Cardinal tutorial

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Slides and code

The R Markdown for this tutorial is available on Github at:

https://github.com/kuwisdelu/asilomar2019

Use R or RStudio to run the document (you may need to update some file paths).

Installation

First make sure `BiocManager` is installed.

Then use `BiocManager` to install `Cardinal`.

```
if ( !requireNamespace("BiocManager") )
  install.packages("BiocManager")

BiocManager::install("Cardinal")
```

For the datasets used in the statistical analysis examples, we'll also install `CardinalWorkflows`.

```
BiocManager::install("CardinalWorkflows")
```

Getting started

Load the Cardinal package with `library()`.

```
library(Cardinal)

register(SerialParam())
```

Note: Cardinal supports parallelization via the `BiocParallel` package. The code above registers a serial (i.e., non-parallel) backend for this session.

Cardinal basics

Example data

To demonstrate reading data and visualization, we'll use example imzML files from imzml.org.

Specifically, we are going to reproduce the first image from the imzml.org website, which comes from the paper *"Histology by mass spectrometry: label-free tissue characterization obtained from high-accuracy bioanalytical imaging"* by Rompp et al.

The data is available from https://www.ebi.ac.uk/pride/archive/projects/PXD001283.

Importing data

We can use `readMSIData()` to import data from an imzML file.

```
file <- file.path("~/Documents/Datasets/PRIDE/PXD001283",
                  "HR2MSI mouse urinary bladder S096.imzML")

mouse <- readMSIData(file, mass.range=c(400, 900),
                     resolution=0.01, units="mz")
```

Providing the mass range and binning resolution will allow faster importing; otherwise, `Cardinal` will try to infer them from reading the data.

```
mouse
```

```
## An object of class 'MSProcessedImagingExperiment'
##   <50001 feature, 34840 pixel> imaging dataset
##     imageData(1): intensity
##     featureData(0):
##     pixelData(0):
##     metadata(16): spectrum representation ibd binary typ
##         files name
##     run(1): HR2MSI mouse urinary bladder S096
##     raster dimensions: 260 x 134
##     coord(2): x = 1..260, y = 1..134
##     mass range: 400 to 900
##     centroided: FALSE
```

Accessing pixel metadata

```
pixelData(mouse)
```

```
## PositionDataFrame with 34840 rows and 0 columns
##                                      :run:   coord:x   coord:y
##                                   <factor> <integer> <integer>
## 1     HR2MSI mouse urinary bladder S096         1         1
## 2     HR2MSI mouse urinary bladder S096         2         1
## 3     HR2MSI mouse urinary bladder S096         3         1
## 4     HR2MSI mouse urinary bladder S096         4         1
## 5     HR2MSI mouse urinary bladder S096         5         1
## ...                                    ...       ...       ...
## 34836 HR2MSI mouse urinary bladder S096       256       134
## 34837 HR2MSI mouse urinary bladder S096       257       134
## 34838 HR2MSI mouse urinary bladder S096       258       134
## 34839 HR2MSI mouse urinary bladder S096       259       134
## 34840 HR2MSI mouse urinary bladder S096       260       134
```

Accessing feature metadata

```
featureData(mouse)
```

```
## MassDataFrame with 50001 rows and 0 columns
##             :mz:
##        <numeric>
## 1            400
## 2         400.01
## 3         400.02
## 4         400.03
## 5         400.04
## ...          ...
## 49997     899.96
## 49998     899.97
## 49999     899.98
## 50000     899.99
## 50001        900
```

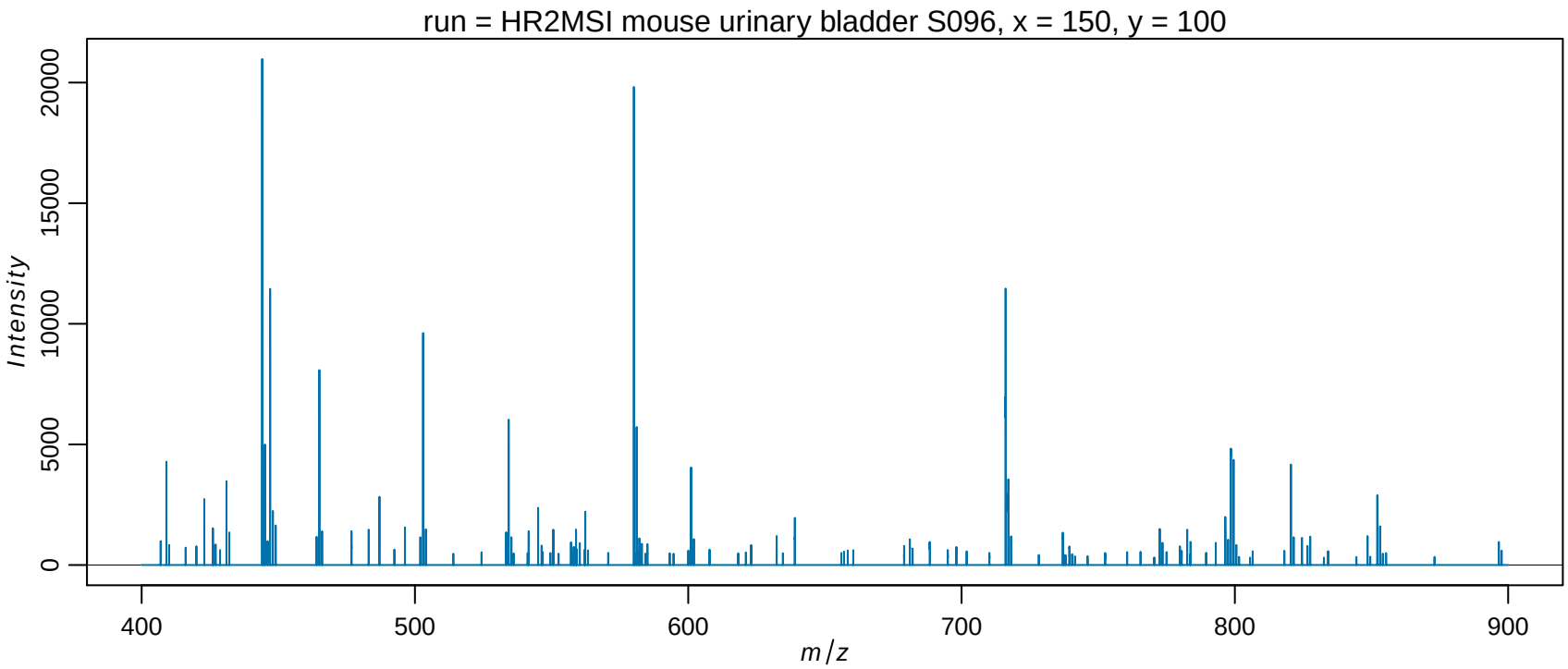
Accessing the spectra matrix

```
spectra(mouse)
```

```
## <50001 row, 34840 column> sparse_matc :: sparse numeric matrix
##           [,1] [,2] [,3] [,4] [,5] [,6] ...
## [400,]       0    0    0    0    0    0 ...
## [400.01,]    0    0    0    0    0    0 ...
## [400.02,]    0    0    0    0    0    0 ...
## [400.03,]    0    0    0    0    0    0 ...
## [400.04,]    0    0    0    0    0    0 ...
## [400.05,]    0    0    0    0    0    0 ...
## ...        ...  ...  ...  ...  ...  ... ...
## (67916471/1742034840 non-zero elements: 3.9% density)
## (2.7 MB real | 815 MB virtual)
```

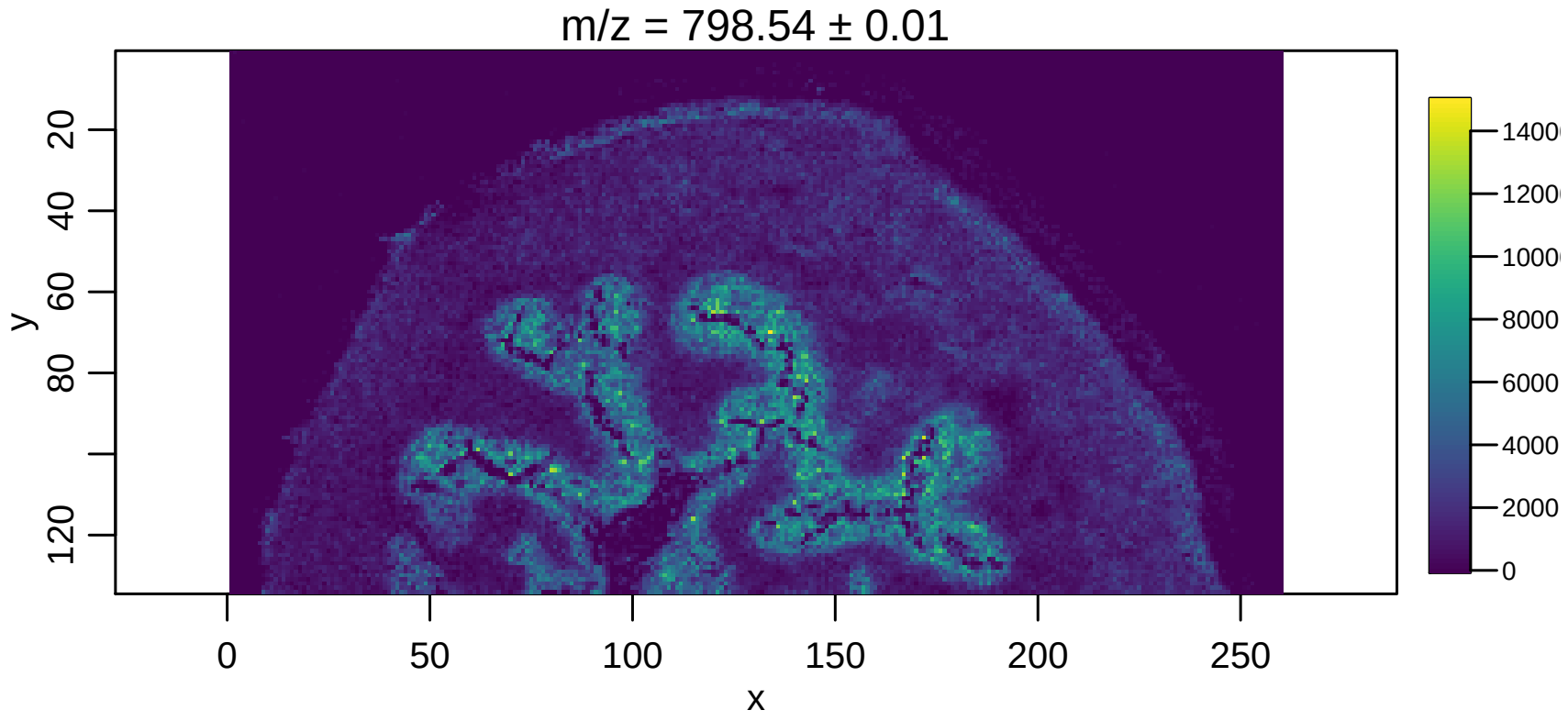
Visualize mass spectra

```
plot(mouse, coord=list(x=150, y=100))
```



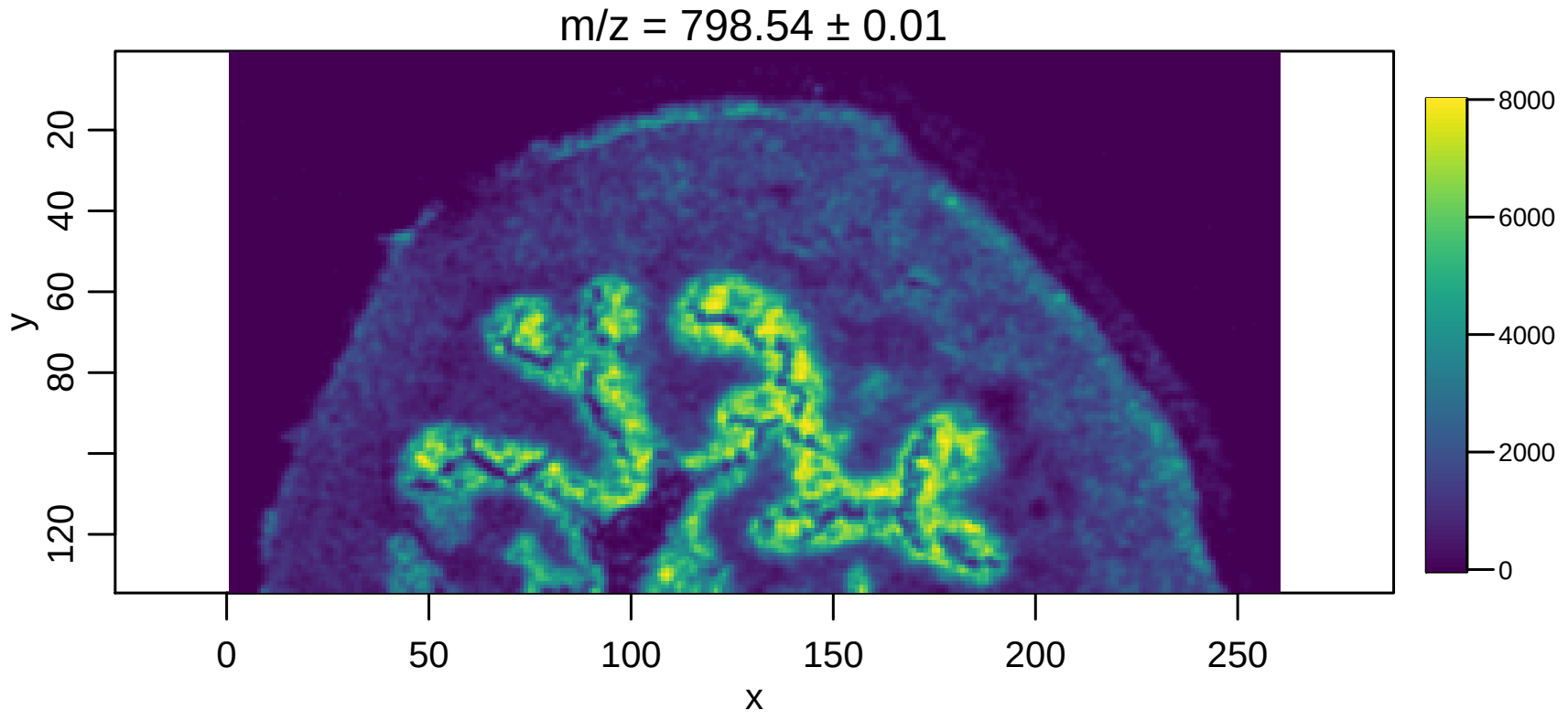
Visualize ion images

```
image(mouse, mz=798.54, plusminus=0.01)
```



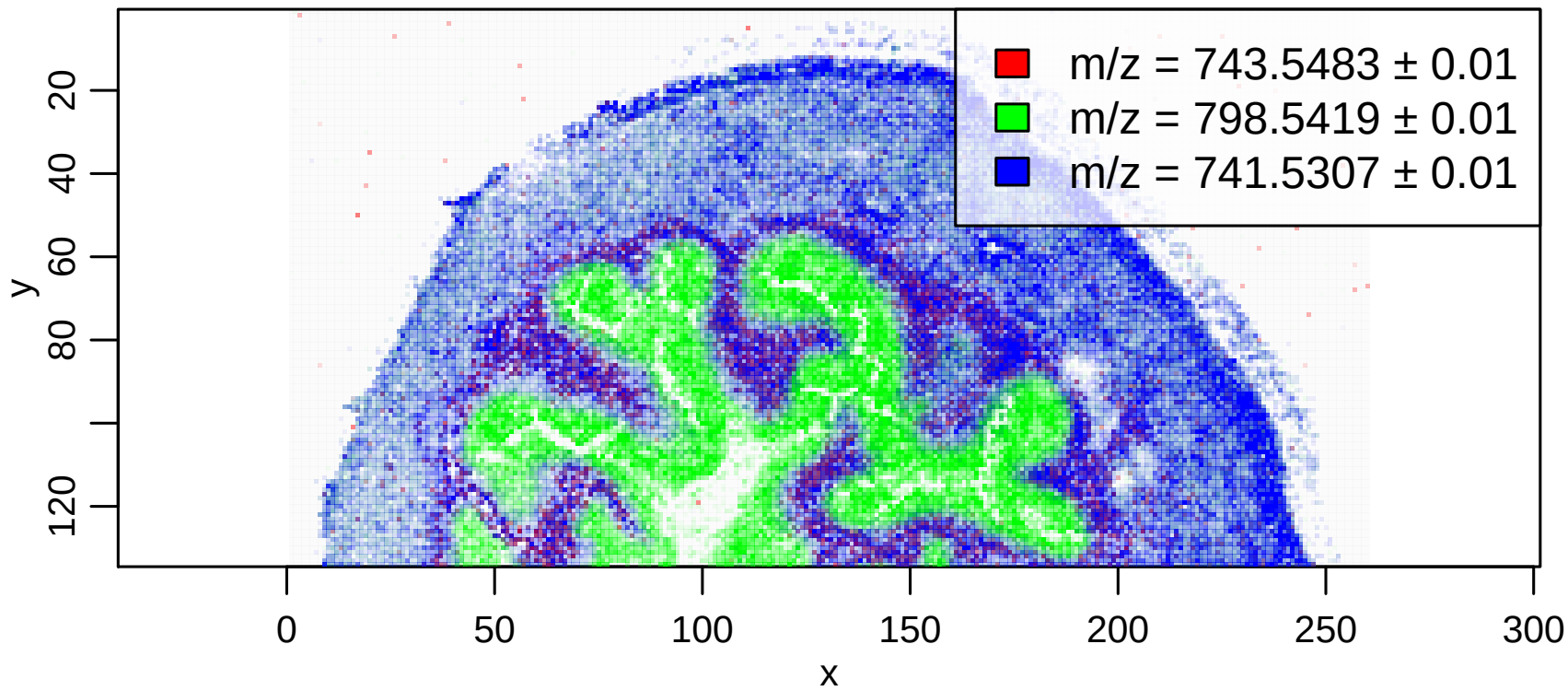
Visualize ion images (w/ enhancement)

```
image(mouse, mz=798.54, plusminus=0.01,
      contrast.enhance="suppress",
      smooth.image="gaussian")
```



Visualize ion images (superposed)

```
image(mouse, mz=c(743.5483, 798.5419, 741.5307),
      plusminus=0.01, superpose=TRUE,
      contrast.enhance="suppress",
      normalize.image="linear",
      col=c("red", "green", "blue"))
```



Segmentation

Pig fetus section

We will use some pre-processed datasets and pre-calculated analysis results from the `CardinalWorkflows` package.

For segmentation, we will use data from a pig fetus cross-section.

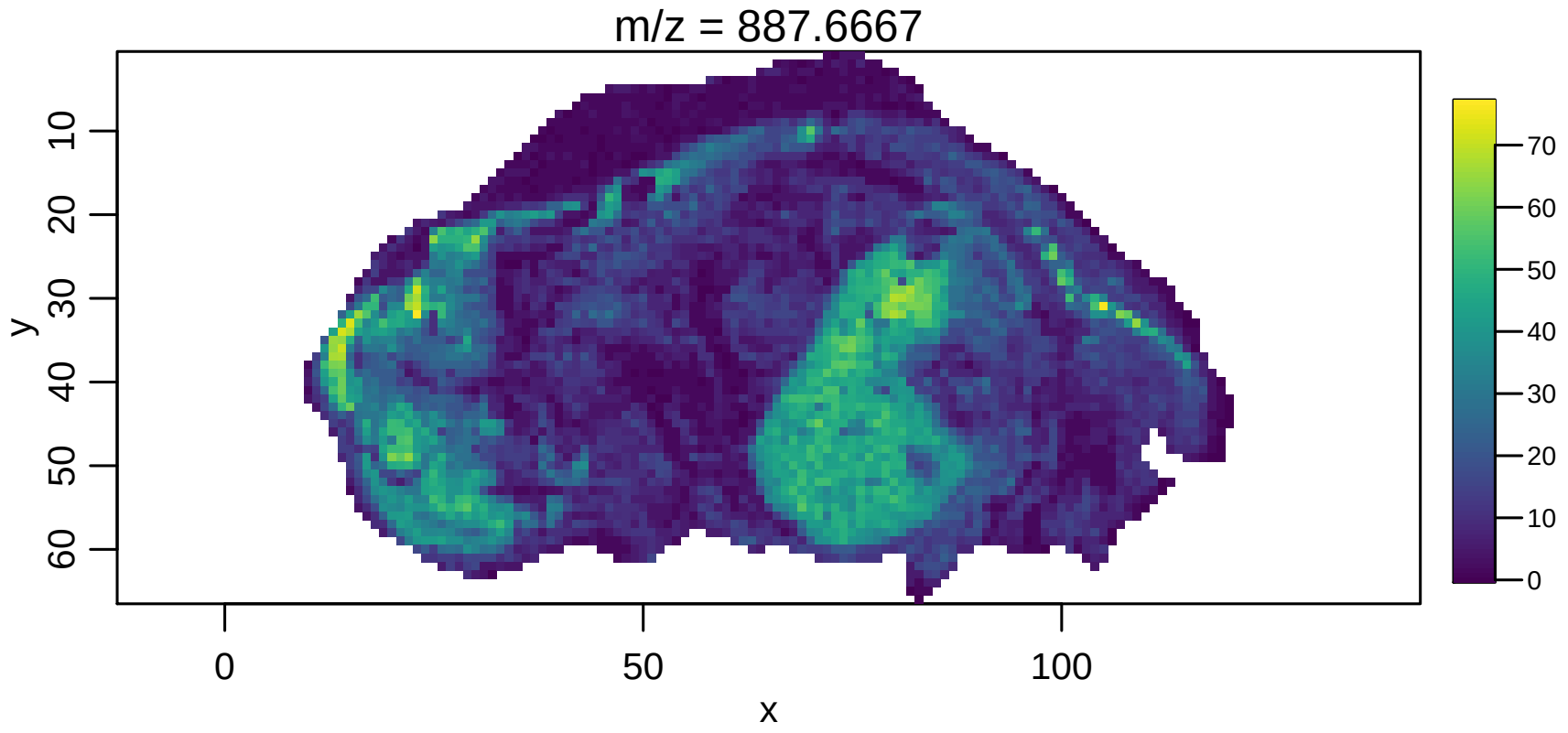
```
data(pig206, package="CardinalWorkflows")

pig206_peaks <- as(pig206.peaks, "MSImagingExperiment")
```

Note: The datasets provided in `CardinalWorkflows` are stored in an older format, so we need to coerce them to an `MSImagingExperiment` using `as()`.

Pig fetus m/z 888

```
image(pig206_peaks, mz=888)
```



Spatial shrunken centroids

We can use `spatialShrunkenCentroids()` to perform spatial shrunken centroids clustering.

This method requires three parameters:

- `r` = smoothing radius
- `k` = initial number of segments
- `s` = sparsity parameter

The method performs automated feature selection controlled by the sparsity parameter `s`.

```
set.seed(2019)
pig206_sscg <- spatialShrunkenCentroids(pig206_peaks,
                                        r=1, k=10,
                                        s=c(0,3,6,9),
                                        method="gaussian")
```

Loading pre-calculated segmentation results

To save time, we will load some pre-calculated segmentation results.

We'll load results for two types of spatial smoothing (`method="gaussian"` and 'method=“adaptive”).

```
data(pig206_analyses, package="CardinalWorkflows")
pig206_sscg <- as(pig206.sscg, "SpatialShrunkenCentroids2")
pig206_ssca <- as(pig206.ssca, "SpatialShrunkenCentroids2")
```

Note: The datasets provided in `CardinalWorkflows` are stored in an older format, so we need to coerce them to an `MSImagingExperiment` using `as()`.

```r
summary(pig206_sscg)
```

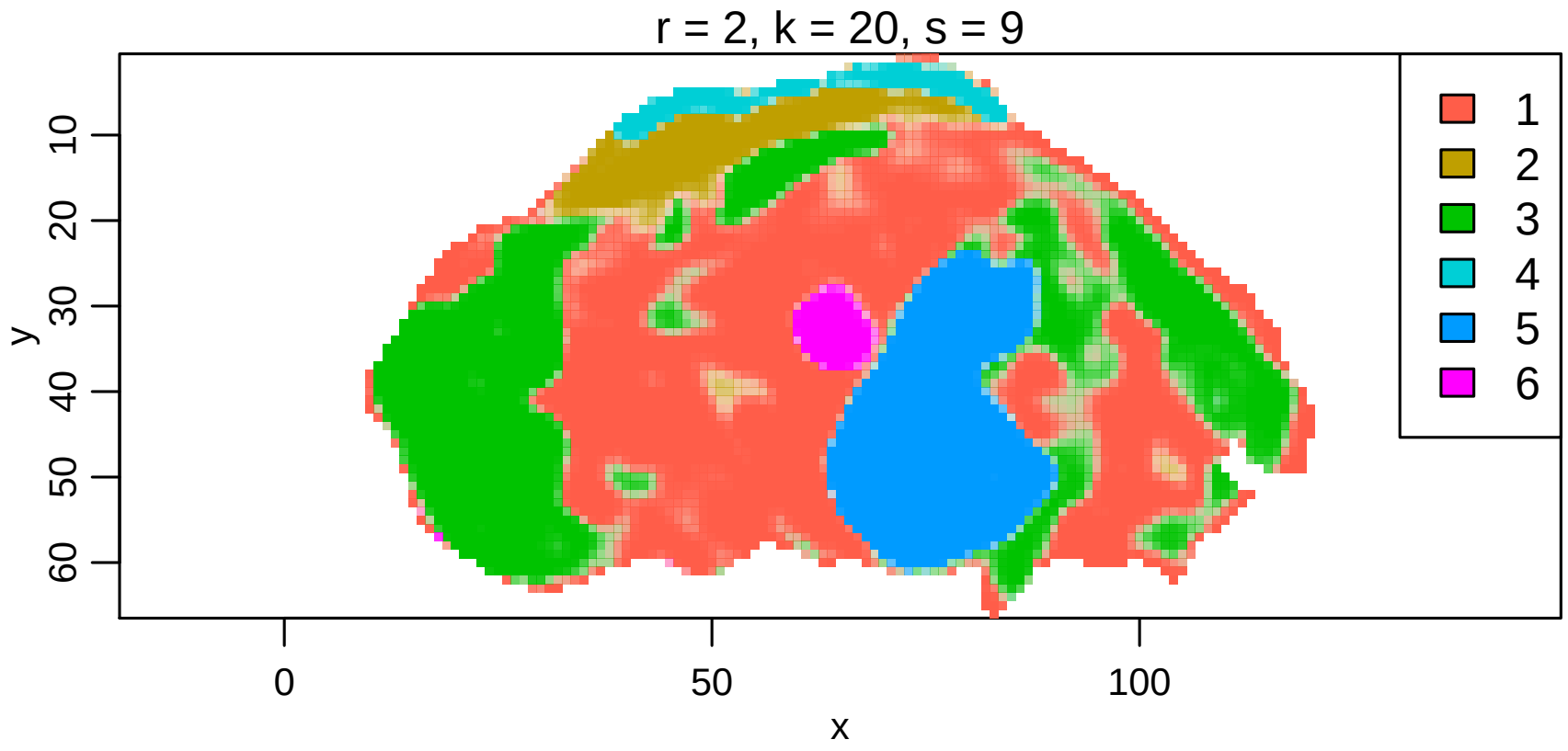
```
## Spatially-aware nearest shrunken centroids:
##
##  Segmentation / clustering
##  Method = gaussian
##  Distance = chebyshev
##
##    Radius (r) Init (k) Sparsity (s) Classes Features/Class       BIC
## 1           1       15             0      15          143.00 20509.345
## 2           1       15             3      10           90.80 10191.825
## 3           1       15             6       7           77.29  6856.784
## 4           1       15             9       6           63.33  5465.215
## 5           1       20             0      19          143.00 25700.733
## 6           1       20             3      11           91.73 11194.021
## 7           1       20             6       8           74.38  7877.644
## 8           1       20             9       6           61.67  5505.197
## 9           2       15             0      13          143.00 18046.962
## 10          2       15             3      10           89.70 10204.595
## 11          2       15             6       6           82.17  6306.085
## 12          2       15             9       6           61.83  5178.595
## 13          2       20             0      18          143.00 24352.247
## 14          2       20             3       9           90.00  9300.596
## 15          2       20             6       6           82.33  6318.644
## 16          2       20             9       6           60.33  5039.033
```

```
summary(pig206_ssca)
```

```
## Spatially-aware nearest shrunken centroids:
##
##  Segmentation / clustering
##  Method = adaptive
##  Distance = chebyshev
##
##    Radius (r) Init (k) Sparsity (s) Classes Features/Class       BIC
## 1           1       15             0      15          143.00 20613.272
## 2           1       15             3       9          100.33  9999.905
## 3           1       15             6       6           85.33  6343.967
## 4           1       15             9       6           62.17  5470.961
## 5           1       20             0      20          143.00 27049.482
## 6           1       20             3      11           83.27 10140.171
## 7           1       20             6       8           68.62  6909.633
## 8           1       20             9       6           61.50  5427.203
## 9           2       15             0      15          143.00 20612.342
## 10          2       15             3       9           99.89 10048.146
## 11          2       15             6       8           69.38  7202.635
## 12          2       15             9       7           55.00  5729.158
## 13          2       20             0      18          143.00 24360.641
## 14          2       20             3      10           90.70 10128.729
## 15          2       20             6       7           78.29  6795.206
## 16          2       20             9       6           65.83  5466.079
```

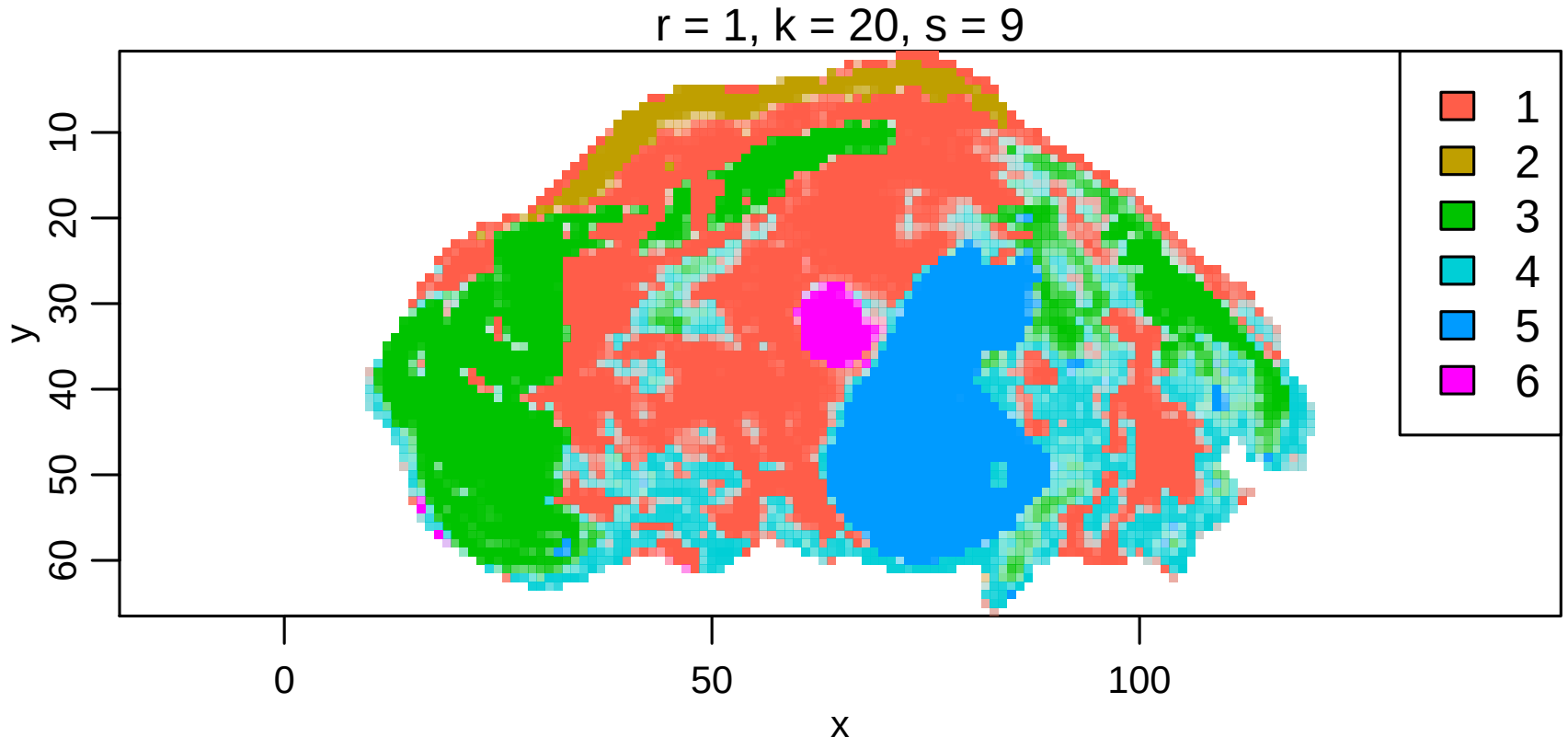
Probability of segment membership (Gaussian smoothing)

```
image(pig206_sscg, model=list(r=2, k=20, s=9),
      values="probability")
```



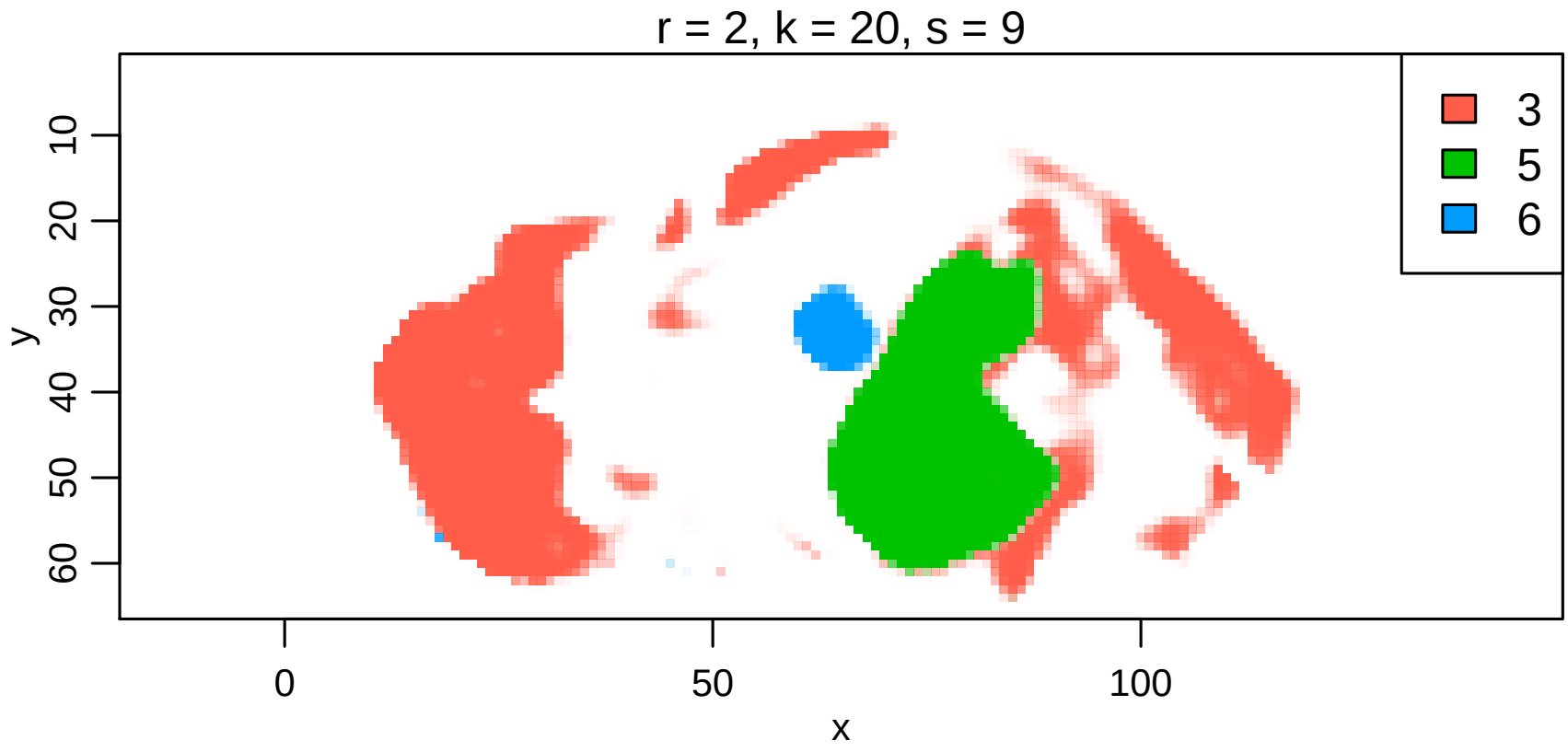
Probability of segment membership (adaptive smoothing)

```
image(pig206_ssca, model=list(r=1, k=20, s=9),
      values="probability")
```



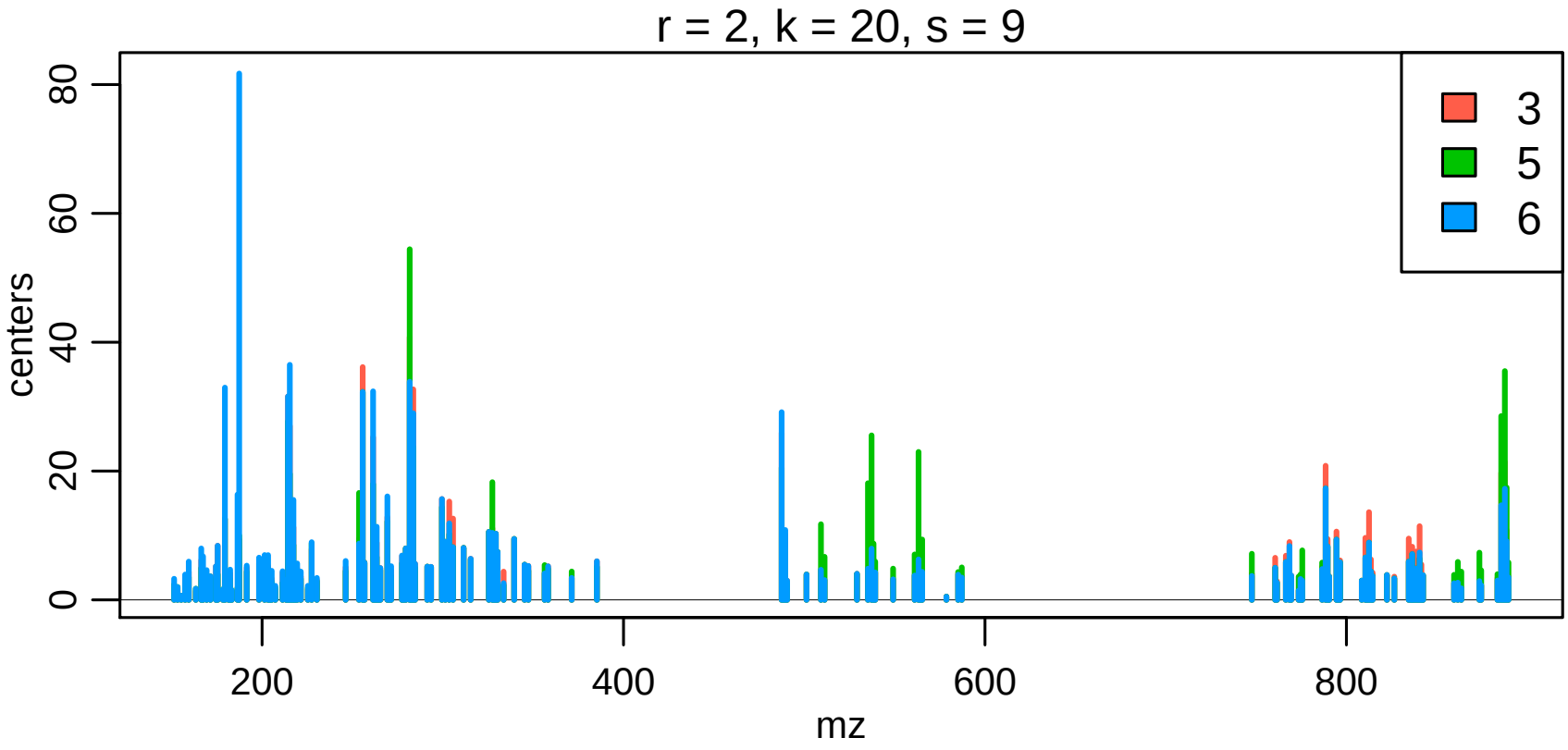
Brain, liver, and heart segments

```
image(pig206_sscg, model=list(r=2, k=20, s=9),
      values="probability", column=c(3,5,6))
```



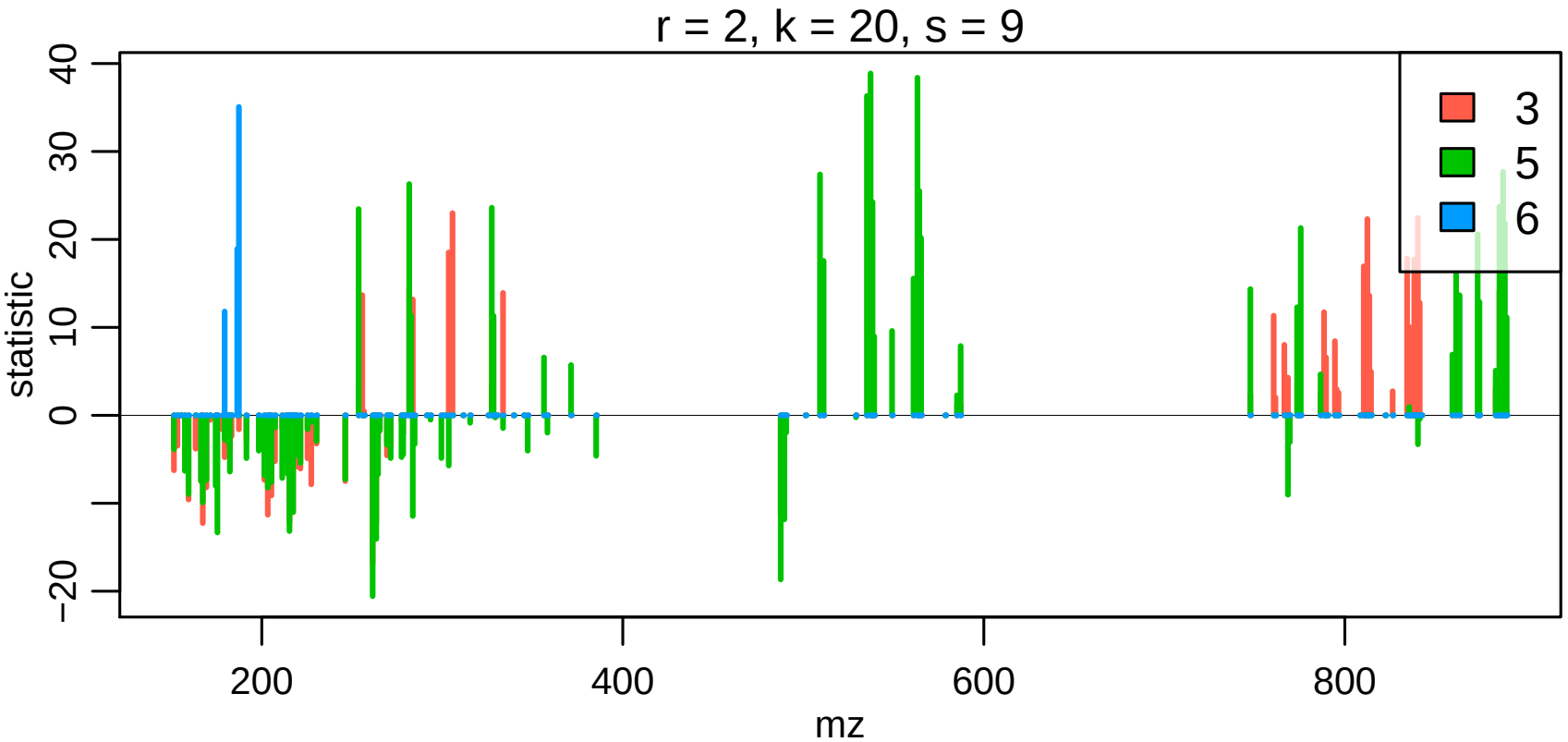
Mean spectra for brain, liver, and heart

```
plot(pig206_sscg, model=list(r=2, k=20, s=9),
     values="centers", column=c(3,5,6), lwd=2)
```



Statistics for brain, liver, and heart

```
plot(pig206_sscg, model=list(r=2, k=20, s=9),
     values="statistic", column=c(3,5,6), lwd=2)
```



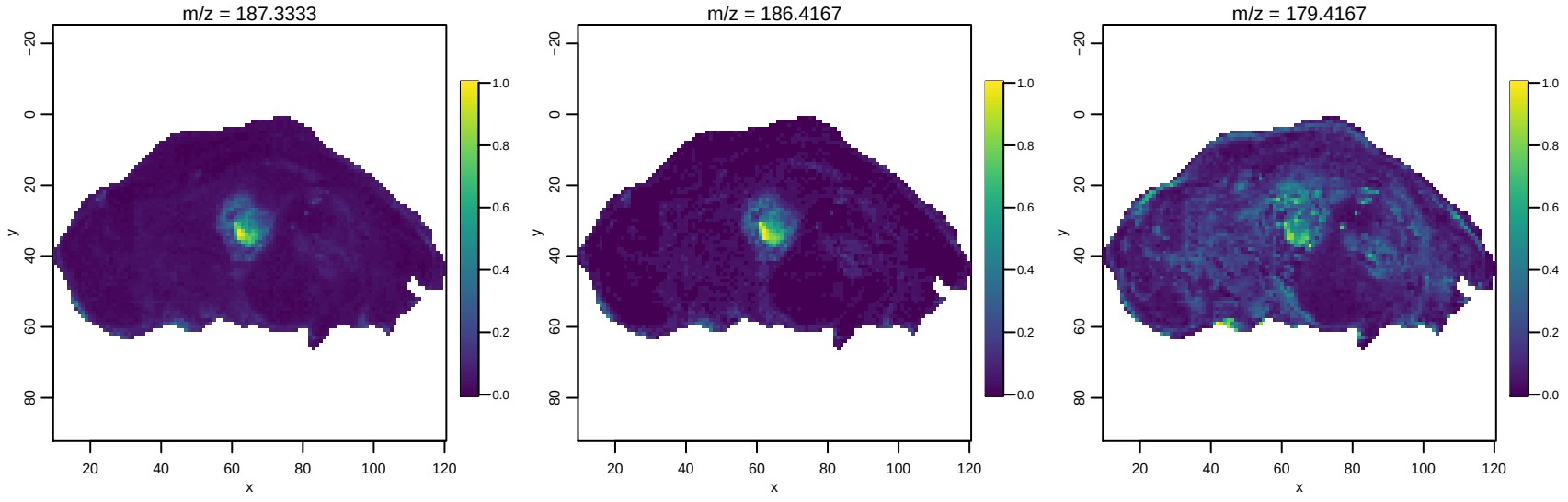
Most distinctive mass features for heart

```
topFeatures(pig206_sscg, model=list(r=2, k=20, s=9), class==6)
```

```
## Top-ranked features:
##          mz r  k s class    centers statistic
## 1  187.3333 2 20 9     6 81.7135934  35.06540
## 2  186.4167 2 20 9     6 16.3548118  18.92717
## 3  179.4167 2 20 9     6 32.9546437  11.79906
## 4  151.3333 2 20 9     6  3.2799443   0.00000
## 5  153.2500 2 20 9     6  2.0027075   0.00000
## 6  155.2500 2 20 9     6  0.6662069   0.00000
## 7  157.4167 2 20 9     6  3.9341399   0.00000
## 8  159.4167 2 20 9     6  5.9435216   0.00000
## 9  163.3333 2 20 9     6  1.7407288   0.00000
## 10 166.3333 2 20 9     6  7.9829617   0.00000
```

Most distinctive mass features for heart

```
image(pig206_peaks, mz=c(187.3, 186.4, 179.4),
      normalize.image="linear", layout=c(1,3))
```



Classification

Renal cell carcinoma (RCC) dataset

For classification, we will look at a dataset with 8 matched pairs of healthy tissue and tumor.

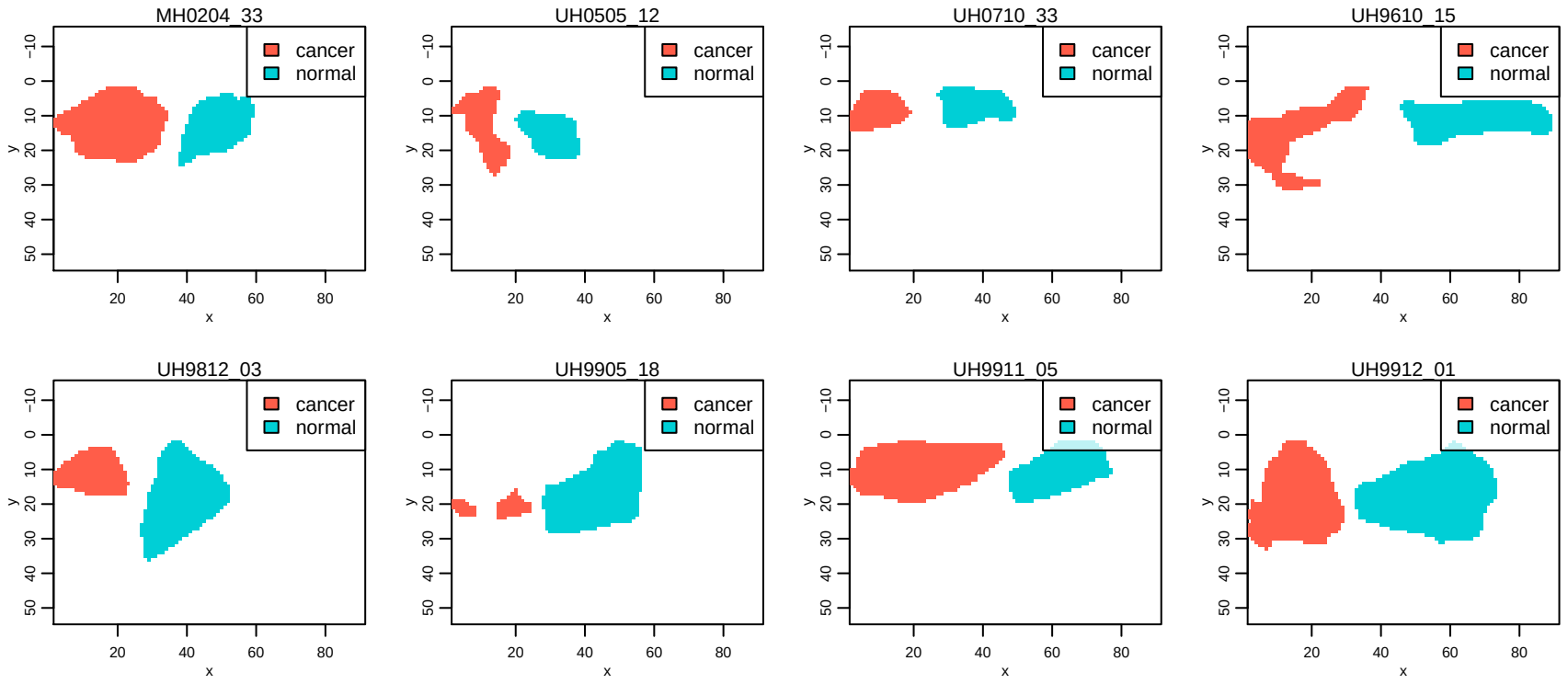
```
data(rcc, package="CardinalWorkflows")

rcc_binned <- as(rcc.small, "MSImagingExperiment")
```

Note: The datasets provided in `CardinalWorkflows` are stored in an older format, so we need to coerce them to an `MSImagingExperiment` using `as()`.

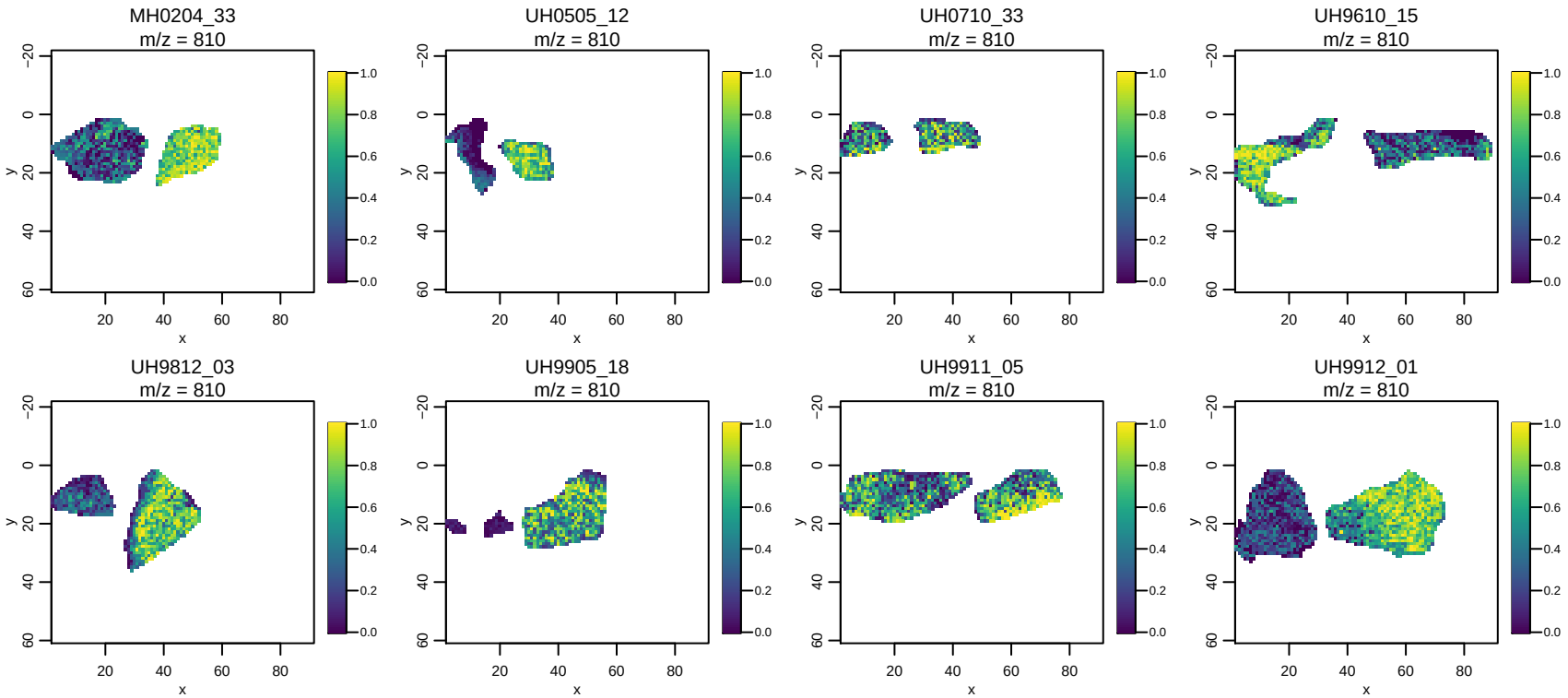
Diagnosis

```
image(rcc_binned, diagnosis ~ x * y,
      layout=c(2,4), key=TRUE)
```



RCC m/z 810

```
image(rcc_binned, mz=810, layout=c(2,4),
      contrast.enhance="histogram",
      normalize.image="linear")
```



Cross-validation

We use `crossValidate()` to perform cross-validation with spatial shrunken centroids classification.

Each run (with a cancer/normal matched pair) is a separate CV fold.

```
rcc_cv <- crossValidate(rcc_binned,
                        .y=rcc_binned$diagnosis,
                        .fun=spatialShrunkenCentroids,
                        r=1, s=0:9,
                        .fold=run(rcc_binned))
```

Loading pre-calculated CV results

To save time, we will load some pre-calculated CV results.

```
data(rcc_analyses, package="CardinalWorkflows")

rcc_cv <- as(rcc.cv.sscg, "CrossValidated2")
rcc_cv$.response <- rcc_binned$diagnosis
```

Note: The CV results provided in `CardinalWorkflows` are stored in an older format, so we need to coerce them to the newer version of the class using `as()`.

```r
summary(rcc_cv)
```

```
## Cross validation:
##
##  Classification on 2 classes: cancer normal
##  Summarized 8 folds: MH0204_33 UH0505_12 ... UH9911_05 UH9912_01
##
##    r k  s  Accuracy Sensitivity Specificity
## 1  1 2  0 0.8429914   0.8476989   0.8159756
## 2  2 2  0 0.8561886   0.8580030   0.8355094
## 3  3 2  0 0.8630894   0.8604488   0.8477564
## 4  1 2  4 0.8490092   0.8483200   0.8264378
## 5  2 2  4 0.8614576   0.8611654   0.8443781
## 6  3 2  4 0.8683942   0.8612681   0.8569161
## 7  1 2  8 0.8547938   0.8488032   0.8424877
## 8  2 2  8 0.8674976   0.8554014   0.8616460
## 9  3 2  8 0.8757184   0.8594661   0.8758228
## 10 1 2 12 0.8594844   0.8458832   0.8524716
## 11 2 2 12 0.8744938   0.8587803   0.8763088
## 12 3 2 12 0.8811212   0.8601993   0.8846880
## 13 1 2 16 0.8657251   0.8499447   0.8669211
## 14 2 2 16 0.8816412   0.8675844   0.8901845
## 15 3 2 16 0.8873204   0.8691277   0.9003744
## 16 1 2 20 0.8687747   0.8452182   0.8850448
## 17 2 2 20 0.8831311   0.8579025   0.9040489
## 18 3 2 20 0.8889099   0.8598918   0.9126307
## 19 1 2 24 0.8642629   0.8229296   0.9028367
## 20 2 2 24 0.8754163   0.8370316   0.9125450
## 21 3 2 24 0.8826314   0.8434021   0.9198243
## 22 1 2 28 0.8127775   0.6927374   0.9346619
## 23 2 2 28 0.8297853   0.7115986   0.9498645
## 24 3 2 28 0.8388546   0.7159251   0.9603586
```

Which is the most accurate model?

```
i <- which.max(summary(rcc_cv)$Accuracy)
summary(rcc_cv)[i,]
```

```
## Cross validation:
##
##  Classification on 2 classes: cancer normal
##  Summarized 8 folds: MH0204_33 UH0505_12 ... UH9911_05 UH9912_01
##
##    r k  s  Accuracy Sensitivity Specificity
## 18 3 2 20 0.8889099   0.8598918   0.9126307
```

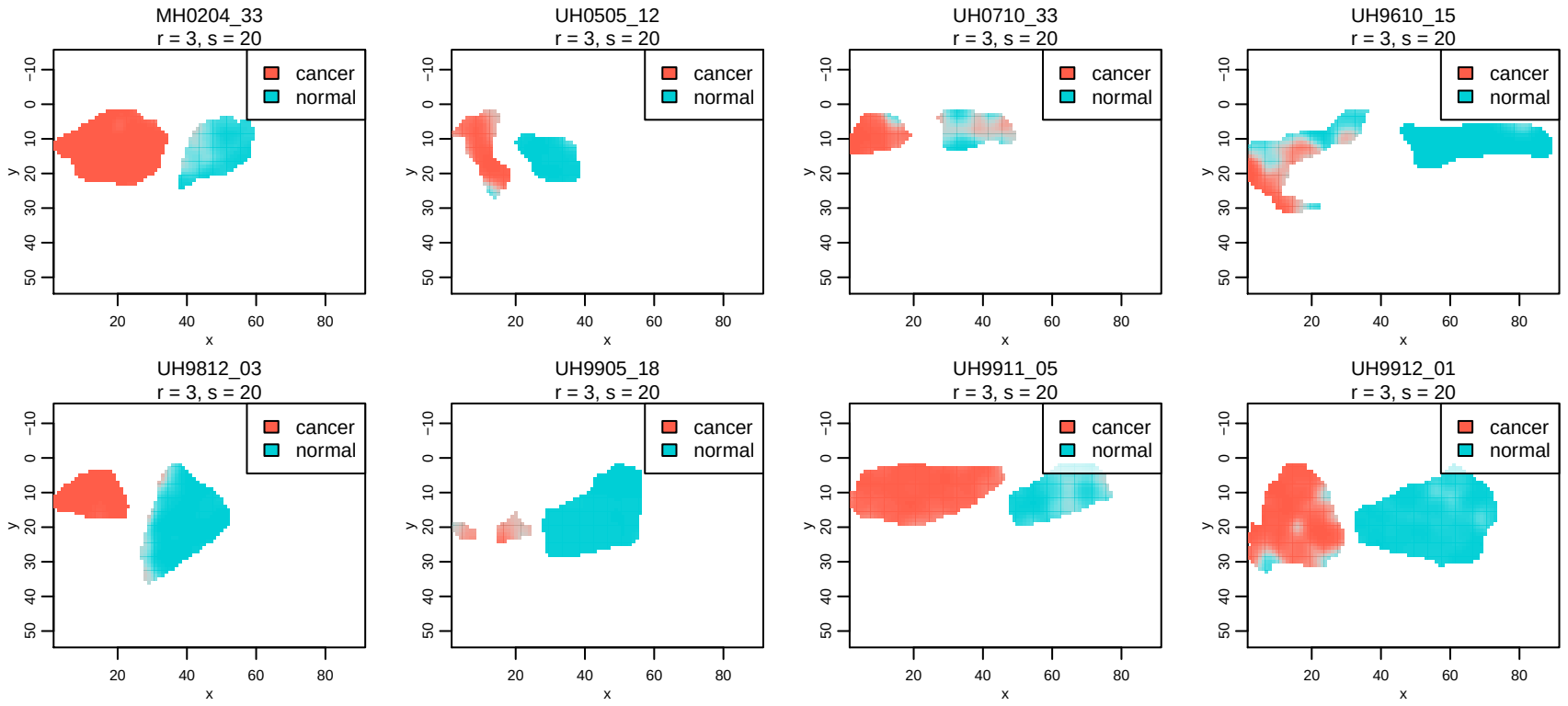
Fit the model with the best parameters to the full dataset.

```
rcc_sscg <- spatialShrunkenCentroids(rcc_binned,
                                     y=rcc_binned$diagnosis,
                                     r=3, s=20)
summary(rcc_sscg)
```

```
## Spatially-aware nearest shrunken centroids:
##
##  Classification on 2 classes: cancer normal
##  Method = gaussian
##  Distance = chebyshev
##
##   Radius (r) Sparsity (s) Features/Class Accuracy Sensitivity Specificity
## 1          3           20              43 0.948988        0.92   0.9733495
```

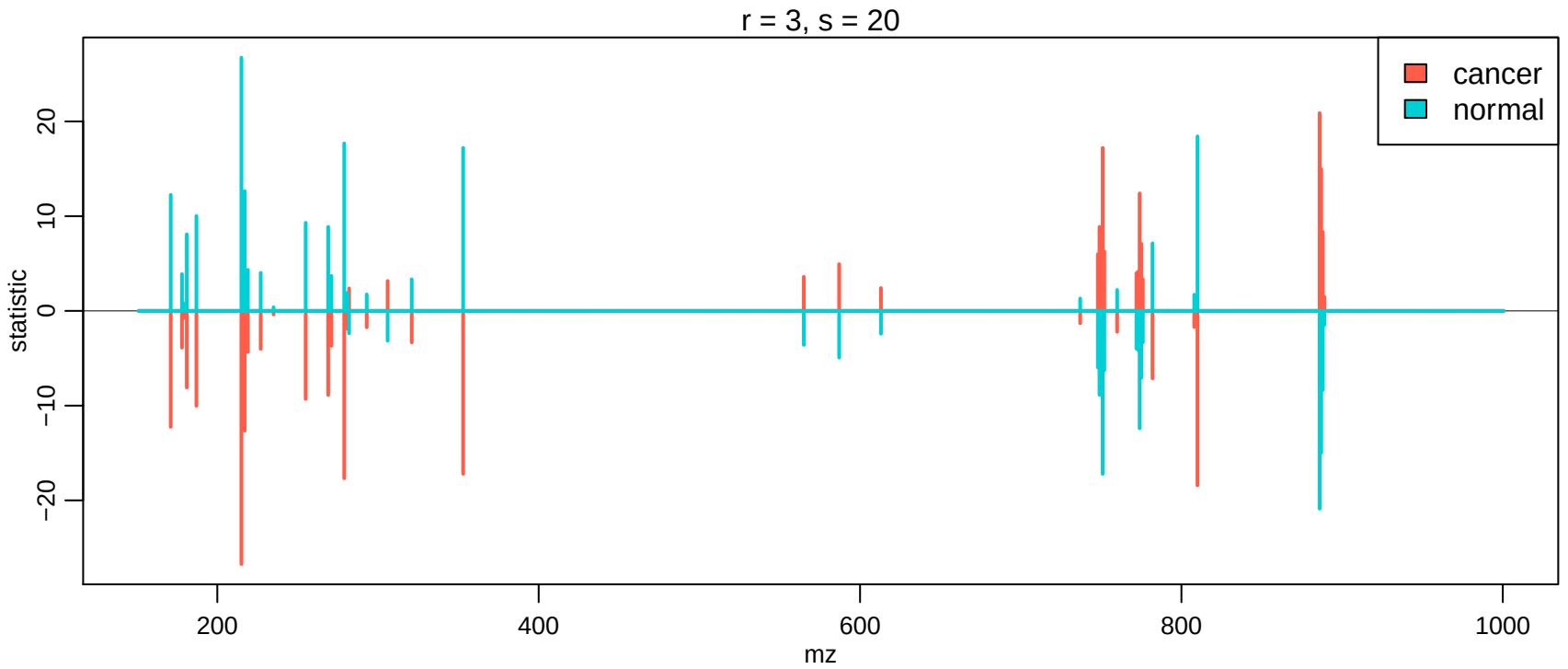
Predicted probability of cancer/normal

```
image(rcc_sscg, values="probability", layout=c(2,4))
```



Mass features over/under-expressed in each class

```
plot(rcc_sscg, values="statistic", lwd=2)
```



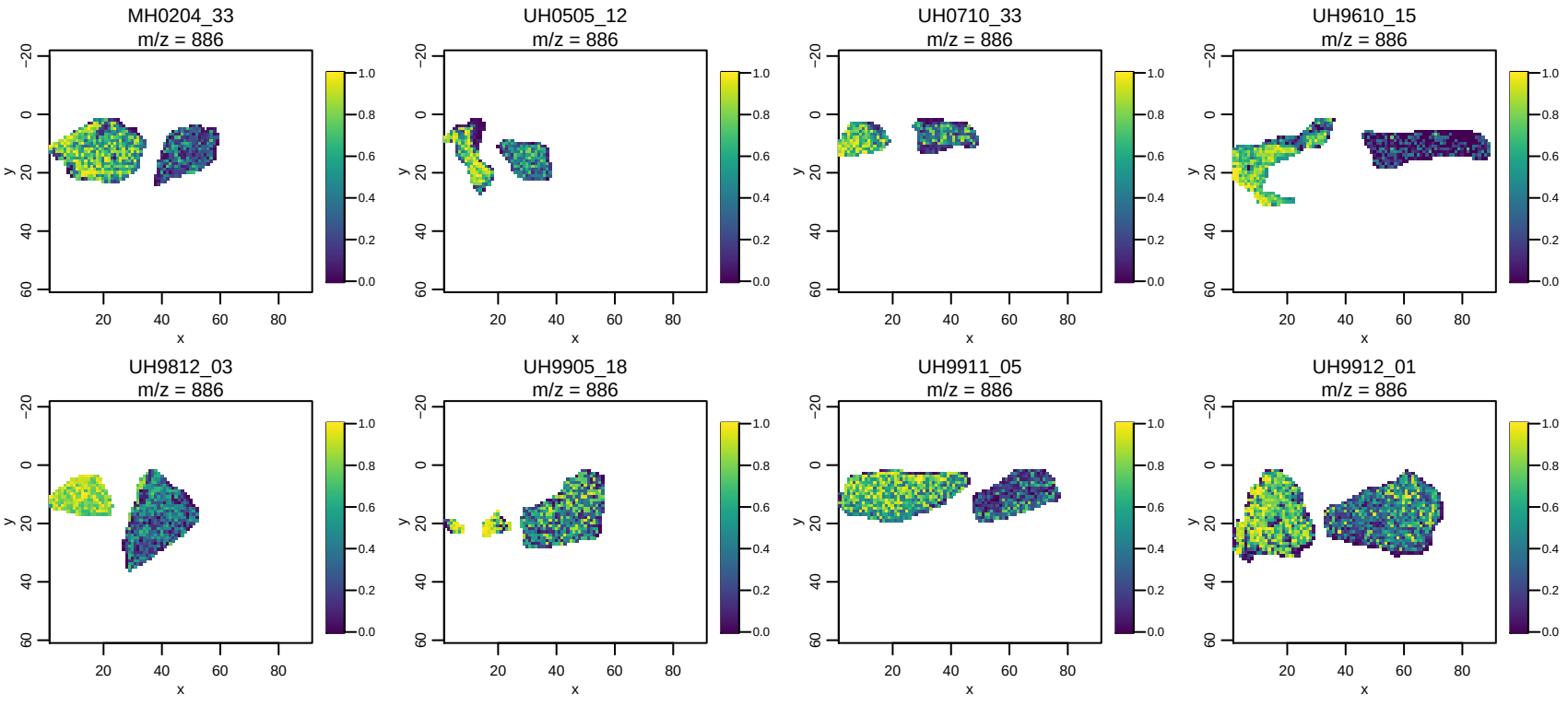
Mass features associated with cancer

```
topFeatures(rcc_sscg, class=="cancer")
```

```
## Top-ranked features:
##      mz r k  s  class   centers statistic
## 1   886 3 2 20 cancer 20.304817 20.877944
## 2   751 3 2 20 cancer  4.202790 17.209928
## 3   887 3 2 20 cancer 10.588845 14.976055
## 4   774 3 2 20 cancer  3.421733 12.399521
## 5   749 3 2 20 cancer  3.866871  8.859075
## 6   750 3 2 20 cancer  2.935949  8.789444
## 7   888 3 2 20 cancer  5.638888  8.341449
## 8   775 3 2 20 cancer  1.937996  7.084207
## 9   752 3 2 20 cancer  1.906379  6.289482
## 10 748 3 2 20 cancer  5.533633  5.977955
```

m/z 886 associated with cancer

```
image(rcc_binned, mz=886, layout=c(2,4),
      contrast.enhance="histogram",
      normalize.image="linear")
```



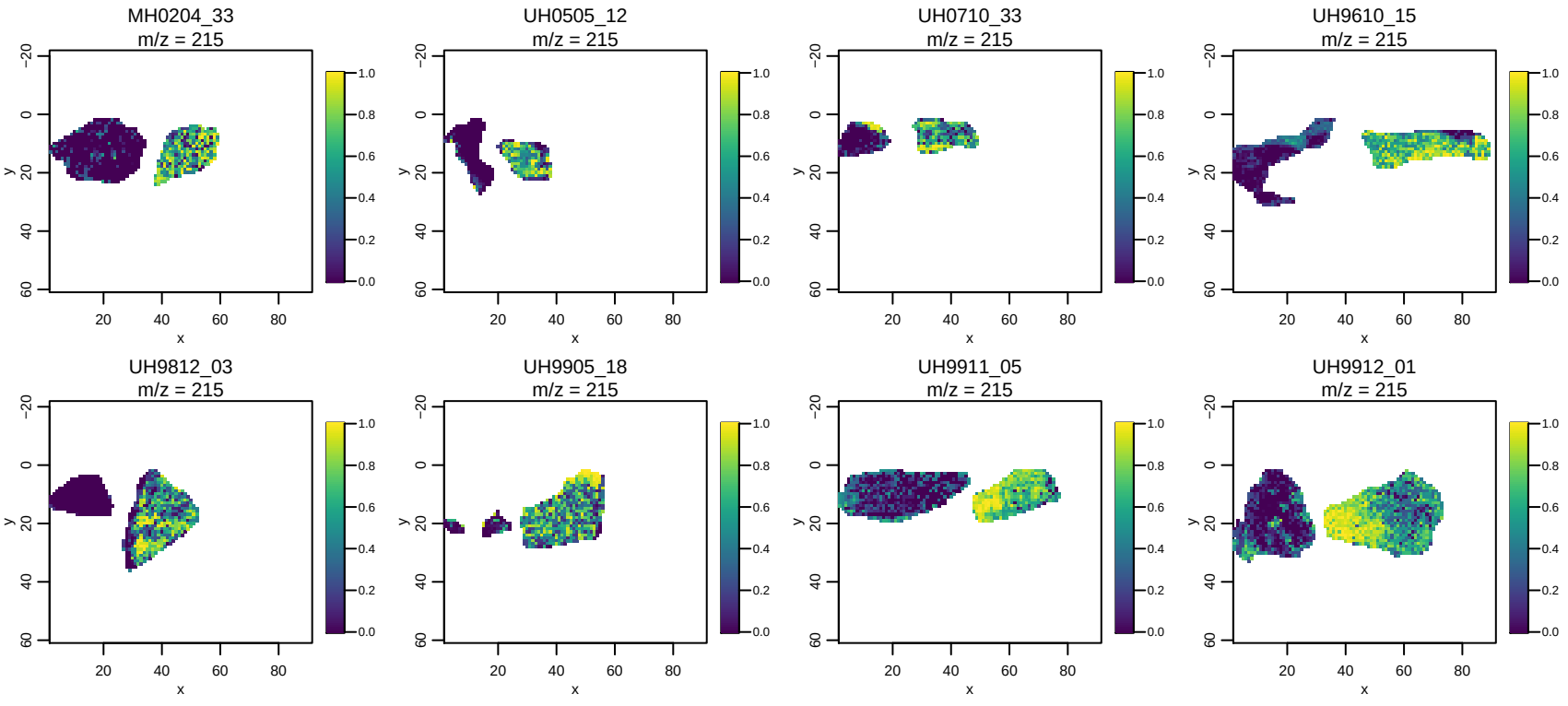
Mass features associated with normal

```
topFeatures(rcc_sscg, class=="normal")
```

```
## Top-ranked features:
##      mz r k  s  class   centers statistic
## 1   215 3 2 20 normal  6.199255 26.722518
## 2   810 3 2 20 normal  8.845353 18.420318
## 3   279 3 2 20 normal  3.719320 17.676717
## 4   353 3 2 20 normal  2.371743 17.202849
## 5   217 3 2 20 normal 10.376391 12.651647
## 6   171 3 2 20 normal  3.432423 12.246610
## 7   187 3 2 20 normal  2.562495 10.017407
## 8   255 3 2 20 normal  4.666685  9.295445
## 9   269 3 2 20 normal  2.174021  8.869836
## 10 181 3 2 20 normal  3.628515  8.084145
```

m/z 215 associated with normal

```
image(rcc_binned, mz=215, layout=c(2,4),
      contrast.enhance="histogram",
      normalize.image="linear")
```



Statistical testing

Statistical testing

Suppose we want to test which mass features are differentially abundant between cancer and normal tissue?

We have N = 8 subjects per condition.

Two approaches we might take using `Cardinal`:

- Average all pixels in each tissue sample and compare means
- Segment each tissue and compare distinctive segments

Let's explore both.

Filter mass features by intensity thresholding

```
rcc_filt <- rcc_binned %>%
  mzFilter(thresh.max=0.25) %>%
  process()

rcc_filt
```

```
## An object of class 'MSContinuousImagingExperiment'
##   <24 feature, 6077 pixel> imaging dataset
##     imageData(1): intensity
##     featureData(0):
##     pixelData(1): diagnosis
##     processing complete(1): mzFilter
##     processing pending(0):
##     run(8): MH0204_33 UH0505_12 ... UH9911_05 UH9912_01
##     raster dimensions: 90 x 36
##     coord(2): x = 2..91, y = 2..37
##     mass range: 179 to 888
##     centroided: FALSE
```

Means-based testing

We need to create a variable for each (subject x condition) combination.

Then we can use `meansTest()` to calculate the mean intensities for each group and fit linear models for each mass feature.

```
rcc_filt$sample <- interaction(run(rcc_filt),
                               rcc_filt$diagnosis)

rcc_mtest <- meansTest(rcc_filt, ~ diagnosis,
                       groups=rcc_filt$sample)
```

```
summary(rcc_mtest)
```

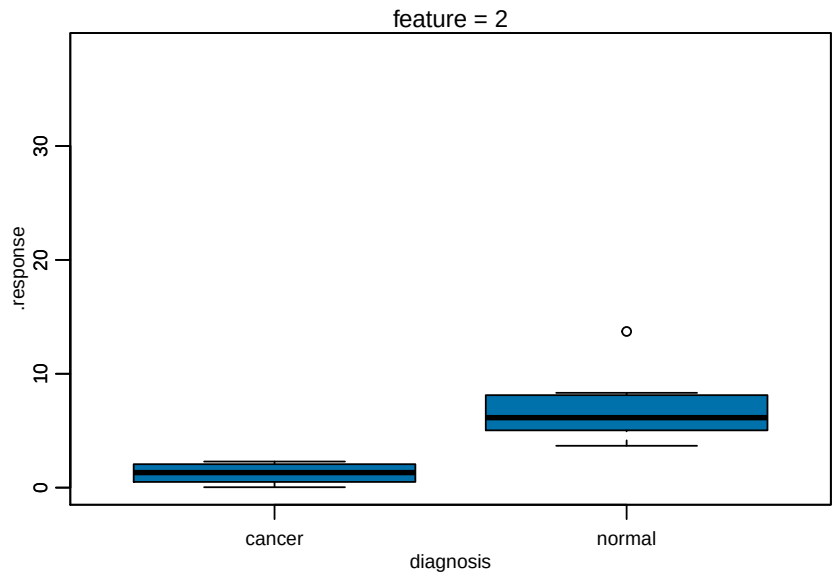
```
## Means-summarized linear model testing:
##
##  Fixed effects: ~diagnosis
##
##  Summarized 16 groups: MH0204_33.cancer UH0505_12.cancer ... UH9911_05.normal
##  Summarized     UH9912_01.normal
##
##  Likelihood ratio test for fixed effects:
##
##    Feature      LR       PValue          FDR
## 1        1  3.0428 8.109691e-02 1.621938e-01
## 2        2 25.0468 5.595576e-07 1.342938e-05
## 3        3  5.3379 2.086668e-02 7.154290e-02
## 4        4  5.7995 1.603029e-02 6.412116e-02
## 5        5  0.1023 7.490393e-01 8.171338e-01
## 6        6  2.4427 1.180745e-01 1.771118e-01
## 7        7  3.6943 5.459962e-02 1.310391e-01
## 8        8  4.8178 2.816663e-02 7.511102e-02
## 9        9  3.2456 7.161508e-02 1.562511e-01
## 10      10  0.0685 7.934699e-01 8.279686e-01
## 11      11  2.1830 1.395439e-01 1.970032e-01
## 12      12  0.0036 9.521536e-01 9.521536e-01
## 13      13  2.6273 1.050369e-01 1.771118e-01
## 14      14  1.3544 2.445037e-01 2.934045e-01
## 15      15  2.4443 1.179504e-01 1.771118e-01
## 16      16  0.6841 4.081620e-01 4.664709e-01
## 17      17  2.0003 1.572729e-01 2.096973e-01
## 18      18  5.0775 2.423862e-02 7.271586e-02
## 19      19 12.5661 3.928116e-04 4.713740e-03
## 20      20  1.7581 1.848666e-01 2.335158e-01
## 21      21  2.6737 1.020161e-01 1.771118e-01
## 22      22 10.1421 1.449245e-03 1.159396e-02
## 23      23  7.4050 6.504441e-03 3.902665e-02
## 24      24  6.0384 1.399762e-02 6.412116e-02
```

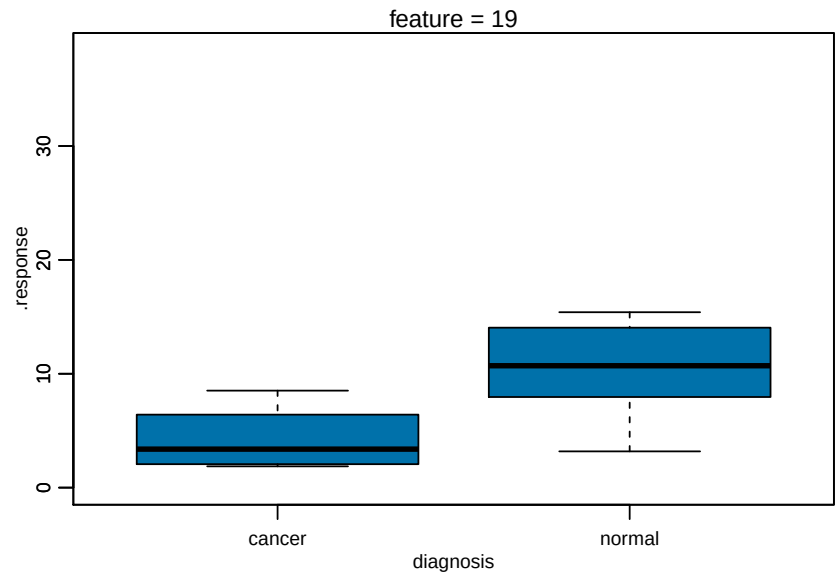
Significant mass features

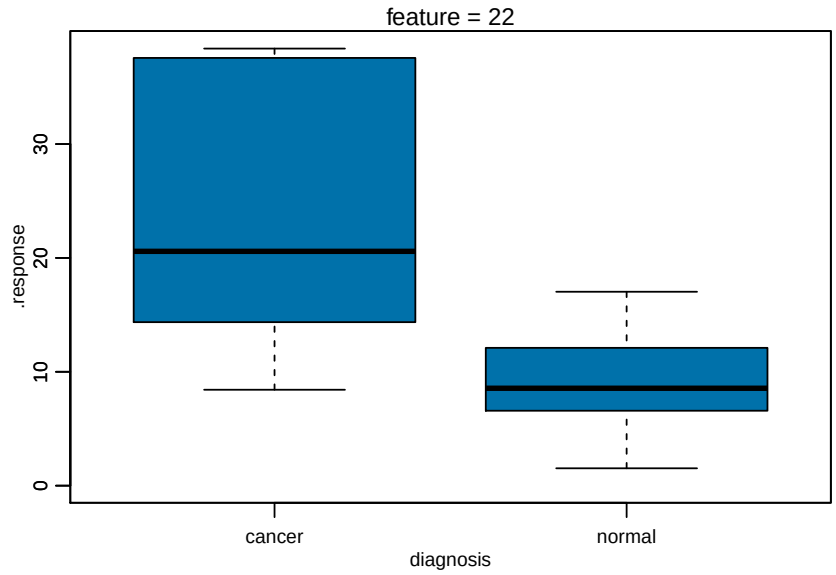
```
topFeatures(rcc_mtest, p.adjust="fdr", AdjP < 0.05)
```

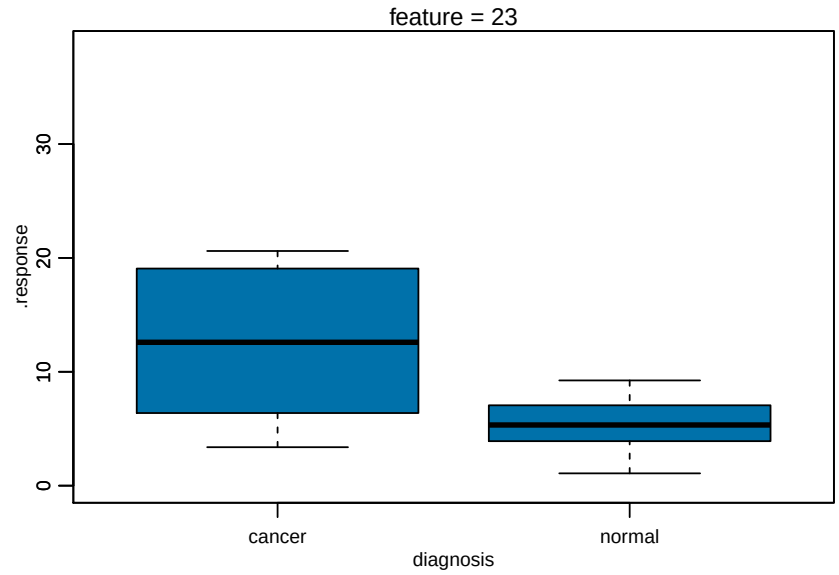
```
## Top-ranked tests: ~diagnosis vs ~1
##    mz feature      LR       PValue         AdjP
## 1 215       2 25.0468 5.595576e-07 1.342938e-05
## 2 810      19 12.5661 3.928116e-04 4.713740e-03
## 3 886      22 10.1421 1.449245e-03 1.159396e-02
## 4 887      23  7.4050 6.504441e-03 3.902665e-02
```

```
plot(rcc_mtest, model=c(2,19,22,23))
```

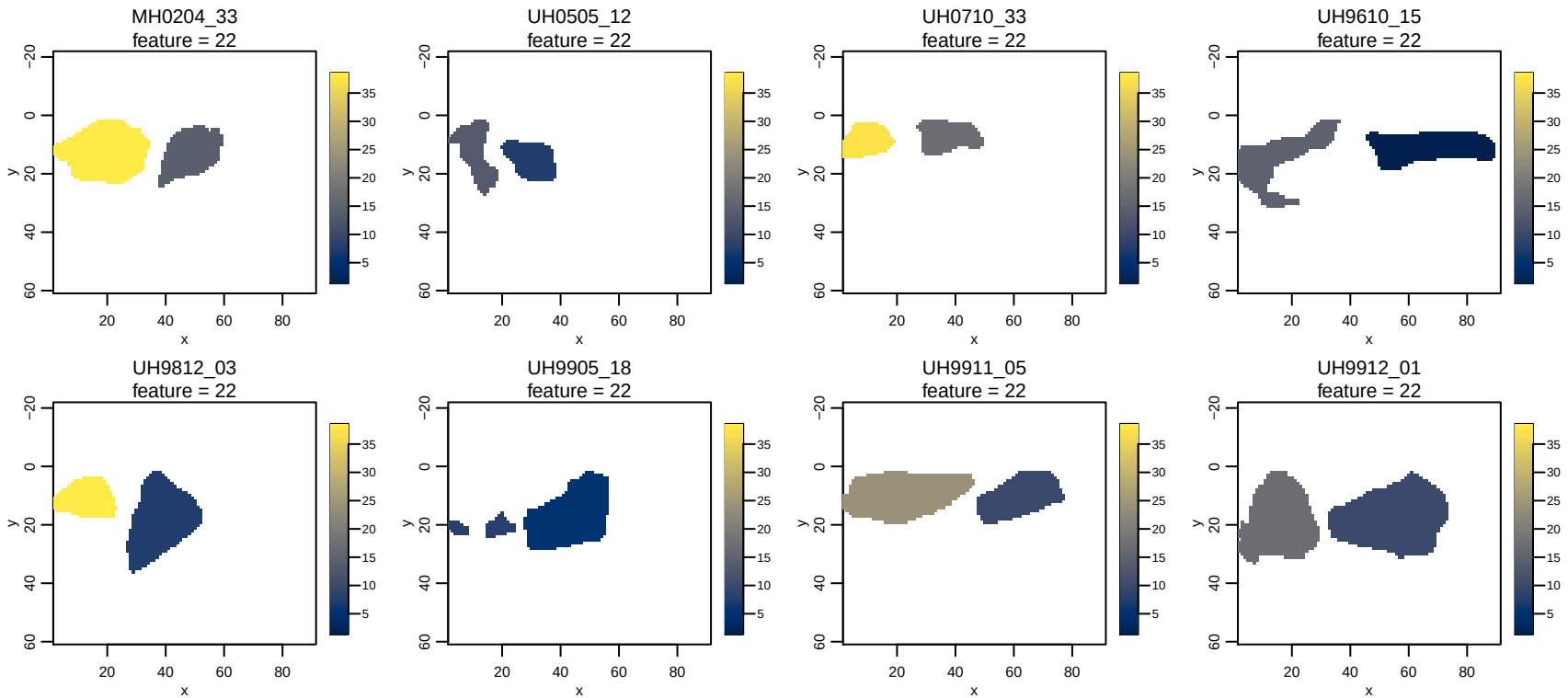








```
image(rcc_mtest, model=22, layout=c(2,4))
```



Segmentation-based testing

To represent potentialy heterogeneity within the tissue, we can segment each tissue first and then compare the most mean intensities for the segments.

First we use `spatialDGMM()` to fit segmentations for each mass feature, and then `segmentationTest()` fits linear models to the segments.

```
rcc_dgmm <- spatialDGMM(rcc_filt,
                        r=1, k=2,
                        groups=rcc_filt$sample)

rcc_stest <- segmentationTest(rcc_dgmm, ~ diagnosis)
```

```
summary(rcc_stest)
```

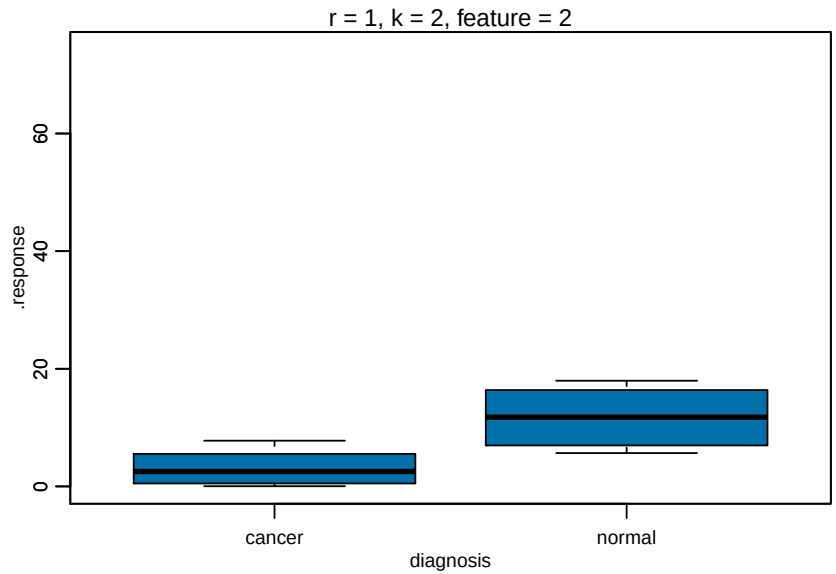
```
## Segmentation-based linear model testing:
##
##  Fixed effects: ~diagnosis
##
##  Summarized 16 groups: MH0204_33.cancer UH0505_12.cancer ... UH9911_05.normal
##  Summarized     UH9912_01.normal
##
##  Likelihood ratio test for fixed effects:
##
##    Radius (r) Init (k) Feature      LR       PValue          FDR
## 1           1        2       1  0.4921 4.830070e-01 0.5796798346
## 2           1        2       2 17.3664 3.082274e-05 0.0003698729
## 3           1        2       3  4.1109 4.260668e-02 0.1690570117
## 4           1        2       4  2.5544 1.099864e-01 0.2030518969
## 5           1        2       5  0.0692 7.925667e-01 0.8270261406
## 6           1        2       6  1.5512 2.129562e-01 0.2839415855
## 7           1        2       7  3.3628 6.668446e-02 0.1690570117
## 8           1        2       8  3.2727 7.044042e-02 0.1690570117
## 9           1        2       9  3.2856 6.989081e-02 0.1690570117
## 10          1        2      10  0.3077 5.791127e-01 0.6317592685
## 11          1        2      11  0.4919 4.830665e-01 0.5796798346
## 12          1        2      12  0.0018 9.657530e-01 0.9657529550
## 13          1        2      13  2.9800 8.429768e-02 0.1839222090
## 14          1        2      14  2.6840 1.013598e-01 0.2027195469
## 15          1        2      15  2.2376 1.346923e-01 0.2155076543
## 16          1        2      16  2.3334 1.266246e-01 0.2155076543
## 17          1        2      17  1.5536 2.126092e-01 0.2839415855
## 18          1        2      18  1.6557 1.981820e-01 0.2839415855
## 19          1        2      19 17.9474 2.270946e-05 0.0003698729
## 20          1        2      20  0.3732 5.412560e-01 0.6185783167
## 21          1        2      21  3.3071 6.898141e-02 0.1690570117
## 22          1        2      22 10.4551 1.223086e-03 0.0097846878
## 23          1        2      23  9.0476 2.630386e-03 0.0157823130
## 24          1        2      24  6.8695 8.768033e-03 0.0420865594
```

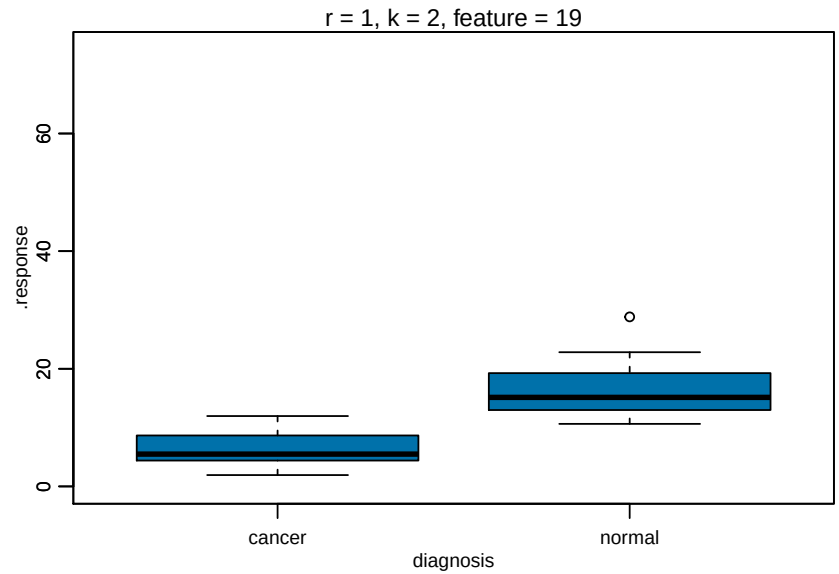
Significant mass features

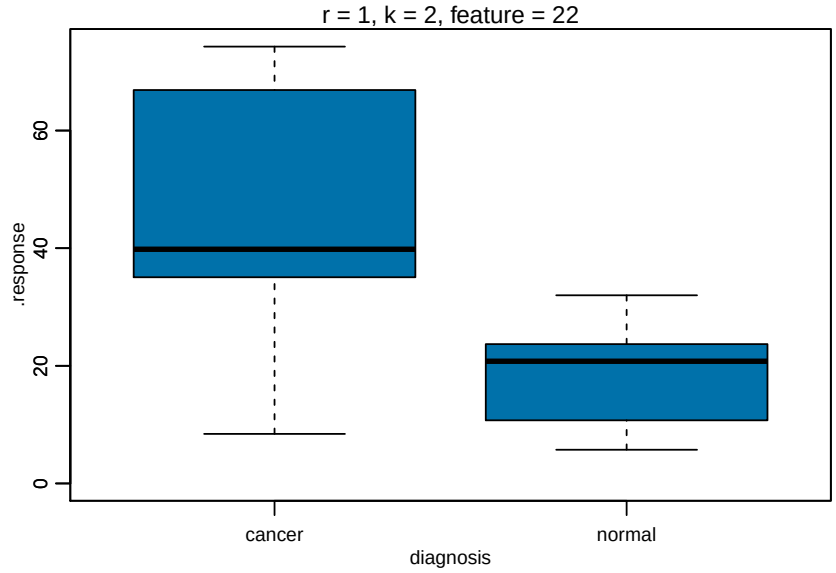
```
topFeatures(rcc_stest, p.adjust="fdr", AdjP < 0.05)
```

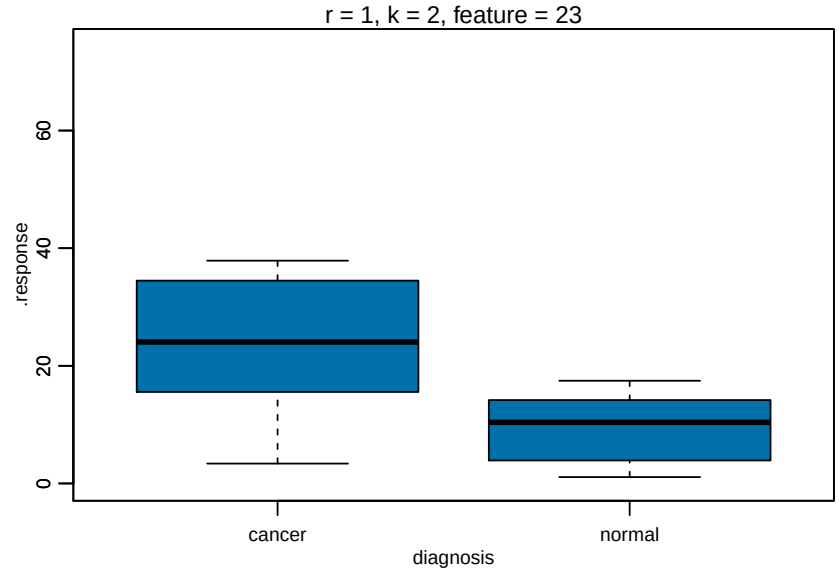
```
## Top-ranked tests: ~diagnosis vs ~1
##    mz r k feature      LR       PValue         AdjP
## 1 215 1 2       2 17.3664 3.082274e-05 0.0003698729
## 2 810 1 2      19 17.9474 2.270946e-05 0.0003698729
## 3 886 1 2      22 10.4551 1.223086e-03 0.0097846878
## 4 887 1 2      23  9.0476 2.630386e-03 0.0157823130
## 5 888 1 2      24  6.8695 8.768033e-03 0.0420865594
```

```
plot(rcc_stest, model=c(2,19,22,23))
```

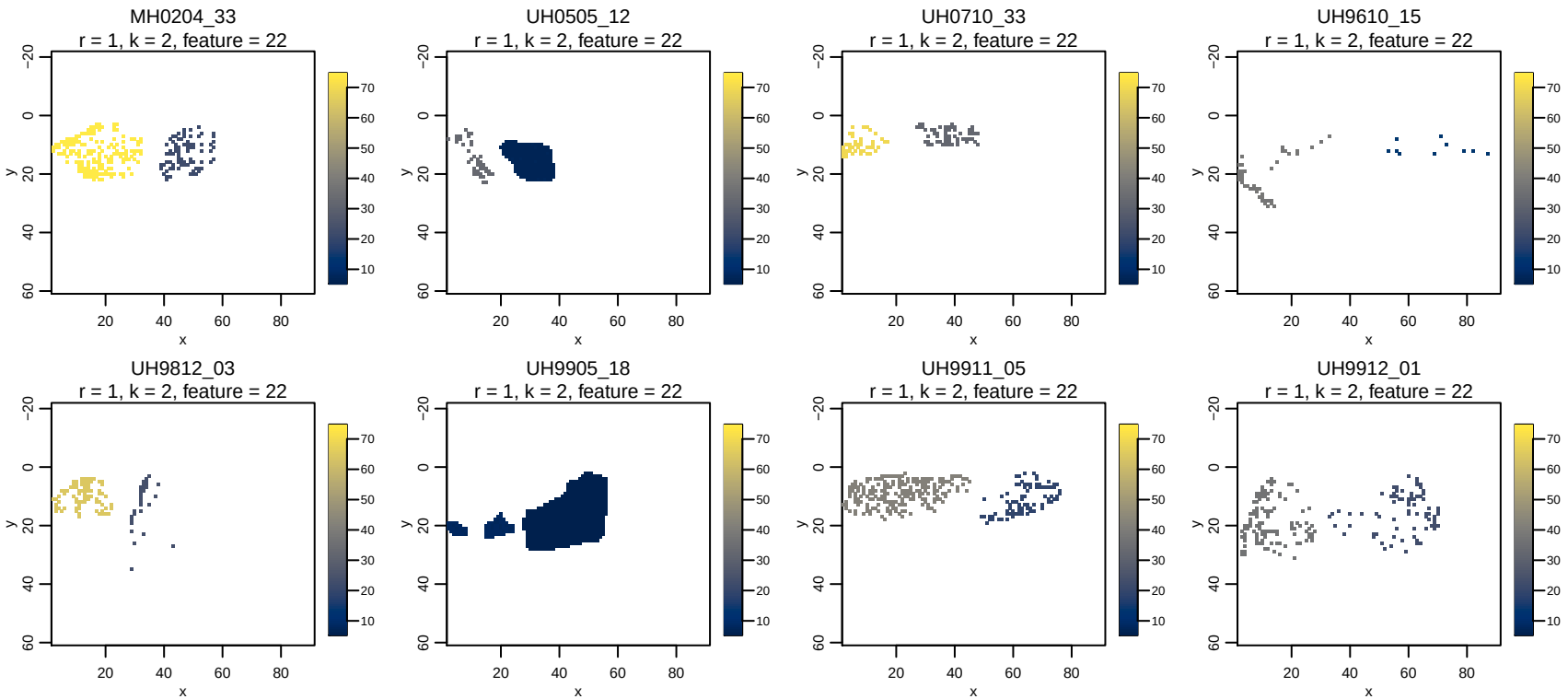








```
image(rcc_stest, model=22, layout=c(2,4))
```



Interactive visualization

Interactive visualization

Shiny interface for Cardinal (under development) on Github...

```
if (!requireNamespace("remotes", quietly = TRUE))
    install.packages("remotes")

BiocManager::install("kuwisdelu/CardinalVis")

library(CardinalVis)

pig206 <- as(pig206, "MSImagingExperiment")

msiVis(pig206)
```

Questions