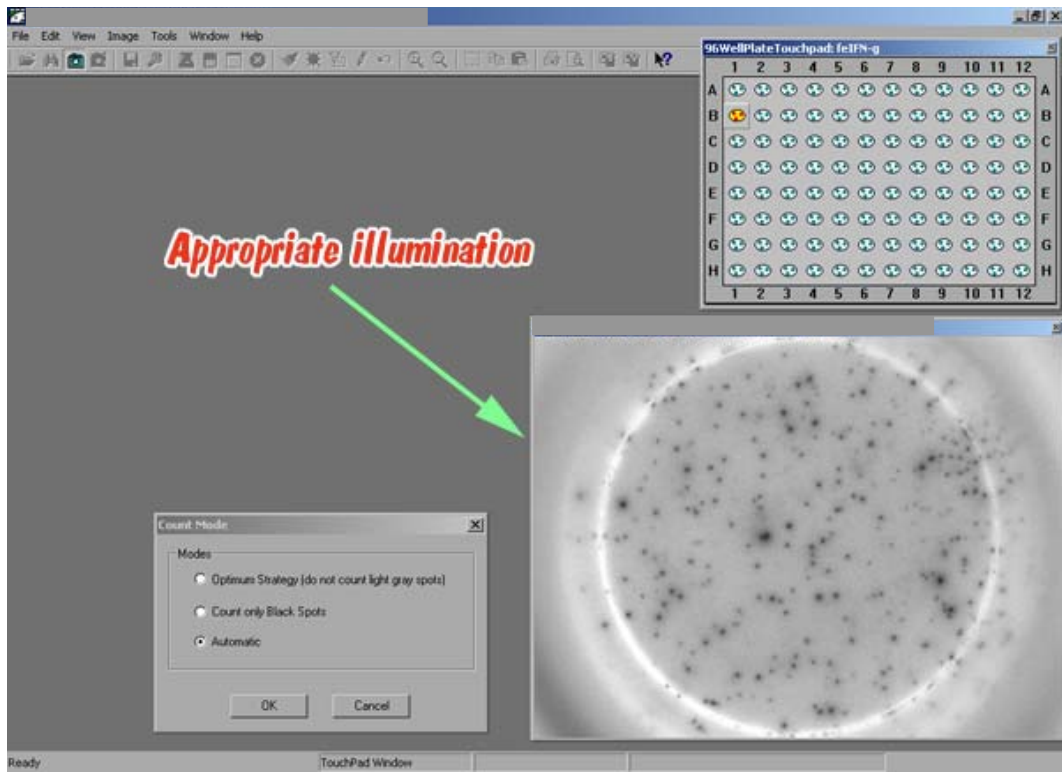
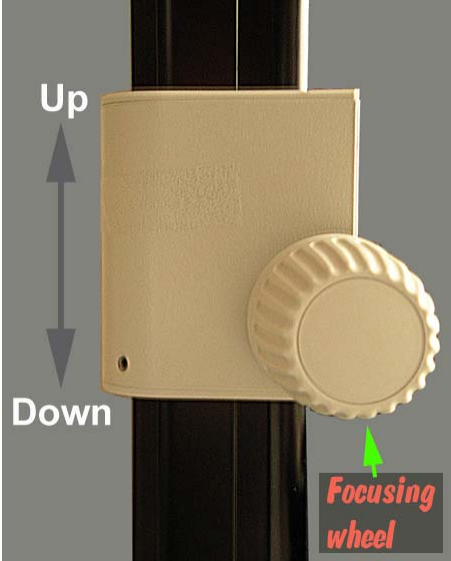


Figure C

It is known that plastic Elispot plates do not have a perfect geometry making some wells to be slightly out of focus (spots may look blurry). To bring such wells back in focus move Sony camera up or down using rotary focusing wheels as shown below (hardware on your systems may be slightly different from the one shown on the image).



Analysis of Elispot Plates and Quantification of Spots

The analysis of Elispot plates is done only on captured images of individual wells. To capture the image of the well it needs to be positioned in the middle of the live image view window (Figure 1).

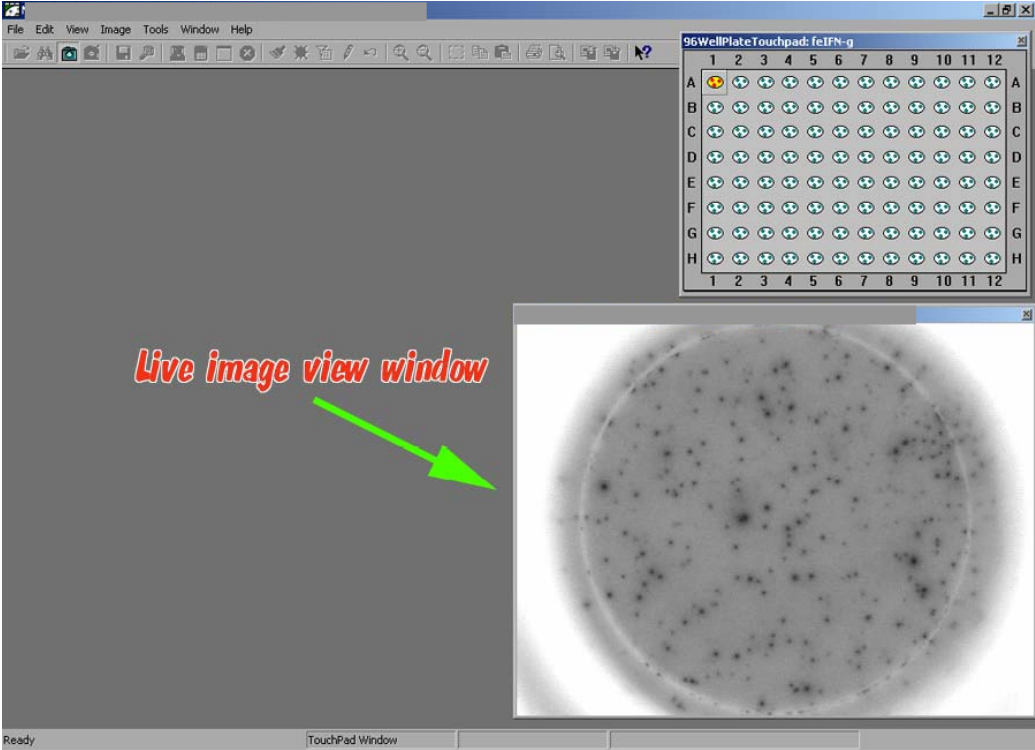


Figure 1

To capture the image of the well click with the **left** mouse button in the Touchpad window on the square box corresponding to coordinates of the well viewed in the Live Image window (e.g. A1; Figure 1). Activated square box on the Touchpad will turn **green** indicating that image in this well has been already captured. Captured image will appear in the Captured Image window in the upper left corner Figure 2).

Notice that the captured image is outlined with the **red** circle – only spots located inside the **red** circle will be counted during the quantification step !

That's why it is important to capture the live image in such a way that real spots are located inside the **red** circle, whereas artificial spots mirrored by the wall of the well are excluded from quantification (e.g. located outside the **red** circle).

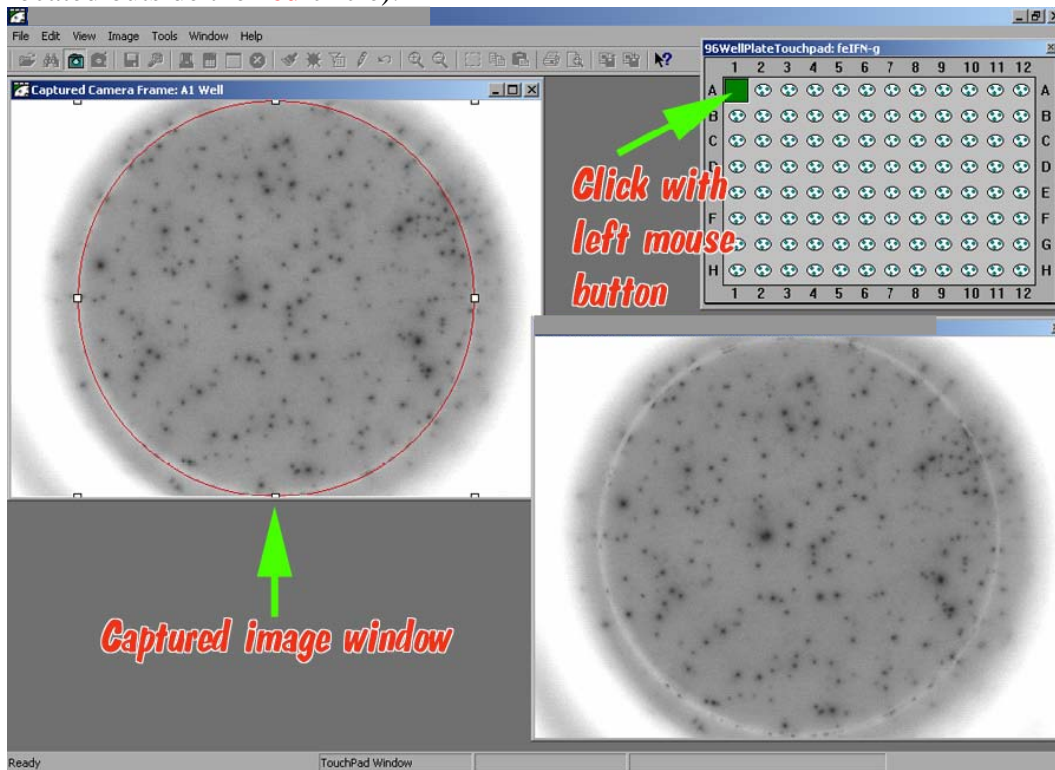


Figure 2

If, after capturing, it turns out that the well was not centered well enough (Figure 3), center it in the Live Image window using X-Y stage positioning wheels and re-capture the image by clicking with the **right** mouse button on the activated **green** square. This will call a drop-down menu (Figure 4): click with the **left** mouse button on **Recapture** button in the dialog box and image will be recaptured (Figure 5).

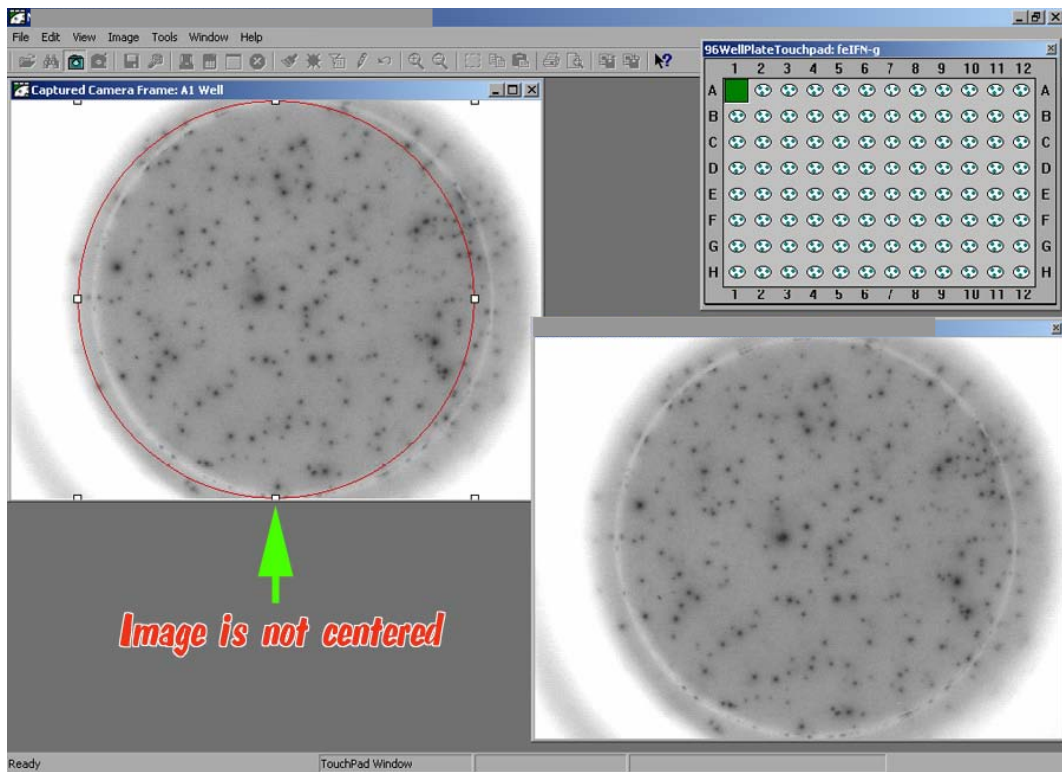


Figure 3

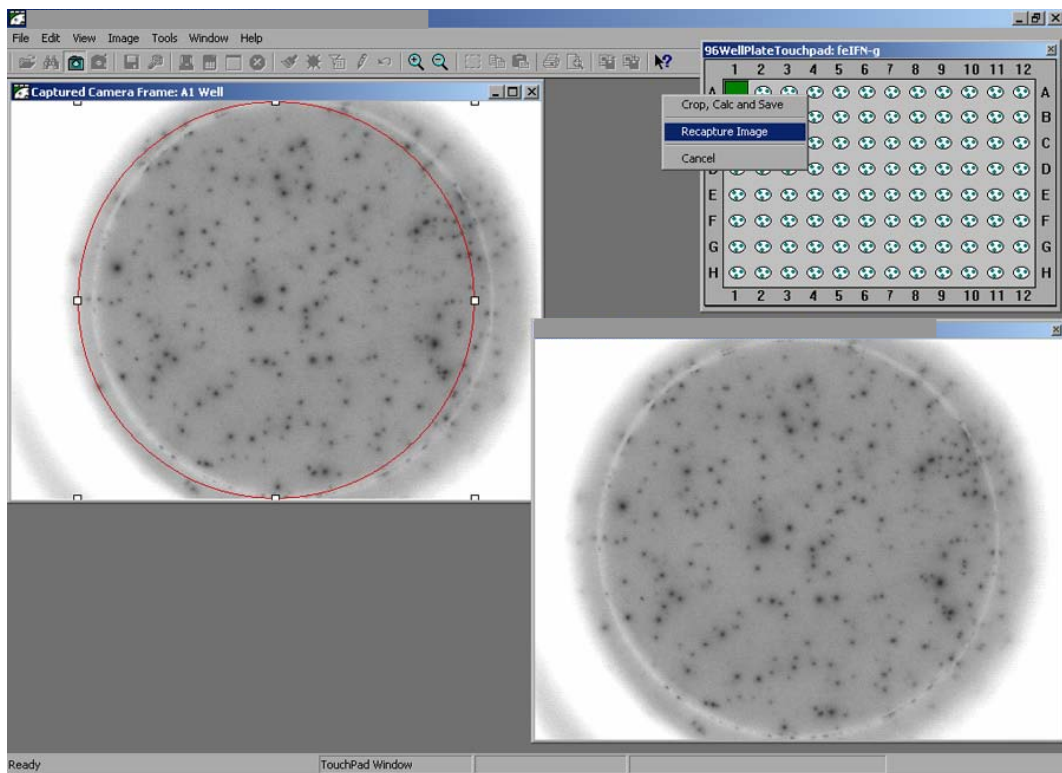


Figure 4

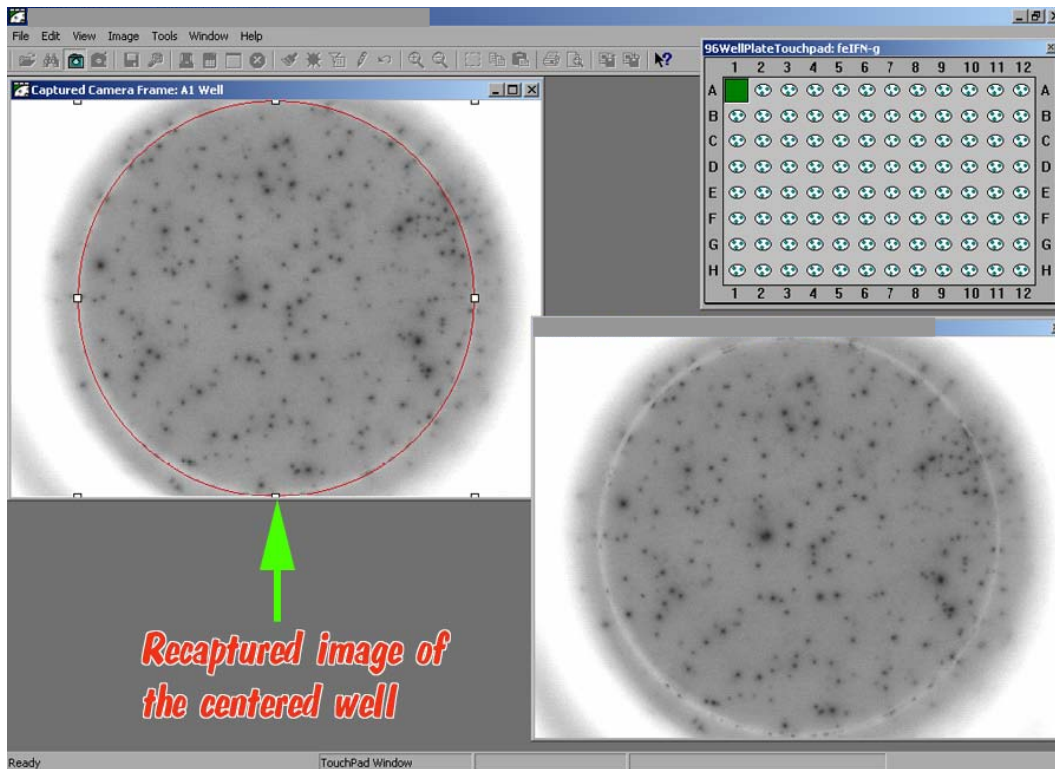


Figure 5

Now spots can be quantified. Click with the **right** mouse button on the activated **green** square. This will call a drop-down menu: click with the **left** mouse button on **Crop, Calc and Save** button (Figure 6). With a single click, captured image will be (a) cropped along the perimeter of the **red** circle, (b) spots will be counted, and (c) image will be saved. Please note, that image will be saved in two formats: a high-resolution publication quality TIFF file and a low-quality JPEG file for e-mail exchange.

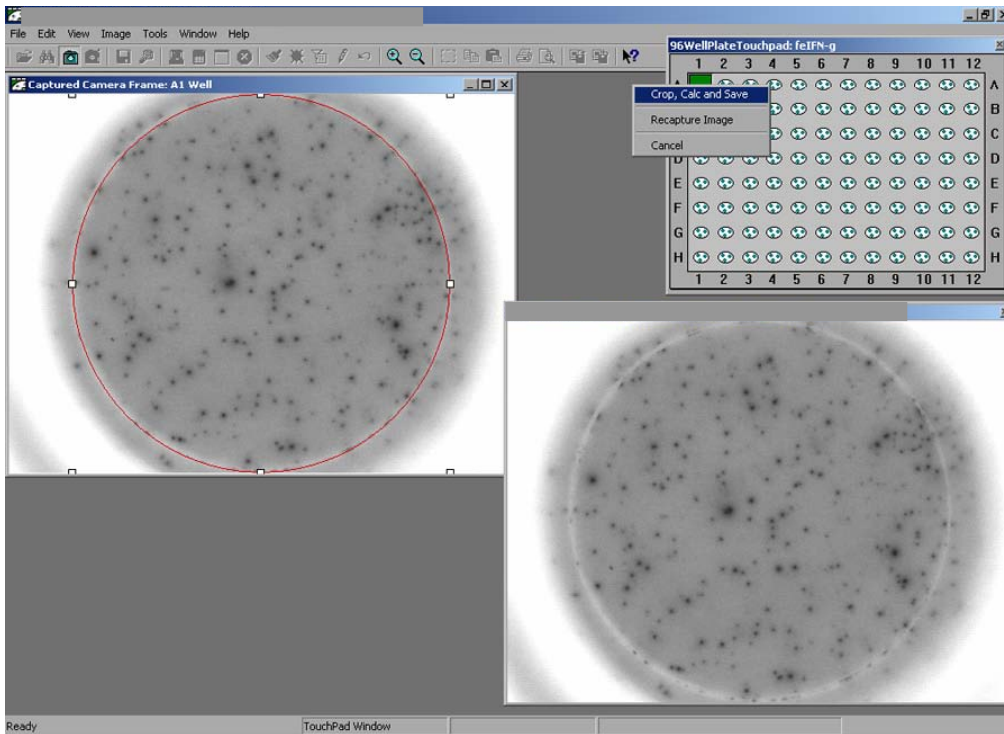


Figure 6

Spots that were counted will be labeled with green check marks in the Captured Image window, and the number of counted spots will appear in the Touchpad window (Figure 7).

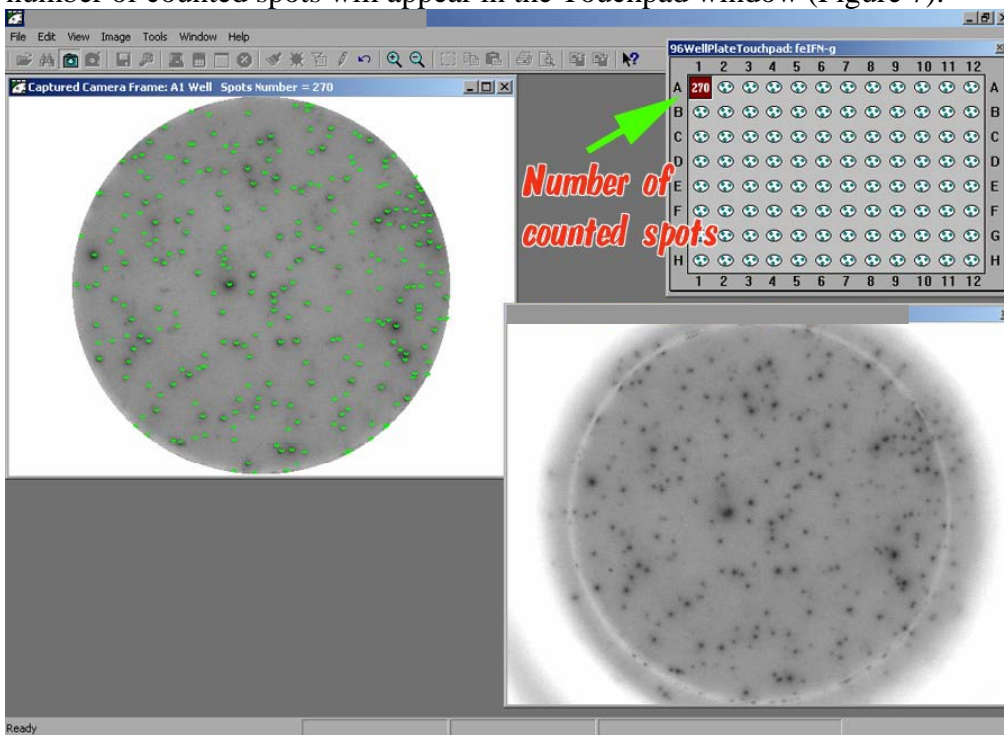


Figure 7

If needed, spots can be re-counted by clicking with the **right** mouse button on the square with numbers in the Touchpad to call a drop-down menu and then clicking with the **left** mouse button on **Undo**

Image button (Figure 8).

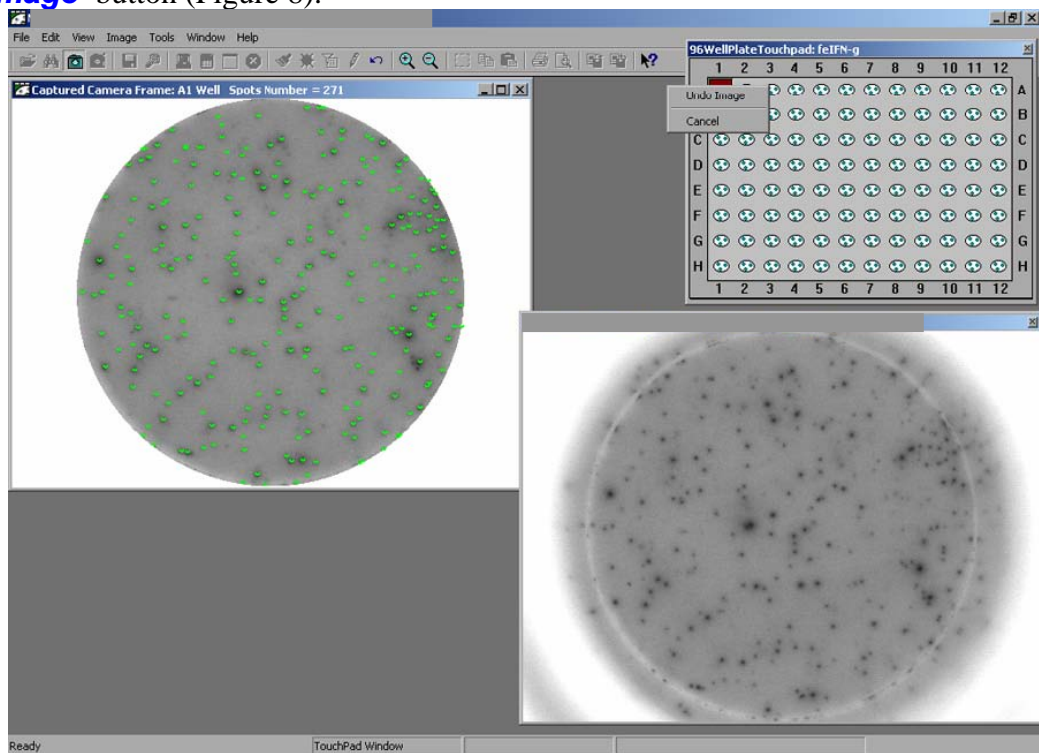


Figure 8

Depending on the quality of staining, the operator can choose one of three quantification modes: “Automatic”, “Count only Black Spots” and “Optimum Strategy (do not count light gray spots)” To choose the mode, click with the **left** mouse button on **Tools > Count Mode...**(Figure 9).

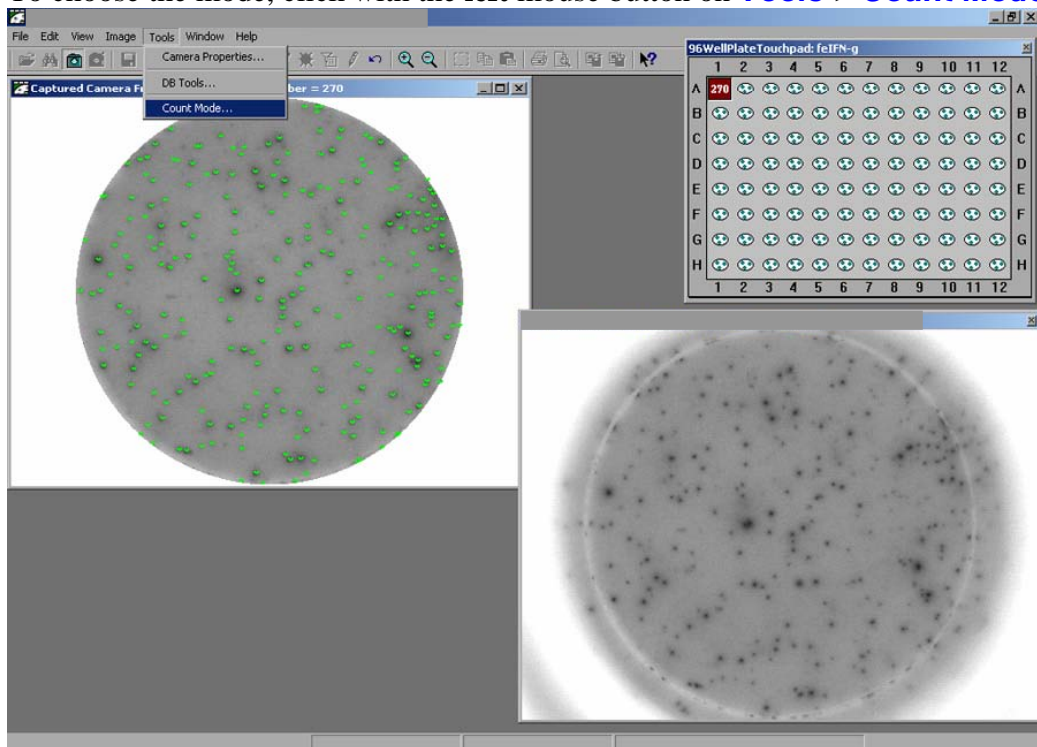


Figure 9

In the Count Mode window (Figure 10) the operator can choose the quantification mode by clicking on it with the **left** mouse button. Depending on the mode selected the number of counted spots in the same well will change. For example, using “Automatic” mode this number can be 270 (Figure 10).

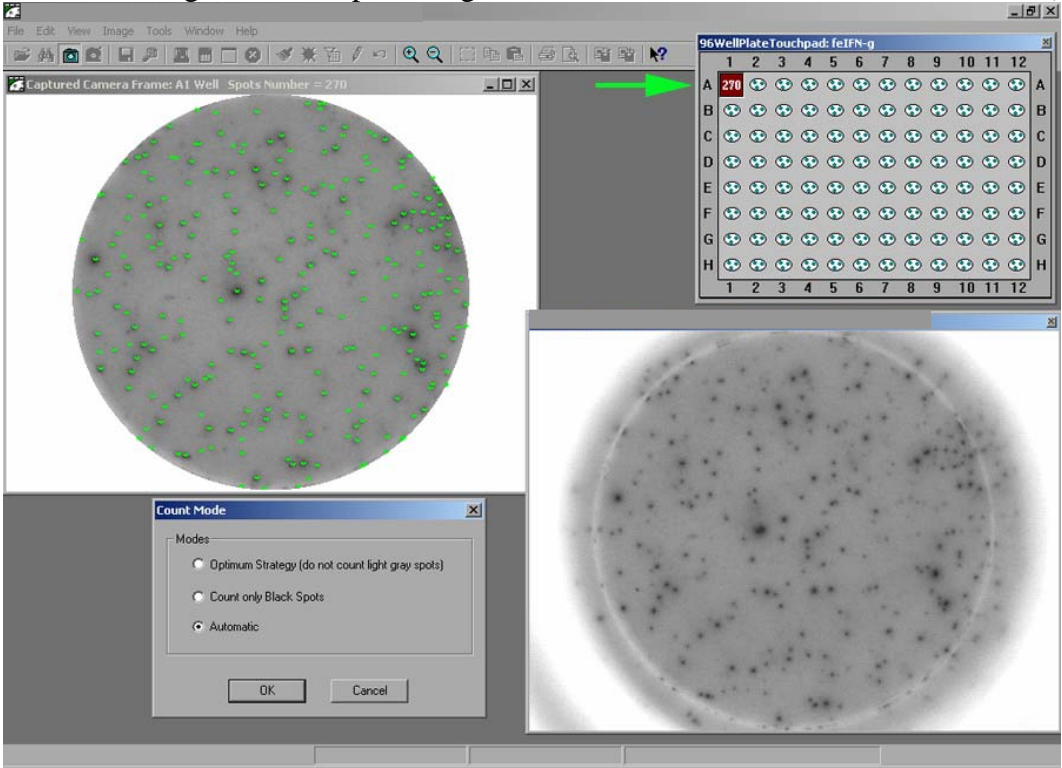


Figure 10

Using “Optimum Strategy” the number of counted spots in the same well will become less – 235 (Figure 11).

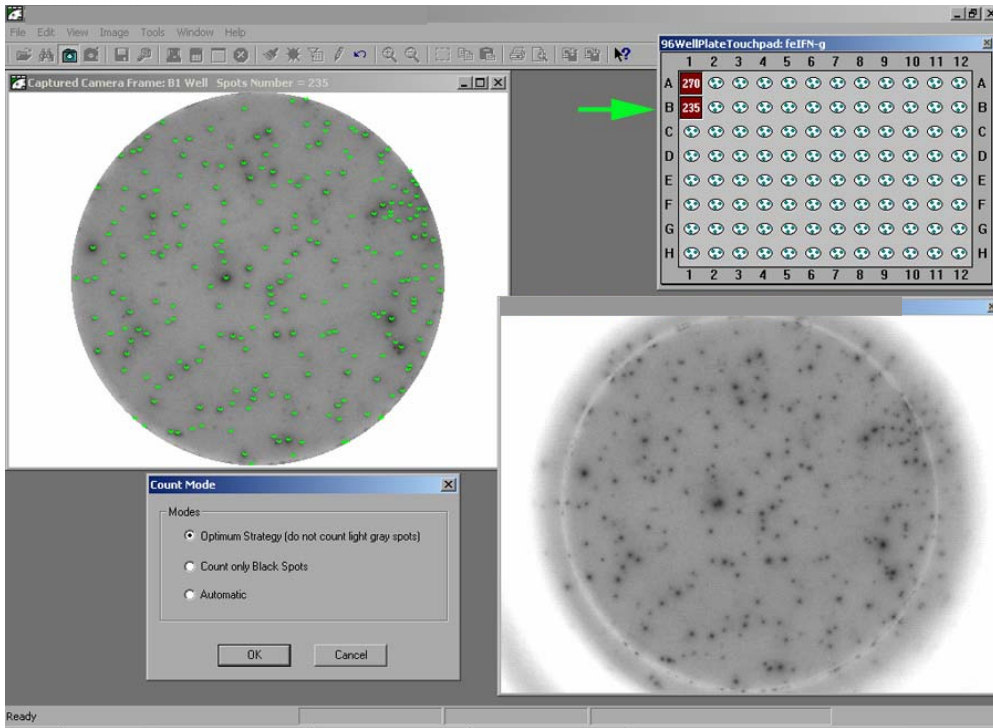


Figure 11

When “Count only Black Spots” mode is selected the number of counted spots in the same well will be 148 (Figure 12).

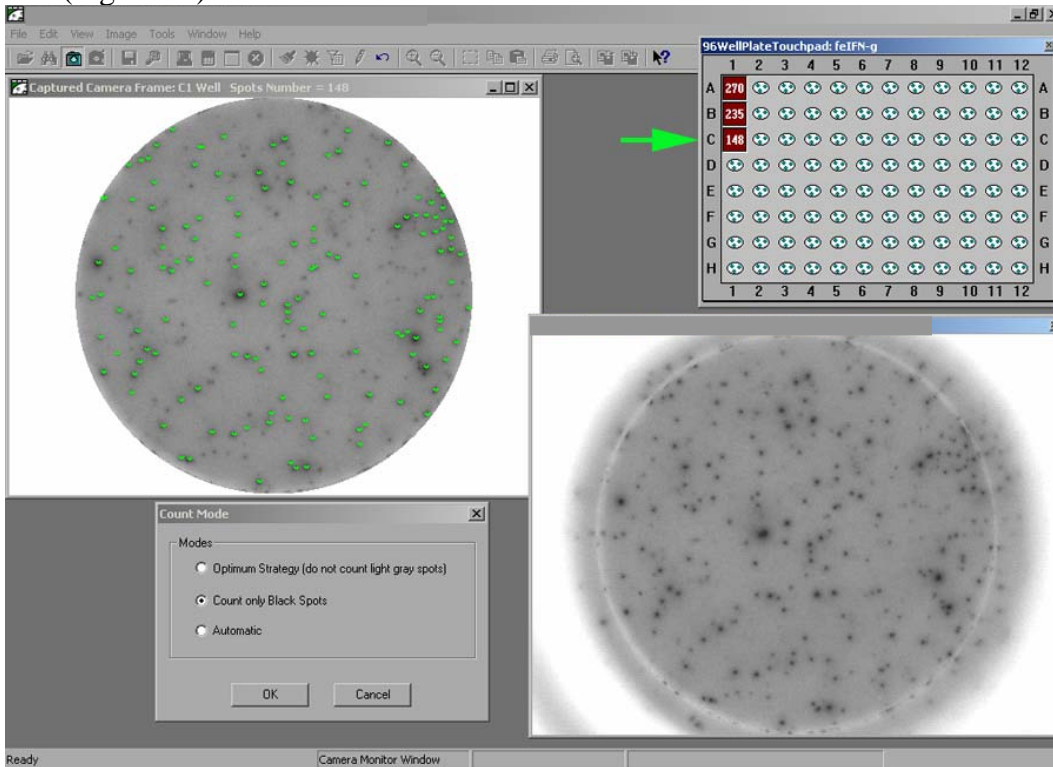


Figure 12

Different quantification modes allow the operator to choose appropriate quantification algorithm matching the staining pattern in the Elispot plate. For example, if in addition to spots there are also cells sticking to the membrane it may be necessary to use a “*Count only Black Spots*” mode to exclude stained cells from quantification. On the other hand, if there are a lot of small-sized specific spots of various intensities it may be advisable to use the most sensitive “*Automatic*” mode to count such spots. “*Optimum Strategy (do not count light gray spots)*” mode is recommended when specific spots have different sizes and intensities. Different modes can be selected not only for analysis of different plates, but also when analyzing wells in the same Elispot plate. In deciding which quantification mode to use, it is helpful to know beforehand what may be a source of artificial spots: reagents’ impurities, contamination of culture media, cell debris, dead cells sticking to membranes, etc.

It is recommended to dry Elispot plates in a clean room away from busy areas to avoid dust from the air getting into the wells. We also recommend keeping a lint and dust remover such as AIR’IT (Fisher Scientific Company) handy to clean wells if needed.

After quantification is finished, results can be exported as tables into Excel (part of MS Office) or Notepad (shortcut to Notepad can be found on the Desktop). To export data click with the **right** mouse button on the window's title bar (green arrow) to call a drop-down menu box and with the **left** mouse button click on **Copy to Clipboard** button as shown on Figure 13.

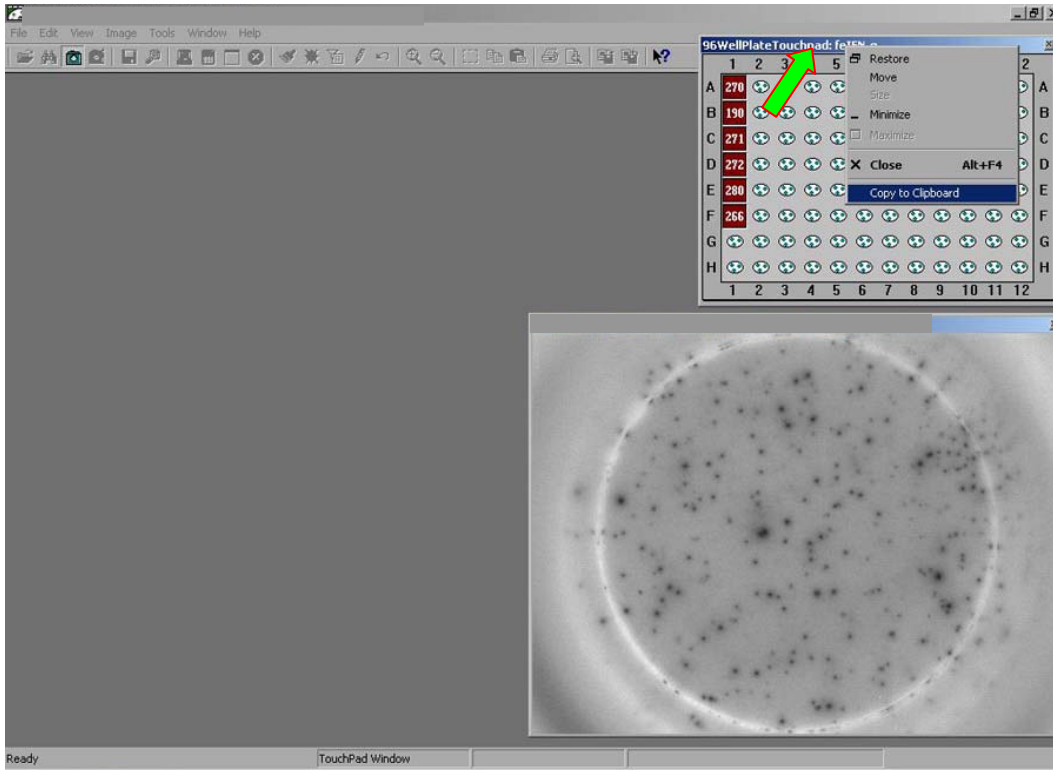


Figure 13

Open, for example, Notepad application, and paste data (Ctrl V) into its window (Figure 14). To avoid losing data, save Notepad table onto hard drive or CD.

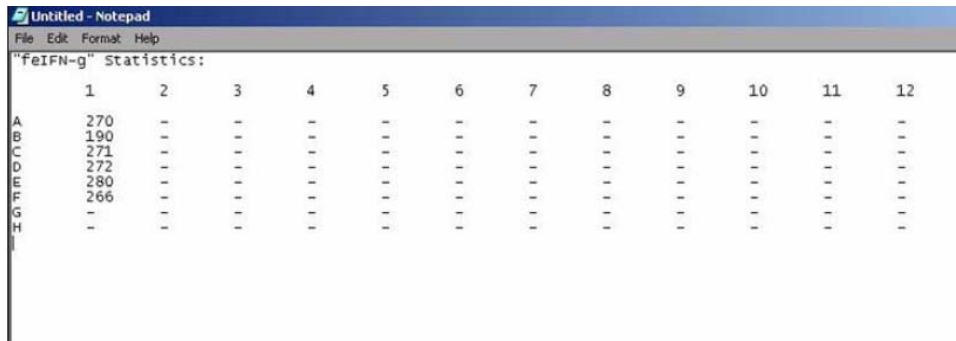


Figure 14

Images of analyzed wells can be found in the project folder created by the operator, which in our case is called "Vaccine" and can be found in:

C:\Program Files\QHubSpoto\DataBase\Vaccine

Vaccine folder contains two folders “Images” and “Slides”: high-resolution publication quality images are stored in the “Image” folder, whereas low-quality images for e-mail exchange are stored in the “Slides” folder (Figure 15).

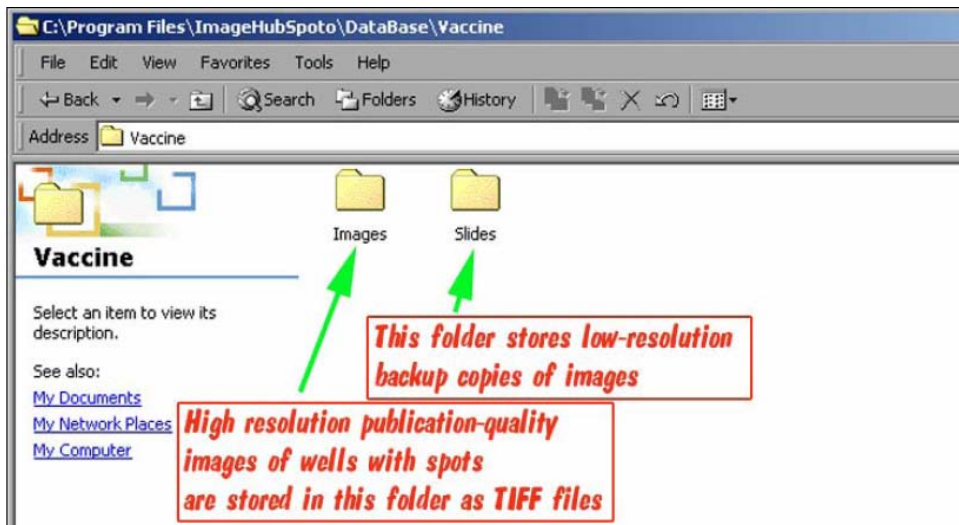


Figure 15

Saved images of wells can be previewed as thumbnails in the open “Images” folder (Figure 16). Image files were given names corresponding to coordinates of wells that were analyzed.

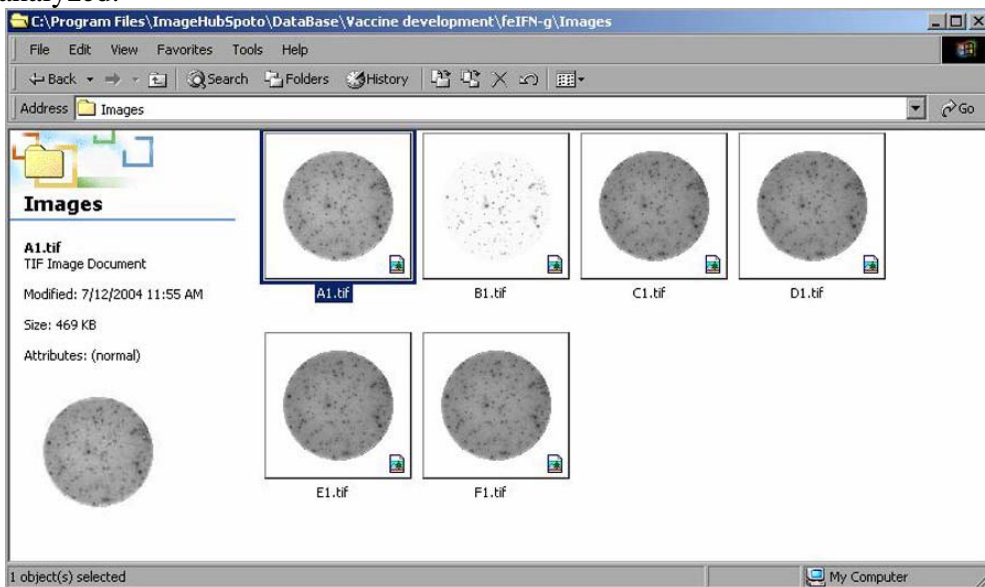


Figure 16