IN Carta

Image Acquisition and Analysis Software

Version 1.14

INPut User Guide

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Chapter 1: IN Carta Image Analysis Software

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The IN Carta™ Image Analysis Software provides powerful analytics combined with an intuitive interface to simplify workflows for advanced phenotypic classification and 3D image analysis.

When used in combination with the MetaXpress® High-Content Image Acquisition and Analysis Software, you can get meaningful data quickly and reliably.

Faster Data

- Intuitive design makes complex analysis accessible with minimal training.
- Shorten analysis time with true parallel processing.

Reliable Data

- Sophisticated algorithms generate reliable data with minimal user input.
- Improved segmentation algorithms represent cellular structures more accurately.

Results that Matter

- See real results quickly—from populations to single cells— using integrated data visualization tools.
- User-friendly interface guides you through your discoveries with continual updates that grow with your needs.

Obtaining Support

Molecular Devices is a leading worldwide manufacturer and distributor of analytical instrumentation, software, and reagents. We are committed to the quality of our products and to fully supporting our customers with the highest level of technical service.

Our Support website—www.moleculardevices.com/service-support—describes the support options offered by Molecular Devices, including service plans and professional services. It also has a link to the Molecular Devices Knowledge Base, which contains documentation, technical notes, software upgrades, safety data sheets, and other resources. If you still need assistance, you can submit a request to Molecular Devices Technical Support.

Technical Support

To contact Molecular Devices Technical Support, submit a support request through the Molecular Devices Knowledge Base at support.moleculardevices.com.

You can also submit a support request by phone. For regional support contact information, go to www.moleculardevices.com/contact.

To expedite support:

- For the IN Carta software, please be prepared to provide the software version and the service tag for the analysis computer. To find the software version, in the IN Carta software, select version in the **System** > **About** in the top right corner of the window. The service tag is on the label on the top of the analysis computer.
- For the MetaXpress software, please be prepared to provide the system ID number, the software version, and the name of the system owner. To find this information, in the MetaXpress software, select **Help** > **About MetaXpress**.

Documentation

Review the product documentation on the Molecular Devices Knowledge Base at support.moleculardevices.com. In addition, online Help is available within the IN Carta software.

Additional Resources

Web-based microscopy courses:

- www.microscopyu.com
- www.ibiology.org/ibioeducation/taking-courses/ibiology-microscopy-short-course.html

The Molecular Probes Handbook offers advice on fluorescent probes and can help you determine if there are better stains available for your analysis:

- www.thermofisher.com/us/en/home/references/molecular-probes-the-handbook.html

Filter information:

- www.semrock.com
- www.chroma.com
- www.omegafilters.com

About This Guide

This guide is intended for the scientist using the IN Carta software. It describes how to use the IN Carta INPut tool to convert image data sets into a format compatible with IN Carta software.

The information in this guide is subject to change without notice. We recommend that you review the guide on the Molecular Devices Knowledge Base at support.moleculardevices.com for the most up-to-date information.

Chapter 2: IN Carta INPut

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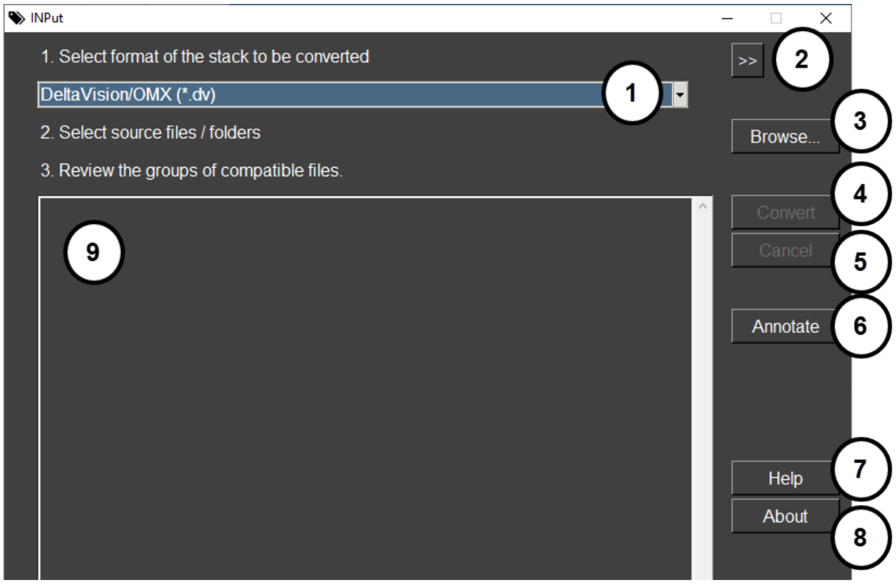
INPut is a tool for converting image data sets into a format compatible with IN Carta analysis software. INPut also allows users to annotate .xdce stacks acquired on IN Cell instrumentation or generated by the INPut converter.

INPut accommodates data sets acquired on or exported from:

- DeltaVision or DeltaVision OMX
- PerkinElmer Operetta/Opera/Columbus

INPut Main Window

The image below shows the initial view of the INPut main window.



1. Select format of the stack to be converted

DeltaVision/OMX (*.dv)

2. Select source files / folders

3. Review the groups of compatible files.

Part	Name	Description
1	Format drop-down list	Allows selection of a format to convert.
2	Expand/Collapse button	Expands/collapses image conversion log window.
3	**Browse** button	Opens file/folder browsing dialog.
4	**Convert** button	Converts selected files into .xdce stack(s).
5	**Cancel** button	Cancels current conversion operation.
6	**Annotate** button	Opens INPut: Annotation Editor.
7	**Help** button	Opens quick guide.
8	**About** button	Opens information about software version.
9	Review panel	Displays information during review step.

Chapter 3: Using INPut to Convert Files

This chapter contains the following instructions for converting files into xdce format:

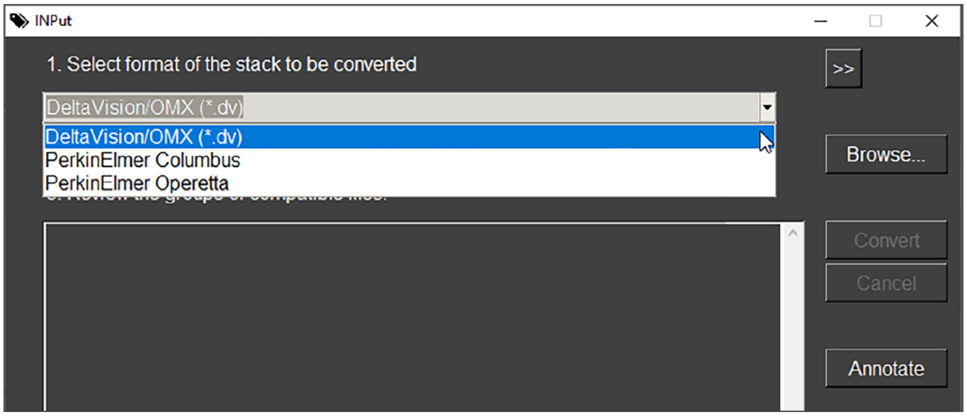
- Converting DeltaVision/OMX Files, see below
- Converting of PerkinElmer Operetta/Columbus Files, see page 14

Converting DeltaVision/OMX Files

Select Files

To select files for conversion:

1. In the main window, select DeltaVision/OMX from the format drop-down list.



1. Select format of the stack to be converted

DeltaVision/OMX (*.dv)

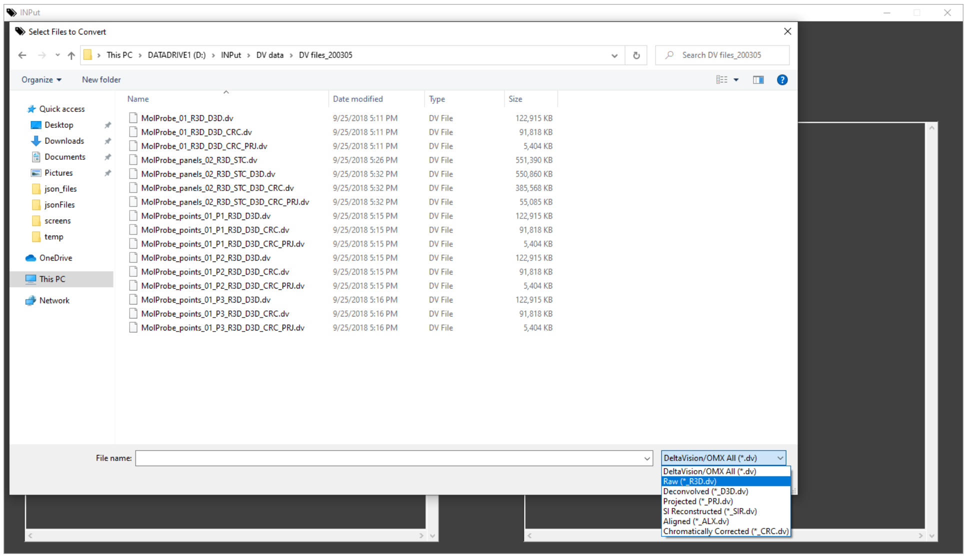
2. Click **Browse**.

   Files with one of the following file extensions appear in the Select Files to Convert dialog.

File Extension	Last Post-Processing Step
DeltaVision/OMX All	*.dv
Raw	*_R3D.dv
Deconvolved	*_D3D.dv
Projected	*_PRJ.dv
SI Reconstructed	*_SIR.dv
Aligned	*_ALX.dv
Chromatically Corrected	*_CRC.dv

3. Select one or more files for conversion.

Note: Files can be filtered by file extension for display based on last post-processing step.



Name	Date modified	Type	Size
MolProbe_01_R3D_D3D.dv	9/25/2018 5:11 PM	DV File	122,915 KB
MolProbe_01_R3D_D3D_CRC.dv	9/25/2018 5:11 PM	DV File	91,818 KB
MolProbe_01_R3D_D3D_CRC_PRJ.dv	9/25/2018 5:11 PM	DV File	5,404 KB
MolProbe_panels_02_R3D_STC.dv	9/25/2018 5:26 PM	DV File	551,390 KB
MolProbe_panels_02_R3D_STC_D3D.dv	9/25/2018 5:32 PM	DV File	550,860 KB
MolProbe_panels_02_R3D_STC_D3D_CRC.dv	9/25/2018 5:32 PM	DV File	385,568 KB
MolProbe_panels_02_R3D_STC_D3D_CRC_PRJ.dv	9/25/2018 5:32 PM	DV File	55,085 KB
MolProbe_points_01_P1_R3D_D3D.dv	9/25/2018 5:15 PM	DV File	122,915 KB
MolProbe_points_01_P1_R3D_D3D_CRC.dv	9/25/2018 5:15 PM	DV File	91,818 KB
MolProbe_points_01_P1_R3D_D3D_CRC_PRJ.dv	9/25/2018 5:15 PM	DV File	5,404 KB
MolProbe_points_01_P2_R3D_D3D.dv	9/25/2018 5:15 PM	DV File	122,915 KB
MolProbe_points_01_P2_R3D_D3D_CRC.dv	9/25/2018 5:15 PM	DV File	91,818 KB
MolProbe_points_01_P2_R3D_D3D_CRC_PRJ.dv	9/25/2018 5:15 PM	DV File	5,404 KB
MolProbe_points_01_P3_R3D_D3D.dv	9/25/2018 5:16 PM	DV File	122,915 KB
MolProbe_points_01_P3_R3D_D3D_CRC.dv	9/25/2018 5:16 PM	DV File	91,818 KB
MolProbe_points_01_P3_R3D_D3D_CRC_PRJ.dv	9/25/2018 5:16 PM	DV File	5,404 KB

Tip: Hold **CTRL** while clicking to select multiple individual files. Hold **SHIFT** while clicking to select a range of files.

4. Click **Open** to import selected files for review.

Review

During the review step the software displays groups of compatible files.

1. Select format of the stack to be converted

DeltaVision/OMX (*.dv)

2. Select source files / folders

3. Review the groups of compatible files.

- ☑ Image Stack: channels = 3, Z planes = 17, T points = 1.
  - 12-well plate
  - ☑ MolProbe_points_01_P1_R3D_D3D_CRC.dv A 1
  - ☑ MolProbe_points_01_P2_R3D_D3D_CRC.dv A 2
  - ☑ MolProbe_points_01_P3_R3D_D3D_CRC.dv A 3
  - ☑ MolProbe_01_R3D_D3D_CRC.dv A 4
- ☑ Image Stack: channels = 3, Z planes = 1, T points = 1.
  - 96-well plate
  - ☑ MolProbe_points_01_P1_R3D_D3D_CRC_PRJ.dv A 1
  - ☑ MolProbe_points_01_P2_R3D_D3D_CRC_PRJ.dv A 2
  - ☑ MolProbe_points_01_P3_R3D_D3D_CRC_PRJ.dv A 3
  - ☑ MolProbe_01_R3D_D3D_CRC_PRJ.dv A 4
- ☑ Image Stack: channels = 3, Z planes = 10, T points = 1.
  - Single slide
  - ☑ MolProbe_panels_02_R3D_STC.dv A 1
- ☑ Image Stack: channels = 3, Z planes = 10, T points = 1.
  - Single slide
  - ☑ MolProbe_panels_02_R3D_STC_D3D.dv A 1
- ☑ Image Stack: channels = 3, Z planes = 7, T points = 1.

Files are grouped into plates based on dimensionality. Default plate dimensions depend on the number of files assigned to a plate. All files assigned to one plate have the same:

- magnification/pixel size
- image size
- number of channels
- number of z planes
- number of time-points

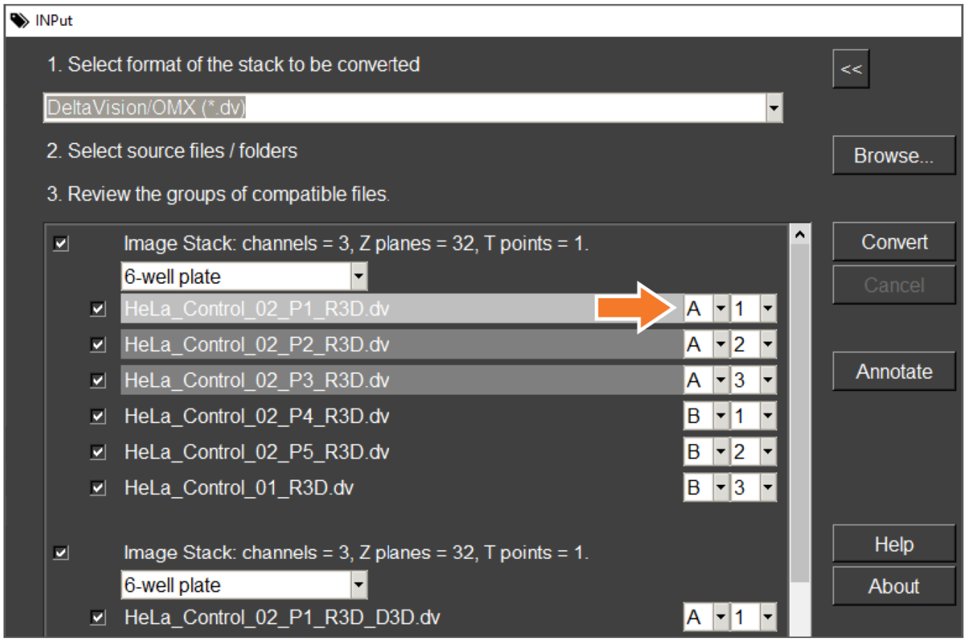
It is possible to modify the plate format or create a virtual plate format for images from a slide. The following table describes the possible actions during the review step.

Action	Description
Display ToolTip with • stack/data set name • image size/magnification	Hover the cursor over the plate heading in the review panel.
Change plate format	Modify using drop-down list under the plate heading. Image files will automatically be assigned well information.
Exclude plate from conversion	Clear the check box for the entire plate to the left of the plate heading.
Exclude individual files from conversion	Clear the check box for the individual file assigned to a plate.
Change well assignment for individual files	Modify row/column selection using drop-down list to the right of the file name.
Change well assignment for a group of files (files will appear as fields within the selected well)	Follow the well-assignment procedure for multiple files, below.

Modify Well Assignment for Multiple Files

To modify the well assignment for multiple files:

1. Left-click and hold the first file in the target range.
2. Drag the cursor up or down to select a range of files.
3. Release the left mouse button.
4. Change the well assignment of the highlighted file in the selected range manually.



DeltaVision/OMX (*.dv)

☑ Image Stack: channels = 3, Z planes = 32, T points = 1.

6-well plate

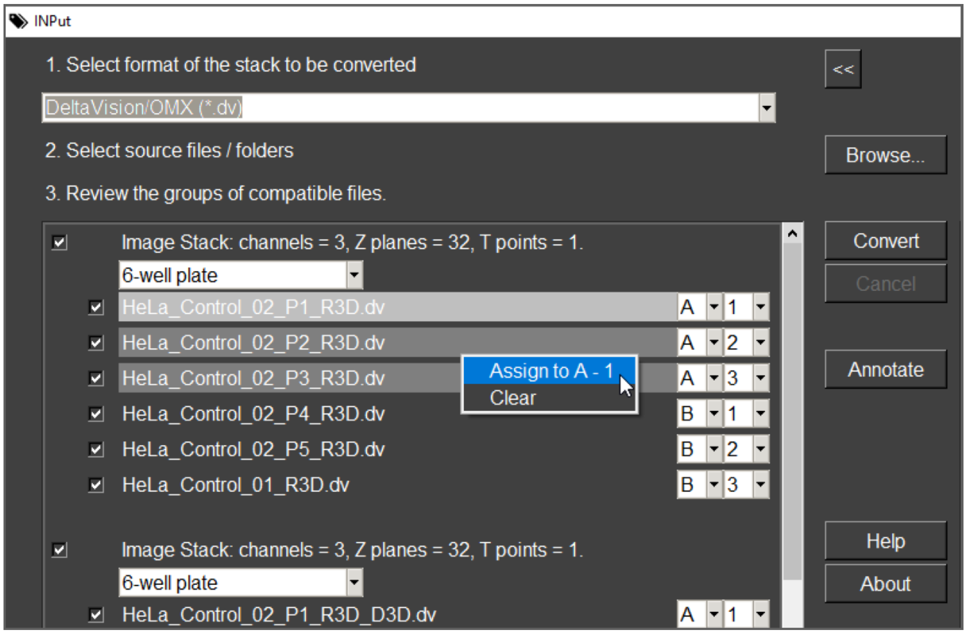
☑ HeLa_Control_02_P1_R3D.dv A 1
☑ HeLa_Control_02_P2_R3D.dv A 2
☑ HeLa_Control_02_P3_R3D.dv A 3
☑ HeLa_Control_02_P4_R3D.dv B 1
☑ HeLa_Control_02_P5_R3D.dv B 2
☑ HeLa_Control_01_R3D.dv B 3

☑ Image Stack: channels = 3, Z planes = 32, T points = 1.

6-well plate

☑ HeLa_Control_02_P1_R3D_D3D.dv A 1

5. Right-click on any file within the selected group.
6. Select **Assign to** to assign all files in the selected group to the well chosen in step 4.



DeltaVision/OMX (*.dv)

☑ Image Stack: channels = 3, Z planes = 32, T points = 1.

6-well plate

☑ HeLa_Control_02_P1_R3D.dv A 1
☑ HeLa_Control_02_P2_R3D.dv A 2
☑ HeLa_Control_02_P3_R3D.dv A 3
☑ HeLa_Control_02_P4_R3D.dv B 1
☑ HeLa_Control_02_P5_R3D.dv B 2
☑ HeLa_Control_01_R3D.dv B 3

☑ Image Stack: channels = 3, Z planes = 32, T points = 1.

6-well plate

☑ HeLa_Control_02_P1_R3D_D3D.dv A 1

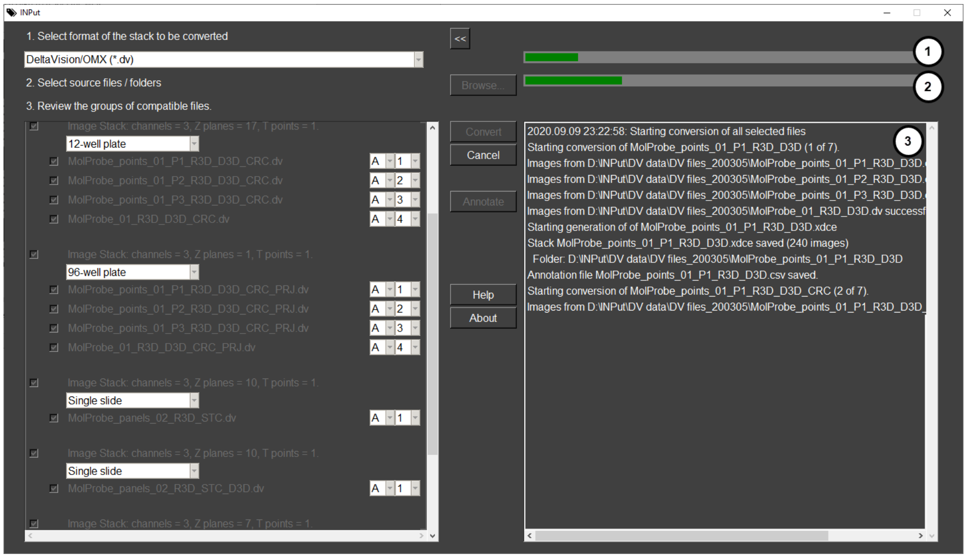
Convert Selected Files

To convert the selected files:

1. Click **Convert**.
   Well assignments for each file are saved in annotation files, which are automatically created for each plate.
2. To cancel the conversion, click **Cancel**.

Note: If the conversion is canceled before it is complete, any newly generated files from the incomplete conversion will be automatically deleted.

During the conversion process, the following information is displayed in the image conversion log window.



1. Select format of the stack to be converted
DeltaVision/OMX (*.dv)
2. Select source files / folders
3. Review the groups of compatible files.

☑ Image Stack: channels = 3, Z planes = 17, T points = 1.
12-well plate
☑ MolProbe_points_01_P1_R3D_D3D_CRC.dv A 1
☑ MolProbe_points_01_P2_R3D_D3D_CRC.dv A 2
☑ MolProbe_points_01_P3_R3D_D3D_CRC.dv A 3
☑ MolProbe_01_R3D_D3D_CRC.dv A 4

☑ Image Stack: channels = 3, Z planes = 1, T points = 1.
96-well plate
☑ MolProbe_points_01_P1_R3D_D3D_CRC_PRJ.dv A 1
☑ MolProbe_points_01_P2_R3D_D3D_CRC_PRJ.dv A 2
☑ MolProbe_points_01_P3_R3D_D3D_CRC_PRJ.dv A 3
☑ MolProbe_01_R3D_D3D_CRC_PRJ.dv A 4

☑ Image Stack: channels = 3, Z planes = 10, T points = 1.
Single slide
☑ MolProbe_panels_02_R3D_STC.dv A 1

☑ Image Stack: channels = 3, Z planes = 10, T points = 1.
Single slide
☑ MolProbe_panels_02_R3D_STC_D3D.dv A 1

☑ Image Stack: channels = 3, Z planes = 7, T points = 1.

```
2020.09.09 23:22:58: Starting conversion of all selected files
Starting conversion of MolProbe_points_01_P1_R3D_D3D (1 of 7).
Images from D:\INPut\DV data\DV files_200305\MolProbe_points_01_P1_R3D_D3D.
Images from D:\INPut\DV data\DV files_200305\MolProbe_points_01_P2_R3D_D3D.
Images from D:\INPut\DV data\DV files_200305\MolProbe_points_01_P3_R3D_D3D.
Images from D:\INPut\DV data\DV files_200305\MolProbe_01_R3D_D3D.dv successf
Starting generation of of MolProbe_points_01_P1_R3D_D3D.xdce
Stack MolProbe_points_01_P1_R3D_D3D.xdce saved (240 images)
  Folder: D:\INPut\DV data\DV files_200305\MolProbe_points_01_P1_R3D_D3D
Annotation file MolProbe_points_01_P1_R3D_D3D.csv saved.
Starting conversion of MolProbe_points_01_P1_R3D_D3D_CRC (2 of 7).
Images from D:\INPut\DV data\DV files_200305\MolProbe_points_01_P1_R3D_D3D_
```

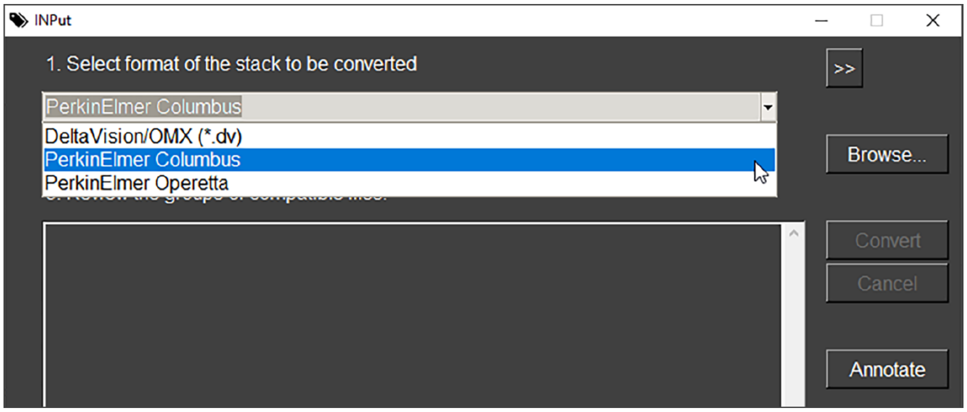
Part	Name	Description
1	Overall progress bar	Shows overall conversion progress
2	Plate progress bar	Shows progress of current plate conversion
3	Log panel	Shows details about conversion process, including final location path of .xdce stack files.

Converting of PerkinElmer Operetta/Columbus Files

Selecting Files

To select files for conversion:

1. In the main window, select PerkinElmer Operetta or PerkinElmer Columbus from the format drop-down list.



1. Select format of the stack to be converted

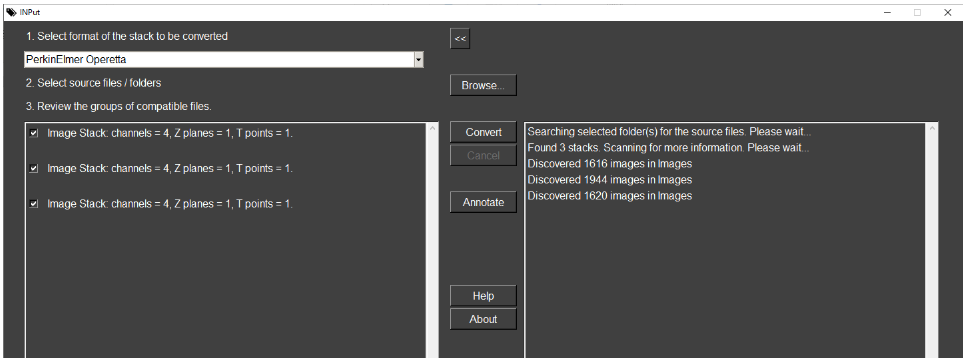
PerkinElmer Columbus

- DeltaVision/OMX (*.dv)
- PerkinElmer Columbus
- PerkinElmer Operetta

2. Click **Browse**.
3. Select a top level folder that contains the image stacks for conversion.

Review

During the review step the software displays the stacks found in the Review panel.



1. Select format of the stack to be converted

PerkinElmer Operetta

2. Select source files / folders

3. Review the groups of compatible files.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

```
Searching selected folder(s) for the source files. Please wait...
Found 3 stacks. Scanning for more information. Please wait...
Discovered 1616 images in Images
Discovered 1944 images in Images
Discovered 1620 images in Images
```

The following table describes the possible actions during the review step.

Action	Description
Display ToolTip with: • stack/data set name • image size • magnification or pixel size depending on data available	Hover the cursor over the plate heading in the review panel
Exclude image stack from conversion	Clear the check box for the image stack to the left of the stack description

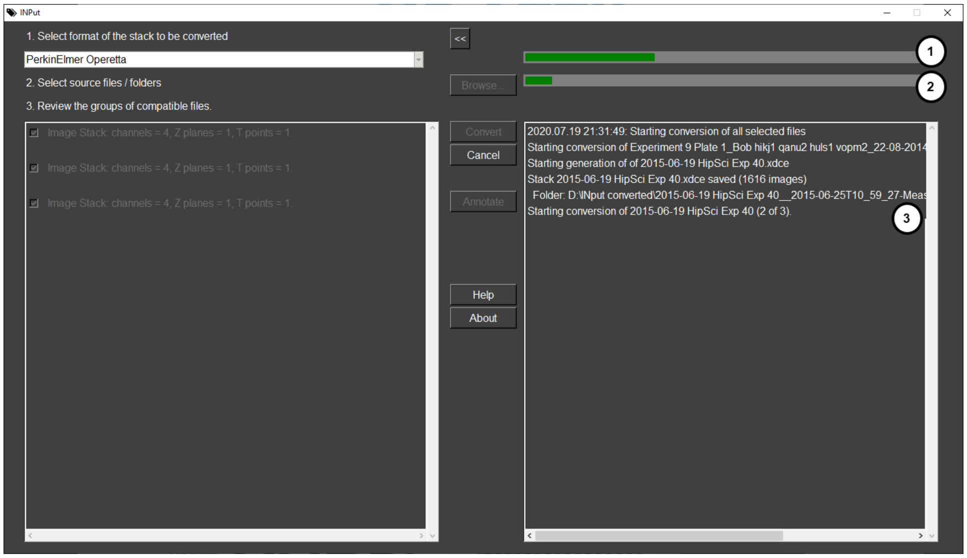
Convert Selected Files

To convert the selected files:

1. Click **Convert**.
2. In the Select Folder dialog, specify the target output folder.
3. Click **Select Folder**.
   INPut initiates conversion of selected data sets into .xdce stacks.
4. To cancel the conversion, click **Cancel**.

> **Note:** If the conversion is canceled before it is complete, any newly generated files from the incomplete conversion will be automatically deleted.

During the conversion process, the following information is displayed in the image conversion log window.



1. Select format of the stack to be converted

PerkinElmer Operetta

2. Select source files / folders

3. Review the groups of compatible files.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

☑ Image Stack: channels = 4, Z planes = 1, T points = 1.

```
2020.07.19 21:31:49: Starting conversion of all selected files
Starting conversion of Experiment 9 Plate 1_Bob hikj1 qanu2 huls1 vopm2_22-08-2014
Starting generation of of 2015-06-19 HipSci Exp 40.xdce
Stack 2015-06-19 HipSci Exp 40.xdce saved (1616 images)
  Folder: D:\INput converted\2015-06-19 HipSci Exp 40__2015-06-25T10_59_27-Meas
Starting conversion of 2015-06-19 HipSci Exp 40 (2 of 3).
```

Part	Name	Description
1	Overall progress bar	Shows overall conversion progress
2	Plate progress bar	Shows progress of current plate conversion
3	Log panel	Shows details about conversion process, including final location path of .xdce stack files.

Chapter 4: Using the INPut Annotation Editor

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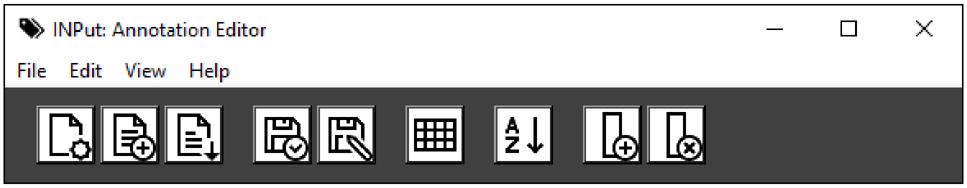
The Annotation Editor tool allows you to create custom plate layouts to store information about each well within a plate. Information about the content of each well can be typed directly into the Annotation Editor widow or pasted from a spreadsheet.

This chapter describes the structure and use of the INPut Annotation Editor tool.

INPut Annotation Editor Main Window

The following image shows the initial view of the INPut: Annotation Editor main window. The following table describes the icons.

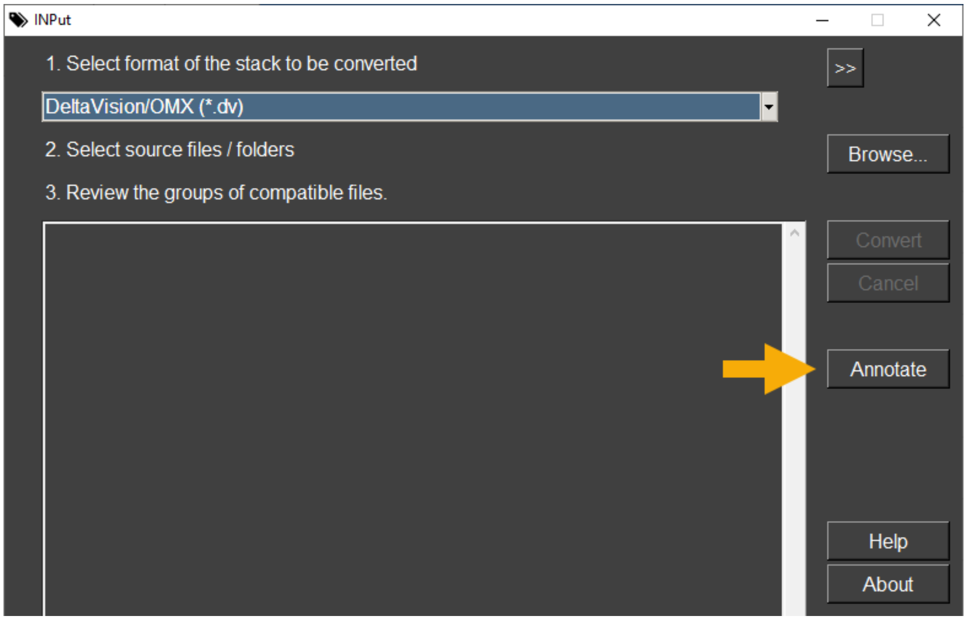


Icon	Description
	Create new annotation file by selecting a predefined sample format or entering a custom format (rows/columns) for the sample.
	Create new annotation based on an .xdce stack. **Note:** Plate format will be automatically recognized after the stack is selected.
	Open existing annotation file for review/edit.
	Save annotation file.
	Save annotation as a new file.
▦	Switch to plate view. **Note:** Icon visible in list view only.
☰	Switch to list view. **Note:** Icon visible in plate view only.
AZ↓	Sort list by row *(default)* or column.
	Add new annotation type selected from a list of predefined descriptors or custom named.
	Remove last annotation column added.

Accessing the INPut Annotation Editor

To access the INPut Annotation Editor:

1. In the INPut main window, click **Annotate**.



The INPut: Annotation Editor window opens.

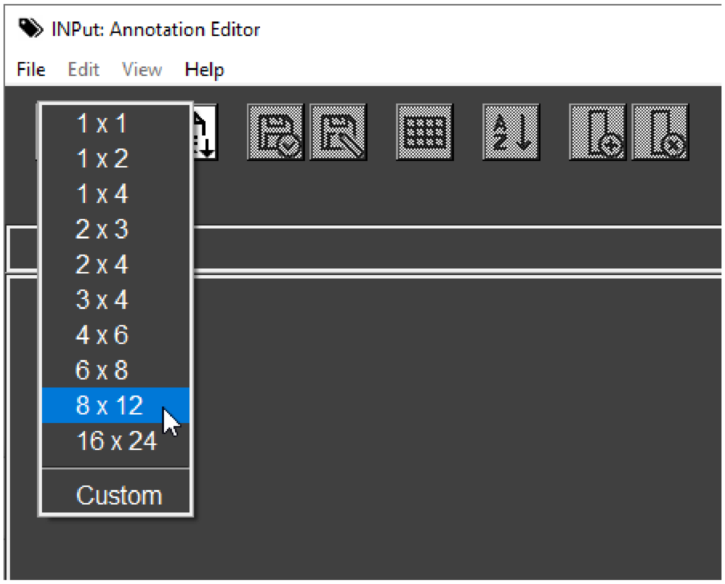
2. Navigate back to the INPut main window by clicking the X in the upper right corner of the INPut: Annotation Editor window.

Note: Save any open annotation file before closing the Annotation Editor.

INPut Annotation Editor Options

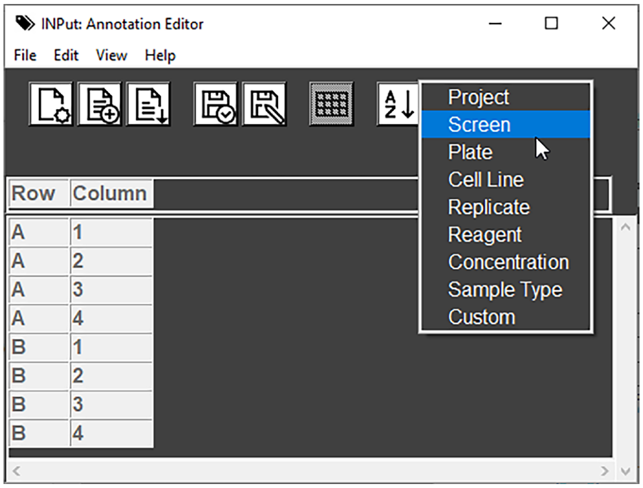
Predefined Sample Formats

The following images shows the specimen holder sample format choices available for new annotations.



Annotation Descriptors

The following image shows the annotation descriptors available when adding new columns. Custom columns are named **Custom** by default. You can rename them as needed.



Row	Column
A	1
A	2
A	3
A	4
B	1
B	2
B	3
B	4

Editing Content

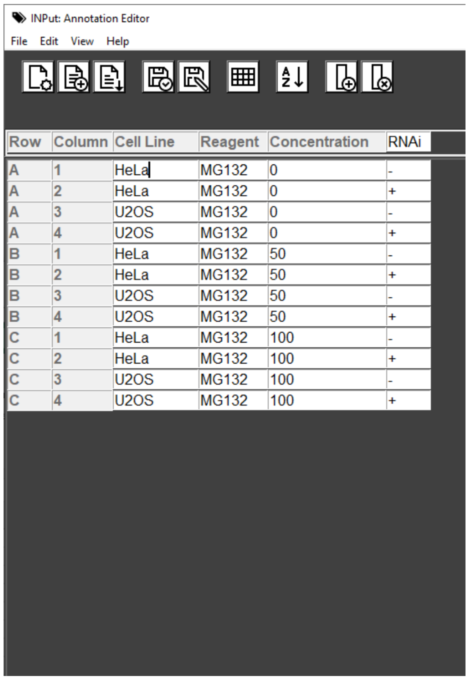
Annotations can be edited by directly typing content in the wells. Edits can also be performed for single or multiple wells according to the following table.

Edit	Action
Select multiple wells	Click and hold while dragging over the target wells.
Repeat information from the top selected well into multiple selected wells below	Right-click selected wells (including the source well) and choose **Fill down** from the drop-down list.
Copy highlighted regions (single or multiple columns)	Right-click selected wells and choose **Copy region** from the drop-down list.
	Use the keyboard shortcut **CTRL+C**.
Paste previously copied regions at cursor position	Right-click on one well and choose **Paste** from the drop-down list.
	Use the keyboard shortcut **CTRL+V**.

View

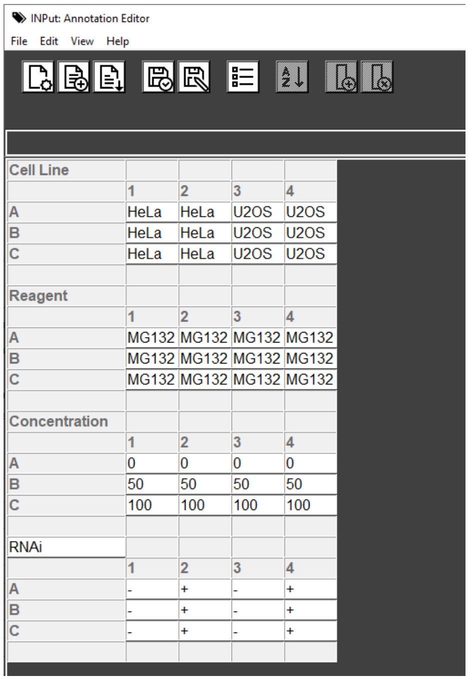
In list view, descriptors are shown as column headings. Sort the data in list view by clicking the sort icon (AZ↓) and choosing column or row.

In plate view, each descriptor is shown in a sample layout.



Row	Column	Cell Line	Reagent	Concentration	RNAi
A	1	HeLa	MG132	0	-
A	2	HeLa	MG132	0	+
A	3	U2OS	MG132	0	-
A	4	U2OS	MG132	0	+
B	1	HeLa	MG132	50	-
B	2	HeLa	MG132	50	+
B	3	U2OS	MG132	50	-
B	4	U2OS	MG132	50	+
C	1	HeLa	MG132	100	-
C	2	HeLa	MG132	100	+
C	3	U2OS	MG132	100	-
C	4	U2OS	MG132	100	+

List View (default)



Cell Line				
	1	2	3	4
A	HeLa	HeLa	U2OS	U2OS
B	HeLa	HeLa	U2OS	U2OS
C	HeLa	HeLa	U2OS	U2OS
Reagent				
	1	2	3	4
A	MG132	MG132	MG132	MG132
B	MG132	MG132	MG132	MG132
C	MG132	MG132	MG132	MG132
Concentration				
	1	2	3	4
A	0	0	0	0
B	50	50	50	50
C	100	100	100	100
RNAi				
	1	2	3	4
A	-	+	-	+
B	-	+	-	+
C	-	+	-	+

Plate View

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