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by *life* technologies

ProtoArray® Prospector Imager v5.2.3

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TRADEMARKS

Contents

Overview

Introduction

The ProtoArray® Prospector Imager (referred to as Imager) is a component of the ProtoArray® Prospector software package. The Imager allows microarray image processing for data analysis.

The Imager combines the functions of image editor and feature signal extraction required to process images acquired with autoradiography or phosphorimaging methods. The Imager combined with ProtoArray® Prospector Analyzer provides a complete workflow for Kinase Substrate Identification (KSI) and Radiolabeled Small Molecule-Protein Interaction Profiling (SMI) applications using ProtoArray® Microarrays.

The Imager offers the following functions:

- Read .TIF file
- Perform image manipulations (brightness, contrast, zoom, rotate, resize)
- Crop image to the desired size
- Read .GAL (GenePix Array List) file, position grid/subarrays on the image
- Analyze image, output signal values in .GPR format
- Advanced functions include automatic grid positioning, block position adjustment
- Identify significant hits and perform cross-array comparison along with ProtoArray® Prospector Analyzer

Important

The ProtoArray® Prospector Imager is designed for use with KSI and radiolabeled-SMI applications only and not for image analysis of fluorescence based applications such as protein-protein interaction (PPI) and immune response biomarker profiling (IRBP). The Imager does not contain functions specific for fluorescent scanner image analysis, such as spot shape detection. You need to use the microarray analysis software that is supplied with the fluorescent scanner such as GenePix® Pro.

Imaging Arrays

You will need a standard desktop image scanner that provides at least 50 μm resolution (>600 dpi) to scan the X-ray film after developing the film to produce a 16-bit TIFF file, or a phosphor screen or phosphorimager that provides at least 50 μm resolution when generating the 16-bit TIFF file.

Supported File Formats

The Imager software supports 8- and 16-bit grayscale TIFF file. 16-bit resolution is recommended for better analysis precision. Other image formats such as .jpg, .bmp, .gif are not supported.

Image Analysis Workflow

The raw image from the image scanner or phosphorimager usually requires some manipulations before the image can be used for signal extraction. You may need to crop the microarray image from the original picture, rotate, invert, or change resolution. Details of image preparation are described in **Raw Image Preparation.**

After preparing the image of a single array, a grid of spots is overlaid onto the image, as described in **Acquiring Signal Values**. The grid is loaded from the .GAL file, which describes the location and identity of all spots on the ProtoArray® Human and Control Protein Microarrays. The .GAL files are available for downloading from ProtoArray® Central at www.lifetechnologies.com/protoarray through the **Online Tools** link under **BioMarker Discovery Resources**. Once the grid is aligned, feature signals are calculated and loaded into ProtoArray® Prospector Analyzer for data analysis.

Installation

Downloading and Installing Imager

The ProtoArray® Prospector Imager installer can be downloaded from the Life Technologies website:

1. Go to www.lifetechnologies.com/protoarray. Click on **Online Tools** link under **BioMarker Discovery Resources**, then click the link to download the **ProtoArray® Prospector** software from the **Online Tools** page.

2. Follow the instructions on the page to download the zipped executable installation file (**Prospector_v5.2.3.zip**) to your computer.

3. After downloading the file, double-click on it to unzip the application. If your computer does not include the WinZip® utility, you can download a version for free from www.winzip.com.

4. In the WinZip® window, double-click on the **ProspectorSetup.exe** file to begin running the installer.

5. The installation program guides you through the installation process.

6. ProtoArray® Prospector is installed in a folder in the Windows **Program Files** directory, and a submenu *Invitrogen\Prospector* is added to the Windows *Start\All Programs* menu**:**
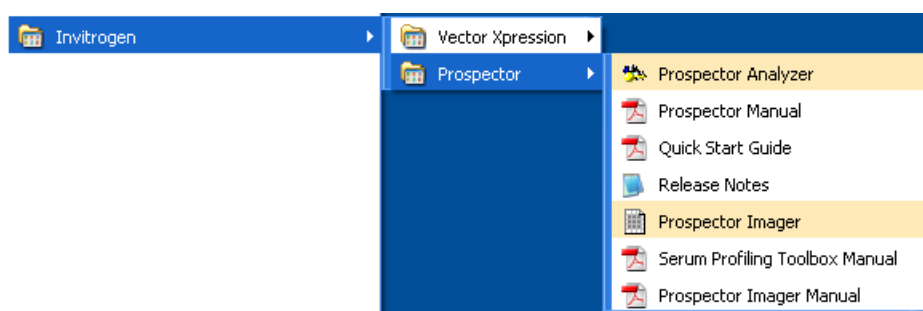
A shortcut for the software is also created on your desktop (shown below):

System Requirements

Supported Operating Systems:	Windows 2000 Service Pack 3, Windows XP Service Pack 2
Required Software:	Windows Installer 3.0, Windows Installer 3.1 or later is recommended.
	Internet explorer 5.01 or later
Disk Space Requirements:	280 MB

Display Settings

The recommended settings for use with Imager are 19"+ monitor, 1024 × 760 or higher resolution and true color.

Note

Installation of Prospector requires Microsoft.NET version 2.0 framework installed on the computer. If .NET is not installed, Prospector helps you download and install the framework. After the framework is installed, start Prospector installation again.

Microsoft® .NET framework redistributable package installs the .NET Framework runtime and associated files required to run applications developed to target the .NET Framework. For details on the .NET framework, visit www.microsoft.com/downloads and click on .NET Framework Version 2.0 Redistributable Package (x86) from the download page.

Upgrading from a Previous Version of ProtoArray® Prospector

1. Install ProtoArray® Prospector as described on the previous page. If you had previous versions installed, old executables are automatically removed, while data and results are retained.

2. If you did not change the default setting for the location of the ProtoArray® Prospector folder during installation, the folder will be located at **\Program Files\Invitrogen\Prospector**. Result subfolders from previous analyses of arrays using ProtoArray® Prospector are located in the same location and are not overwritten.

Automatic Update of ProtoArray® Prospector

ProtoArray® Prospector can be configured to check for available version updates and download and install any newer versions.

This feature checks the Life Technologies website for newer versions of the application. If a newer version is available the version information is displayed and the option is given to download the newer version. When the newer version is downloaded the option is given to install the upgrade immediately, or install the upgrade the next time the application is started.

A manual version check can be performed by selecting the *Tools > Check for software update* option.

To configure this feature, select the **Options** icon on the menu bar. Within the **Options** dialog box select the **Web Parameters** tab. Check the box labeled *Auto check for version updates*. This setting enables the software to automatically check for updates on a regular interval. If this option is not selected the user is still able to check for newer versions via the *Tools > Check for software update* option.

Getting Started

Starting the Program

To begin running Imager, select the program from the **_Windows Start Menu > Programs_** list, or double-click on the application shortcut on your desktop.

The interface consists of a menu bar, toolbars, a status bar, an information panel, and an image window
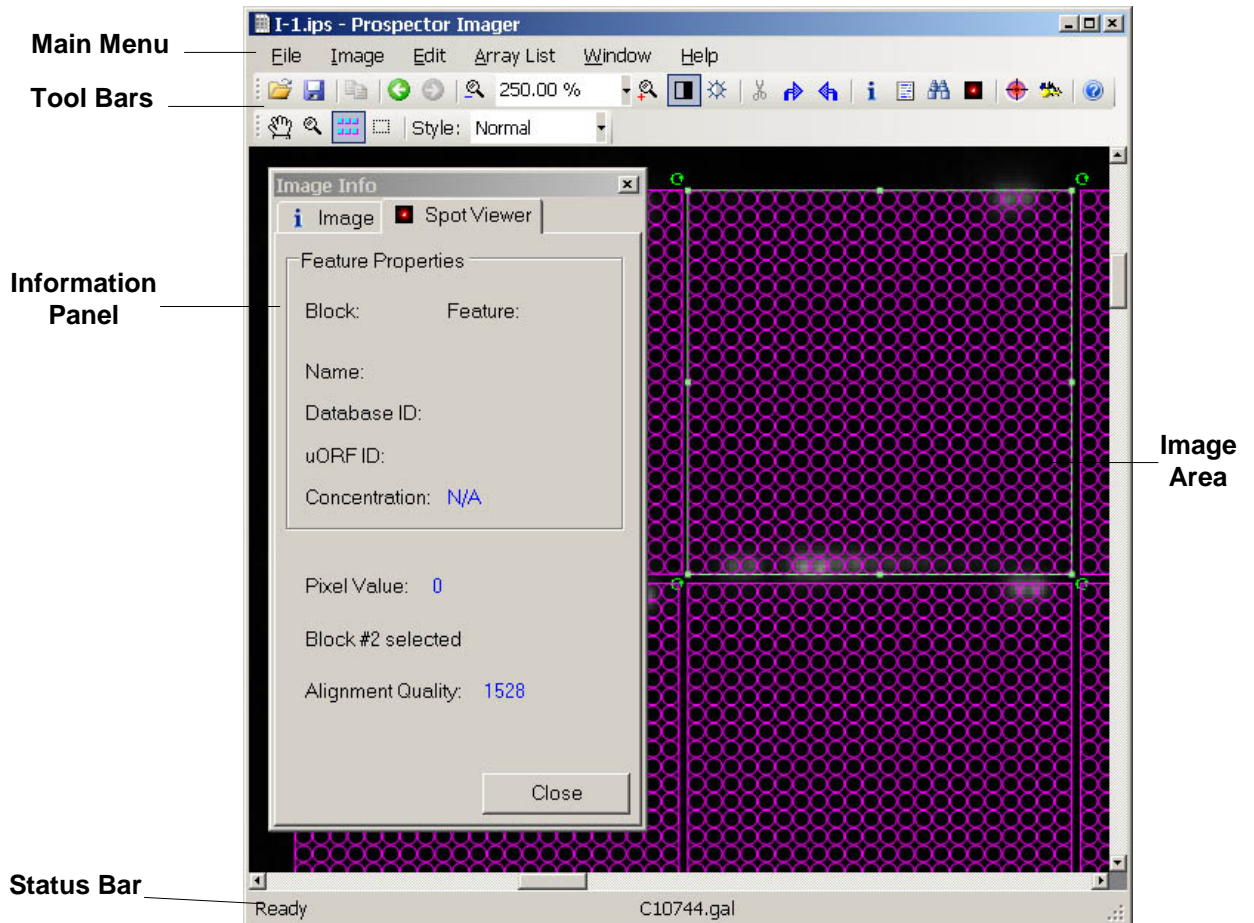
The main menu contains all functions to manipulate an image. Most frequent used functions are also available as buttons on toolbars. Move the mouse cursor over a button to see a short description of its function. The following sections describe typical workflows to process an image and explains each operation in more details. Most functions are referred to by a small picture (for example ☼), which indicates that such a function is available both as a button and a menu item.

Image Area Manipulations

Many image manipulations are done with the mouse. The keyboard may be also convenient for some operations, such as grid position adjustments. Details for each manipulation are described below.

Modes of the Image Area

There are four modes of image area manipulation, which affect appearance of the image area and the way mouse operates. Use the toolbar buttons shown below to select the modes:

- **Hand Tool**

  In this mode, the mouse drags the image, scrolling the image over the image area.

- **Magnify Tool**

  In this mode, the mouse is used to zoom in on the image. Click once on any image point to zoom in. Select with mouse the image area of interest to zoom to that area.

- **Array Mode**

  This mode is used to position a grid of spots on the image. A standard ProtoArray® contains 48 blocks of spots. When the grid is first loaded from the .GAL file, all blocks are selected. To select a single block, click on the block with the left mouse button. To select multiple blocks, hold the Shift key and click or you can hold the left mouse and drag the cursor over the blocks. To select all blocks, click outside the grid area. You may also use menu commands to select/unselect blocks.

  Selected blocks may be dragged with the mouse or keyboard arrow keys. Corners and sides of block have "handles" to stretch/rotate a block or the whole grid, if necessary.

- **Select Mode**

  This mode is used to select a part of the image. Two types of selection are available:

  - Normal

    With this style selection, the rectangle size is selected with mouse.

  - Fixed size.

    With this style selection, the size is specified in pixels, and only selection rectangle position and angle may be changed with the mouse. This style may be convenient if you know the physical size of array (1'x3' for a standard ProtoArray®) and image resolution. For example, if the resolution is 600 dpi, set selection size to 600 × 1800 pixels to select the whole array.

    To adjust selection angle, press and hold the Ctrl key and drag with mouse any angle of the selection rectangle as shown below:
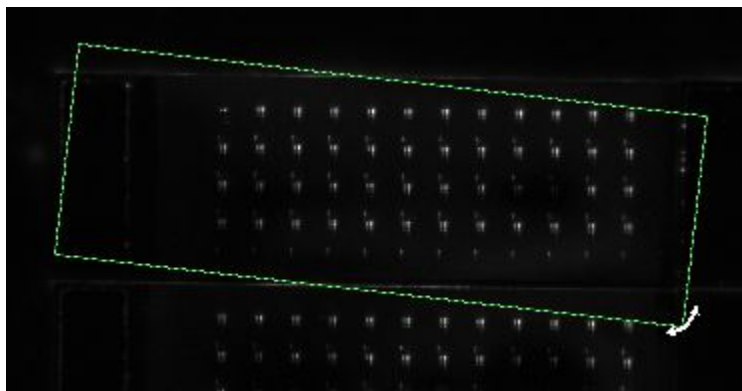
Keyboard Hot Keys

The keyboard hot keys are listed below:

Key	Function
Ctrl +	Zoom in
Ctrl -	Zoom out
Ctrl *	Zoom to screen
Arrows (←↑↓→)	Move selected block(s) by 1 pixel
Ctrl Arrow	Move selected block(s) by 10 pixels
Ctrl +A	Select all image
Ctrl + D	Deselect image
Ctrl + Mouse	Rotate selection
Ctrl + O	Open project
Ctrl + S	Save project
Ctrl + Z	Undo
Ctrl + Y	Redo

Raw Image Preparation

Introduction

The raw image from the film scanner or phosphor imager usually contains images of several arrays. The various steps involved in the image preparation are:

- Invert colors to white on black, if necessary
- Crop a single array image
- Correctly orient the image on the screen

Preparing the Image

To prepare the microarray image for analysis:

1. Start ProtoArray® Prospector Imager from the Windows Start menu: **Start > Programs > Invitrogen > Prospector > Prospector Imager** or double-click on the application shortcut on your desktop.

2. Open .TIF image file using the ![folder button] button or select **Open\Image** from the **File** menu

3. Select the image in the **Open dialog.**

4. Adjust the visual appearance of the image using Image Parameters ![icon] window, if necessary by adjusting the image resolution or brightness/contrast feature. Note that this function only affects a visual appearance of the image and does not change pixel intensities.
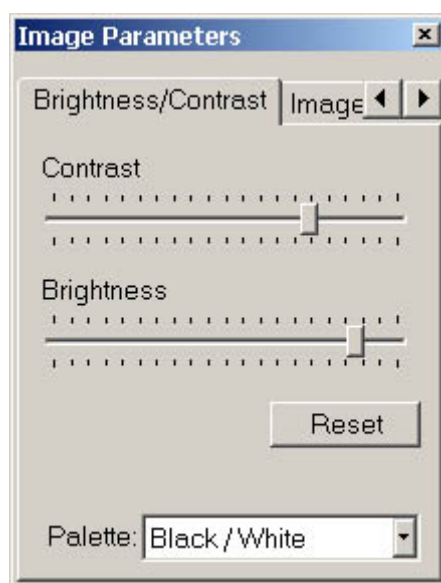

5. Invert the image using the invert button ![invert button] from the toolbar. This switches the image to negative and back, which sometimes helps to visually identify obscure feature spots. Note that this function only affects the visual appearance of the image and does not change pixel intensities. Use the **Save Inverted Image As…** function to invert the actual pixel values.

6. Using the Hand Tool ![hand tool] from the toolbar, drag the image with the mouse to the center of the area of interest.

7. To adjust the zoom level, use the Magnify Tool ![magnify tool] from the toolbar and use the mouse to select the image area to magnify. You may also use ![zoom out] and ![zoom in] buttons.

8. Rotate image with rotate tool ![rotate tool] on the toolbar such that the array image is vertical and the barcode is located at the bottom.
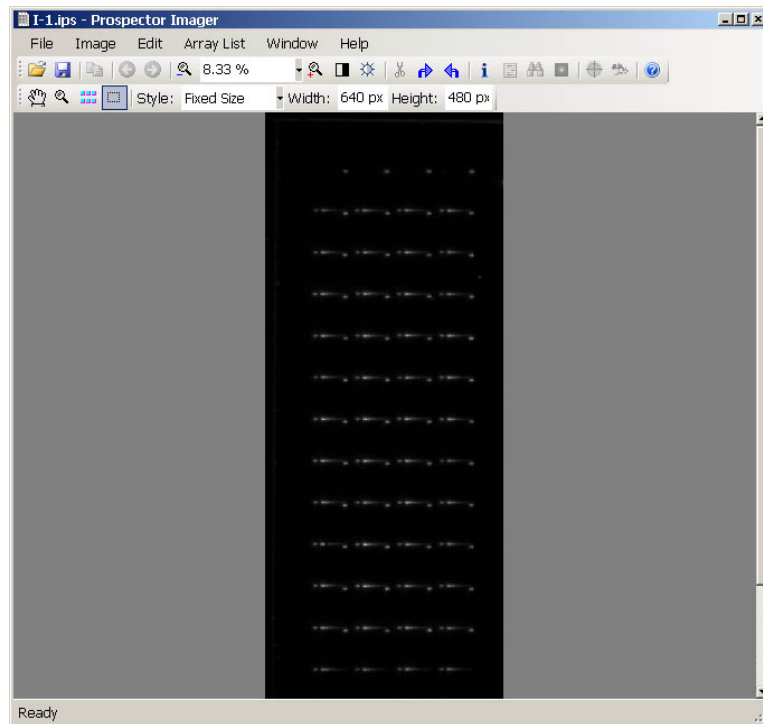
9. Switch to Select Mode tool ![select mode] to set the selection style. We recommend setting the selection style to **Fixed Size** and specify selection size 1800 × 600 or 600 × 1800 depending on the image orientation (assuming that the scanner resolution is 600 dpi and microarray slide is 1" × 3").

    Note: If the image is scanned at a different dpi, set the fixed rectangular area accordingly. For example, if the image is scanned at 1200 dpi, set the fixed rectangular area to 1200 × 3600 pixels to cover the 1" × 3" array area.

10. If the spots are not aligned vertically, rotate the selection rectangle by pressing and holding the Ctrl key, and rotating the selection angle with the mouse. Use the Zoom in tool ![zoom in tool] at this step, if needed. First rotate and align the rectangle against the Alignment Control Kinase spots, release the Ctrl key and move the rectangle to cover the whole array area.
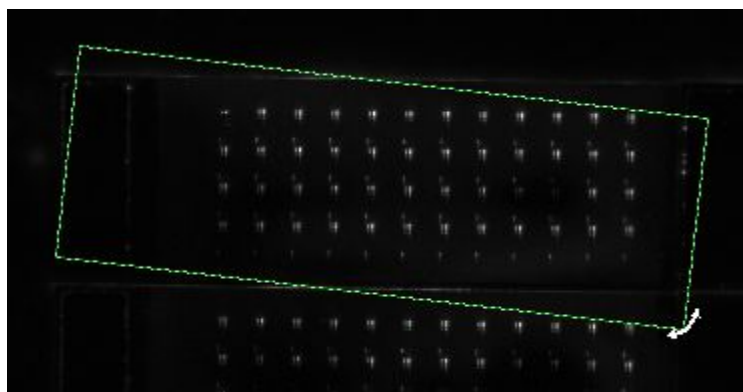
11. Crop the selected rectangle which should correspond to the perimeter of the array using the Crop tool ✄.

12. Optionally you may resize image to a bigger size. This may increase signal values precision, but slows down analysis and increases image file size. Use **Image > Resize** menu command. We recommend stretching the image integer number of times, *i.e.* 3x or 4x, and constrain proportions.

13. To save the prepared image as a .TIF file use **Image | Save As…** or **Image | Save Inverted Image As…** menu. Specify the file name and location. Be sure to include the barcode in the name of the image.

14. You may now repeat this workflow to prepare another image or proceed to the next section for signal quantification.

Note: Each function that changes the image, such as Crop, Rotate, Resize, can be undone using undo ⬅ and redo tools ➡ from the toolbar. However functions that change the image appearance such as Zoom, Scroll or Invert do not require undo capability.

Data Acquisition

Introduction

After the image of a single array is prepared, overlay a grid of spots onto the image. The grid is loaded from the .GAL file (GenePix® Array List), which describes the location and identity of all spots on the ProtoArray® Human, Yeast, and Control Microarrays. Once the grid is aligned, feature signals are calculated and loaded into ProtoArray® Prospector Analyzer for data analysis.

Acquiring Signal Values

1. Open a prepared single-array image using the button or select **Open\Image** from the **File** menu.

2. Download the .GAL file from ProtoArray® Central at www.lifetechnologies.com/protoarray by clicking on the **Online Tools** link. On the **Online Tools** page, click the link under **ProtoArray® Lot Specific Information** and use the lot number to download the array specific information as directed on the web page. Save the .GAL file to a specific location.

3. Select the Array Mode tool from the toolbar. You can also load an array list using the button or **Open\Array List** command from the **File** menu. A standard file open dialog opens for selecting the .GAL file from the appropriate location. If no array list was loaded, the screen automatically shows a standard file open dialog to load the array list from a .GAL file.
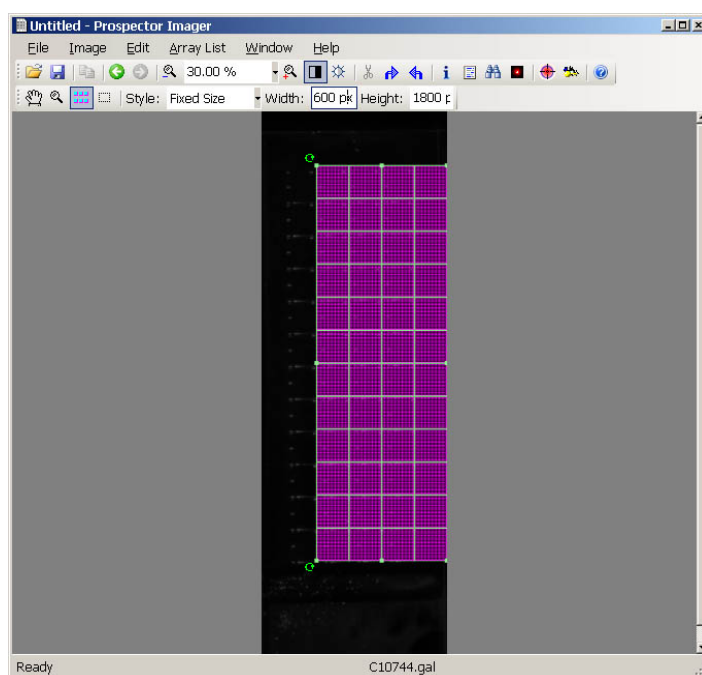
4. Adjust a scaling factor corresponding to a resolution of your scanner, if necessary. Use menu item *Window > Show Image Parameters > Image Resolution* to specify scanner resolution (usually 600 dpi, but may be different for your scanner).

5. Set the feature diameter to 190 µm using menu *Array List > Block Properties...*
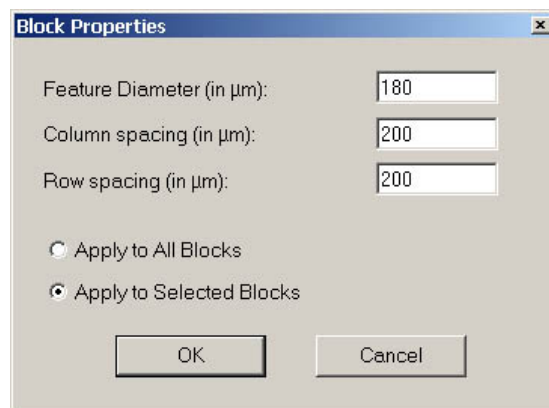
6. *Using the mouse and keyboard arrow keys, adjust the grid over the image manually. Use spots corresponding to the Alignment Control Kinase (PKCeta) as reference spots to orient the microarray image. Click to select individual subarray to move, hold Shift key and click to select multiple subarrays (you can also hold the left mouse and drag the cursor to cross the blocks) or click outside the grid area to select all subarrays. Scroll through the image to ensure that the grid is in the proper location for each subarray.*

   See **Image Area Manipulations** for details of keyboard and mouse operations, and **Additional Functions** for automated grid positioning tools.

7. After the grid is adjusted properly and all features are aligned, use **Save Project As...** on the **File** menu to save the project (including the image, grid position and parameters for future references). Specify the file name and location.

8. When all subarrays are positioned correctly over the image, select menu item *Array List > Analyze Results...* Specify the file name to store calculated signal values and select the KSI application.
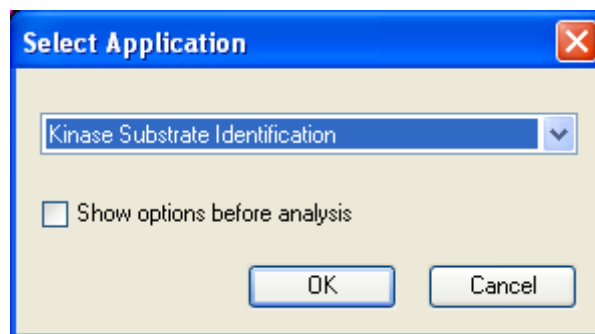
9. Press **OK**. Imager automatically opens the Analyzer component of Prospector for data analysis and the file is now opened and processed by ProtoArray® Prospector Analyzer.
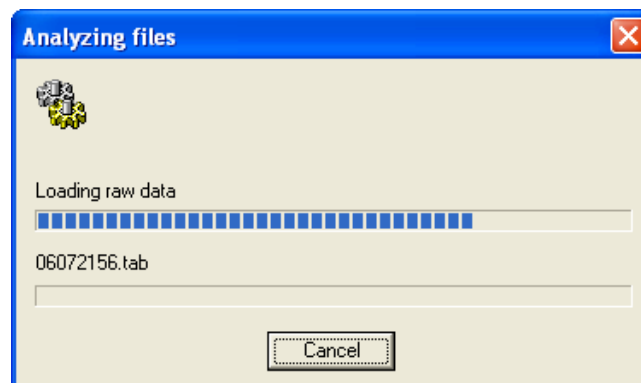
10. The required PAI (ProtoArray Application Information) files for v5.0 and v5.1 microarrays are now included with installation of the ProtoArray® Prospector v5.2.3. Prospector automatically downloads .PAI files from Life Technologies ProtoArray® Central if the required file is not found in the **Application-Lot Specific Files** subfolder.

11. Specify experiment type: Step 1 (Control Kinase) or Step 2 (Customer Kinase) for control microarray, or Step 3 (Buffer) or Step 4 (Customer Kinase) for experimental microarray.
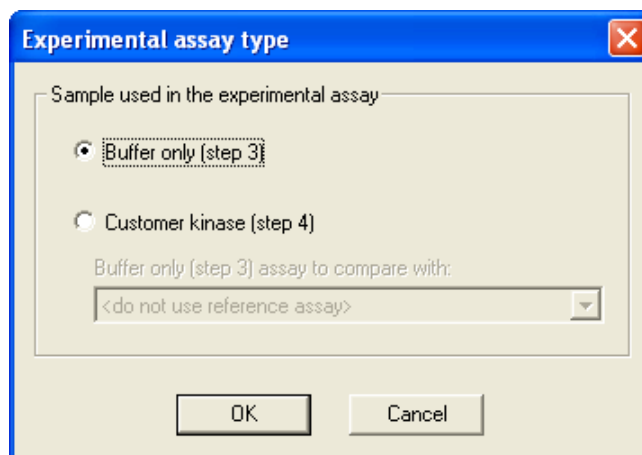
12. ProtoArray® Prospector Analyzer shows a summary of analysis completed in the right view. See Prospector manual for detailed description. You may now return to Imager to verify hits identified by the software are also observed on the actual image.

Additional Functions

Save Inverted Image

The Imager assumes that zero pixel value corresponds to black color. If raw image is negative (black on white), you need to invert the image, changing each pixel value V to 65535-V. To do this use menu function Save Inverted Image, and then open this saved image. Note that the Invert function ▮ only changes a visual appearance of the image (some spots are easier identified when drawn as black on white) and does not affect the actual pixel values, or results of signal acquisition.

Save Project As

A Project is a human-readable .xml file which contains links to an image and array list files, as well as parameters such as grid position. We recommended saving the project before analyzing results to simplify further verification of hits identified by ProtoArray® Prospector Analyzer.

Find Features

The Find Features function is available on the *Array List* submenu or from the toolbar 🔍 and allows finding one or more features with specified name or Database ID.
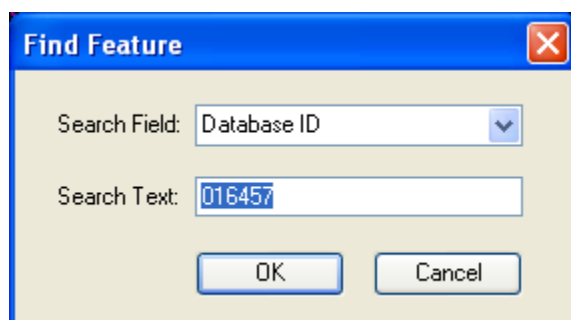
All features with search field containing specified search text are found and listed.
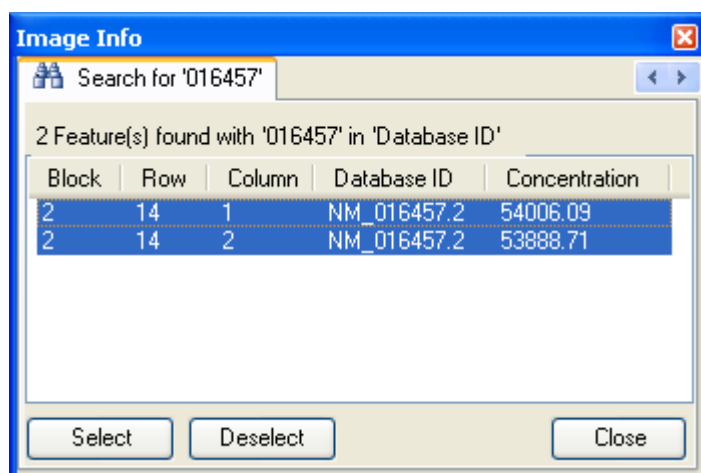
When one or more rows are selected in the list, the corresponding spots are highlighted on the array image.
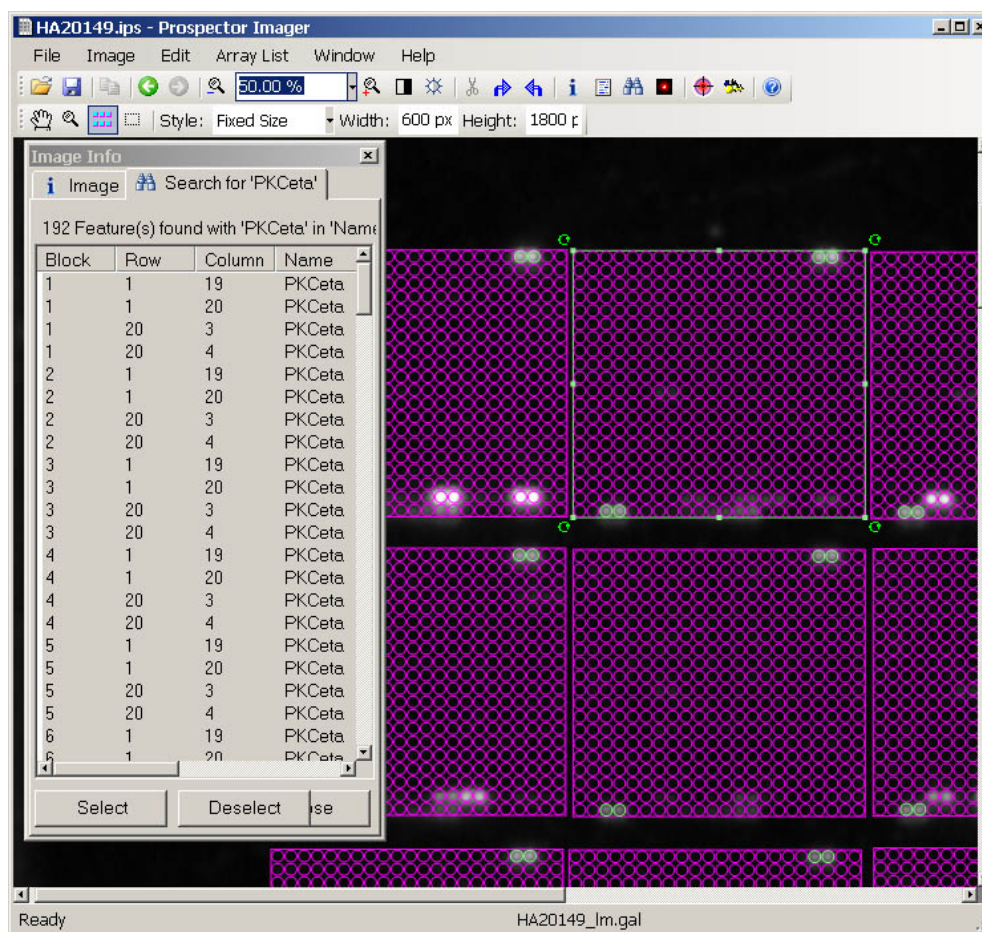
Auto Position

The Auto Position function is available on the **Array List** submenu or from the toolbar ⊕ and tries to automatically detect the whole grid position, size and angle. It does not adjust individual block positions. Protein array images are hard for automated positioning because most features have

signals close to a background. If this function fails, you may use Undo ⬅ to restore previous grid layout.

Auto Align Blocks

The **Auto Align Blocks** function is available on the **Array List** submenu and automatically adjusts the position of each selected block (subarray). Imager shifts each block in every direction within one spot distance trying to find optimal location of the block. Position the blocks close enough before running this function.

Technical Support

Obtaining support For the latest services and support information for all locations, go to www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities

- Search through frequently asked questions (FAQs)

- Submit a question directly to Technical Support (techsupport@lifetech.com)

- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents

- Obtain information about customer training

- Download software updates and patches

Safety Data Sheets (SDS) Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/support.

Certificate of Analysis The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available from www.lifetechnologies.com

Limited warranty Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at http://www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies.

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