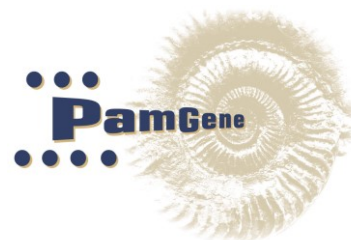




BioNavigator

Image Quantification and Statistical Analysis of Data





BioNavigator User Manual
Version 2.3, 2020

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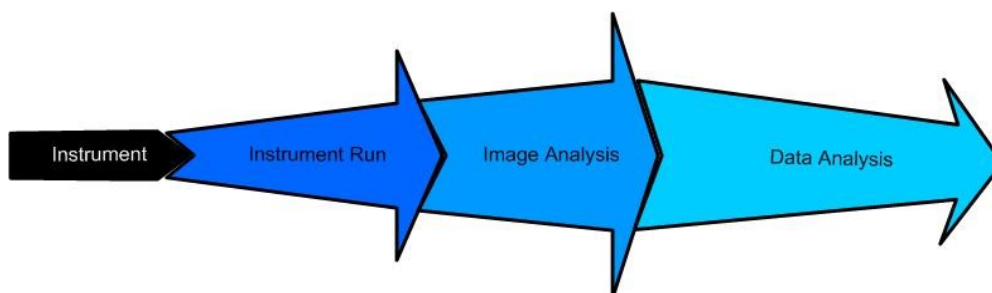
1 INTRODUCTION

1.1 Intended use

BioNavigator is intended to be used in combination with PamChip® microarray data to analyse images and perform statistical analysis on microarray data

1.2 Description of the BioNavigator

With Evolve and the different types of PamStations® PamGene International B.V. provides a powerful combination to generate a lot of valuable research data. For analyzing these large amounts of data PamGene developed a powerful multi variate analysis tool named BioNavigator 6. The image below illustrates the simplified research process. BioNavigator starts with converting the images into numeric data for statistical analysis. This conversion from images to numbers can be done for 1 or more PamChip® Arrays.



Containing a large set of statistical data analysis methods BioNavigator version 6.x supports the scientist in data analysis process steps.

BioNavigator is the software package which performs image analysis and data analysis for a PamStation® using PamChip® Arrays. It is capable of processing one or more PamChip® Arrays at a time. For a concept overview to BioNavigator, please see [concepts](#) (see section 2.1). For a features introduction to BioNavigator (see section 2).

BioNavigator comprises of two main parts:

- [BioNavigator editor](#) for the making of a [data protocol](#) and
- [PamCloud services](#) to help share and discuss with the PamChip® community

This manual will uniquely deal with the BioNavigator editor.

2 INTRODUCTION TO FEATURES

The following are the main features and benefits of BioNavigator 6. They are categorized by four main sections:

- Visualization
- Computation
- Storage
- Sharing

To fully understand the features mentioned below it is important to understand the fundamental [concepts of BioNavigator](#) (see section 2.1).

A tabular overview of features is given below:

Category	Feature	User Benefit
Visualization	Drag and Drop (e.g. cross-tab view)	Very easy to explore data
	Multiple views (e.g. cross-tab view)	User can customize the view
	Deep inspection (e.g. cross-tab view)	User quality control back to the original image
	Overlay biological knowledge (e.g. cross-tab view , enrichment files)	User can compare biological interpretation with existing knowledge databases
Computation	Highly automated conversion of images to numbers (e.g. image analysis)	User spends less time with data analysis and more time with science
	Any step by step sequence of computation (e.g. data protocol)	User does not lose the overview
	What you see is what you compute e.g. (editor)	User easily customizes the data analysis
	Connection to a library of computation steps (e.g. PamApps Store)	User gets empowered with new methods
	Tightly integrate with R and Matlab analysis tools (i.e. BioNavigator and R)	User uses existing in-house code

Storage	Upload and Download to a central repository (e.g. Repository)	User easily finds and integrate data and information
	Perform multi PamChip® analysis (e.g. Repository)	User easily integrates many instrument runs quickly from storage
	Flexible interface to retrieve data (e.g. Repository Viewer Step)	User selects only what is required
	Store a wide range of content types in central location (e.g. Repository)	User easily stores documents, data protocols and images
	Visually navigate through the stored content (e.g. PamExplorer , Repository)	User finds data and documents easily
Sharing	Export any data from the workflow (e.g. export formats , Export Step)	User shares any data with others or any external programs
	Tight integration with the PamCloud (e.g. Home Window , PamCloud services)	User enters discussion and sharing quickly
	On-line documentation is available with one click (e.g. PamWiki)	User gets the latest and best documentation there is
	Extensive community support (e.g. PamCloud services)	User asks for help and receives it

2.1 Main concepts in BioNavigator

Editor

The [Editor](#) is part of the [BioNavigator](#) software. Its primary purpose is to modify a [data protocol](#).

Data Protocol

A [data protocol](#) is a connected series of data analysis [steps](#). These data analysis [steps](#) can be run and modified by the [Editor](#) (see section 4.1).

What is a Cube?

A cube is the data structure which is used by data protocol to store its data. Each data step holds a cube and since a data protocol may have multiple data steps there can be many cubes contained in a data protocol. The cube is composed of three dimensions (Spot, Array, Quantitation Type).

Viewing a Cube

The contents of a cube can be viewed using different visualization modes. The default view allows a cube to be viewed as an excel-like sheet where each spreadsheet contains spot measurement as rows and the column represents each image taken of an array. Each sheet represents a different measurement or computation type called a quantitation type.

Transforming a cube

A protocol is composed of data steps and each data step takes an input as a cube ([inCube](#)) and computes an output cube ([outCube](#)). The computation is defined in the data step is defined by a data transformation. Each transformation is defined by a set of groups, filters and operators.

Data provenance (tracking cubes)

Since a data protocol is composed of a chain of data steps and each data steps has an associated input and output cube, it is possible to track back all computation. Therefore if we were to calculate say an average in a data step, it is possible to view how the average was calculated by looking at the input data cube and tracking the source data points. All computed values in data protocol can be traced back in this manner; we term this as data provenance. Data provenance is essential when dealing with scientific applications.

Exporting a cube

Any cube within a data protocol can be exported. This allows for the user to define and determine exactly what output he or she requires.

3 DATA PROTOCOL








A **data protocol** is a work flow of [data protocol steps](#). It may or may not contain data. A **data protocol** file contains the definition of the **data protocol**. It is stored as a [file](#) with an extension *.bn6. A **data protocol** is represented as a simple hierarchical tree structure. The top of the tree represents the beginning of the work flow and the branches the successive data manipulation steps. There are no limits to the number of trees a **data protocol** can contain. The nodes of the tree are called [data steps](#) and are used to perform the processing on the data. The links of connecting the nodes is the data and is termed a [data cube](#).

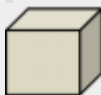













4 BIONAVIGATOR EDITOR

The editor module of BioNavigator allows for users to modify and run data protocols. Before a data protocol can be edited or run you require launching the editor, this will launch a main editor window.

4.1 Description of protocol steps

Short descriptions of the different protocol steps are given in the overview table below.

Name of Step	Icon	Description
Text Step		Allows capture of text.
File Step		Allows the selection of a file.
Tab Step		Allows the selection of a tab delimited file. e.g. Selecting the array layout file for the Image Analysis Step .
Folder Step		Allows the selection of a folder. e.g. Selecting all images.
Image Overview Step		Allows the browsing of images .
Image Analysis Step		Performs image analysis .
R Data Step		Performs a computation using a R script. Uses the same principles as the Data Step .

Data Step		Performs a data computation.
Export Step		Allows for the export of data.
Repository Import Step		Allows for the import of data.
Repository Viewer Step		Allows for the browsing of data in the repository.
R Step		Performs a computation using a R script. e.g. Import of non PamStation data is performed using a R script.
Import Images Step		Allows for images to be embedded in the protocol in order.
Union Step		Allows for two data cubes to be joined.
Merge Step		Allows for two data cubes to be merged. Used with the Union Step .
Data Enrichment Step		Allows for additional annotation to be imported onto the data.
Sub Protocol Step		Allows for sub protocols to be created within the protocol.
Input Step		Allows incoming data to be visible to the sub protocol
Output Step		Allows outgoing data from a sub protocol to be visible to the protocol
Matlab Step		Performs computation using Matlab. Uses similar structure as the Data Step
Wizard Step		Allows for the creation of wizards. Wizards are one manner in which sub protocols are used.

[Data View Step](#)

Allows for the definition of a visualization within a [sub protocols](#).

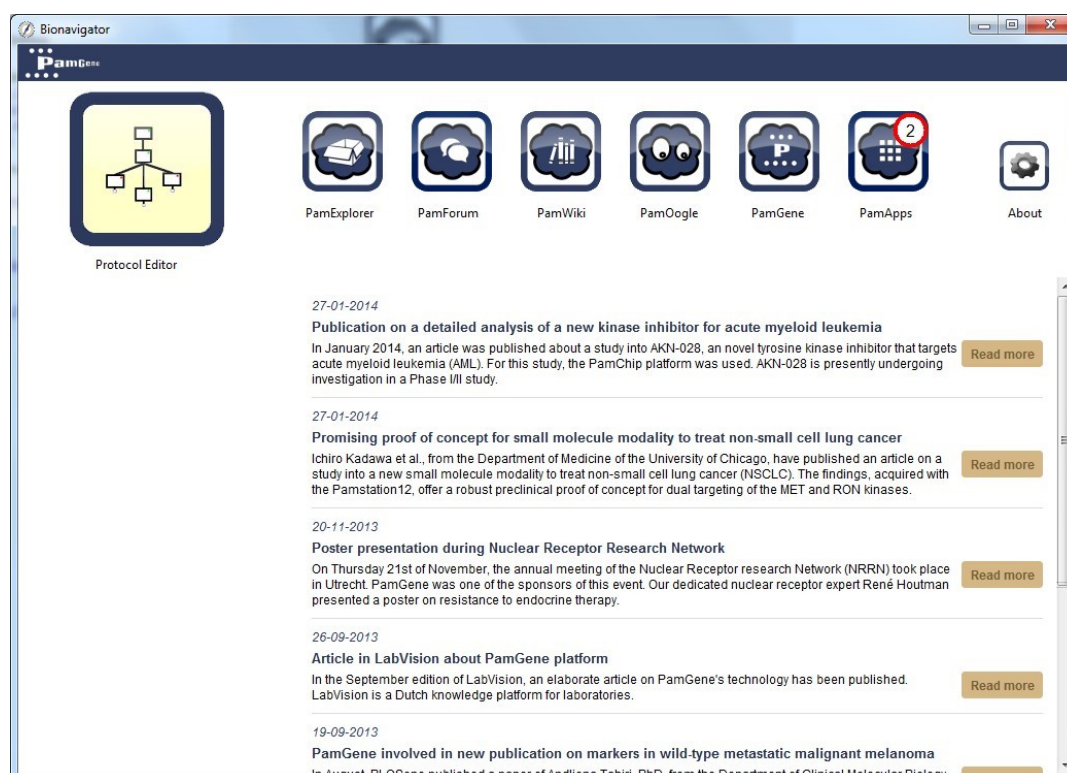
4.2 Launching BioNavigator Editor

To launch the editor you require to double click the BioNavigator Icon:



Figure 1: BioNavigator6 icon

The main home window will open:



Now double click on the Protocol Editor. The main editor window will open now (see below).

4.3 Editor Main Window

The initial editor window looks like this:

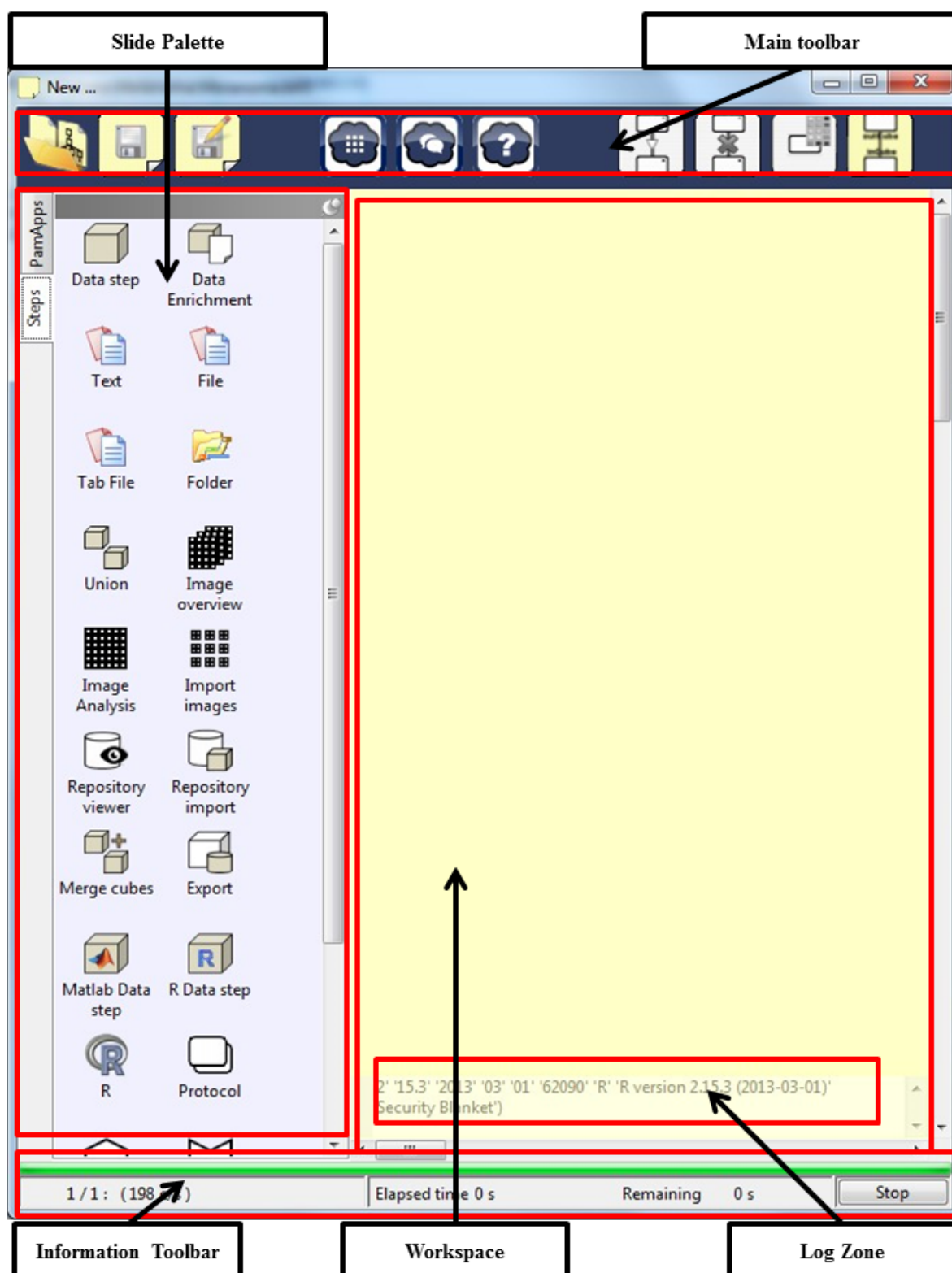


Figure 2: The Editor Main window








The **Editor Main Window** of [BioNavigator](#) allows for users to edit and run [data protocols](#). Before a [data protocol](#) can be edited or run you require to click on [Editor Icon](#) in the [Home Window](#), this will launch a [Editor Main Window](#).




The **Editor Main Window** consists of the following parts:

Editor Main Toolbar	(for opening and saving data protocol , quick links to the PamCloud services , creating data protocol steps)
Step Palette	(for choosing protocol steps)
PamApp Palette	(for choosing PamApp steps)
Editor Workspace	(for creating a data protocol)
Log Zone	(for logging of actions)
Information Bar	(for logging elapsed time, remaining time and file size)

4.4 Main Editor Toolbar

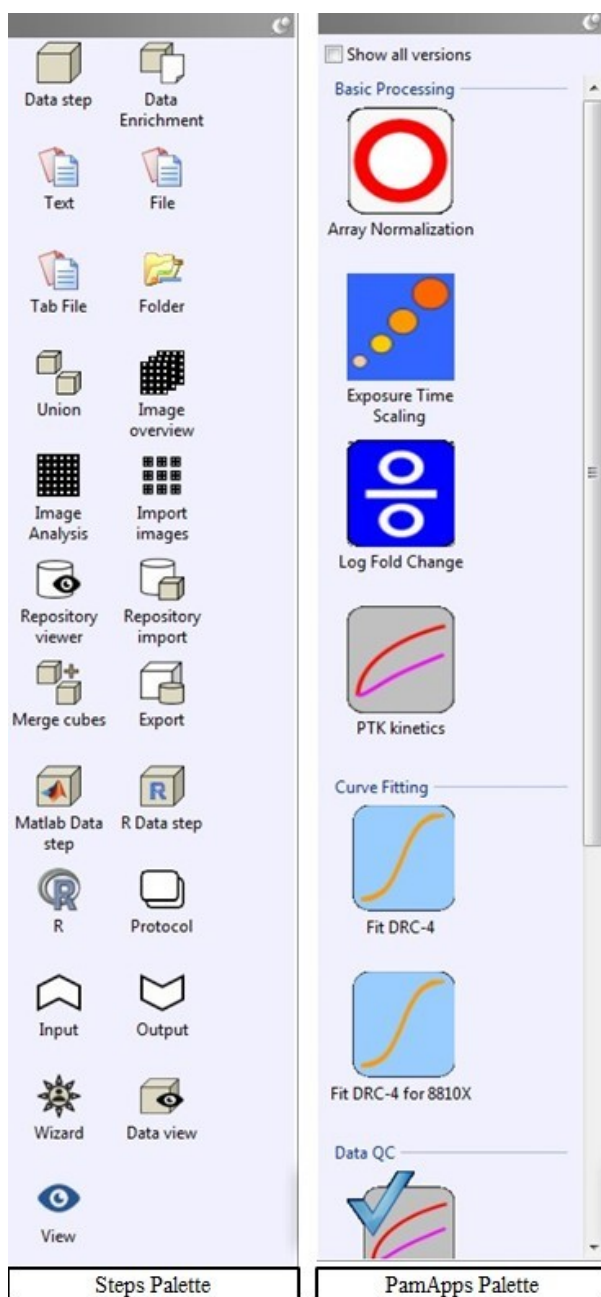
The **Editor Toolbar** allows the user to click on the buttons to perform actions described below, it is part of the [Editor Main Window](#):

Button Name	Icon	Description
OpenNew Button		Open new and existing data protocols
Save Button		Save the data protocol
Save Document As Button		Save and rename the data protocol
PamApps Button		Brings you to the PamApp Store
Feedback Button		Brings you to a feedback page on the PamCloud
Help Button		Brings you to a help page on the PamCloud
Link Button		Create a link between two data steps

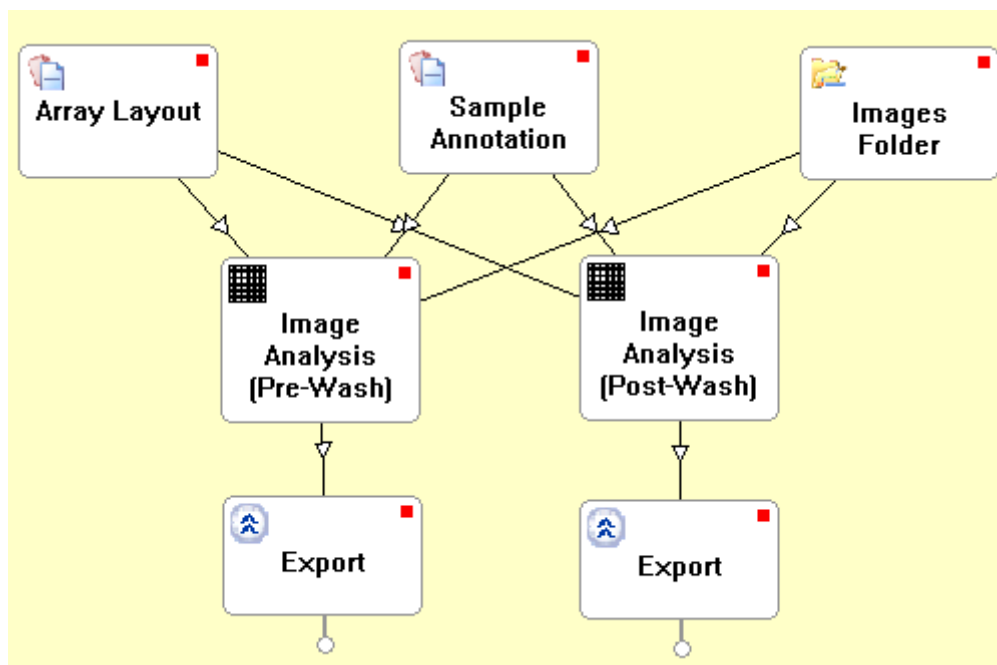
Remove Button		Deletes a step or link between two steps
Color Button		Colors the boundary line of a step
Show simplified Button		Show/Toggles extra information of the steps i.e. ports

4.5 Slide Palette

On the left side of the protocol editor screen, a palette of symbols is presented. These symbols represent the different steps of the protocol. These steps can be added by dragging them to the workspace and so form the desired protocol. These steps are known as data protocol steps if they originate from the step palette and PamApp steps if they originate from the PamApps palette.



4.6 Editor Workspace



The **Editor Workspace** can be recognized by the yellow background. The [data protocol steps](#) can be dragged to the **Editor Workspace** from the slide palette. The linking between the [data protocol steps](#) can be made with the [Editor Main Toolbar](#).

4.7 Editor Information Bar

Information Bar allows users to estimate the rate and time of completion of a step, it is composed of two parts, the **Speed Information Zone** and **Time Information Zone** (see below). In addition the user may interrupt the [step](#) using the [Stop Button](#).

Speed Information Zone allows users to see the rate of processing in terms of elements/second. An element can be any item in the list it is processing (e.g. [Images](#), [Cubes](#)). **Time Information Zone** allows users to estimate the time of completion of a [step](#). It is given as [Elapsed time](#) and [Remaining time](#). [Remaining time](#) is a naive estimation based on the elapsed time. The **Information Bar** is a part of the [Editor Main Window](#).



Figure 3: Editor Information Bar

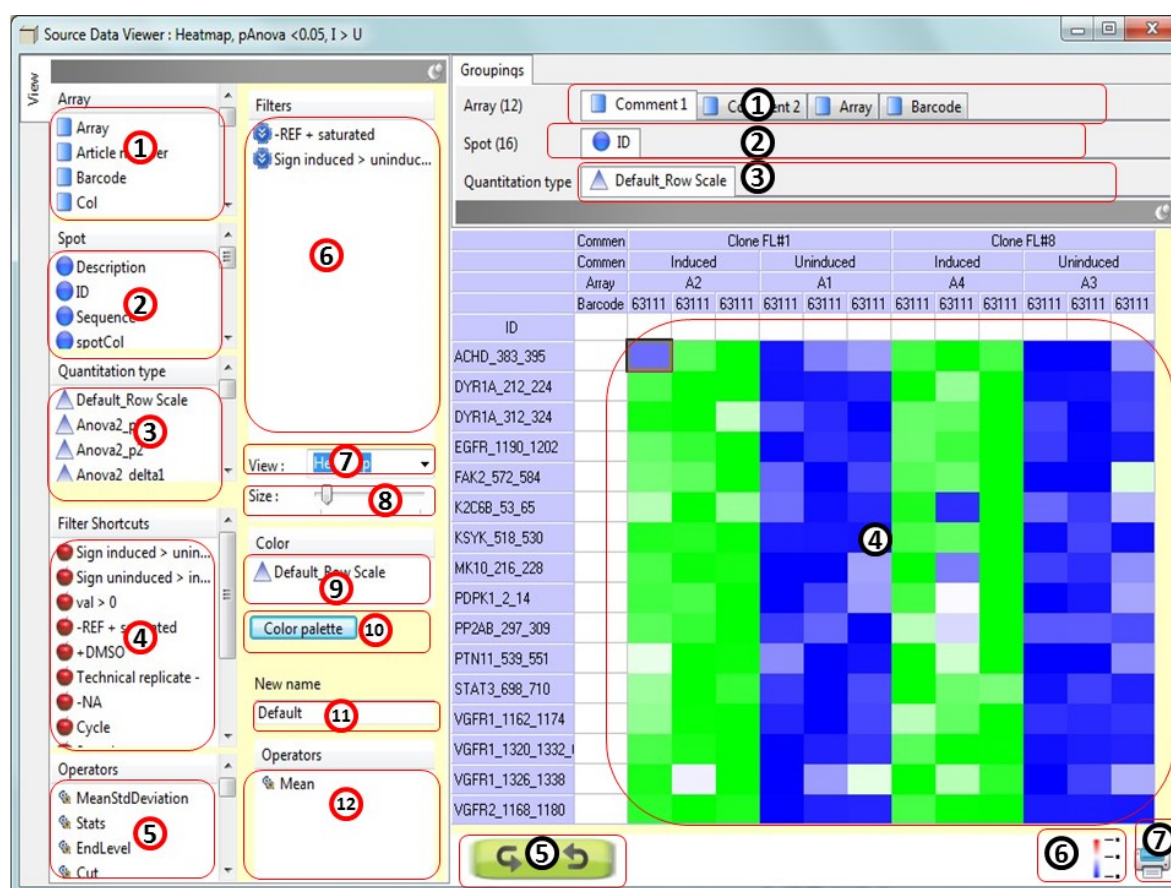
4.8 Editor Log Zone

The editor log zone gives a survey of actions for BioNavigator.

```
4:19:13 PM : BioNavigator Editor started.
4:19:13 PM : #('i386-pc-mingw32' 'i386' 'mingw32' 'i386, mingw32' " '2' '6.2' '2008' '02' '08' '44383' 'R' 'R version 2.6.2 (2008-02-08)')
4:20:30 PM : Loading protocol : C:\Documents and Settings\data1\Desktop\gro-erik3b.pdp
```

4.9 Cross tab window

The **Cross-tab Window** is the main visualization window in BioNavigator. It is generic and appears when the user double clicks on a [link](#), or a [step](#). The **Cross-tab Window** may be configured to visualize a wide range of [views](#). It is composed of two main panels, **Left Panel** which can slide in/out and the **Main panel** where the data is presented.



Left Panel

1	Array Box	Available existing Array factors user selects from
2	Spot Box	Available existing Spot factors user selects from
3	Quantitation Type Box	Available existing Quantitation types user selects from

④	Filter Shortcut Box	Available existing filters user selects from
⑤	Operator Box	Available existing operators user selects from
⑥	Filters Box	Active filters select to filter the Data Grid
⑦	View Type Menu	Active View Type selection for the Data Grid
⑧	Size Slide Bar	Active Size of View Type
⑨	Color Box	Active coloring parameter to be applied to the Data Grid
⑩	Color Palette Button	Active color palette to be applied to the Data Grid
⑪	New Name Box	Definition of the new name computed by the operators on the Data Cell of the Data Grid .
⑫	Selected Operators Box	Active operator to be applied to the Data Cell of the Data Grid .

Main Panel#

①	Array Bar	Active Array grouping user defined for the Data Column
②	Spot Bar	Active Spot grouping user defined for the Data Row
③	Quantitation Type Bar	Active Quantitation type value presented in the Data Cell .
④	Data Grid	Data values are presented
⑤	Refresh Button	Refreshes the current view of the Data Grid
⑥	Legend Button	Opens the Legend Window
⑦	Print Button	Prints the view to the Printer.

5 GLOSSARY

5.1 Array Layout File

Array layout file is a text file defining the content (i.e peptides) of the [Array](#). The name (i.e. [ID](#)) of the peptides, the position of peptides (i.e. [Row](#), [Col](#)), and amino acid sequence (i.e. [Sequence](#)).

The **Array layout file** is usually located in a [Evolve results folder](#). The **Array layout file** is delivered by PamGene for each of the [PamChip](#) types, and is based on the [article number](#).

5.2 Barcode

A numeric identifier(9 digits), usually found on the PamChip itself.

PamChip Type	Example
PamChip 4	001234456
PamChip 96	002589687

There is a structure to the **Barcode** name, it is composed of the following parts (see table below).

Example: 631003101

Name type	digits length	Example
Article number	3 digits	631
Batch number	3 digits	003
Carrier number	1 digits	1
Chip number	2 digits	01

5.3 Data cube

A **cube** is the data structure which is used by [data protocol](#) and [Repository](#) to store and represent data. Each [step](#) represents a cube and since a [data protocol](#) may have multiple [steps](#) there can be many **cubes** contained in a data protocol. The **cube** is composed of three dimensions ([Spot dimension](#), [Array dimension](#), [Quantitation type dimension](#)).

5.4 Data protocol

A **data protocol** is a work flow of [data protocol steps](#). It may or may not contain data. A **data protocol** file contains the definition of the **data protocol**. It is stored as a [file](#) with an extension *.bn6. A **data protocol** is represented as a simple hierarchical tree structure. The top of the tree represents the beginning of the work flow and the branches the successive data manipulation steps. There are no limits to the number of trees a **data protocol** can contain. The nodes of the tree are called [data steps](#) and are used to perform the computational processing on the data. The links of connecting the nodes is the data and is termed a [data cube](#).

6 BIONAVIGATOR 6 FAQ

There is an extensive range of FAQs on the PamCloud. Please refer to this resource as it is continually updated. All links below are clickable, be sure to have an internet connection and your pamcloud login and password to view the answers to the FAQ questions.

6.1 [BioNavigator 6 FAQ](#)

- Introduction
 - [What are the resources available to learn BioNavigator](#)
 - [Can BioNavigator run on a Mac?](#)
 - [Can I put BioNavigator on my laptop?](#)
 - [Is BioNavigator included with a PamStation?](#)
- General
 - [Why can I not change the view of Step?](#)
 - [How can I avoid having to reset a run step in order to modify the view of the step in question?](#)
 - [What are the laptop or desktop requirements for BioNavigator?](#)
 - [What are the PamCloud services available in BioNavigator 6.0?](#)
 - [How do I get automatic updates of the PamCloud content?](#)
 - [What is an enrichment file?](#)
 - [How to merge two cubes?](#)
 - [How do I get error bars shown with my data?](#)
 - [How do I change colors?](#)
 - [How do I export a peptide list with values?](#)
- Image processing
 - [Why do some of my grids for my spot seem out of position?](#)
 - [How can I correct my grid\(s\)?](#)
 - [How do I combine multiple experiments?](#)
- Using cross-tab view
 - [How to set the y axis in the cross-tab view?](#)
 - [How to move a column in the cross-tab view?](#)
 - [Why do some of my data points disappear when I add a QT filter?](#)
 - [How do I color my volcano plot with a specific peptide list?](#)
- Making and customizing protocols

- [How do you make sure on of the order of the operands when doing a subtraction or divide operator?](#)
 - [How do you know which order the operands will be used in a cell?](#)
 - [Are there BioNavigator demonstration protocols?](#)
 - Filters
 - [How do I create a filter?](#)
 - [How do I save a filter?](#)
 - [How do I load a filter?](#)
 - [View Templates](#)
 - [How do I create a view template?](#)
 - [How do I save a view template?](#)
 - [How do I load a view template?](#)
 - Protocol Templates
 - [How do I create a protocol template?](#)
 - [How do I save a protocol template?](#)
 - [How do I load a protocol template?](#)
- Importing
 - [How do I import my own data into BioNavigator?](#)
- Exporting
 - [How do I save a picture from BioNavigator?](#)
 - [How to export a graph from a single cell in the Cross Tab view?](#)
- Enrichments
 - [How to perform sample enrichment?](#)
 - [How to perform peptide enrichment?](#)
 - [How to perform peptide enrichment\(1 to N\)?](#)
 - [How do I build an enrichment file from data?](#)
 - [How do I import the enrichment file into my protocol?](#)
 - [Where do I connect a Data Enrichment Step?](#)
 - [Why I am missing quantitation types after a Data Enrichment Step?](#)
- Conversion
 - [How to convert a factor value to a quantitation type?](#)
- Repository Lite
 - [How to start the repository server?](#)
 - [How to stop the repository server?](#)
 - [How to delete entries \(i.e cubes\) in the repository?](#)
 - [How to check the indexing engine \(i.e solr\)?](#)
 - [How to open microsoft services window?](#)

- [PamExplorer](#)
 - [Do I need BioNavigator to view a file or folder on the PamCloud Repository?](#)
- Support
 - [How to find out what BioNavigator version name you have?](#)
 - [How to check your R project setup for BioNavigator?](#)
 - [What internet connection does BioNavigator6 require?](#)
- [PamApps](#)
 - [How can I build a PamApp?](#)
 - [How to make a PamApp out of your data protocol or sub protocol?](#)
 - [How do I install a PamApp?](#)

6.2 [BioNavigator and R](#)

- [What R packages are necessary to run BioNavigator?](#)
- [What version of R should I use with BioNavigator?](#)
- [How can I update to a newer version of a RStepOperator](#)
- [How to install R version 2.15.3](#)
- [How to install the snow package](#)
- [How to modify the PATH variable for R](#)
- [What R packages are necessary to run BioNavigator](#)
- [What version of R should I use with BioNavigator](#)
- [How can I update to a newer version of a RStepOperator](#)
- How can I [configure R for use with Bionavigator](#)

6.3 [Data Analysis](#)

- Image Analysis
 - [How is background calculated?](#)
 - [How is the median SigmBg calculated?](#)
 - [Why do I get negative values in my SigmBg?](#)
 - [What does the Signal_pValue represent?](#)
 - [What is an article number?](#)
 - [How do I remove NOK \(Visual QC\) from the data analysis?](#)
 - [How do I correct for ATP dependent peptides?](#)
 - [How do I solve gridding issues?](#)
- Pathway Analysis
 - [In cases when few pathways are involved, can the software predict the pathways with high certainty?](#)



- [How do I upload data to MetaCore from BioNavigator?](#)
- Viewing
 - [What is the best view for viewing images with spot information?](#)
 - [Can I export my views](#)

6.4 [PamExplorer](#)

- [How to upload data into the PamCloud?](#)

7 SUPPORT

PamGene aims to provide total support to enable customers to fully realize the benefits of the PamGene technology and to maximize use of the PamStation®12 system.

The software components and data handling are essential interfaces to the system and are supported by a dedicated software support team through email, telephone and on site visits, if required.

Please contact support on:

+31(0) 73 615 8900

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8 RIGHTS AND RESTRICTIONS

8.1 Intellectual property rights

The use of The PamChip® microarrays is licensed among others under patent and patent applications issued to PamGene B.V.

Any patents, patent applications and other proprietary rights named in this manual are included for reference purposes only. No licenses to use the processes or products covered by such patents, patent applications or proprietary rights are granted by or implied by PamGene International B.V. It is the responsibility of the user to determine whether a license is required and obtained.

8.2 Trademarks

PamStation®12 and PamChip® are trademarks of PamGene BV, registered in one or more countries.

8.3 Disclaimer

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Although this manual has been carefully prepared with every precaution to ensure accuracy, **PamGene International B.V.** can assume no liability for any errors or omissions, or for any direct or indirect damages resulting from application of this information.

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