ACGT MICROARRAY FACILITY	
STANDARD OPERATING PROCEDURE	
TITLE: Data capture	PAGE 1 of 5
SOP#: MA014	REVISION LEVEL:. 1 DATE: 15 January 2007
AUTHOR: L Solomon	REVIEWER: Nicky Olivier

Purpose:

Once an image has been acquired by scanning a slide, the next step is to measure and record the signal intensity of each feature

Scope:

This protocol outlines the steps required to generate a genepix result (.gpr) file consisting of a spreadsheet of spot intensities suitable for further analysis, from a scanned image. The software used at the ACGT Microarray Facility is GenePix Pro 5.1.

Procedure:

Opening an image:

- If you have just completed scanning a slide, the image will be displayed on the screen ready to analyse.

- To open a previously saved image:
    - Click the *File…* button on the right hand side of the GenePix Pro main window
    - Select *Open Images*.
    - Navigate to a directory that contains your previously saved TIFF images
    - Select the two images corresponding to one slide (_532 and _635) and click the *Open* button.

If you have an image open you can change the display properties of the image:

- To switch between single-wavelength and ratio images:
    - Select the view you need from the buttons in the *Image* group at the top left of the Image tab

- To change the single-wavelength and ratio colours:
    - Click the *Color* button in the *Tools* group on the Image tab
    - Select from the list of colour combinations on the menu
    - Select *Invert Color* at the bottom of the menu to invert

Color

ACGT MICROARRAY FACILITY
STANDARD OPERATING PROCEDURE

TITLE: Data capture		PAGE 2 of 5
SOP#: MA014	REVISION LEVEL:. 1	DATE: 15 January 2007
AUTHOR: L Solomon	REVIEWER: Nicky Olivier	

Try experimenting with various display settings to find the one that is most comfortable for you. For example, green-blue with inverted color is good for distinguishing features from background at high magnification.

- To change the brightness and contrast of the image:
- Click the *Auto Scale Display Settings* button to set brightness and contrast to their optimum settings
- Move the individual brightness and contrast sliders for custom settings
- Click the reset button to return to default setting

- To zoom in and out:
- Zoom in by selecting *Zoom Mode* from the *Tools* group on the left hand side of the Image tab
- Left click and drag to draw a rectangle around the area that you want magnified, or just left click to zoom in step by step
- Zoom out by clicking the *Undo Zoom* button from the *Tools* group on the left hand side of the Image tab.
- Return to the full-scale image by clicking the *Full Scale* button from the *Tools* group on the left hand side of the Image tab.

Once you are satisfied with the way the image looks you can start with detecting the features on the slide. In GenePix Pro each individual spot that is deposited on a slide is assigned a *feature indicator*. A grid of feature indicators is called a *block*. There can be several blocks on a single array depending on the arrayer setup during slide printing.

The easiest way to generate blocks for analysis is to load the information contained in a GenePix Array List (GAL) file. GAL files link the information from the arraying process to the analysis, by providing identification information on each spot printed on the slide.

If you need to generate your own GAL file, access the help files in GenePix Pro by clicking on the *Help* button on the right hand side of the screen. If your slides were printed at the ACGT Microarray Facility at the University of Pretoria, South Africa you will be supplied with the GAL file for your slides.

- To load a GAL file:

- Click on the File… button on the right hand side of the screen and select *Load Array List…*
- Navigate to where your GAL file is saved, select it and click on *Open*

GenePix has alignment algorithms for finding the location of complete blocks on an image and for aligning feature indicators within blocks with features on the image.

- To place blocks over features:
  - Click the *Align Blocks* button on the left hand side of the screen and select F*ind All Blocks* (Hotkeys: Shift+F7) to place each block in its optimal starting position for the alignment of features

- To align features
  - Click the *Align Blocks* button and select *Align Features in All Blocks* (Hotkeys: Shift+F5) to align each feature indicator with the nearest feature

If the circles are not neatly lined up over each spot, you may need to start again by loading the GAL file as before and manually shifting the blocks into approximately the correct position before trying to align blocks and features.

- To move blocks manually:
  - First ensure that you are in 'Block Mode' by clicking on the Block Mode button from the *Tools* group on the left hand side of the Image tab.
  - Blocks can now be clicked and dragged into position as well as stretched or shrunk from the sides and corners.

Once you have successfully arranged your blocks and features you may want to fine-tune the feature alignment manually. For example, it may be necessary to align manually features that are flagged *Not Found* by *Align Blocks*, or to move features that have aligned over bright background spots caused by dust or scratching.

- To manually move, resize and fine-tune feature indicators:
  - First select *Feature Mode* from the *Tools* group on the left hand side of the Image tab.
  - Feature mode has two sub-modes, *rectangle* and *lasso*, for making multiple selections. Once you are in Feature mode, click the Feature mode button again to display a choice of rectangle or lasso
    - o In Rectangle mode, if you hold down the mouse and drag it, every feature in the bounding rectangle of the region is selected.
    - o In Lasso mode, hold down the mouse and draw an arbitrary closed region to select all the features in the region.
  - Once you have selected a feature or features you can move them with the arrow keys, or resize using <Ctrl>+arrow keys

Before beginning analysis of your slide you may need to mark or *flag* individual feature indicators. For example, you may find some features should be ignored during your final analysis because of imperfections on the array.

- To flag a feature on the Image tab:
  - Select *Feature Mode* as above
  - Select a feature that you wish to flag by clicking on it
  - Right click and select a flag to apply to the feature – *Good*, *Bad*, *Not Found* or *Absent*

GenePix Pro will also flag features automatically, for example if *Align Blocks* fails to find a feature, the feature is flagged *Not Found*. All flags are stored in the final Results table and are exported with numerical equivalents.

- To save your block positions and other settings
  - Click the *File…* button on the right hand side of the GenePix Pro main window
  - Select *Save Settings As* from the menu
  - Enter the target directory *(to be issued by the ACGT Microarray Facility)*
  - Enter a file name for the settings and click *OK*

Saving the settings will save the position of the array as well as display settings such as contrast and brightness.

- After aligning the analysis array and flagging any features:
  - perform the analysis of the image by pressing the *Analyze* button.

The analysis extracts data from the single wavelength images in each feature indicator, and the results are sent to the Results tab spreadsheet.

- Once the analysis is completed, remember to save your data:
  - Click the *File…* button on the right hand side of the GenePix Pro main window
  - Select *Save Results As*
  - Enter the target directory *(to be issued by the ACGT Microarray Facility)*
  - Enter a file name in following format: ccyy-mm-dd_barcode_description
  - From the group of *Naming* options, check the boxes to add prefixes and suffixes to the name (for example date and barcode number)
  - Ensure that the data is saved as a Genepix Results File (.gpr)
  - In addition, if you wish to save JPEG images of the analyzed image, please ensure that the *Save a JPEG image containing all analyzed features* checkbox is ticked (**This is compulsory for all users of the ACGT Microarray Facility at the University of Pretoria for subsequent analysis using the SpotVerify program**).
  - Click *OK*