Case study 2: microbial culture natural product dataset

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Table of contents

1 Overview

In this case study, we perform differential abundance analysis on a MALDI-TIMS-MS1 natural products dataset of bacterial-fungal co-culture. First we processed the data in TIMSImaging, followed by a non-spaital Wilcoxon rank-sum test to get results comparable to the literature. Then we explore further using mixed effect models in R, contrast 1) non-spatial and spatial modeling, 2) processing with and without ion mobility. The purpose of this case study is to show that TIMSImaging creates informative features, which enables selecting precursors associated with specific regions as prioritized targets in the following MS2 imaging experiment design.

The dataset is from Laura Sanchez Lab and available on MASSIVE(MSV000097837).
Shepherd, R. A., G. T. Luu and L. M. Sanchez (2025). "MALDI-TIMS-MS(2) Imaging and Annotation of Natural Products in Fungal-Bacterial Coculture." Anal Chem 97(35): 18867-18872.

2 Data processing in TIMSImaging and rank-sum test

2.1 Introcution

In this part, we process the MALDI-TIMS-MS1 dataset of bacterial-fungal co-culture, then find features with high intensity in the microbioal region, and export the processed data in the open imzML format.

```
import timsimaging

# enable visualization in the Jupyter notebook
from bokeh.io import show, output_notebook
output_notebook()
# disable FutureWarning
import warnings
warnings.filterwarnings('ignore')
```

```
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```
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```

2.1.1 Load MALDI-TIMS-TOF raw data

```
bruker_d_folder_name = r"D:\dataset\Laura_Gordon\250321_JB182_Pen12.d"
dataset = timsimaging.spectrum.MSIDataset(bruker_d_folder_name)
dataset
```

```
MSIDataset with 12173 pixels
        mz range: 99.999-1100.005
        mobility range: 0.400-1.800
```

2.2 Understanding experiment setting

As the TIC image shows, there are 4 regions: *G.arilaitensis* + *P.solitum* co-culuture(top), *P.solitum*(bottom left), *G.arilaitensis*(bottom middle) and the media/matrix(bottom right)

```
dataset.image()
```

```
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```
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```

2.2.1 Peak processing

The first step is to extract features. Due to the heterogeneity of regions, we set `sampling_ratio` to 1 so that all pixels are used for mean spectrum calculation.

```
results = dataset.process(sampling_ratio=1, frequency_threshold=0.05, intensity_threshold=0.0
```

```
Computing mean spectrum...
Traversing graph...
Finding local maxima...
Summarizing...
```

Here we get 784 features in total, each of them corresponds to an ion image.

```
table, _ = timsimaging.plotting.feature_list(results["peak_list"])
```

```
show(table)
```

```
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```

```
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```

```
show(results["viz"])
```

```
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```

2.2.2 Differential analysis using Wilcoxon rank-sum test

For precursor targets in following MS2 experiments, we want to select ions relevant to cell culture and exclude matrix ions. Specifically, a desired precursor should be associate with the microbioal culture region, with minimum intensity in the matrix region. We referred the paper below, which uses a non-spatial Wilcoxon rank-sum test.
Rauser, S., C. Marquardt, B. Balluff, S.-O. Deininger, C. Albers, E. Belau, R. Hartmer, D. Suckau, K. Specht, M. P. Ebert, M. Schmitt, M. Aubele, H. Höfler and A. Walch (2010). "Classification of HER2 Receptor Status in Breast Cancer Tissues by MALDI Imaging Mass Spectrometry." Journal of Proteome Research 9(4): 1854-1863.

```
import numpy as np
from scipy.stats import mannwhitneyu
```

```
intensity_array = results["intensity_array"]
```

Here we use the same ROI setting as the original paper: the media/matrix region as group 1, while all other region as group 2, treat pixels as samples and test if there is a signifcant intensity difference between two groups.

```
dataset.set_ROI("matrix", xmin=200, ymin=100)
matrix = intensity_array.loc[dataset.rois["matrix"]]
cultures = intensity_array.loc[np.setdiff1d(intensity_array.index, dataset.rois["matrix"])]
```

Apply RMS normalization to each group before the test:

```
# RMS normalization
rms = np.sqrt(np.mean(np.square(intensity_array), axis=1))
intensity_array_norm = intensity_array.div(rms, axis=0)

matrix = intensity_array_norm.loc[dataset.rois["matrix"]]
cultures = intensity_array_norm.loc[np.setdiff1d(intensity_array.index, dataset.rois["matrix"
```

Compute the statistics and fold change.

```
stat, p = mannwhitneyu(cultures, matrix)
stats = results["peak_list"].copy()
stats["culture_mean"] = np.mean(cultures, axis=0).to_numpy()
stats["matrix_mean"] = np.mean(matrix, axis=0).to_numpy()
stats["log2foldchange"] = np.log2(np.mean(cultures, axis=0)/np.mean(matrix, axis=0)).to_numpy
stats["neg_log10_pvalue"] = -np.log10(p)
stats
```

	mz_values	mobility_values	total_intensity	culture_mean	matrix_mean	log2foldchange	neg
26	172.040229	0.627523	2306.307730	0.059480	0.068297	-0.199415	3.8
41	187.055019	0.614947	1350.618829	0.042400	0.027598	0.619482	13.
45	189.070660	0.618844	3353.983734	0.101712	0.074469	0.449772	13.
47	190.050757	0.964792	884.310605	0.025086	0.027849	-0.150782	4.6
75	201.059263	0.651327	1019.046168	0.033806	0.020592	0.715197	15.
...	...	...	...	...	...	...	...
6602	1079.112242	1.511486	4353.308634	0.138639	0.126743	0.129429	1.3
6614	1088.066520	1.557763	1397.371889	0.044244	0.015218	1.539681	62.
6618	1088.067282	1.464957	1019.910129	0.033066	0.011269	1.553035	51.
6623	1089.070123	1.484550	835.687998	0.026375	0.009408	1.487155	49.
6636	1094.083050	1.511502	3821.645773	0.119431	0.066028	0.855034	45.

2.2.3 Visualize results in a volcano plot

```
from bokeh.plotting import figure,show
from bokeh.models import ColumnDataSource, HoverTool
from bokeh.transform import factor_cmap
```

Now we can make a volcano plot. The features to be selected is on the top right, that present high signal-to-noise ratio and high significance score.

```
f = figure(
    title="Differential abundance",
    match_aspect=True,
    toolbar_location="right",
    x_axis_label="log2foldchange",
    y_axis_label="neg_log10_pvalue",
    x_range=(-10,10)
)
source = ColumnDataSource(stats)
volcano = f.scatter(x="log2foldchange",
          y="neg_log10_pvalue",
          source = source)
hover = HoverTool(renderers=[volcano], tooltips=[
            ("m/z", "@mz_values{0.0000}"),
            ("1/K0", "@mobility_values{0.0000}"),
            ("index", "$index"),
        ],)
```

```
f.add_tools(hover)
show(f)
```

```
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```

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```

2.2.4 Corroborate differential features in literature

Here is the features reported in the literature, using the "find discriminating" funtion in SCiLS Lab. We retrieve them in the feature list and look where they are in the volcano plot.

```
target = np.array([
    417.2348,
    425.2625,
    428.2594,
    475.2532,
    532.3086,
    588.3737,
    615.3198,
    627.3479,
    655.2726,
])
```

All those features were detected by TIMSImaging, with minimal m/z differences.

```
indices = []
for m in target:
    mz_tol = m * 50 * 1e-6
    index = np.nonzero(stats['mz_values'].between(m-mz_tol, m+mz_tol))[0]
    indices.append(index[0])
stats.iloc[indices]
```

	mz_values	mobility_values	total_intensity	culture_mean	matrix_mean	log2foldchange	neg_
1757	417.245748	0.948254	2343.955229	0.079047	0.000924	6.418199	152.6
1860	425.261619	0.975195	3731.217038	0.117055	0.002412	5.600804	129.7
1900	428.261729	0.960216	3326.308223	0.111457	0.001042	6.741408	172.4
2509	475.254336	1.006788	2615.506695	0.085422	0.002050	5.381051	154.7
3280	532.309080	1.081939	1673.549659	0.056178	0.004467	3.652642	102.5

	mz_values	mobility_values	total_intensity	culture_mean	matrix_mean	log2foldchange	neg_
4028	588.373038	1.151386	1445.407788	0.048771	0.001496	5.026967	95.4
4378	615.320932	1.144504	1024.922287	0.036958	0.001301	4.828456	115.
4544	627.353146	1.177115	958.086092	0.031236	0.001508	4.372415	132.
4843	655.273642	1.272451	2910.554342	0.091324	0.002353	5.278412	140.

```
stats["target"] = "No"
stats["target"].iloc[indices] = "Yes"
```

Now we can view the position of these precursors in the volcano plot:

```
#func = lambda df: (df.log2foldchange>4)&(df.neg_log10_pvalue>50)&(df.matrix_mean<1000)
#source.add(np.where(func(stats), "Orange", "Steelblue"), name="color")
source = ColumnDataSource(stats)
a = figure(
    title="Differential abundance",
    match_aspect=True,
    toolbar_location="right",
    x_axis_label="log2foldchange",
    y_axis_label="neg_log10_pvalue",
    x_range=(-10,10)
)
volc = a.scatter(x="log2foldchange",
          y="neg_log10_pvalue",

          color = factor_cmap("target", ["Steelblue", "Orange"], ["No", "Yes"]),
          #color = 'color',
          source = source)
hover = HoverTool(renderers=[volcano], tooltips=[
            ("m/z", "@mz_values{0.0000}"),
            ("1/K0", "@mobility_values{0.0000}"),
            ("index", "$index"),
        ],)
a.add_tools(hover)
show(a)
```

```
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```

```
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```

Similar to the internal function in SCiLS, Wilcoxon rank-sum test outputs significant p values for these features. Deisotoping and applying an intensity filtration would result a more concrete precursor candidate list.

2.2.5 Export the processed data in imzML format

Finally, export processed data for further differential analysis in R.

```
timsimaging.spectrum.export_imzML(dataset, path=r"D:\dataset\laura_gordon", peaks=results)
```

3 Differential abundance analysis in R

3.1 Introduction

In this part, we continue to perform differential abundance analysis on the processed imzML from Section 1 to find precursors that present high intensity in the microbial culture region. First we apply a non-spatial linear fixed effects model, then explore modeling with spatial correlation. In addition, we also contrast differential analysis on features with and without ion mobility.

3.1.1 Package setup

We will use `Cardinal` for data import and normalization, `spaMM` for differential analysis and `ggplot2` for visualization. If the packages are not yet installed, run following code in a R session:

```
if (!require("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("Cardinal")
package.install("spaMM")
package.install("ggplot2")
```

Then load the packages:

```
library(Cardinal)
library(spaMM)
library(ggplot2)
```

3.1.2 Load processed dataset

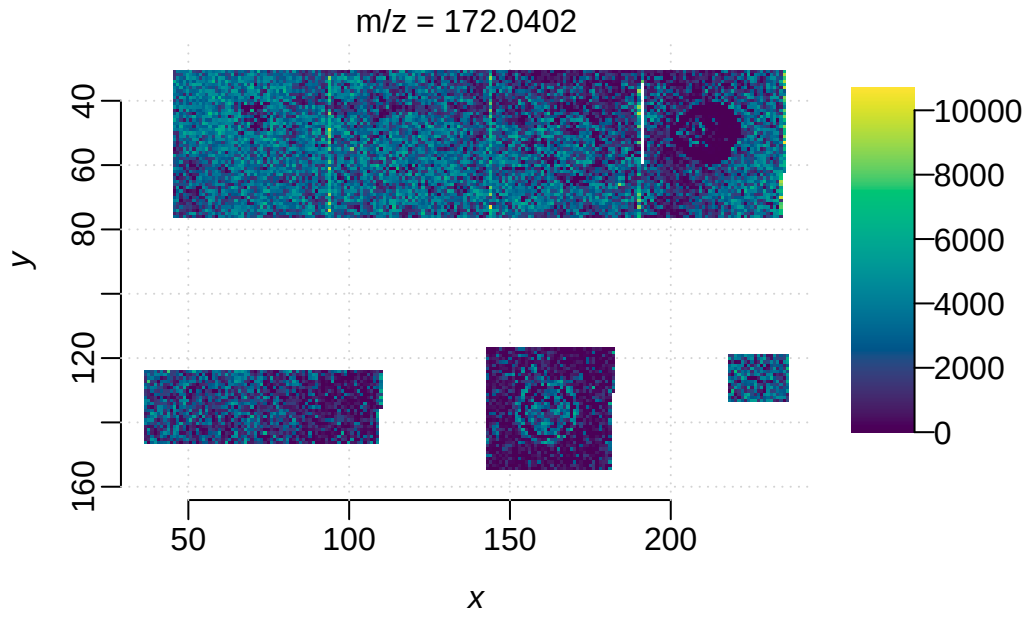
First we load the processd imzML from TIMSImaging into Cardinal:

```
msa <- readMSIData("D:\\dataset\\Laura_Gordon\\laura_gordon.imzML", as="MSImagingArrays", ext
mse <- convertMSImagingArrays2Experiment(msa)
mse
```

```
MSImagingExperiment with 781 features and 12173 spectra
spectraData(1): intensity
featureData(1): mz
pixelData(3): x, y, run
coord(2): x = 37...236, y = 31...154
runNames(1): laura_gordon
experimentData(5): spectrumType, spectrumRepresentation, lineScanSequence, scanType, lineScan
mass range:  172.0402 to 1094.0830
centroided: TRUE
```

check the regions:

```
image(mse)
```



3.1.3 Data preparation

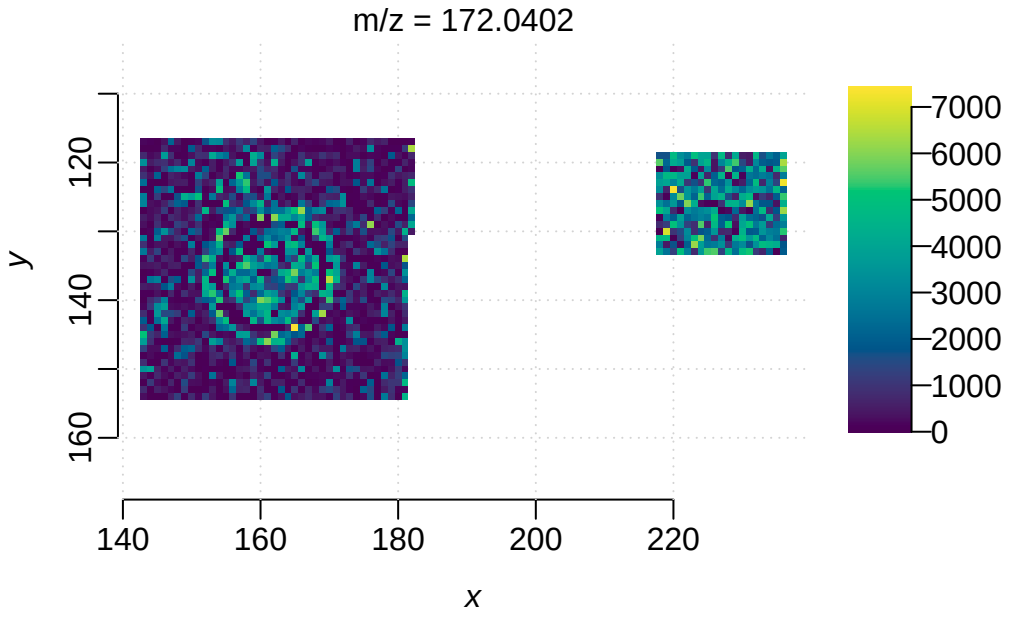
Then we label 3 microbioal regions as a group(`culture`) and the matrix region as the other group(`media`), which is consistent with the literature.

```
coords <- coord(mse)
pixel_label <- ifelse(coords$x > 200 & coords$y > 100, "media", "culture")
pData(mse)$label <- factor(pixel_label)
pData(mse)
```

```
PositionDataFrame with 12173 rows and 4 columns
                       x         y          run    label
               <numeric> <numeric>     <factor> <factor>
spectrum=1            46        31 laura_gordon  culture
spectrum=2            47        31 laura_gordon  culture
spectrum=3            48        31 laura_gordon  culture
spectrum=4            49        31 laura_gordon  culture
spectrum=5            50        31 laura_gordon  culture
...                  ...       ...          ...      ...
spectrum=12169       232       133 laura_gordon    media
spectrum=12170       233       133 laura_gordon    media
spectrum=12171       234       133 laura_gordon    media
spectrum=12172       235       133 laura_gordon    media
spectrum=12173       236       133 laura_gordon    media
coord(2): x, y
run(1): run
```

To reduce the complexity of spatial modeling, we just compare the *G.arilaitensis*(bottom middle) region(`culture`) and the media/matrix region(`media`). For targets in following MS2 acquisition, we want to exclude matrix/media ions and select precursors spatially associated with the microbioal culture region. Specifically, a desired precursor should present high intensity in the microbioal culture region and minimum intensity in the matrix/media region.

```
mse_subset <- subsetPixels(mse, x>120, y>100)
image(mse_subset)
```

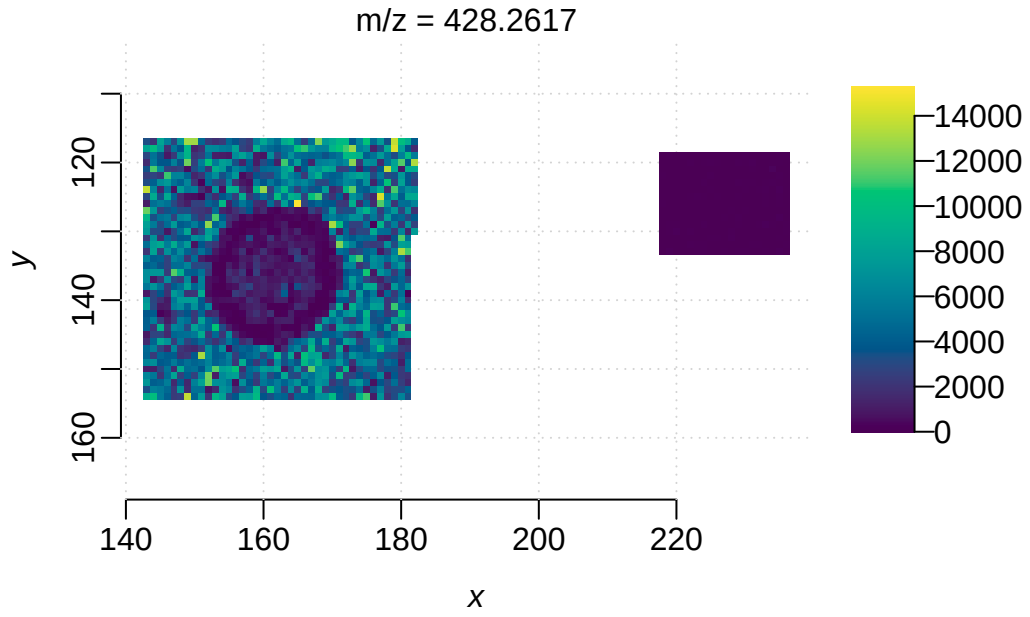


First we try differential abundance analysis on a feature(mz=428.26) reported in the paper. Extract the ion image as a data frame, with pixels labeled as either in 'media' or 'culture' region.

```
m <- 428.26
i <- findInterval(m, mz(mse_subset))+1
intensity <- spectraData(mse_subset)$intensity[i,]
df <- data.frame(
  intensity = intensity,
  label = pData(mse_subset)$label,
  x = pData(mse_subset)$x,
  y = pData(mse_subset)$y
)
# set the media group as the baseline
df$label <- relevel(df$label, ref="media")
```

Plot its ion image:

```
image(mse_subset, i=i)
```



3.1.4 Fitting a non-spatial model

Now we fit a non-spatial linear fixed effect model on this feature. Let s be a pixel position, k be a group label, then the intensity is a linear response to group label:

$$y_s = \mu_k + \varepsilon_s$$

where y_s is the intensity at s, the fixed effect μ_k is the mean intensity in group k, ε_s is the random error.

```
model <- fitme(
    intensity ~ label,
    data = df,
    method = "REML",
)
summary.HLfit(model, details=c(p_value="Wald"))
```

```
formula: intensity ~ label
Estimation of fixed effects by ML.
Estimation of phi by 'outer' REML, maximizing restricted logL.
family: gaussian( link = identity )
 ------------ Fixed effects (beta) ------------
```

```
             Estimate Cond. SE t-value p-value
(Intercept)     35.59    162.1  0.2195  0.8263
labelculture  4070.59    176.9 23.0093  0.0000
 -------------- Residual variance  ------------
phi estimate was 7492370
 ------------- Likelihood values  -------------
                        logLik
logL               : -16622.21
log restricted-lik : -16611.02
```

The result shows that ion m/z=428.26 present higher intensity in the culture region(`labelculture`) than the media region(`intercept`) with high significance score. However, in the non-spatial modeling the pixels were considered as independent samples, which is not true(pixels are highly correlated), the degree of freedom is overstimated and resulted in extremely small p-vales(type-I error).

3.1.5 Fitting a spatial model

Next, we try a spatial linear mixed effect model with Matern correlation. The model could be expressed as

$$y_s = \mu_k + U_s + \varepsilon_s$$

where y_s is the intensity at s, the fixed effect μ_k is the mean intensity in group k, the random effect U_s is the Matérn random field at s, and ε_s is the random error.

```r
model <- fitme(
    intensity ~ label + Matern(1|x+y),
    data = df,
    method = "REML",
)
summary.HLfit(model, details=c(p_value="Wald"))
```

```
formula: intensity ~ label + Matern(1 | x + y)
REML: Estimation of corrPars, lambda and phi by REML.
      Estimation of fixed effects by ML.
Estimation of lambda and phi by 'outer' REML, maximizing restricted logL.
family: gaussian( link = identity )
 ------------ Fixed effects (beta) ------------
             Estimate Cond. SE t-value   p-value
(Intercept)     26.59     1347 0.01974 0.9842505
labelculture  5279.35     1578 3.34540 0.0008216
 --------------- Random effects ---------------
```

```
Family: gaussian( link = identity )
                    --- Correlation parameters:
      1.nu      1.rho
0.19882987 0.04176932
           --- Variance parameters ('lambda'):
lambda = var(u) for u ~ Gaussian;
   x + y  :  5112000
# of obs: 1781; # of groups: x + y, 1781
 -------------- Residual variance  ------------
phi estimate was 3272180
 ------------- Likelihood values  -------------
                         logLik
logL       (p_v(h)): -16315.52
Re.logL  (p_b,v(h)): -16299.60
```

The spatial model also shows the differential abundance between two regions, but with more reasonable p-value.

###Fitting non-spatial and spatial model on all features Then we can loop over all features by fitting a model on each of them:

```
nonspatial_de <-function(mse){
  mz_values <- mz(mse)
  fit_results <- lapply(seq_along(mz_values), function(i) {
  intensity_i <- spectraData(mse)$intensity[i, ]

  # create a subset data frame
  sub_df <- data.frame(
    intensity = intensity_i,
    label = pData(mse)$label,
    x = coord(mse)$x,
    y = coord(mse)$y
  )
  # set the media group as the baseline
  sub_df$label <- relevel(sub_df$label, ref="media")
  # fit a linear fixed effect model
  model <- tryCatch({
    fitme(intensity ~ label,
          data = sub_df,
          method = "REML")
  }, error = function(e) NULL)

  # summarize the results
```

```
  if (!is.null(model)) {
    #coefs <- fixed.effects(model)
    stats <- summary.HLfit(model, details=c(p_value="Wald"), verbose=FALSE)[['beta_table']]

    return(data.frame(
      mz = mz_values[i],
      index = i,
      intercept = stats[1,1],
      label_effect = stats[2,1],
      t_value = stats[2,3],
      p_value = stats[2,4]
    ))
  } else {
    return(NULL)
  }
})
# combine all results
fit_results_df <- do.call(rbind, fit_results)
fit_results_df$intensity <- fit_results_df$intercept+fit_results_df$label_effect
fit_results_df$log2_foldchange <- log2((fit_results_df$intercept+fit_results_df$label_effect)
fit_results_df$neg_log10_p <- pmin(-log10(fit_results_df$p_value), 20)
return(fit_results_df)
}
```

Summarize the results:

```
fit_results_df <- nonspatial_de(mse_subset)
head(fit_results_df, 10)
```

```
         mz index intercept label_effect    t_value p_value intensity
1  172.0402     1 2743.7439   -1577.9945 -16.517216       0 1165.7493
2  187.0550     2 1008.0175     887.5974  13.303868       0 1895.6150
3  189.0707     3 2753.1754    1225.5625  10.933209       0 3978.7380
4  190.0508     4 1054.0000    -403.6845 -13.734408       0  650.3155
5  201.0593     5  754.3474     813.6653  14.013560       0 1568.0127
6  202.0783     6  777.3088     654.8363  13.810138       0 1432.1451
7  203.0734     7  776.4386     601.9418  13.041554       0 1378.3803
8  204.0812     8 1640.6281    1148.1520  13.707021       0 2788.7801
9  205.0654     9 1014.4842     374.1428   8.819151       0 1388.6270
10 214.0656    10 4392.1018    4708.4363  14.635449       0 9100.5381
   log2_foldchange neg_log10_p
```

```
1       -1.2348882          20
2        0.9111452          20
3        0.5312143          20
4       -0.6966631          20
5        1.0556363          20
6        0.8816179          20
7        0.8280303          20
8        0.7653860          20
9        0.4529127          20
10       1.0510404          20
```

Here is the code for spatial model:

```r
spatial_de <- function(mse){
  mz_values <- mz(mse)
  fit_results <- lapply(seq_along(mz_values), function(i) {
    message("Fitting feature", i)
    intensity_i <- spectraData(mse)$intensity[i, ]

    sub_df <- data.frame(
      intensity = intensity_i,
      label = pData(mse)$label,
      x = coord(mse)$x,
      y = coord(mse)$y
    )
    sub_df$label <- relevel(sub_df$label, ref="media")

    model <- tryCatch({
      fitme(intensity ~ label + Matern(1|x+y),
            data = sub_df,
            fixed = list(nu=0.5),
            control.HLfit = list(algebra="spcorr", NbThreads=8),
            method = "REML")
    }, error = function(e) NULL)

    if (!is.null(model)) {
      coefs <- fixed.effects(model)
      stats <- summary.HLfit(model, details=c(p_value="Wald"), verbose=FALSE)[['beta_table']]

      return(data.frame(
        mz = mz_values[i],
        intercept = coefs[1],
```

```
        label_effect = coefs[2],
        t_value = stats[2,3],
        p_value = stats[2,4]
      ))
    } else {
      return(NULL)
    }
  })
  fit_results_df <- do.call(rbind, fit_results)
  fit_results_df$log2_foldchange <- log2((fit_results_df$intercept+fit_results_df$label_effe
  fit_results_df$neg_log10_p <- pmin(-log10(fit_results_df$p_value), 20)
  return(fit_results_df)
}
```

It takes hours to run the spatial model on all the features. For presentation here we load the pre-computed results:

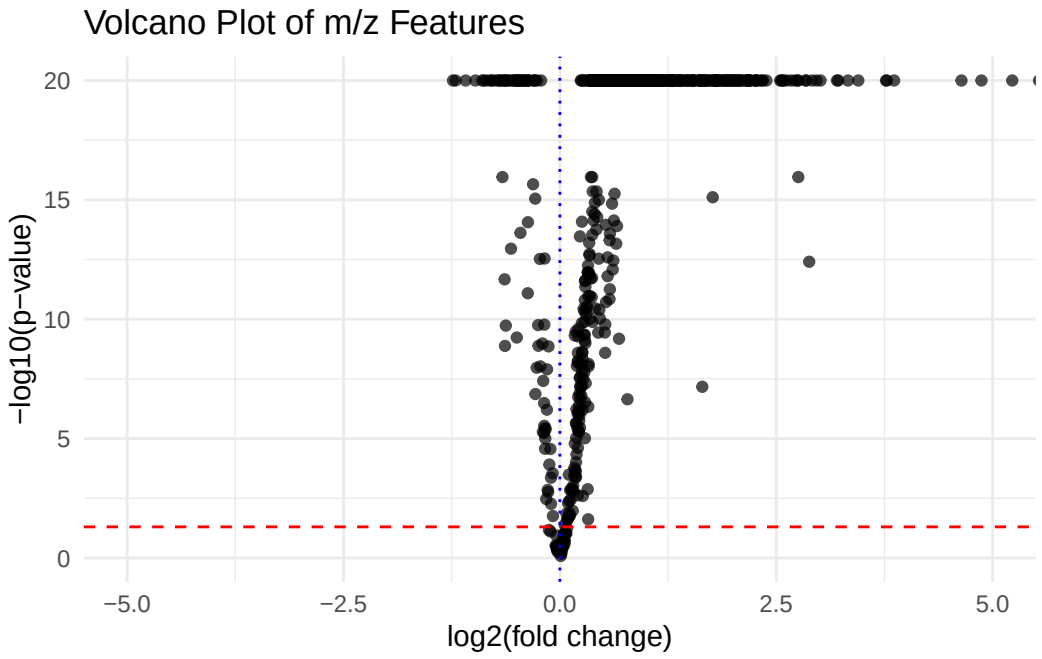
```
fit_results_df_sp <- read.csv("D:\\dataset\\Laura_Gordon\\spatial_model_results.csv")
head(fit_results_df_sp, 10)
```

```
         mz intercept label_effect   t_value      p_value log2_foldchange
1  172.0402 2870.8918   -1852.1692 -3.813199 0.0001371793      -1.4947377
2  187.0550 1032.1892    1143.4519  2.324345 0.0201070065       1.0757331
3  189.0707 3142.2868    1406.1612  1.569304 0.1165770829       0.5335595
4  190.0508 1066.5681    -513.1645 -2.867744 0.0041340929      -0.9465722
5  201.0593  826.7352     983.1938  2.384268 0.0171131320       1.1304359
6  202.0783  916.4583     686.2478  1.955258 0.0505525712       0.8063687
7  203.0734  913.1006     671.3881  2.131018 0.0330876572       0.7951717
8  204.0812 1765.4276    1434.6161  2.329726 0.0198206294       0.8580740
9  205.0654 1267.1642     472.7607  1.076814 0.2815634507       0.4574216
10 214.0656 5172.3841    5307.5780  2.215906 0.0266979208       1.0187322
   neg_log10_p
1    3.8627114
2    1.6966526
3    0.9333868
4    2.3836198
5    1.7666705
6    1.2962568
7    1.4803340
8    1.7028826
9    0.5504237
10   1.5735226
```

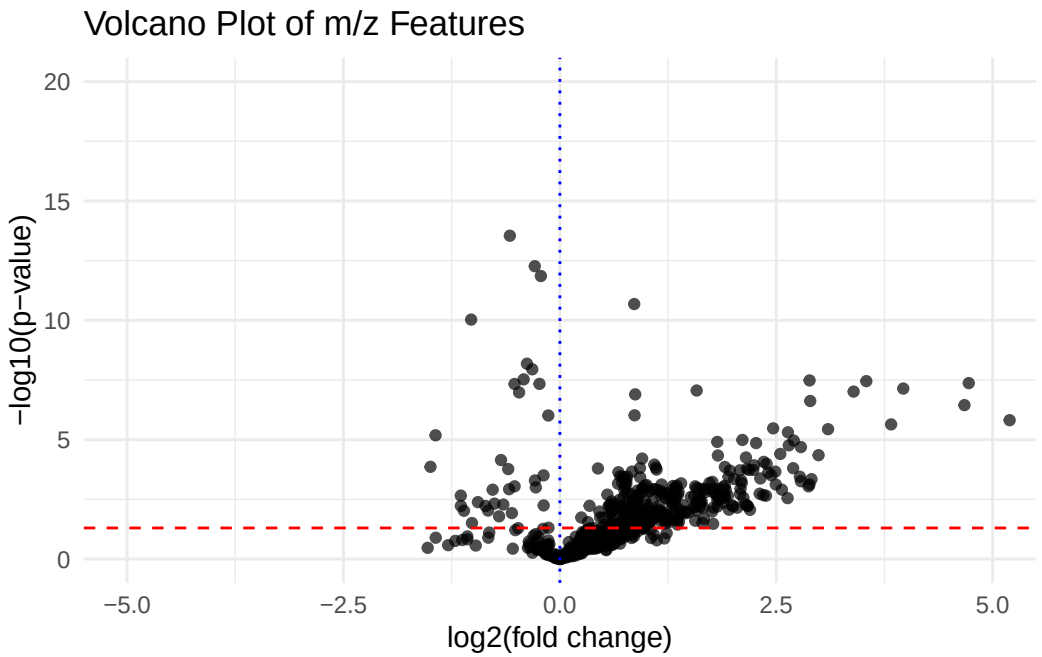
Now we can visualize the results with volcano plots.

```
volcano_plot <- function(df){
  p <- ggplot(df, aes(x = log2_foldchange, y = neg_log10_p)) +
    geom_point(alpha = 0.7) +
    geom_hline(yintercept = -log10(0.05), color = "red", linetype = "dashed") +  # p=0.05 thr
    geom_vline(xintercept = 0, color = "blue", linetype = "dotted") +
    labs(
      title = "Volcano Plot of m/z Features",
      x = "log2(fold change)",
      y = "-log10(p-value)"
    ) +
    coord_cartesian(xlim = c(-5, 5), ylim=c(0,20)) +
    theme_minimal()
  return(p)
}
```

```
volcano_plot(fit_results_df)
```




```
volcano_plot(fit_results_df_sp)
```



The spatial modeling resulted in a more reasonable volcano plot. Points on the top right(significantly up-expressed in the microbial culture region with high fold change) are the candidate precursors. For example,

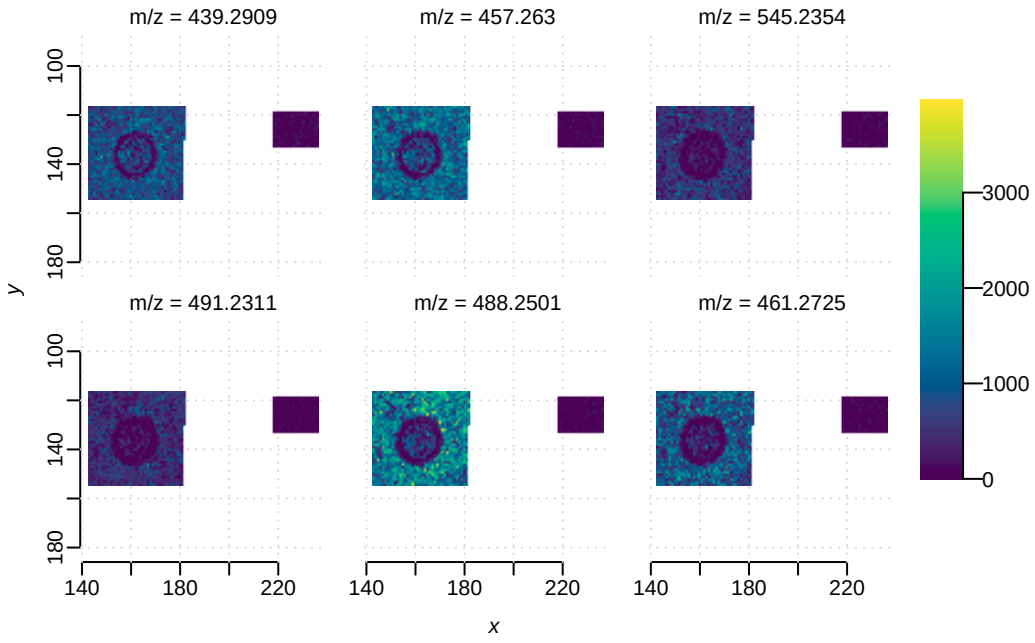
```
selected_precursors <- subset(fit_results_df_sp, (log2_foldchange>2) & (p_value<0.05))
selected_precursors <- selected_precursors[order(selected_precursors$neg_log10_p, decreasing
head(selected_precursors, 10)
```

```
          mz intercept label_effect   t_value      p_value log2_foldchange
294 439.2909  87.68310     699.8994 12.207582 0.000000e+00        3.167060
319 457.2630  81.43585    1054.3189  8.500808 0.000000e+00        3.801844
446 545.2354  65.71369     419.6170  5.524842 3.297832e-08        2.884702
374 491.2311  37.80553     402.0757  5.512629 3.535136e-08        3.540445
367 488.2501  53.84855    1369.3657  5.479604 4.262793e-08        4.724101
328 461.2725  56.87537     832.7660  5.385277 7.233332e-08        3.967348
353 479.2486 141.22950    1343.3214  5.332992 9.660731e-08        3.393913
409 523.2550 105.22080     676.1195  5.165406 2.399180e-07        2.892531
397 510.2310  77.99742    1913.4726  5.090496 3.571283e-07        4.674263
292 439.1950  99.20545    4620.6606  4.890073 1.007983e-06        5.572183
    neg_log10_p
294   50.000000
319   50.000000
446    7.481772
```

```
374    7.451594
367    7.370306
328    7.140662
353    7.014990
409    6.619937
397    6.447176
292    5.996547
```

Plot ion images for some example precursors with differential abundance:

```
indices = as.numeric(row.names(selected_precursors)[1:6])
image(mse_subset, i=indices)
```



3.1.6 Processing TIMSCONVERT data

In order to show ion mobility also benefits differential abundance analysis, we contrast the results from processing without ion mobility.

```
msa_timsconvert <- readMSIData("D:\\dataset\\Laura_Gordon\\250321_JB182_Pen12.imzML")
mse_timsconvert_binned <- bin(msa_timsconvert, resolution=20, units="ppm")
centroided(mse_timsconvert_binned)<-FALSE
```

```
set.seed(42, kind="L'Ecuyer-CMRG")
peaks_timsconvert <- peakProcess(mse_timsconvert_binned, SNR=6, tolerance=400, units="ppm")
```

Then we filtered the features by intensity and got 378 features.

```
peaks_timsconvert <- summarizeFeatures(peaks_timsconvert)
max_intensity <- max(fData(peaks_timsconvert)$mean)
intensity_filter <- fData(peaks_timsconvert)$mean > 0.01*max_intensity
peaks_timsconvert <- subsetFeatures(peaks_timsconvert, select=intensity_filter)
peaks_timsconvert
```

```
MSImagingExperiment with 378 features and 12173 spectra
spectraData(1): intensity
featureData(4): mz, count, freq, mean
pixelData(3): x, y, run
coord(2): x = 37...236, y = 31...154
runNames(1): 250321_JB182_Pen12
metadata(2): processing_20260106134446, processing_20260106134549
experimentData(5): spectrumType, spectrumRepresentation, lineScanSequence, scanType, lineSca
mass range:  172.0407 to 1095.0892
centroided: TRUE
```

Here is the code to run spatial-aware differential abundance analysis on these features, for time being we also use pre-computed results.

```
pData(peaks_timsconvert)$label <- factor(pixel_label)
peaks_timsconvert_subset <- subsetPixels(peaks_timsconvert, x>120, y>100)
fit_results_df_timsconvert <- nonspatial_de(peaks_timsconvert_subset)
```

```
fit_results_df_timsconvert <- read.csv("D:\\dataset\\Laura_Gordon\\spatial_timsconvert.csv")
```

Next we find the matching features between with and without ion mobility. Since there could be multiple isobaric features differentiated by ion mobility and detected by TIMSImaging, a `mz_noim` value might match with multiple `mz_im` values.

```
mz_im <- fit_results_df_sp$mz
mz_noim <- fit_results_df_timsconvert$mz
idx <- sapply(mz_im, function(v) which.min(abs(mz_noim - v)))
spatialde_results <- data.frame(
  mz_im = mz_im,
```

```
  mz_noim = mz_noim[idx],
  mob = spectraData(msa)$mobility$'spectrum=1',
  tolerance = (mz_im-mz_noim[idx])/mz_im,
  fc_im = fit_results_df_sp$log2_foldchange,
  fc_noim = fit_results_df_timsconvert$log2_foldchange[idx],
  pvalue_im = fit_results_df_sp$neg_log10_p,
  pvalue_noim = fit_results_df_timsconvert$neg_log10_p[idx]
)
# find features with high fold change
spatialde_results <- subset(spatialde_results, (abs(tolerance)<5e-5) & (fc_im>1))
head(spatialde_results, 10)
```

```
      mz_im  mz_noim       mob     tolerance    fc_im   fc_noim pvalue_im
10 214.0656 214.0648 0.6694399  3.764268e-06 1.018732 1.0773983  1.573523
11 215.0710 215.0724 0.6675574 -6.546154e-06 1.030314 1.0101238  1.560817
18 227.0735 227.0738 0.6857717 -1.464568e-06 1.252852 1.2403257  1.656022
19 228.0797 228.0818 0.6865217 -8.946302e-06 1.211128 1.0259938  1.838233
20 229.0762 229.0776 0.6973090 -5.781674e-06 2.196807 2.5045678  2.060626
32 241.0760 241.0707 0.7123838  2.184876e-05 2.170429 2.0054843  2.216579
46 256.0758 256.0762 0.7366803 -1.479089e-06 1.081612 1.1283892  1.865507
48 257.0697 257.0766 0.7420743 -2.689380e-05 2.049206 0.9206352  2.151130
49 257.0804 257.0766 0.7354503  1.487710e-05 1.222383 0.9206352  1.684749
51 258.0785 258.0811 0.7425315 -9.830324e-06 1.888017 1.9253027  2.351527
   pvalue_noim
10    1.683095
11    1.575105
18    1.509311
19    1.363297
20    1.913232
32    2.152838
46    1.768733
48    1.820350
49    1.820350
51    2.255194
```

Now we can contrast the p-values for matched features. The x axis is the p-value processing without ion mobility, and the y axis is correponding ion moblity-aware p-value. Most points are above the diagonal, showing that the ion mobility-aware approach of TIMSImaging produced smaller p-values for differential abundance analysis.

```r
ggplot(spatialde_results, aes(x = pvalue_noim, y = pvalue_im)) +
  geom_point(alpha = 0.7) +
  geom_abline(intercept = 0, slope = 1, color = "red", linetype = "dashed")+
  labs(
    title = "Contrast of p-values",
    x = "without ion mobility",
    y = "with ion mobility"
  ) +
  coord_fixed(ratio=1, xlim = c(0, 10), ylim=c(0,10))
```