First steps FTIR spectra	the same for MALDI and	2 Hierarchia	cal Clu
Clover MS Data Analysis Software Marrie: Study View Name: Study 1 Data management: Over MS Data Analysis Software Name: Study 1 Data management: Over MS Data Analysis Software New study Spectra files Studies St	▲ User Demo [userdemo@cloverbiosoft com] Premium It Clover Blooft ♦ Logott ● Main Construction ● Main Construction ● Main Construction ● Prediction Models ● Mabritec Central Identification ● Puela Delgado Manzano [27:07:2023 15:39:39 9:39:39 ● Samples: 521 Paula Delgado Manzano [27:07:2023 15:39:40 ● Puela Delgado Manzano [27:07:2023 15:39:40 15:39:40 ● Samples: 1829 ● Samples: 1829 ● New Peak Matrix 15:39:40 * New Peak Matrix ● Samples: 367 © 1 1; ● Samples: 367 © 2 1 1; ● Samples: 367 © 3 10:1 ● Samples: 367	Image: Signappin Signappi	Listering. Solution of your studies: Solution
Create Categories Choose an Experiment	Create a Peak Matrix	4. Select the experiment and peak matrix desire	the ed to be
		analysed.	
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How to perform Hierarchical Clustering **CLOVER MS Data Analysis Software**



Category name 🗕 1	Number of samples 🖨	Cluster 1 🌄 🖨	Cluster 2 🎦 🖨
Category I	34	17.65 %	82.35 %
Category II	47	93.62 %	6.38 %

ing Analysis	2.1 1. Appl
<text>er ver ver ver ver ver ver ver ver ver v</text>	 Clover MS Date of Home My profile Data management Upload files Spectra files New study Studies Projects Analysis R Bio Analysis Bio Analysis Chooose a for metrices Cut-off is with the O
Hierarchical Clustering - Select Peak Matrix	categories
Ramón y Cajal Please, select an experiment and then a peak matrix: Search Q E. hormaechei 64 files VIII vs VI By Mass jesus.jimenez@cloverbiosoft.com 07-08-2023 Image: Comparison of the second secon	Hierarchical Clustering result Set the cut-off to change the number of clusters of the desired categories. Category I Cut off Manual Optimal 12,094 12,094 Apply cut off

selecting in **Display** section above:

(i)



PCA

- If PCA was selected, dendrograms are realized with PCA components rather than peaks.

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Scale (or not) your data. oly (or not) PCA. alvsis Software 은 User Demo [userdemo@cloverbiosoft.com] Premium 🏛 Clover Biosoft 🕞 Logou lierarchical Clustering Study Ramón y Cajal 🔷 Experiment E. hormaechei 🔷 Peak Matrix VIII vs V Parameters Performs the Hierarchical Clustering analysis selecting between the different options Method for distance between Method for metric

method to calculate distance between clusters and s. Then, press Calculate.

s calculated Manually by default (A), but can be done Optimal algorithm taking into account the desired es (B).

btained. You can modify it manually or with the optimal algorithm taking into ac	Hierarchical Clustering result Set the cut-off to change the number of clusters obtained. You can modify it manually or with the optimal algorithm taking into account the desired categories.)
Category II	Known Categories Category I Category I Category I	
	Cut off Manual Optime! Based on the SDI×mC algorithm. Simpson's diversity index (SDI) and mean coherence (mC) for the categories selected at legend group 1 Applied cutoff: 14.391 SDI: 0.478 mC: 0.789 SDI x mC: 0.378	
	Calculate optimal cut off	



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😑 🏂 Clover MS Data Analysis Software

Home	Experiment
🚑 My profile	Study Study 1 Experimen
Data management	Name: Experiment 1
🚹 Upload files	Description: -
🖹 Spectra files	Created by: User Demo [use
New study	Preprocessed spectra
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4. Select the experin matrix desired to be

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cal Clustering	Analy	sis				
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	Dartial Lo	A Contract Squares Discriminant	1. C)pen (Classificatio	n section
traction with Principal A) if desired and cluster the by hierarchical clustering.	A category w peak matrix. relations betw categories, b scores assign	ill be assigned to each spectrum on the Then, the PLS-DA method finds the ween the intensity values and those eing the result a scatter plot with the ned to each spectrum.	2. (Hi	Click to erarch	o open nical Cluste	ring.
^{k matrix} Data Analysis Software	Analysis runs Ouser Demo [use	on one peak matrix rdemo@cloverbiosoft.com] Premium	፹ Clover Biosoft € Logo	out		
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1. Apply (or not) PCA.



Calculate.

4. Cut-off is calculated Manually by default (A), but can be done with the Optimal algorithm taking into account the desired categories (B).



7

Known Categories	Known Categories
Category I	Category I
Cut off	Cut off
Manual Optimal 3	Manual Optimal
12.094	Based on the SDI×mC algorithm. Simpson's div Applied cutoff: 14.391 SDI: 0.478
12.094	Calculate optimal cut off
Apply cut off	

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2. Scale (or not) your data.

3. Choose a method to calculate distance between clusters and for metrics. Then press



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Category name 🗕 1	Number of samples 🖨	Cluster 1 🌄 🖨	Cluster 2 🎦 🖨
Category I	34	17.65 %	82.35 %
Category II	47	93.62 %	6.38 %

This table shows the **percentage** of samples that belong to the same category in each of the clusters created by the Hierarchical Clustering. For example, there are 47 samples in Category II, of which 93.62%

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