

1. Turn on the computer
2. Login on windows with your Agendo Credentials: username – **Your email** and password – **Your email's password**
3. Open **IDEAS** Software and click on the **Start Analysis** option
4. On **File menu** select **Open** to open your files

(.rif = Raw image file; .cif = Compensated image file; .daf = Data analysis file)

5. Select an analysis template (.ast file)
6. Select the used channels and the **Image Gallery Display** properties will be applied to the images
7. Select the **Wizard** for you objective and follow the indicated steps
8. To add additional analysis use the histogram and dot plot icons or select **Guided Analysis**, choose **Build Blocks** and automatically create plots to the analysis
9. When the analysis is complete save the data file (.daf) and analysis template (.ast)
10. When the Wizard is complete, validate the regions by opening the control files using the saved analysis template and compensation matrix. Also verify the data in the statistics report under **Reports** and **Define statistics report**

Batch the analysis

- a. Complete you analysis and ensure that everything is correctly analysed
- b. On **Tools** menu select **Batch data files**, then select **Add Batch** and after **Add Files**. Select all files to be batch processed (you can batch .rif, .cif or .daf)
- c. If data is not already compensated select the **Compensation Matrix** to be applied
- d. Select the **Template** to apply to the batched data
- e. Choose **Submit Batches** and wait for the IDEAS to process data

Generate the report

- a. Reporting **Images**
 - i. Select the **Gallery Display** icon
 - ii. Select the channel to adjust and optimize the image contrast
 - iii. Create composite overlays
 - iv. Create Gallery view
 - v. Select images for you figure, save them as a population and display in the image gallery

- vi. Right-click and select **Copy / Save Gallery**. Edit display to include measurement tool feature values, object number, channel names and paste into reporting software
- f. Reporting **plots**
 - i. Right-click on plot and select **Graph Properties**
 - ii. Select **Statistics** icon and add / remove statistics
 - iii. Right-click on the plot and select **Copy / Save Graph**
 - iv. Save to clipboard and paste into reporting software

Histogram overlays

- v. In IDEAS
 1. Select **Tools / merge .cif**
 2. Ctrl-select the data files to overlay
 3. Open the merged .cif in the experiment template
 4. Create the population to overlay for each sample in the merged data file
 5. Create a histogram and in the histogram properties Ctrl-select each population to overlay
- vi. In Microsoft Office Word
 1. Copy / Paste each histogram into Word
 2. Select the top plot and set transparent color and repeat for each histogram making each below visible

Images

11. To merge **Raw Image Files**, on Tools menu, select **Merge .rif Files** and **add files** to merge
12. To save an **analysis template** file, from the File drop down option select Save as template file (.ast)

Compensation

1. To create a compensation matrix
 - a. Select **New matrix** when opening a .rif select **Compensation**, then **Create New Matrix**
 - b. Select the control files for compensation, which are the ones that are recorded as “-noBF”. Click **next** to load the files
 - c. Validate the compensation matrix, checking for high values, that will be highlighted in red and optimize the matrix, correcting first the biggest ones:

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- i. Double-click the red value in the matrix and the Matrix coefficient intensity plot is displayed
 - ii. Use a region tool to select a new positive population that excludes any outlier and assign the new population to its channel
 - iii. A new matrix is calculated and should be re-evaluated
 2. Click **Finish** and save the compensation matrix