Addendum 2005-09-22

Scanning Membranes

1 Loading a membrane

When scanning membranes, the cassette with permanently mounted glass plate - Mounted Glass Cassette - should be used. To achieve optimal calibration when scanning, the black plastic hold down plate should be placed on top of the membranes.

Note: *Wear powder free gloves when handling the cassette and membranes. The powder used in laboratory gloves can fluoresce and may also scatter light affecting image quality.*

To load a cassette

1 Clean the glass plate with distilled water and dry it using a lint free cloth. Since fluorescent material has come into direct contact with the glass plate we recommend using a lint free tissue moistened with 10% hydrogen peroxide to remove this material, followed by cleaning with distilled water.

2 Place the membrane(s) directly on the plate with the blotting side facing down. If the membrane(s) is wet make sure to exclude air bubbles when positioning it.

3 Note the positions of the membrane boundaries on the plate. You will use this information to determine the area(s) to be scanned, see *2 Set Up a membrane scan*.

4 Place the hold down plate in the cassette on top of the membranes.

5 Put the lid on the cassette (see *Fig. 3-4* in the User Manual), and close the cassette by turning the locking cams up.

6 Insert the cassette into the scanner as described in *3.2.3 Inserting a cassette* in the User Manual.

2 Set Up a membrane scan

Start the Scanner Software by double-clicking the **Ettan™ DIGE Imager** icon on the workstation desktop, or by using the start menu: **Start:Programs/GE Healthcare/Ettan DIGE Imager**. The scanner software Ettan DIGE Imager window opens in Setup View.

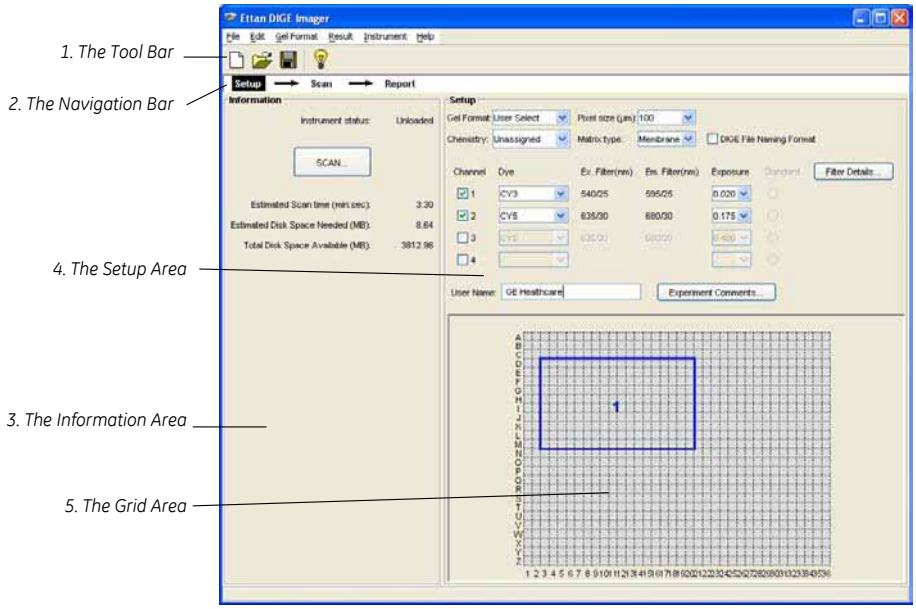

Fig 1. The Ettan DIGE Imager Setup View

1 The **Tool Bar** is used to manage scan templates and to turn on the scanner light.

2 The **Navigation Bar** shows the status of the scan workflow.

3 The **Information Area** shows the instrument status, displays information about the scan, and includes the **SCAN** button that is used to start a scan.

4 The **Setup Area** contains the parameters and settings for the scan.

5 The **Grid Area** defines the area or areas to scan. The grid coordinates are aligned with those on the cassette.

To set up a scan

In the Setup Area of the Setup View, the parameters and settings for the scan are set.

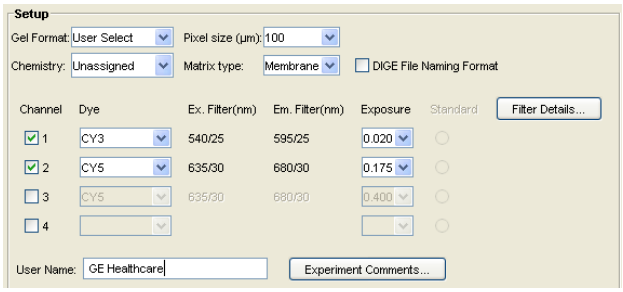

Fig 2. Parameters and settings in the Setup Area

Matrix type:

Choose membrane from the **Matrix Type** list.

The steps below do not have to be performed in a strict order.

1 **Gel format**

The **Gel format** list is used to set the scan area. In the **Gel Format** list in the Setup Area choose **User Select**. In this mode, you can manually set the area to be scanned. Then use the mouse and drag a box from the positions of the membrane boundaries. The scan area is shown as a blue box in the Grid Area.

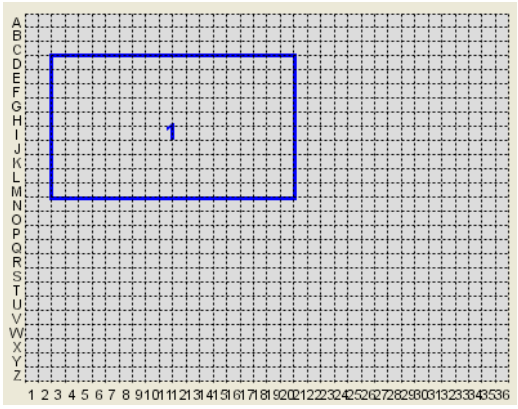

Fig 3. Grid Area

2 **Chemistry**
When scanning membranes the chemistry should be **Unassigned**.

3 **Pixel Size**
Select the pixel size, i.e. resolution to use, in the **Pixel size** list. 100 µm should be used.

4 **Channels and dyes**
Select the number of channels to be scanned by clicking the **Channel check** box. Between one and four channels can be programmed. Selection of the Channel check boxes must be performed in sequential numerical order. For each selected channel, select a dye to be scanned in that channel. When selecting dye, the excitation and emission filter to be used for that dye are automatically presented by the software. The excitation and emission filter combinations have been selected to give the optimum results with minimal crosstalk.

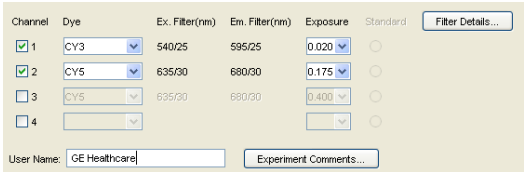

Fig 4. Filter settings

5 **Exposure**
Select the exposure to be used for each channel in the **Exposure list**. Generally low exposures are used for membranes. However, a quick test scan on a small area should be performed to identify an optimal exposure. See *3.4.1 Finding the optimal settings* in the User Manual.

6 **Comments**
It is optional to add comments to your data. To add comments to the data:

1 On the bottom of the Setup Area, click **Experiment Comments** to open the Experiment Comments dialog.

2 Type in the **Membrane ID**, the **Individual Source**, and the **Sample ID**.

3 Type in **Comments** for the scan. To use the same comments for all the gels in this scan, select **Use this Comment for all Gels**. (This option only applies to the Comments field. All other fields are always specific to each gel area.)

4 Click **Close**.

To start the scan and for more information see *3.4.2 Start a scan* and following chapters in the User Manual.