ACGT MICROARRAY FACILITY	
STANDARD OPERATING PROCEDURE	
TITLE: Scanning using the Genepix 4000B scanner	**PAGE 1 of 4**
SOP#: MA013	**REVISION LEVEL:. 1**	**DATE: 15 January 2007**
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Purpose:

This SOP describes the procedure for scanning dual-labeled hybridized slides to obtain image files for spot capture and data analysis using a Genepix 4000B scanner.

Procedure

For users of the ACGT Microarray Facility:

Access to the data server is necessary prior to generating and storing data. Follow the "Request data server access" link on the ACGT microarray website (www.microarray.up.ac.za), enter the required information, and a password will be issued. Access to this data server is restricted to users of the ACGT Microarray Facility, and is only accessible from the campus of the University of Pretoria.

- Switch scanner on, first at the wall plug and then on the scanner itself (the lasers have to warm up for at least 15 minutes prior to scanning to allow the lasers' output to stabilize)
- Start the Genepix Pro 5.1 program

Note: The scanner needs to be switched on to use the GenePix Pro 5.1 program in full mode.

Loading a slide

- Open the scanner by sliding the scanner door to the left.
- Gently lift the tab on the front of slide holder to raise the top half.
- With one finger, hold open the adjustment arm on the inside left edge of the slide holder. Insert the slide face down into the slide holder frame with the top edge towards the hinge of the slide holder. A slight flick of the adjustment arm will ensure proper positioning of the slide (do not push the slide into position).

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TITLE: Scanning using the Genepix 4000B scanner	PAGE 2 of 4
SOP#: MA013 REVISION LEVEL:. 1	DATE: 15 January 2007
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- Gently close the slide holder lid until it snaps shut, and close the door.

Performing a preview scan

Note: Use the 40 µm preview scan to identify the region on the array that is to be scanned at high resolution (5-10 µm).

- Switch to the *Histogram* tab
- Set the image balance at a minimum intensity of 500 and a maximum intensity of 65530.
- Switch to the *Image* tab
- Press the *Preview Scan* button on the right hand side of the GenePix Pro main window

Note: The gains for both photomultiplier tubes need to be balanced to obtain an equal amount of green and red signal in each channel. This results in a pixel ratio (635 nm/532 nm) of approximately 1.0

After the Preview scan is completed, switch to the *Histogram* tab. The count ratio, indicated on the left-hand side of the main window, must approximate 1.0.

For count ratios larger than 1.0:

- Open the *Hardware Settings* dialog box on the right hand side of the main window (this window can remain open during the scan).
- Increase the PMT gain for the 635 nm channel, or decrease the PMT gain for the 532 nm channel.
- Switch to the *Image* tab and perform the Preview Scan again.
- Repeat these steps until the count ratio approximates 1.0.

For count ratios smaller than 1.0:

- Open the *Hardware Settings* dialog box on the right hand side of the main window (this window can remain open during the scan).

- Increase the PMT gain for the 532 nm channel, or decrease the PMT gain for the 635 nm channel.

- Switch to the *Image* tab and perform the Preview Scan again.

- Repeat these steps until the count ratio approximates 1.0.

Drawing a scan area

Following the preview scan, draw a scan area around the features/area of interest (only this area is scanned during the data scan).

- Select the *Scan Area* tool from the *Tools* group on the left-hand side of the main window.

- Move the mouse cursor to the top left of the features on the image

- Holding in the mouse button, drag a rectangle around the region of interest, including the barcode (if available).

Changing laser power

Note: Laser power cannot be adjusted while a scan is in progress

- Open the *Hardware Settings* dialog box on the right-hand side of the main window

- Change the *Power (%)* field for each wavelength to the desired value (10, 30 or 100)

Note: The Power field for both wavelengths must be the same.

Performing a data scan

- Press the *Data Scan* button on the right-hand side of the main window

Saving images

Note: The raw image data must be saved for data analysis purposes, since the ratio image cannot be used for this purpose. The data can be saved as multi-image or single-image TIFF files, with single-image TIFF files being the recommended format since it is accessible from most data analysis programs. Raw data should not be saved in the JPEG format, since this is not suitable for data analysis.

Following the Data Scan, the images have to be saved.

- Click on the *File* button on the right-hand side of the main window

- Select *Save Images*

- Select the target directory (*will be issued by the ACGT Microarray Facility*)

- Type in the file name:

    - The recommended format is: ccyy-mm-dd_barcode_description

- Select the image type as Single-image TIFF files (*.tif)

- Press the *Save* button