

English







EXPLORING LIFE .....





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## **Analyzing Chromatograms in just 5 Steps**

## Setup Project

Setup your experiment by importing your GCxGC images into GasPedal and arrange replicates in groups.

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### Warp Images

Setup a warping strategy, use GasPedal's automatic warping, and review warpings.



## Detect and Quantify Spots

Do consistent spot detection across the whole project.



## Analyze Spot Profiles

Find interesting volatiles.



#### Present Results

Export results to other applications for presentation or further analysis.

Note:

If you have not already done so, please download the example data that we use in this guide at www.decodon.com/gaspedal-getting-started.html. Unpack the archive. It contains the following images and match map files for the warping:

Group	Image Files (two replicates per group)
DJP	DJP_01.cdf, DJP_02.cdf
CR	CR_01.cdf, CR_02.cdf
JRP	JRP_01.cdf, JRP_02.cdf







## Create a new data pool

- 1. Click on the button **'Change Pool'** and *navigate* to a folder where you want to store your new pool.
- 2. Right click and select 'Create Folder', then click on the new folder to *rename* it. Type in a name for your new pool.
- 3. Click on or and confirm the following dialog with .

## Create and open a new project

The 'Projects' dialog appears again showing an empty list of available projects.

- 1. Click on the swew button to create a new project.
- 2. Provide at least a name for your new project and confirm with or.
- 3. Select the new project from the list and press Open.

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GasPedal's Projects dialog.

## Set up groups for the images

We will now create the *three groups* for our experiment - one for each of the three replicate pairs. Let's first of all use the two groups already present – as you should see in the Light Table:

- 1. Right-click on the first group and choose 'Properties' from the context menu.
- 2. Replace the group's name by 'DJP'. If you want, choose another color and confirm with or.
- 3. Repeat steps 1 and 2 for the second group assigning the name 'CR'.



GasPedal 1.0.0 - My first project (C:\Users\Schorsch\.GasPedal\demonool)	
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1 Setup Project 🛞	
My first project	• DJP
Import your images into the pool.	
Open the Light Table to organize images in replicate groups.	
Groups (add a group)	
• DJP (no images, add)	
• Group 2 (no images, add)	
Use the Image Attributes clalog to assign samples, channels, and runs for your images.	Group 2
2 Warp Images 🛞	

GasPedal's Workflow and Light Table - showing an empty project.

Create a new group '*JRP*', by clicking on the link 'Add a new group...' in the Workflow.

## Add images to your groups

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GasPedal's image Import Manager.

- 1. In the Workflow click on the 'Add...' link next to the *DJP* group.
- 2. Click on Import Images and select the two images 'DJP\_01' and 'DJP\_02' from the *example data* by using the left mouse button in combination with the CTRL-Key. Click on Next.
- Now you can edit name and other descriptive data, set up Time Offset (2sec) and Modulation Period (6sec). Click on Next again to repeat this for the next image.

4. Click on **Finish** to save assignments and close the dialog.

Please import the sample GCxGC images 'CR\_01' and 'CR\_02' as well as 'JRP\_01' and 'JRP\_02' into their respective groups.









Two GCxGC images, overlaid into a dual channel image, not warped.

Two GCxGC images, overlaid into a dual channel image, after warping. Differences in spots are clearly visible.

## Define a warping strategy

- 1. Switch to the second step of the 'Workflow' called 'Warp Images'.
- 2. Click on the 'Warp Strategy...' link to open the 'Warping Strategy Manager'.
- 3. Select the 'Group Warping Strategy', set the warp modes to 'identical' within groups and to 'automatic' between groups, and confirm the default settings with OK.

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Warp Mode Within Groups      Judentical  Warp Mode Between Groups	
Automatic	~
	OK Cancel

GasPedal's Warping Strategy manager.

4. Open the **'Warping Setup'** by clicking on the link 'Setup direct warpings' in the Workflow. It should look similar to the following image:







Warping Setup window after applying the Group Warping Strategy.

## Use GasPedal's Automatic Warping

- 1. Click on the 'Job Manager' link.
- 2. Just click the 'Start' button in the 'Job Manager'. It will monitor and perform all automatic warping jobs.

## **Review Warpings**

Review dire Double click or	ct warpings n any pair in the lis	t to ope	n it in the	Dual View.
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DJP_01	JRP_01	٢	ок	

Review direct warpings (Step 2 of the Workflow).

- Double-click on a row in the table below 'Review Direct Warpings' where the symbol looks like .
- 2. A **'Dual View'** window opens and shows the overlay of the respective two GCxGC images.
- 3. Please also check if the image background has been hidden by using the 'Show/Hide Background' button ( ) in the tool bar.





#### 🝗 Note:

To work with match vectors, select the Match Vector Tool (top-most button in the left vertical tool panel of the Dual View).

To ease the use you might want to switch on "Snap Match Vectors To Spots". This can be done at 'Tools' > 'Options' > 'GasPedal' > 'Match Vectors'. Temporarily switch it off with CTRL key.

If there are regions *where match vectors seem to be incorrect*, you can set and change all match vectors manually:

- Select a match vector just left-click on the vector.
- Select multiple match vectors drag a rectangle with the mouse (keep the left mouse button pressed). All match vectors in that rectangle will be selected.
- Delete match vectors right click on a match vector and select 'Delete'.
- Set a new match vector first click on the spot in the orange, then on the corresponding spot on the blue image. Use the 'Spectra comparison' (right click on vector) to compare the nearest spectra at start and end point of the match vector. Use TIC, spectra and additional database information for best results.
- Change a match vector drag one end of the match vector to change its position.

Note: You can undo match vector operations by clicking the 'Undo' button .

Click on the <sup>O Warp</sup> button to apply your new vectors. You can iteratively add, correct or delete match vectors until the result is satisfying.





## **Detect and Quantify Spots**

## Generate a fusion image

- 1. Switch to the workflow step called 'Detect and Quantify Spots' and click on the 'Fuse all images' link to open the 'Image Fusion' dialog.
- 2. Click on Fuse to use the default settings and let GasPedal create a new image.

## Detect spots on the fusion image

Click on the link 'Detect Spots on Fused Image...' in the Workflow. After detection is finished, the fused image will appear with detected spot boundaries.

## Edit spots on the fusion

- 1. Open the new fusion image by clicking on 'Open Fused Image using Union...'.
- 2. A 'Dual View' window opens showing the single view of the fused image only.

Note:

Select the 'Spot Editing Tool' (fourth button in the left vertical tool panel in the Dual View) to edit spots. We strongly recommend to edit spots ON

THE FUSION IMAGE ONLY.

#### Splitting a Spot



Two spots, detected as one and split. (Click once into the center of the spot that was not detected separately).

#### Adding a new Spot



Image region before / after adding a spot. (Just click on the center of an undetected spot to add it.)

#### **Merging Spots**



Example for joined spots. (Drag a line from one spot center to the other.)









#### **Move Spot Markers**

Spot markers can be moved by dragging with the left mouse button.

#### **Delete Spot Markers**

To delete a spot marker you can *right-click* on it.



#### **Remove Spots**

- 1. Activate the 'Spot Selection Tool' and select one spot or a group of spots.
- 2. Right-click and select 'Cancel Spot'.

To see all canceled spots with dotted line spot boundaries, select 'Spots' > 'Show Canceled Spots' from the Dual View's menu.

## **Transfer Spots**

- 1. In the Workflow step 'Detect and Quantify Spots' click on the link 'Transfer Spots from Fused Image...'.
- 2. The **'Transfer Spots'** dialog opens. Confirm the default settings with **w**.



Spot boundaries after spot transfer. Choose the 'Image Regions' link in the workflow to open this view. The essential spot pattern is the same on all images, making 100% spot matching possible.





## **Analyze Spot Profiles**

## Filtering for Interesting volatiles

#### Note: -

The Quantitation Table is synchronized with all other views of GasPedal: Selecting a row in the table will select the spot e. g. in the Dual View.



Filter Dialog

Let's show spots with a difference of factor 2 or bigger:

- 1. Open the 'Quantitation Table' and switch to the 'Statistics' tab.
- 2. Look for the ratio column in the CR group 'Ratio mean % volume CR / mean % volume DJP'.
- 3. Click on the top part of the column header labeled 'Filter'.
- 4. Insert the filter borders 'Show values from' '0.5' and 'to' '2'.
- 5. The 'Filter active' will be checked

automatically, additionally check the box 'Negated'. The latter will change the filter dialog's text to 'Hide values from' '0.5' and 'to' '2'. Confirm with  $\square$ .

6. Open the **'Dual View'** with the two images 'DJP\_01' and 'CR\_01'. Only spots meeting our criteria will still have spot boundaries.

## Advanced Statistical Analysis

GasPedal includes advanced multivariate statistics for the analysis of 2D GcxGC images, among them:

- Heat map display of spot profiles
- Various methods of clustering including Hierarchical Clustering, k-means / medians Clustering
- Numerous variations of t-Test
- Analysis of Variance (ANOVA)
- Non-Parametric tests, incl. Wilcoxon / Mann-Whitney, Kruskal-Wallis, Mack-Skillings, and Fisher-Exact test
- Pavlidis Template Matching for defined contents of volatiles in the image (PTM)
- Principal Component Analysis (PCA)

#### Note:-

The example project is actually too small to rely on results but still helps to understand how statistical analysis in GasPedal works.





#### Getting a high level overview of spot data - heat map



Heat map for the example project.

#### Discovering patterns in content profiles

1. Press the 🐱 button in the toolbar. Choose 'Gene Tree', 'Euclidian Distance' and 'Complete Linkage'.

#### 2. To confirm press or.

The tree represents similarities between spot profiles.

If you do this for the 'Sample Tree', all replicates of the same sample should appear in the same cluster (subtree). If not, this indicates that there are outliers in your project - due to e.g. experimental problems the image is very different from the other replicates. You should consider removing this image from your project. You can control visibility of images by clicking on in GasPedal's main tool bar. After changes, please close and re-open Analysis.

In the workflow step 'Analyze Spot Profiles' click the link 'Analysis'.

The *legend* across the top shows the color code for spot intensities. Each column contains all spot intensities of one image. Each row shows the volume of one spot (volatile) matching over all different GCxGC images in your project. The normalized data is standardized by default.



Hierarchical Clustering for Spot Profiles.





#### Finding different spots of volatiles: Statistical Tests

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t-Test parameters.

- 1. Click on the **TEST** button.
- 2. Select 'p-values based on permutations' (first red arrow).
- 3. In the 'False discovery control' part of the dialog define limits for the number of false positive spots in the result set using the 'number of false positive genes should not exceed' or 'proportion of false positive genes should not exceed' (second red arrow).
- 4. Confirm with or to see a heatmap display of significant spots only.

Note: -

There is a lot more to know about statistical analysis in GasPedal. Please read on in the TMeV manual or contact us at support@decodon.com for a free live web session.







#### Create interactive HTML-Reports

Choose from one of the following reports from the 'Reports' menu:





General information about samples, groups, images, etc.

Detailed information for selected / List all labels or those marked spots. Click on the *barchart / id* for selected / marked to select the spot in GasPedal and open a spots incl. data. detailed report for it.

#### Note:

To mark spots choose 'Mark' > 'Mark selected spots' in the Quantitation Table or right click and select 'Mark spot' in the Dual View.

Export Tables to spreadsheet application (MS Excel, Libre Office Calc,...)

- 1. Open the 'Quantitation Table'.
- 2. Choose 'Export' > 'Export Spreadsheet' from the table's menu.

Export Image Views into presentation application (MS PowerPoint, Libre Office Impress, )

- 1. Open the 'Dual View' for any image pair.
- 2. Choose 'Export' > 'Export to Presentation Slide' and confirm with OK.
- 3. Follow the instructions on the screen to create the presentation.





## Where Can I Find Out More about GasPedal?

The easiest way to learn more is going to <u>http://www.decodon.com</u> or contacting us per Email to <u>support@decodon.com</u>. We will be happy to answer your questions or to show you more in a live web demo.

#### Your DECODON Team



If you have any comment or suggestion regarding this 'Getting Started with GasPedal Guide' or any other of our documents we welcome hearing from you.







#### Request you personal demo today!

**Want to know more?** Contact us today to arrange your personal live web demo. All you need is a web browser and a phone – an expert will show you how you can apply GasPedal to your specific GCxGC analysis needs.

You can download an evaluation version of GasPedal from www.decodon.com. Your questions and remarks are welcome, call us at +49 3834 515230 or send an email to info@decodon.com.

#### Technical data:

**Supported GCxGC data formats: GasPedal** supports the most important data file formats CDF and mzML.

**Supported image file formats: GasPedal** supports virtually all calibrated and uncalibrated image file formats on the market today, including tiff (8 bit, 12 bit, 16 bit), JPEG, BMP, GIF, PNG, PNM.

Supported Operating Systems (\*recommended): GasPedal runs on Windows 7\* / 8 / 10 at 32 or 64\* bit, Mac OS X 10.6 (Snow Leopard) / 10.7 (Lion) / 10.8\* (Mountain Lion) and most flavours of 32 bit or 64 bit\* Linux.

#### Hardware Requirements:

Minimum Hardware: Pentium III, 800 MHz, 4 GB RAM or Intel-based Mac, 4 GB Recommended Hardware: Dual or Quad Core Processor with 2 GHz or better, 8+ GB RAM, in combination with a 64-Bit operating system

# DECODON

DECODON GmbH Walther-Rathenau-Str. 49a 17489 Greifswald, Germany

www.decodon.com info@decodon.com phone: +49(0)3834 515230 fax: +49(0)3834 515239

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