

Spectronaut™ Tutorial Series

Conditions setup within the DIA analysis wizard

Annotate samples during the DIA analysis setup, in order to perform statistical testing in Post Analysis

Intro on Conditions setup in Spectronaut

❖ Scope of the Condition Setup

- The Experimental Condition Setup (specifying conditions, replicates) is useful for obtaining condition-wise metrics as well as for Statistical testing within the post-analysis processing steps.

1. Condition-wise metrics

These metrics summaries the data per condition and can be accessed in the Post-Analysis perspective. They include: Coefficients of variation and Binned Identifications.

2. Statistical testing in Spectronaut

The statistical testing for differential abundance is performed by paired Student's t-test. Please have in mind that the paired Student's t-test consist of matched pairs of experimental samples, or one group of samples that has been tested twice (such as before and after treatment).

❖ You will need:

- Meta-data on the experimental design
- Sample run files (DIA)
- Optionally: Conditions setup table file (Excel table)

Overview of the Conditions Setup panel during DIA Analysis set-up

Order number
Can be modified

Condition Identifier name

Fraction
Unique identifier
In case of off-line sample fractionation

Quantity Correction
A priori sample Quantity ratio

Color
Used for plots

Specify conditions in order to perform statistical tests during post analysis.

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correc...	Label	Color	File Name
1	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	1	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
2	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	2	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
3	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	3	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
4	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	4	1	Not Defined	Color [Gray]	liver-healthy-p1-...
5	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	5	1	Not Defined	Color [Gray]	liver-healthy-p1-...
6	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	6	1	Not Defined	Color [Gray]	liver-healthy-p1-...

Reference
used only for plots
In the Post Analysis:
Conditions panel

Condition ID name

Replicate ID number

Label
For display purposes
in plots
(a short-hand label
per condition)

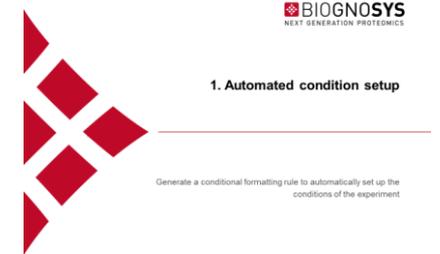
File name
unmodifiable

Default un-annotated conditions setup panel

Three ways to perform condition setup

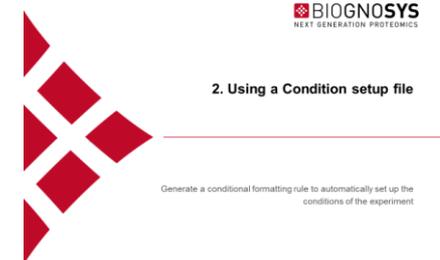
1. Automated condition setup

Generate a conditional formatting rule to automatically set up the conditions of the experiment



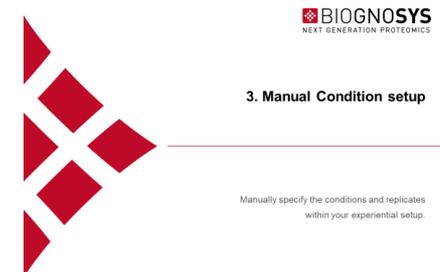
2. Condition setup by using a Condition setup table

Create a Condition setup table file that annotates all the samples in the experiment



3. Manual Condition setup

Specify the experimental conditions manually in the DIA analysis wizard



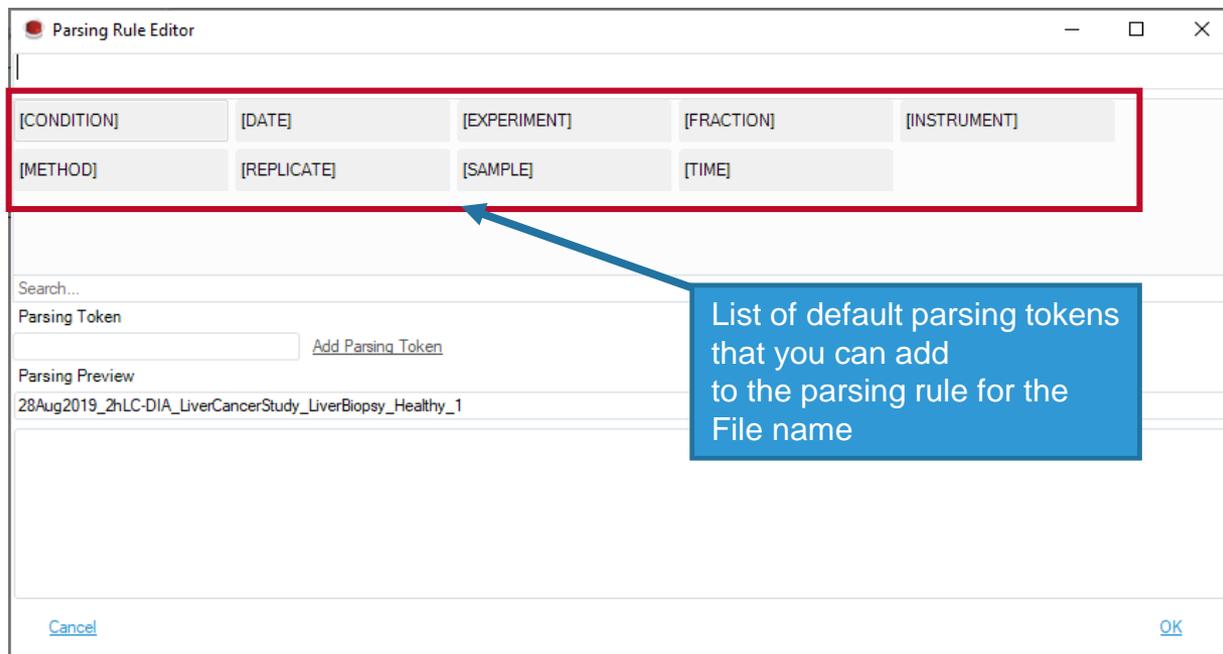
1. Automated condition setup

Generate a conditional formatting rule to automatically set up the conditions of the experiment directly from the file name

Automated condition setup: Specify a File name parsing schema

The screenshot displays the Spectronaut software interface. The top menu bar includes icons for Library, Analysis, Post Analysis, Report, QC, Pipeline, Databases, Settings (marked with a red circle and '1'), and About. Below the menu bar, the 'Global' settings tab is selected (marked with a red circle and '2'). The main settings area is divided into two panes. The left pane shows a tree view of settings categories: BGS Factory Settings, General, Directories, Plotting, and Reporting. The right pane displays various settings, including CPU Affinity (checkboxes for CPU 0-6), High Performance Mode (checked), Experiment Naming Strategy (Longest block of letters strategy), File Name Parsing Schema (highlighted with a red box and marked with a red circle and '3'), QC Plot History Length (100), Remove Common Filename Pattern (Never remove common pattern), Check for Updates on Startup (checked), and Protein UI Label ([Accession]). A blue callout box with an arrow points to the File Name Parsing Schema field, containing the text 'Click on the Parsing Rule to edit'.

Automated condition setup: Specify a Filename Parsing Rule



- ❖ A parsing rule is a set of instructions that inform Spectronaut what type of information you want to extract from the file name and how it is encoded.

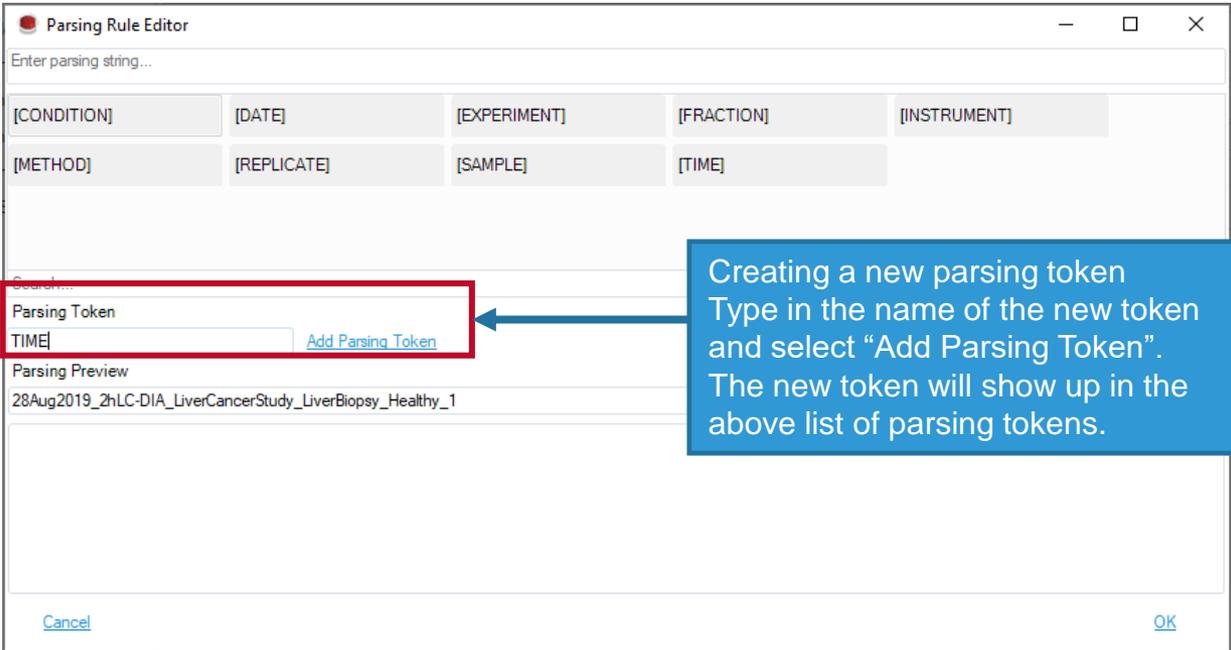
Automated condition setup: Use a Filename Parsing Rule

The screenshot shows the 'Parsing Rule Editor' window. At the top, a red box highlights the current parsing rule: [DATE] x _ [METHOD] x _ [EXPERIMENT] x _ [SAMPLE] x _ [CONDITION] x _ [REPLICATE] x. Below this, there are buttons for [FRACTION] and [INSTRUMENT]. A search bar is present with the text 'Search...'. Below the search bar is a 'Parsing Token' input field and an 'Add Parsing Token' button. The 'Parsing Preview' section shows a red box around the text: 28Aug2019_2hLC-DIA_LiverCancerStudy_LiverBiopsy_Healthy_1. Below the preview, the following fields are listed: DATE = 28Aug2019, METHOD = 2hLC-DIA, EXPERIMENT = LiverCancerStudy, SAMPLE = LiverBiopsy, CONDITION = Healthy, and REPLICATE = 1. At the bottom, there are 'Cancel' and 'OK' buttons. Two blue callout boxes with arrows point to the highlighted rule and the preview text.

Current Parsing Rule Created by selecting Parsing tokens to be used in the File name

Current Parsing Preview Of a file name Based on the specified Parsing rule

Automated condition setup: Adding a Parsing Token



The screenshot shows the 'Parsing Rule Editor' window. At the top, there is a text input field labeled 'Enter parsing string...'. Below this is a grid of parsing tokens: [CONDITION], [DATE], [EXPERIMENT], [FRACTION], [INSTRUMENT], [METHOD], [REPLICATE], [SAMPLE], and [TIME]. A search bar is located below the grid. In the search bar, the text 'Parsing Token' is entered, and the token '[TIME]' is displayed in a dropdown menu. A blue arrow points from a text box to the 'Add Parsing Token' button next to the search bar. Below the search bar is a 'Parsing Preview' section showing the example string '28Aug2019_2hLC-DIA_LiverCancerStudy_LiverBiopsy_Healthy_1'. At the bottom of the window are 'Cancel' and 'OK' buttons.

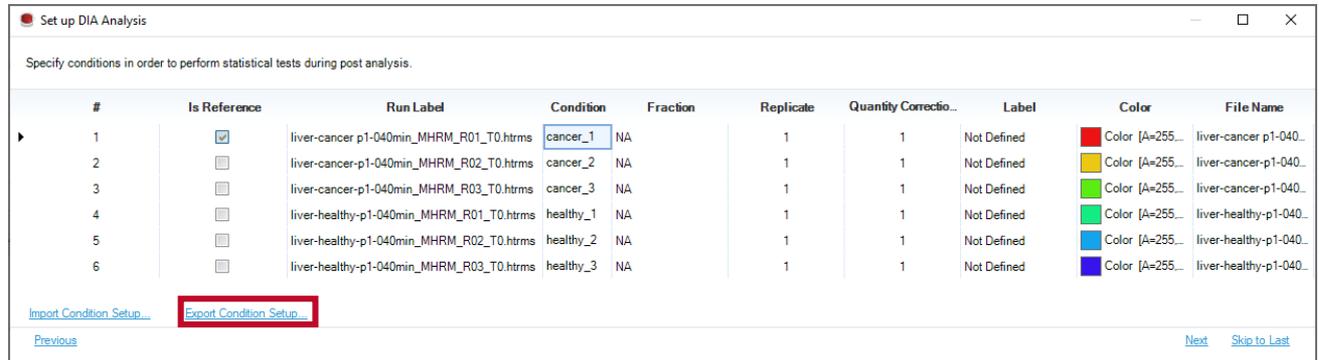
Creating a new parsing token
Type in the name of the new token
and select "Add Parsing Token".
The new token will show up in the
above list of parsing tokens.

2. Using a Condition setup file

Generate a conditional formatting rule to automatically set up the conditions of the experiment

Using a Condition setup file

1. Export Condition Setup...



2. Open the exported file

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correction Factor	Label	Color	File Name
1	TRUE	liver-cancer-p1-040min_MHRM_R01_T0.htrms	cancer_1	NA	1	1	Not Defined	#EC1313	liver-cancer-p1-040min_MHRM_R01_T0
2	FALSE	liver-cancer-p1-040min_MHRM_R02_T0.htrms	cancer_2	NA	1	1	Not Defined	#ECC813	liver-cancer-p1-040min_MHRM_R02_T0
3	FALSE	liver-cancer-p1-040min_MHRM_R03_T0.htrms	cancer_3	NA	1	1	Not Defined	#5BEC13	liver-cancer-p1-040min_MHRM_R03_T0
4	FALSE	liver-healthy-p1-040min_MHRM_R01_T0.htrms	healthy_1	NA	1	1	Not Defined	#13EC80	liver-healthy-p1-040min_MHRM_R01_T0
5	FALSE	liver-healthy-p1-040min_MHRM_R02_T0.htrms	healthy_2	NA	1	1	Not Defined	#13A4EC	liver-healthy-p1-040min_MHRM_R02_T0
6	FALSE	liver-healthy-p1-040min_MHRM_R03_T0.htrms	healthy_3	NA	1	1	Not Defined	#3713EC	liver-healthy-p1-040min_MHRM_R03_T0

3. Edit the corresponding columns and save

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correction Factor	Label	Color	File Name
2	TRUE	liver-cancer-p1-040min_MHRM_R01_T0.htrms	cancer	NA	1	1	Not Defined	#EC1313	liver-cancer-p1-040min_MHRM_R01_T0
3	FALSE	liver-cancer-p1-040min_MHRM_R02_T0.htrms	cancer	NA	2	1	Not Defined	#ECC813	liver-cancer-p1-040min_MHRM_R02_T0
4	FALSE	liver-cancer-p1-040min_MHRM_R03_T0.htrms	cancer	NA	3	1	Not Defined	#5BEC13	liver-cancer-p1-040min_MHRM_R03_T0
5	FALSE	liver-healthy-p1-040min_MHRM_R01_T0.htrms	healthy	NA	1	1	Not Defined	#13EC80	liver-healthy-p1-040min_MHRM_R01_T0
6	FALSE	liver-healthy-p1-040min_MHRM_R02_T0.htrms	healthy	NA	2	1	Not Defined	#13A4EC	liver-healthy-p1-040min_MHRM_R02_T0
7	FALSE	liver-healthy-p1-040min_MHRM_R03_T0.htrms	healthy	NA	3	1	Not Defined	#3713EC	liver-healthy-p1-040min_MHRM_R03_T0

Using a Condition setup file

4. Import Condition Setup...

Import Condition Setup

Source

Path: [Browse...](#)

Separator: auto

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correction Factor	Label	Color	File Name
1	TRUE	liver-cancer-p1-040min_MHRM_R01_T0.htms	cancer	NA	1	1	Not Defined	#EC1313	liver-cancer-p1-040min_MHRM_R01_T0
2	FALSE	liver-cancer-p1-040min_MHRM_R02_T0.htms	cancer	NA	2	1	Not Defined	#ECC813	liver-cancer-p1-040min_MHRM_R02_T0
3	FALSE	liver-cancer-p1-040min_MHRM_R03_T0.htms	cancer	NA	3	1	Not Defined	#5BEC13	liver-cancer-p1-040min_MHRM_R03_T0
4	FALSE	liver-healthy-p1-040min_MHRM_R01_T0.htms	healthy	NA	1	1	Not Defined	#13EC80	liver-healthy-p1-040min_MHRM_R01_T0
5	FALSE	liver-healthy-p1-040min_MHRM_R02_T0.htms	healthy	NA	2	1	Not Defined	#13A4EC	liver-healthy-p1-040min_MHRM_R02_T0
6	FALSE	liver-healthy-p1-040min_MHRM_R03_T0.htms	healthy	NA	3	1	Not Defined	#3713EC	liver-healthy-p1-040min_MHRM_R03_T0

[Cancel](#) [Load](#)

Result: Annotated Conditions setup panel

Set up DIA Analysis

Specify conditions in order to perform statistical tests during post analysis.

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correctio...	Label	Color	File Name
1	<input checked="" type="checkbox"/>	liver-cancer-p1-040min_MHRM_R01_T0.htms	cancer	NA	1	1	Not Defined	Color [A=255...	liver-cancer-p1-040...
2	<input checked="" type="checkbox"/>	liver-cancer-p1-040min_MHRM_R02_T0.htms	cancer	NA	2	1	Not Defined	Color [A=255...	liver-cancer-p1-040...
3	<input checked="" type="checkbox"/>	liver-cancer-p1-040min_MHRM_R03_T0.htms	cancer	NA	3	1	Not Defined	Color [A=255...	liver-cancer-p1-040...
4	<input type="checkbox"/>	liver-healthy-p1-040min_MHRM_R01_T0.htms	healthy	NA	1	1	Not Defined	Color [A=255...	liver-healthy-p1-040...
5	<input type="checkbox"/>	liver-healthy-p1-040min_MHRM_R02_T0.htms	healthy	NA	2	1	Not Defined	Color [A=255...	liver-healthy-p1-040...
6	<input type="checkbox"/>	liver-healthy-p1-040min_MHRM_R03_T0.htms	healthy	NA	3	1	Not Defined	Color [A=255...	liver-healthy-p1-040...

[Import Condition Setup...](#) [Export Condition Setup...](#)

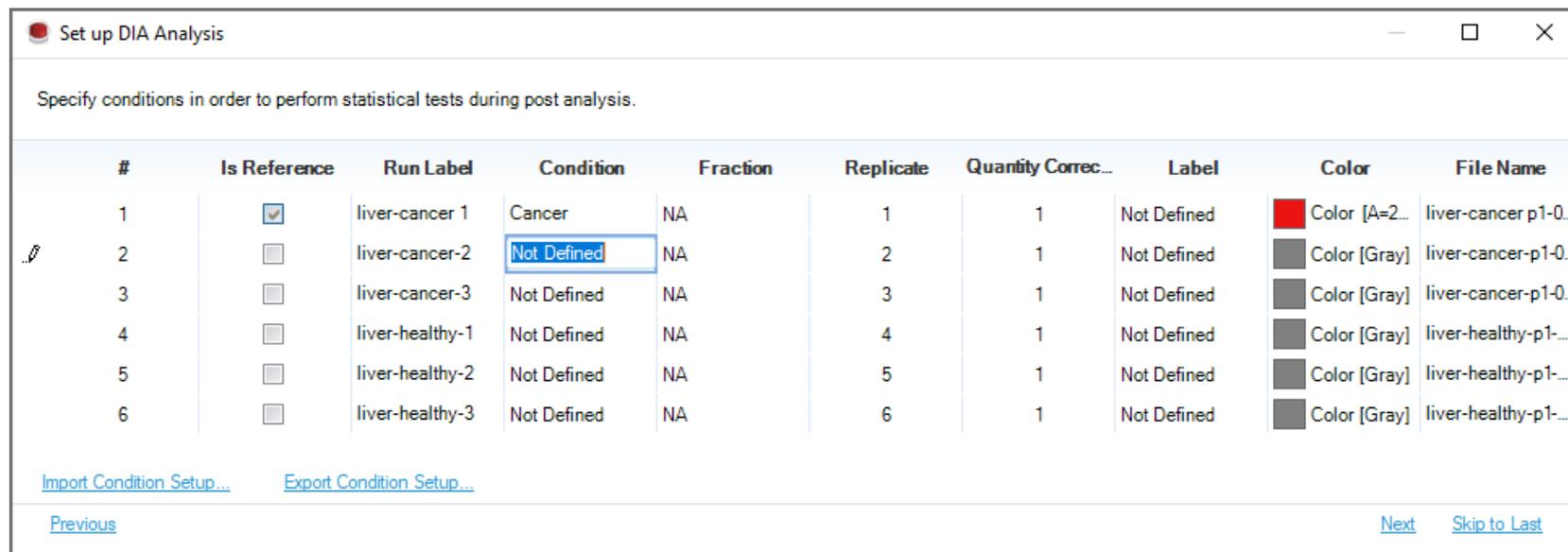
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3. Manual Condition setup

Manually specify the conditions and replicates
within your experiential setup.

The Conditions Setup panel during DIA Analysis set-up

Start manually annotating the table by clicking on a specific field



Set up DIA Analysis

Specify conditions in order to perform statistical tests during post analysis.

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correc...	Label	Color	File Name
1	<input checked="" type="checkbox"/>	liver-cancer 1	Cancer	NA	1	1	Not Defined	Color [A=2...	liver-cancer p1-0...
2	<input type="checkbox"/>	liver-cancer-2	Not Defined	NA	2	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
3	<input type="checkbox"/>	liver-cancer-3	Not Defined	NA	3	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
4	<input type="checkbox"/>	liver-healthy-1	Not Defined	NA	4	1	Not Defined	Color [Gray]	liver-healthy-p1-...
5	<input type="checkbox"/>	liver-healthy-2	Not Defined	NA	5	1	Not Defined	Color [Gray]	liver-healthy-p1-...
6	<input type="checkbox"/>	liver-healthy-3	Not Defined	NA	6	1	Not Defined	Color [Gray]	liver-healthy-p1-...

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Manually specify the conditions and replicates

Default
unannotated

Set up DIA Analysis

Specify conditions in order to perform statistical tests during post analysis.

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correc..	Label	Color	File Name
1	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	1	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
2	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	2	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
3	<input checked="" type="checkbox"/>	liver-cancer-p1-...	Not Defined	NA	3	1	Not Defined	Color [Gray]	liver-cancer-p1-0...
4	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	4	1	Not Defined	Color [Gray]	liver-healthy-p1-...
5	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	5	1	Not Defined	Color [Gray]	liver-healthy-p1-...
6	<input checked="" type="checkbox"/>	liver-healthy-p1-...	Not Defined	NA	6	1	Not Defined	Color [Gray]	liver-healthy-p1-...

[Import Condition Setup...](#) [Export Condition Setup...](#)

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After manual
Setup
Fully annotated

Set up DIA Analysis

Specify conditions in order to perform statistical tests during post analysis.

#	Is Reference	Run Label	Condition	Fraction	Replicate	Quantity Correc..	Label	Color	File Name
1	<input checked="" type="checkbox"/>	liver-cancer 1	Cancer	NA	1	1	C	Color [A=2...]	liver-cancer-p1-0...
3	<input checked="" type="checkbox"/>	liver-cancer-2	Cancer	NA	2	1	C	Color [A=2...]	liver-cancer-p1-0...
2	<input checked="" type="checkbox"/>	liver-cancer-3	Cancer	NA	3	1	C	Color [A=2...]	liver-cancer-p1-0...
4	<input type="checkbox"/>	liver-healthy-1	Healthy	NA	1	1	H	Color [A=2...]	liver-healthy-p1-...
5	<input type="checkbox"/>	liver-healthy-2	Healthy	NA	2	1	H	Color [A=2...]	liver-healthy-p1-...
6	<input type="checkbox"/>	liver-healthy-3	Healthy	NA	3	1	H	Color [A=2...]	liver-healthy-p1-...

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NB! Please have in mind that the paired Student's t-test has matched pairs of samples (for example: Condition1_replicate2 will be matched with Condition5_replicate2).



Additional information

For more information on DIA data analysis, please have a look at our additional resources

- ❖ Our Knowledge Base articles on DIA analysis:
<https://help.biognosys.com/help/search?keyword=DIA+analysis>
- ❖ Our youtube channel with webinars and tutorials:
<https://www.youtube.com/user/BiognosysAG>
- ❖ The Spectronaut User Manual
<https://biognosys.com/media.ashx/spectronautmanual.pdf>

Final Remarks

- ❖ This tutorial covers the use of experimental conditions setup as a brief guide.
- ❖ If you have further questions or suggestions, please do not hesitate to contact us at support@biognosys.com.
- ❖ Your feedback is very valuable for us.

Your Biognosys Team