Batch Coordinator V 1.0 User Manual



1 Introduction

Batch Coordinator is an easy to use software designed specially for effective image processing and analyses. It simplifies and automates image processing and analysis by executing the specified and customized commands on each image within a multiple image data set.

By applying the specific command sequence to large numbers of images, Batch Coordinator allows benefits of:

- Significantly reducing multiple image analysis and processing time
- Increasing reproducibility of repetitive procedures by decreasing risk of inconsistency
- Facilitating comparison and evaluation of multiple data sets by standardized output
- Improving and optimizing overall system utilizations by reducing resource consumption
- Increasing system flexibility and reusability by decoupling the software components

1.1 Preface

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1.2 Product Overview

Batch Coordinator collects commands, that you might ordinarily issue in Imaris, into a single batch job and execute them together, rather than entering each command individually and processing each image separately.

Batch Coordinator ensures that image analysis and processing is carried out in exactly the same way, every time for every single image in a large data set.

The Batch job has four stages:

- 1. To start Batch processing a command sequence is created in Imaris with adjusted parameter values. By default, Batch commands will be launched in Batch Coordinator automatically each time they are submitted using Imaris.
- 2. Selecting images for processing and analysis using Batch Coordinator. Images are placed in an input file list and added to the Processing Queue. Output file names and directory were automatically generated and placed in the output file list.
- 3. Running Batch Coordinator, executing the complete command sequence on selected input files and collecting results in the output file list.
- 4. Reviewing the results either in Imaris or in Excel.

Batch Coordinator Features

The Batch Coordinator has following features:

- · Customize and standardize command sequence performed on each input image within a large data set.
- Generate automatically output file directory and names for each Batch job
- Pause, resume, cancel, and obtain the status for any submitted batch and/or any individual job
- Manage batch job by add or delete commands and fine-tuning parameters values
- Launch externals applications to inspect input / output data files (i.e. Imaris, or Excel)
- Runs both on Mac and Windows.

Command Sequence

A command sequence can include following commands:

- Flip, rotate, invert, convert, crop Data Set, etc.
- Crop, resample, add / delete, swap channels and time points etc.
- Change the data type, voxel size and time stamps
- Apply a Gaussian or Median filter
- Threshold Adjustment by Baseline and Background Subtraction, Threshold Cut-off, Connective Baseline
- Contrast change by Linear Stretch, Gamma Correction, Normalize Intensity
- Display Adjustment
- Spots and Surfaces analysis with Detection, Tracking and Statistics

1.3 About this Manual

This manual describes all the features of Batch Coordinator. This User Manual provides a description of all menu entries, display modes, functions and parameters. The manual is organized into 14 Chapters.

Chapter 1. Provides an <u>introduction</u> to the Batch Coordinator. It describes the function and demonstrates basic capabilities and features of the Batch Coordinator.

Chapter 2. Describes installation procedure, system requirements and licensing options.

Chapter 3. Display Main screen and gives an overall look of the major areas and toolbars.

Chapter 4. Provides details of Batch job creation. It consider the principles in Command sequence design, with an explanation and benefits of <u>creating Command sequence using Imaris</u>. An <u>example</u> with screenshots, describing a procedure of creating Command sequence and executing a Batch job, is also included in this chapter.

Chapter 5. Describes <u>Menu File</u>. Examines techniques for saving and implementing Command sequences and considers options allowing the submission and the execution of a Batch job on different machines.

Chapter 6 Contain information about Menu Edit, and describes creation/deletion of Batch and addition/removal of batch Files.

Chapter 7. Gives an overall look of Menu Control and explain how a Batch job can be executed.

Chapter 8. Describes Menu Help and presents commands that display documentation or other information about the Batch Coordinator.

Chapter 9. Describes in details **Batch Area** and summarize Batch jobs specific elements.

Chapter 10. Discuss frequently used commands presented in **Batch Control Toolbars**.

Chapter 11. Describes <u>Command/Parameter area</u>. Defines all <u>commands</u> recognized by Batch Coordinator. It provides guidance for parameter <u>values</u> modifications and emphasizes importance of incorporating the correct parameter values.

Chapter 12. Discusses control shortcuts of <u>Command/Parameter Toolbars</u> used for managing commands in Command Parameter Area.

Chapter 13 Provides an explanations of unique features of commands **Spots and Surfaces**.

Chapter 14. Describes the output <u>Statistic</u> file. It provides a screenshot example of managing statistical files after executing Batch job with commands Spots and Surfaces.

2 Getting Started

This chapter describes:

Installation

Licence

Starting Batch Coordinator

2.1 Installation

The software is delivered on a standard CD or downloaded from www.bitplane.com. The CD includes a folder containing the necessary manuals, or the manuals can be downloaded.

Minimum hardware/software requirements are:

- Windows XP, Vista or higher
- Mac OS x10.40 or higher
- CD-ROM
- · Network facilities for image import from the microscope
- 512 MB RAM (> 1 GB recommended)

Bitplane also recommends:

• A database for storing images (e.g., Image Access)

Installation

To install the software, please proceed as follows:

- Insert your Batch Coordinator CD-Rom in the computer.
- Follow the instructions on the screen.
- The installation is completed automatically.

2.2 Licensing Options

Batch Coordinator is designed as a high-throughput high-availability software that targets both individuals as well as large institutes and corporate. It provides the confidence of high-assurance trustworthy systems, in addition to the benefit of low-cost software support and maintenance, plus guaranteed long-term support through participation from a booming Imaris community. The Imaris community benefits from frequent updates, better portability to different computer systems and the ability to directly contribute through software enhancements and improvements.

Stand Alone

This licensing option allows Batch Coordinator to be placed on Mac or Windows computer system. Imaris does not have to be present and this version of Batch Coordinator does not use or depend on the licenses of Imaris. Stand alone Batch Coordinator is available in the following configurations:

- Stand-Alone up to 2 parallel jobs
- Stand-Alone up to 4 parallel jobs
- Stand-Alone up to 8 parallel jobs
- Stand-Alone up to 16 parallel jobs

- Stand-Alone up to 32 parallel jobs

Imaris Dependent

This licensing option allows Imaris batch to be placed on Mac or Windows computer system, but utilizes licenses of Imaris and its modules as part of its operation. For example, if you want to perform batch job that require tracking and the output of the statistics from those tracks, this version of Batch Coordinator would use the Imaris, Imaris MeasurementPro, and ImarisTrack licenses. The advantage of this option is that it is lower cost than the stand alone version. The Imaris dependent version of Batch Coordinator comes in two configurations:

- Imaris dependent up to 2 parallel jobs
- Imaris dependent up to 4 parallel jobs

2.3 Starting Batch Coordinator

Starting Batch Coordinator

It is advisable to start the Batch Coordinator either before or concurrently with the Imaris.

To open Batch Coordinator double-click on the Batch Coordinator icon.

The software opens with the main screen.

Supported File Formats

Batch Coordinator can read the following file formats, i.e. it can read the image and the parameters.

- Andor: Multi-TIFF series (*.tif, *.tiff)
- Applied Precision, Inc: DeltaVision (*.i3d, *.dv)
- Biorad: MRC-600, MRC-1024 (*.pic)
- BioVision: IPLab Mac (*.ipm)
- Bitplane: Imaris 5.5 (*.ims)
- Bitplane: Imaris 3.0 (*.ims)
- Bitplane: Imaris 2.7 Classic/Old (*.ims)
- Bitplane: Imaris Scene File (*.imx)
- Carl Zeiss: LSM 510 (*.lsm)
- Carl Zeiss: LSM 410, LSM 310 (*.tif, *.tiff)
- Carl Zeiss: Axiovision (*.zvi)
- Image Cytometry Standard: ICS used by Nikon, Huygens, and others (*.ics, *.ids)
- Leica: TCS-NT (*.tif, *.tiff)
- Leica: LCS (*.lei, *.raw, *.tif, *.tiff)
- Leica: series (*.inf, *.info, *.tif, *.tiff)
- Leica: Image File Format (*.lif)
- Molecular Devices: Metamorph STK (series) (*.stk)
- MRC primarily electron density volumes as in cryo-EM (*.mrc, *.st, *.rec)
- Olympus: FluoView TIFF (*.tif, *.tiff)
- Olympus: FluoView 1000 OIF (*.oif)
- Olympus: FluoView 1000 OIB (*.oib)
- Olympus: Cell^R 1.1/standard (*.tif, *.tiff)
- Open Microscopy Environment XML (*.ome)
- Open Microscopy Environment TIF (*.tif, *.tiff)
- Perkin Elmer: UltraView (*.tim, *.zpo)
- Scanalytics: IPLab (*.ipl)
- TILL Photonics: TILLvisION (*.rbinf)

Plus it can read general TIFF series (or BMP series).

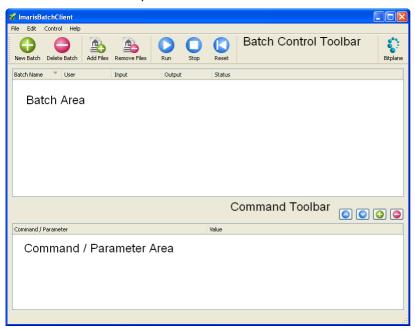
3 Main Screen

The main screen in Batch Coordinator provides the facility to build, submit and process batch jobs. It has two main areas and two Toolbar buttons sections:

- Batch Area
- Command Parameter Area
- Batch Control Toolbar
- Command Toolbar

From this screen all of the batch component can be accessed, edit and managed. The menu bar at the top of the window contains the entries: File, Edit, Control and Help.

Each Main screen component is documented in more detail in the following sections.



Batch area

The central area of the screen is Batch area. Batch area provides detailed specification of Batch. It contains all the information of Batch: Batch name, with input and output files names, user name and Batch status information.

Batch Control Toolbar

Batch Control Toolbar contains shortcut buttons named New Batch, Delete Batch, Add Files, Remove Files, Run, Stop, Reset. These shortcuts represent the core tools available to the user. The same action can be accessed via the Menu File, Edit or Control.

Command-Parameter Area

For selected Batch, in the Command -Parameter area all Commands Sequence and commands parameters with specific Values are displayed.

Command Toolbar

The Controls toolbars let you add or delete commands or edit command sequence arrangement by moving selected commands up and down.

4 Batch Creation

Batch consist of **Command Sequence** applied to selected **input files** as a singe command unit, and the results **output files**, collected in a newly created image file or statistical files. Command sequence contain the commands with the specified parameters values. A command describes the operation you want execute on an input dataset and specifications that a command uses to perform its action are called parameters. Batch Coordinator offers a variety of available commands for Batch processing, some most frequently used ones for image processing (Gaussian filter, Threshold adjustment) and some more complex like Surface and Spot detection.

A Command sequence can be created either using Imaris or directly in Batch Coordinator.

4.1 Creating a Batch Command Sequence in Imaris

From Imaris commands with Add to Batch button Add to Batch button and be submitted for Batch processing. Imaris command that can be submitted to Batch are in Menu Image Processing and Menu Edit. For Commands Surfaces and Spots, Creation Wizard must be used for selecting and adjusting parameters and for submitting Tab Creation .

Using following procedure, Command sequence is set up in Imaris and submitted to Batch Coordinator.

- 1. Open an image in Imaris.
- 2. Select the first command that will be applied to all images.
- 3. Adjust commands parameters either in Command's window or throughout the Creation Wizard for Spots and Surfaces. The effect of the modification and parameter adjusting can be seen instantly in the image as changes are applied.
- 4. Submit the adjusted command to Batch coordinator by click on **Add to Batch button** Add to batch.
 - Add to batch button is available at the bottom of the command window.
- For Spots and Surface select Tab Creation and **Add to batch** button is available at the bottom of the Tab **Creation** $\frac{1}{4}$ panel.
- 5. Repeat process for series of command that you would like to be execute in Batch Coordinator.

Note: Bear in mind that commands are added in Command Sequence into the Batch Coordinator by the order they are entered in Imaris. For example, if command Flip and Rotate commands were add to the batch, the Flip action will be executed first and then the Rotate. So before progressing to the next step make sure the batch commands are ordered properly and all parameters are confirmed.

- 6. The last step is selecting command **Save as** adding the command **Save** in Batch command sequence.
- 7. Open Batch Coordinator.
- In Batch area a New Batch will be created with automatically created <u>User name</u> and <u>Batch Name</u>. A Batch

Name always match the name of the first submitted Imaris command (default option). In command/parameter area the Command Sequence will contain the list of Imaris selected commands following command Open. The last command in the Command Sequence should be command Save.

4.2 Creating a Batch Command Sequence in Batch Coordinator

Batch job can be created alternatively in Batch coordinator, rather than selecting commands from Imaris. Select command New Batch from Menu File or press New Batch button and default command sequence is created. In command/parameter window command sequence will contain following commands:

Open Save

To modify the commands of New Batch double click on the command line. An arrow appears indicating a drop down window. From a list of commands select a new command. The command sequence is changed in accordance with a selection.

Note: In contrast to the command sequence creation by Imaris, creating a command sequence directly by Batch Coordinator sets parameters at default values. As a result, parameters will not be adjusted and optimized to fit the Batch process and analysis specific requirements. Therefore, it is highly advisable to create Batch job with command sequence and adjusted parameters using Imaris. Please refer to the Chapter <u>Values</u> for additional reference.

4.3 Batch job

To complete Batch job input files must be added by clicking on the button <u>Add Input files</u>. All selected files will be added to the <u>Input file list</u> and the Output files will be generated automatically and added to the <u>Output file list</u>.

To initialize a Batch select command <u>Run</u>. The Command sequence will be executed on the all input files. The resulting image and statistical files will be collected and saved in the output file list.

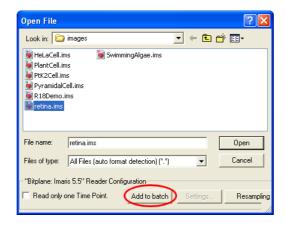
4.4 Example

In this example the compete procedure for setting and executing Batch will be presented. You will start with creating the Command sequence containing commands Rotate and Gaussian Filter with adjusted parameters in Imaris. Than selection of Input files and the output file list creation will be described, followed by description of starting a Batch job. The last part will illustrate how the batch resulting files can be reviewed.

Command Sequence Creation

Open Imaris. In Menu File select command Open. Select image Retina. Press Add to Batch button and than open the image Retina.

A Retina image is displayed in Imaris Main screen.



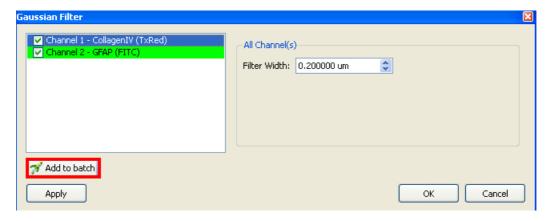
Open Menu Image Processing and select command Rotate.

A Rotate Channels window will appear. For Rotation select all channels along Z axis in Counter Clockwise orientation.

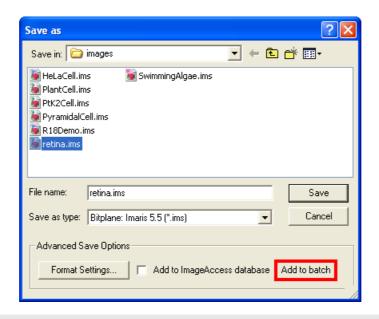
Click on Add to Batch button Add to batch submits the command into Batch Coordinator.



Open Menu Image Processing and select command Gaussian filter. In Gaussian filter window select both channels and enter 0.2um as a Filter Width value. Click on the Add to Batch button submits the command into Batch Coordinator.



The last step in creating the Command Sequence in Imaris is selecting a commands Save as. Click on the Add to batch button and command Save added the command Sequence in Batch Coordinator.



Batch Coordinator

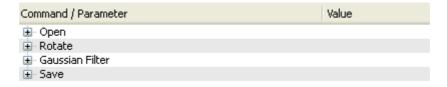


Open a Batch Coordinator

A New Batch with User Name and Batch Name-Rotate is automatically created and displayed in Batch area.



In Command Parameter area the Command Sequence is displayed. The command order is the same as commands were selected and submitted in Imaris.



To demonstrate the principle of submitting the parameter values from Imaris to Batch coordinator click on the (+) symbol next to the command **Gaussian filter**. On expanded command line all parameter values are available. The value for parameter **Sigma** is set to be 0.2 um as set by Imaris for **Filter Width** in Gaussian filter window. Value for Parameters Channel is on on as filter be applied to both channels.



Input files

The next step in is selecting all files that will be processed with created Command sequence. Click on the

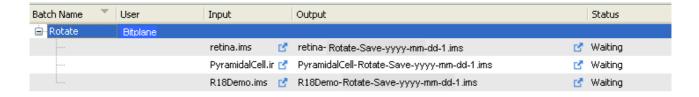
Add files Button and Choose input files winnow appears.



Select files for Batch execution and click on the Open. All selected files will be added to the Input list in Batch area.

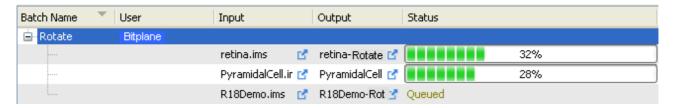
Output Files

The Output files names and directory will be generated automatically and added to the Output file list. All output files will be placed in the same directory as the input file, in the **new folder**, called **batch output**. The output files names are created in accordance with the rule of output filename.

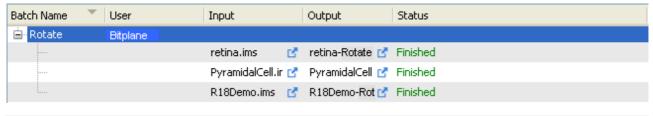


Starting Batch Job

To start a Batch job select click on the icon Run . By Running Batch Coordinator, the complete command sequence is executing on all input files and Batch processing results are collected and stored in the output file list. In status line massage Queued for all file waiting to be executed.

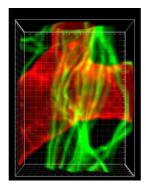


Completed batch job are marked Finished and remain in the batch display.

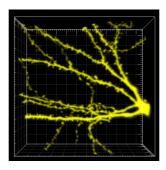


Reviewing results

To verify results click on the icon displayed. In a Retina output file. Imaris as externals applications will be launched processed Retina will be displayed.



In addition to opening output Batch files directly in Batch Coordinator, resulting files can be open in Imaris by selecting and opening Batch file from the file list. Open PyramidallCel-BATCH-year-month-day.ims and the Pyramidal cell image is displayed in the viewing area after the batch processing.



5 Menu File

The Menu File has the following commands and shortcuts:

(Ctrl+N or Cmd+N) **New Batch** New Batch from command Sequence (Ctrl+O or Cmd+O) (Ctrl+S or Cmd+S) Save command sequence

Import Batch Export Batch

Exit (Ctrl+ Q or Cmd+Q)

5.1 **New Batch**

Open the File Menu and then click on the New Batch. Batch structure will be created in Batch area with automatically created Batch Name- and user name. In command/parameter window command sequence will contain following commands:

Open Save.



The same action can be achieved with selecting New Batch New Batch button in the Toolbar

Note: To initialize a Batch job input-files must selected and added.

5.2 **New Batch from Command Sequence**

To set up a new Batch on an existing Command Sequence select New Batch from command Sequence in Menu File. The window Open command sequence will appear.

Select a command sequence from the list and click **Open** or double-click on the requested file entry.

Creates a new Batch structure in Batch area based on command Sequence already saved.

Please refer to the Chapter Commands for more information about organization and modification of Commands Sequence.

5.3 Save Command Sequence

Save command sequence saves a current command sequence with set of parameter values. It provides the advantage of a faster creating new Batch and the possibility of reusing standardized and complex Commands Sequences, therefore saving time and increasing reproducibility of data processing.

Select option in File Menu Save command sequence. This will open the Save command sequence window.

Select the directory and enter the name for the command sequence to be saved or confirm the suggestion. Click Save.

Command sequence is saved and available to be later used in creating New Batch process.

The default command sequence extension is *. bcx.

5.4 Import Batch

This option allow to rerun the same **Batch** or to import data from outside your Batch Coordinator system. Select Import Batch in Menu File. An import Batch window will appear. Click Open or double-click on the select Batch from the list.

A new Batch job is containing command sequence with the input and output file list added to Batch area. The default Batch file extension for is *bjx.

5.5 Export Batch

Export Batch allows Batch to be saved and letter executed, exchanged with another Batch user or mailed for a question for the support team.

Select the Batch you would like to **Export**. Select **Export Batch** in Menu **File** and the Export Batch window will be open.

Select the directory and enter the name for the Batch to be exported or confirm the suggestion and then click **Save**.

The default Batch file extension for is *bjx.

5.6 Exit

Terminates Batch Coordinator.

Note: Batch Coordinator saves automatically current setting (Command Sequences and files) even after exiting the program.

6 Menu Edit

The Menu **Edit** has the following commands and shortcuts:

(Ctrl+N or Cmd+N) **New Batch**

Delete Batch

Add Files... (Ctrl+M or Cmd+M)

Remove Files

Select All (Ctrl+A or Cmd+A)

Select None **Preferences**

(Ctrl+P or Cmd+P)

6.1 **New Batch**

Open the Edit Menu and then click on the New Batch. Batch structure will be created in Batch area with automatically created Batch Name-New Batch and user name. In command/parameter window command sequence will contain following commands:

Open

Save.



The same action can be achieved with selecting New Batch New Batch button in the Toolbar.

Note: To initialize a Batch job input-files must selected and added.

6.2 **Delete Batch**

This option allows deleting selected Batch.

Select Batch to be deleted. The batch will be highlighted in Batch area.

In **Edit** Menu select option **Delete Batch**. Selected Batch will be removed.

The same action can be achieved with selecting Delete Batch button in the Toolbar.

6.3 **Add Files**

To Add files for Batch processing select in Menu Edit and choose Add Files. Choose input files window appears. Select the single file to batch or multiple files with ctrl or shift key. All selected files will be added to the Input list and the output files will be generated automatically and added to the Output file list.

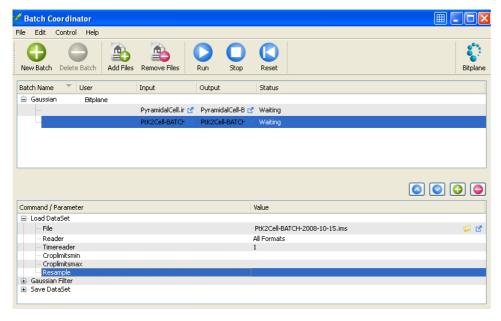
Refer to the Chapter Preference for changing automatic option for defining output name and directory.

The same action can be achieved with selecting (a) Add Files button in the Toolbar.



To modify the input file files list click on the line to select file and either press Remove File Button or in Edit Menu select option Remove File.

Note: Input files added to the Batch Coordinators have properties defined by acquisition. These image properties effect how files could be processed.



6.4 Remove Files

This option allows deleting selected Files.

Select Batch and within Files to be deleted. The files will be highlighted in Batch area.

In Edit Menu select option Delete Files. Selected files will be removed from Input and Output columns.

The same action can be achieved with selecting Remove Files Button from Batch Toolbar.

6.5 Select All

The **Select All** command will create a new selection that includes all Batch in **Batch Area**.

6.6 Select None

Select None cancels all selections. If nothing is selected, this function will perform no action.

6.7 Preferences

The Preferences dialog allows you to customize various settings and to customize Batch environment setting.

Any changes you make to the Batch Preferences settings take effect in image processing.

Batch Agent Path

The preference option is set in conjunction with Imaris at the time of installation. It is advised that only the system administrator has access to it, since any change can have serious effect on the system if incorrectly set.

Browse

Click Browse to choose the new directory

Number of commands

Number of commands will verify altered directory of Batch Agent Path.

Reload

Data Cache limit (MB):

Batch Coordinator uses a data caching mechanism that allows you to process images that are significantly larger than the physical memory (RAM) installed in the computer system. This mechanism writes image data blocks to the disk and reads them back into the physical memory when they are needed.

Memory Limit (MB)

The value of "data cache" limit controls the amount of data blocks Batch Coordinator will keep in memory at any time.

Maximum Number of instances

Default setting defined by license type and by default is set to be maximum available number. Inserting a smaller number limits the numbers of CPU and permitting use of another applications.

Batch setting

Batch Job path

The preference option is set in conjunction with Imaris at the time of installation. It is advised that only the system administrator has access to it, since any change can have serious effect on the system if incorrectly set.

Browse

Click Browse to choose the new directory

Coordinator path

The preference option is set in conjunction with Imaris at the time of installation. It is advised that only the system administrator has access to it, since any change can have serious effect on the system if incorrectly set.

Browse

Click Browse to choose the new directory

Remote address installation

The preference option is set in conjunction with Imaris at the time of installation. It is advised that only the system administrator has access to it, since any change can have serious effect on the system if incorrectly set.

Default output name

This preference can be adapted and changed by all users. Preferences allow you to define default directory of output files as well default output file name. The default location is the input file directory. In a input file

directory, a new folder is created with the name is **batch output**. The default output file name is created by input file name with addition of Batch and command name, date stamp and file number. The output file extension is predetermined by the type of batch file being created.

<source_path>/batch-output/<source_name>-<batch_name>-<command_name>-<year>-<month>-<day>-<fi
le_no>.<default_ext>

Select option Default Output Name and you can save output file in the directory you specify.

All Output filename will be changed according to the rule user has selected.

Note Both forward (/) and backward slashes can be used (\) and be mixed in the same filename without problems.

Jobs file name

The preference option is set in conjunction with Imaris at the time of installation. It is advised that only the system administrator has access to it, since any change can have serious effect on the system if incorrectly set.

Browse

Click **Browse** to choose the new directory

OK button

To confirm any changes in Preference setting press **OK** button.

Cancel

If you make changes but decide not to use them, click Cancel.

7 Menu Control

The Menu **Edit** has the following commands and shortcuts:

 Run
 (Ctrl+R or Cmd+R)

 Pause
 (Ctrl+U or Cmd+U)

 Stop
 (Ctrl+T or Cmd+T)

 Reset
 (Ctrl+D or Cmd+D)

The **Control** commands will be performed on selected entry either files or entire Batch job.

7.1 Run

Start an execution of batch process by selecting command **Run** from Command Menu. A Command sequence will be executed on all of the input data set files.

The same action can be achieved with selecting Run Button in Batch Toolbar.

7.2 Pause

Pause a batch process by selecting Pause command in Menu Command.

When batch is running, it can be paused by selecting the **Pause** command. In Status column Paused with percentage of finished job will be displayed.

7.3 Stop

When batch is running by selecting **Stop** in Menu **Command** an execution of batch process is stopped. Current running Batch jobs will be canceled and returned to "un-executed" state. In Status column Waiting will be displayed.

The same action can be achieved with clicking on the **Stop button** in Batch Toolbar.

7.4 Reset

In Menu **Command** select **Reset** and finished batch process will be restored to an "un-executed" state. This option allows modifying Batch job by inserting new commands in command sequence or new input files.

8 Menu Help

The Menu **Help** has the following commands:

User Manual

Bitplane AG

About Batch Coordinator

8.1 User Manual

The User Manual provides you with the basic information about Batch Coordinator: The menu(s), the different Batch Coordinator views and all adjustable parameters in the program. To find a specific parameter use the "Search function" in the online version of the User Manual.

8.2 Bitplane AG

If your computer is connected to the Internet, Batch Coordinator can directly open the Bitplane AG homepage (www.bitplane.com).

8.3 About Batch Coordinator



9 Batch Area

Batch area summarize all information related to Batch jobs. It contains following columns:

Batch Name
User Name
Input file list
Output file list
Status column

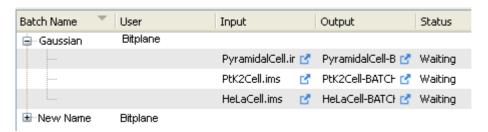
9.1 Overview

In **Batch Area** the hierarchical structure provides information on current and completed batches.

To expand the Batch and display Batch specific details, click the plus symbol (+) next to <u>Batch Name</u>. The selected Batch is expanded, displaying the batch name, all input and output file names within the batch, the name of the user who submitted the batch, and status line.

Click on the minus symbol (–) next to Batch name to collapse the content and only Batch and user names will be displayed.

Command Sequence of currently selected Batch is displayed in the Command Parameter area.



9.2 Batch Name

In the case when Batch and Command sequence is created using Imaris, by default a **Batch Name** is corresponding to the name of first selected command.

In the case when Batch and Command sequence is directly created Batch coordinator using Menu <u>File</u>, <u>Edit</u> or <u>New Batch button</u>, default option for Batch name is **New Batch**.

In order to change Batch Name double click on it and enter a new, customized name.

9.3 User

Automatically generated each time when new batch is created by user login session.

9.4 Input file

Displays the list of all files selected for Batch processing.

To verify and confirm input file selections double click on the icon react to file name. It open input files with Imaris (or standard application) in a new window.

To remove file from the input file list click on the line to select file and either press **Remove File** button or in Edit Menu select option Remove File.

Advance users option

Input files column could be also modified in Command Area. Expend command line Open by clicking on the

plus symbol (+) next to command name. Command is expended and Parameter **File** displays current file name in **Value** column.

Input file name or directory could be changed either by:

- 1. Entering in the Value column the full path to the new directory along with a new file name, or
- 2. Double clicking open file icon . Open File Window will allow selection of new file name or new destination folder.

Please refer to the Chapter Commands-Open for additional information about input files.

9.5 Output File

The Batch Coordinator can generate a variety of different output files and therefore rule must be adopted to prevent output file names from colliding. Secondly, in the Batch Coordinator the organization of creating output file names differ.

These two issues have combined resulted in the sophisticated but straightforward method in which Batch Coordinator handles the <u>output file names</u> creation.

Output file column

Displays the list of all pending or created files as a result of Batch processing.

To view and re-examine the changes in output file double click on the icon external application in a new window.

9.5.1 Output File Names

In Output column file names and directory are automatically generated in accordance with the rule of output filename.

Output Files Directory

All output files will be placed in the same directory as the input file, in the **new folder**, called **batch output**.

Output File Names

The Output file names generated by using a naming rule comprised of the following:

By default the output file name is automatically generated from **input file name** using suffixes **batch name**, **command name**, **date stamp** and **file number**.

The output files inherit the **input file name** as a fist part of output file name.

The next part in output file name will be replaced by the **Batch Name**. Please refer to the Chapter Batch Area-<u>Batch Name</u> for complete details on setting Batch name.

The next part of the output file name is command name. Only commands linked with the generating output files can appear as a **command name**. Therefore, only commands **Save**, **Spots** or **Surfaces** (which generate output files) can appear as a command name in the output file name. Please refer below to Output file types and formats for more details.

The output file name is determined by **date stamp**, the year (yyyy), month (mm) and day (dd) of the files added in the Batch in format **yyyy-mm-dd**, and **number** of the output files crated.

User can change value of this column by changing the rule in Menu Edit-Preference, or edit items one by one

in Command Parameter Area.

Output file types and formats

Output file types and formats depend on the commands specified to be executed in Batch job. Formats the output files are:

- 1. Images with extension IMS for Imaris files
- 2. Images with extension IMX for Imaris Scene
- 3. Statistical files with the extension CSV for data presented as comma separated values.

All files with extension IMS are generated by command <u>Save</u> regardless of preceding commands in the command sequence. The output file name of files with extension ims will contain Save as a command name in the output file name.

All files with extension IMX and CSV are generated either by command Spots or Surfaces regardless of preceding commands in the command sequence.

The output file name of files with extension IMX and CSV will contain Spots or Surfaces as a command name in the output file name.

All files with extension CSV are statistical files and generated either by command Spots or Surfaces.

Advance users option

Files in Output column could be also modified in Command Area. Expend command line Save by clicking on the plus sign next to command name. Command is expended and Parameter **File** displays current file name in **Value** column.

Output file name or directory could be changed either by:

- 1. Entering in the Value column the full path to the new directory along with a new file name, or
- 2. Double clicking open file icon . Open File Window will allow selection of new file name or new destination folder.

Multiple output files (statistical files and Imaris scene) can be created by selecting commands: <u>Spots or Surfaces</u>.

Please refer to the Chapter <u>Statistics</u> for additional information about statistical files presentation and Chapter <u>Commands-Save</u> for output file presentation and arrangement.

9.6 Status

The Status line displays and reports the full status messages on any batch.

For unprocessed files in Status line message is Waiting.

After starting a batch job percentage of execution is displayed with bars indicate the amount of progress. When the progress bar reaches 100 percent, file has been successfully processed, all commands executed and output file saved in the designated folder. Completed batch job are marked **Finished** and remain in the batch display.

Unsuccessful batch and job items are marked **Crashed** and remain in the batch display.

In status line massage **Queued** for all file waiting to be executed.

10 **Batch Control Toolbars**

Batch Control Toolbars has following buttons:



10.1 **New Batch**

Click on the **New Batch** icon . Batch structure will be created in **Batch area** with automatically created Batch Name Gaussian (default option) and user name. In command/parameter window command sequence will contain following commands:

Open Gaussian Filter Save.

The same action can be achieved with selecting New Batch command in Menu File or Edit.

Note: To initialize a Batch job input-files must selected and added.

10.2 **Delete Batch**

This option allows deleting selected Batch.

Select Batch to be deleted. The batch will be highlighted in Batch area. Click on the Delete Batch



button and selected Batch will be removed.

The same action can be achieved with selecting **Delete Batch** in the Menu **Edit**.

10.3 Add Files

To Add files for Batch processing click on the **Add Files** Button . Choose input files window appears.

Select the single file to batch or multiple files with ctrl or shift key. All selected files will be added to the Input list and the output files will be generated automatically and added to the Output file list.

The same action can be achieved with selecting Add Files in Menu Edit.

To remove input file click on the line to select a file and either press Remove File Button are in Menu

Edit select option Remove File.

Note: Input files added to the Batch Coordinators have properties defined by acquisition conditions and they affect how files could be processed.

10.4 Remove Files

This option allows deleting selected Files.

Select Batch and Files to be deleted. The files will be highlighted in Batch area. Click on the **Remove Files** button . Selected files will be removed from the Input and the Output file list.

The same action can be achieved with selecting option **Remove Files** in **Edit** Menu.

10.5 Run

Start an execution of batch process. Click on the **Run Button** and a Command sequence will be executed on all of the input data set files.

The same action can be achieved with selecting Run from Menu Command.

10.6 Stop

When batch is running by clicking on **Stop** button an execution of batch process is stopped. Current running Batch jobs will be canceled and returned to "un-executed" state. In **Status** column Waiting will be displayed.

The same action can be achieved with selecting Stop from Menu Command.

10.7 Reset

Click on the **Reset** button and finished batch process will be restored to an "un-executed" state. This option allows modifying Batch job by inserting new commands in command sequence or new input files. The same action can be achieved with selecting **Reset** in Menu **Command**.

11 Command/Parameter Area

This chapter describes all commands that are recognized by Batch Coordinator.

Commands could be defined as a set of actions executed on each file in the input file list. Information and specifications that a command uses to perform its action are called parameters. In simple terms, the command is what is done; the parameter is how it does it.

All commands supported by Batch Coordinator are closely associated with commands in Imaris.

11.1 Overview

In the Command Parameter Area a Command Sequence of selected Batch is displayed.

A full list of the commands is accessible by double clicking on the command line. Double click on the command line and an arrow appears indicating a drop down window with a list of all Batch Coordinator available commands names.

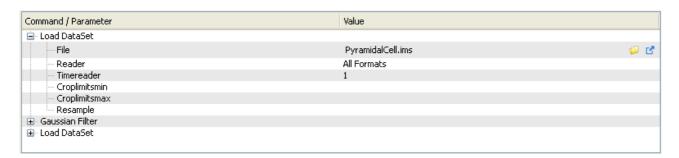
In Commands Parameter Area the hierarchical structure of Command Sequence provides information on currently selected command.

To expand the Command sequence, click the (+) symbol next to Command Name. The selected command line is expanded, displaying the all the parameters values specific for selected command. Click on the (-) symbol next to Command name to collapse the content and only Command name is shown.

To change order of Commands in sequence the **Up** and **Down** buttons of <u>Command/Parameter</u> <u>Toolbars</u> can be used.

When command is expanded double click on the Value column (in parameter line) to fine-tune the values within the command.

Note: For each command in the Parameters collection, a **Value** must be set from a specific list of choices. Please refer to the Chapter <u>Values</u> for possibilities and risks of modifying command values.



11.2 Commands

Following Commands are available in Batch Coordinator.

Open
Save
Flip
Rotate
Channel Shift
Gaussian Filter
Median Filter
Linear Stretch
Gamma Correction
Invert

Normalize Layers

Baseline Subtraction

Threshold Cutoff

Background Subtraction

Connective Baseline

Display Adjustment

Set Coordinates

Set Time Points

Add Slices

Add Channels

Add Time Points

Delete Slices

Delete Channels

Delete Time Points

Crop 3D

Crop Time

Resample Time

Resample 3D

Change Data Type

Swap Time and Channels

Swap Time and Z

Spots

Surfaces

Matlab

Merge Csv Statistics Post

Please refer for a more detailed description of the commands and parameters to the Imaris Reference Manual .

11.2.1 Open

By default set to be a first command in command sequence created either by Batch job Creation in Imaris or directly in Batch Coordinator.

Expend command line Open by clicking on the plus sign next to command name. Command is expended and Parameter File displays current file name in Value column.

Input file name or directory could be modified either by:

- 1. Entering in the Value column the full path to the new directory along with a new file name, or
- 2. Double clicking open file icon . Open File Window will allow selection of new file name or new destination folder.

To verify and confirm input file double click on the icon react to file name. It open input files with Imaris (standard application) in a new window.

11.2.2 Save

In order to save output file from Batch job it is necessary to include Save as a last command in command sequence. Command Save is determining extension and part of the file name of the output files.

All files with extension **IMS** are generated by command Save regardless of preceding commands in the command sequence.

The output file name of files with extension **IMS** will contain **Save** as a **command name** in the output file name.

Expend command line Save by clicking on the plus sign next to command name. Command is expended and Parameter File displays current file name in Value column.

Output file name or directory could be changed either by:

- 1. Entering in the Value column the full path to the new directory along with a new file name, or
- 2. Double clicking open file icon . Open File Window will allow selection of new file name or new destination folder.

To verify and confirm output file double click on the icon real next to file name. It open output files with Imaris (standard application) in a new window.

11.2.3 Flip

Flip image channels either as a group or individual channels in x, y z direction.

11.2.4 Rotate

Rotates image channels either as a group or separately in Counter Clockwise, Clockwise orientation.

11.2.5 Channel Shift

Allows moving channels relative to one another.

11.2.6 Gaussian filter

The Gaussian filter smoothes the image. The filter value can be applied separately to any of channels or to all of them.

11.2.7 Median Filter

The Median Filter replaces the intensity of each voxel with the statistical median of the intensities of neighboring voxels. The filter value can be applied separately to any of channels or to all of them.

11.2.8 Linear Stretch

Linearly extending image contrast to the new limits. Minimum and Maximum value can be applied separately to any of channels or to all of them.

11.2.9 Gamma Correction

Intensifying the gray value of a specific range of voxels.

11.2.10 Invert

Inverting all channels individually or all together.

11.2.11 Normalize Layers

Normalize Layers individually or all together.

11.2.12 Baseline Subtraction

Baseline Subtraction subtracts the baseline value from the intensity of every voxel in the image. The value can be applied separately to any of channels or to all of them.

11.2.13 Threshold Cutoff

Threshold compares the intensity of every voxel in the image to the threshold value. The voxel intensity values below are assigned new intensity, a threshold value new. The Threshold Cutoff values for the channels may differ.

11.2.14 Background Subtraction

Applies a Gaussian filter to define the background at each voxel and then performs a Baseline Subtraction of this variable background. The filter value can be applied separately to any of channels or to all of them.

11.2.15 Connective Baseline

The Connective Baseline works with two, a lower and an upper, threshold values. It applies these thresholds to divide the image into regions and then processes each region with a Baseline Subtraction using either the lower or the upper threshold. The threshold values can be applied separately to any of channels or to all of them.

11.2.16 Display Adjustment

The Display Adjustment manages channel visibility, controls opacity and limits color contrast range.

11.2.17 Set Coordinates

Defines image coordinates.

11.2.18 Set Time Point

Manages file time stamp, modification and last accessed time.

11.2.19 Add Slices

Adding image slices. Data sets must correspond in image dimension, channels and time points.

11.2.20 Add Channels

Adding channels extend or complete the data set information. Data sets must correspond in image dimension, channels and time points.

11.2.21 Add Time points

Adding time points to the data set. Data sets must correspond in image dimension, channels and time points.

11.2.22 Delete Slices

Deletes slices from data set.

11.2.23 Delete Channels

Deletes channels from Data set.

11.2.24 Delete Time Points

Deletes time points from Data set.

11.2.25 Crop 3D

Cropping unwanted parts of an image reducing data set down to the region of interest.

11.2.26 Crop Time

Reducing the total number of time points at the beginning or end of the series of images.

11.2.27 Resample Time

Reducing the total number of time points, displaying the images at a faster speed.

11.2.28 Resample 3D

Reducing the voxel density in a data set, but keeping the original relationship between the voxels.

11.2.29 Change Data Type

Changing data type from current display to 8, 16 or 32 bit.

11.2.30 Swap Time and Channels

Exchanging the t-axis and the channels and re-formatting the image.

11.2.31 Swap Time and Z

Exchanging the t-axis and the z-axis and re-formatting the image that the time dimension is mapped onto the z-axis and the z-dimension is mapped onto the t-axis.

11.2.32 Spots

Multiple step procedure for automatic detection of point-like structures. Please refer to the Chapter <u>Spots</u> and <u>Surfaces</u> for Batch command more application details and Imaris Reference Manual for Spots Creation Wizard.

Command Spots is determining extension and part of the file name of the output files.

All files with extension **IMX** and **CSV** are generated by command Spots regardless of preceding commands in the command sequence.

The output file name of files with extension **IMX** and **CSV** will contain **Spots** as a **command name** in the output file name.

11.2.33 Surfaces

Multiple step procedure for automatic detection and creation of artificial solid objects. Please refer to the Chapter <u>Spots and Surfaces</u> for Batch command more application details and Imaris Reference Manual for Spots Creation Wizard.

Command Surfaces is determining extension and part of the file name of the output files.

All files with extension **IMX** and **CSV** are generated by command Surfaces regardless of preceding commands in the command sequence.

The output file name of files with extension **IMX** and **CSV** will contain Surfaces as a **command name** in the output file name.

11.2.34 Matlab

Developing and integrating of custom algorithms specifically tailored to scientific applications. A variety of function is offered: Data set, Spot, Filament, Track, Surface, Camera, Stereology functions. Available only for Windows.

11.2.35 Merge Cvs Statistics Post

Collects all batch statistical files in to one combined file. Please refer for a more detailed description of the command to the Chapter <u>Statistics</u>.

11.3 Values

Batch should be able to be run unattended. To obtain correct values for batch processing, it is important to ensure that the values are corresponding correctly to the image setting in every regard. Therefore, it is absolutely critical that values are checked in Imaris. Test and verify all command parameter values in the Imaris before placing them in a batch process. The simplest method of setting correct variables for a command sequence is to open Imaris and create a New Batch with a selected Imaris Command sequence. Please refer to the Chapter <u>Batch Creation</u> for additional references.

Risks and Cautions

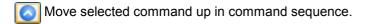
Avoid editing batch commands values directly in Batch Coordinator. In order for the batch file to work correctly, parameters must be set in accordance with **image acquisition** and **exactly fitting processing** and **analysis requirements**. Replacing and adjusting variables directly in a batch file could result in Batch job not execute properly, as the batch command act in accordance with the parameters entered. Because batch process is operating automatically, if parameter values are used incorrectly, any command can stop the process without necessarily giving the user any indication there is a problem.

Note: Parameter values are case sensitive.

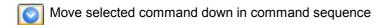
12 Command/Parameter Toolbars

Bringing users the control of the Command/ Parameter area. Use command toolbars to move up or down commands through a list of commands or to add or delete commands in command sequence. Changes can be seen directly in the command list.

Up



Down



Plus

Add a new command to existing command sequence. A new added command by default is Open.

Minus

Remove command from command sequence.

Please refer to the Chapter Commands <u>Value</u> for additional information and advices.

13 Spots and Surfaces

The commands Spots and Surfaces have many similarities to other Batch commands, but there are some distinct differences that need to be taken into account when creating Batch job containing the Command sequence with commands Spots or Surfaces.

Due to Commands complexity, it is essential to use **Imaris** Spots and Surfaces **Creation Wizard** for creating Batch Commands sequence **Spot** and **Surface**.

In Imaris, adjust commands parameters throughout the Creation Wizard 🤾 Create for Spots and Surfaces.

The effect of the modification and parameter adjusting can be seen instantly in the image as changes are applied.

Complete Creation Wizard.

Select Tab Creation \\ and all parameters values are once more displayed.

Click on Add to Batch button Add to batch at the bottom of the Tab Creation panel.

Like other commands, Spot (or Surface) is added in Command sequence and listed in the Command/Parameter area.

Please refer to the Chapter **Batch Creation** for additional references.

Note: Commands Spots or Surfaces are creating multiple output files from a single input file.

Two generated output files are: Imaris scene with, extension IMX, and Statistical file, with extension CSV for comma-separated values.

Please refer to the Chapter <u>Statistics</u> for additional information about statistical files and Chapter <u>Output File</u> for output file presentation and arrangement.

14 Statistics

Files with the CSV (comma separated values) extension are used to save tabular statistical data and can be opened by the many spreadsheet applications(Excel OpenOffice Calc). All files with extension CSV are statistical files and generated either by command Spots or Surfaces.

CSV folder Statistical File Output

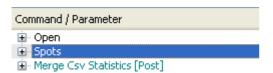
For each input file new **CSV folder** is created within **batch output** folder. (Refer to the Chapter <u>Output files</u> for rule for generating <u>output files</u> name and <u>directory</u>). The folder will contain all statistical files created after executing Batch job, one for each individual statistical variable. Hence, Batch job, containing the commands Spots or Surfaces, even for a single input file will produce the multiple output files. As a consequence a large number of statistical files must be processed and analyzed.

Batch Coordinator provides option for combining multiple CSV statistical files.

A merger of CSV-files is facilitated by the uniform structure within the individual statistical report folders and standardized files naming. All **file names** with matching **names of statistical variables** (area, volume, intensity) are fused together.

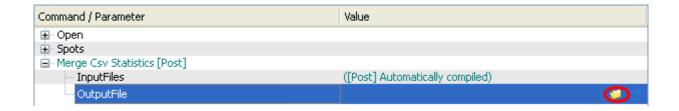
Command Merge Csv Statistics Post

To merge CSV Output files of all jobs within a Batch, a new command must be inserted into a Command Sequence. In Command/Parameter Tool bar click on the **Add command** Button . A new command (Open), appears to be very last command in the Command Sequence. Double click on added command line and an arrow appears indicating a drop down window. From a list of commands select a command **Merge Csv Statistics Post**.



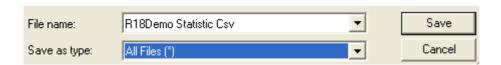
Command merge will combine datasets with a common statistical variable and incorporate them in one common file containing the specific variable of all input files.

Expand command Merge Csv Statistics Post by clicking on the (+) symbol. Two parameters are available, **InputFiles** and **OutputFiles**.



A value for Input files is automatically generated from individual statistical files. A value for the merged Output folder name must be created.

Click on the open file icon and a new window will be open. Specify the folder name for merged Statistical CSV file and save it. Alternatively, the new directory and a new file name can be entered directly in the parameter OutputFile Value line.



Statistical CSV file name and directory will be written as a value of parameter OutputFile.



Command merge will identify and retrieve from the individual CSV folders all files with the same specific file name (all files that share a common statistical variable the same statistical variable) and merged in one common file. As a result, a new, merged file will be created containing combined specific statistical variables. The names of new merged files will correspond to names of statistical variables from individual files.

The resulting merged folder will have the same number of statistical files as the number of calculated statistical variables.

If commands spots or surfaces are not present in the Command Sequence, Merge Csv Statistics will be ignored.

Excel files

Besides CSV format, statistical data can be also created as XLS file (Excel files).

The following steps are used to change format of statistical file. Please proceed as follows:

In Batch area select Batch containing Command Sequence with commands Spots or Surfaces.

Expand command Spot (or Surfaces) by clicking on the (+) symbol.

The type of statistical output files is defined in parameter **Statisticswriter** and value is set to be by default **bpStatisticsWriterCsv**.

Change value of StatisticWriter parameter from **bpStatisticsWriterCsv** to **bpStatisticsWriterExceXIs**.

Note: Parameter values are case sensitive.

As a result, for every input file an Excel file will be created and saved. The statistical variables will be organized in spreadsheets.