# IrisSpatialFeatures - An R package to quantify the tumor microenvironment based on multiplex IF data

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## Short example on how to use the IrisSpatialFeatures package

### Reading the dataset

This is a toy example, based on 2 sample with 2 coordinates each. The 20x images were acquired on the Mantra system of PerkinElmer, analyzed in inForm. There are three different phenotypes present in this example: melanoma cells (SOX10+ cells), CD8 cells indicating cytotoxic T-cells and other or undefined cells which have neither SOX10 or CD8 protein expression, but show up as cells according to the nuclear stain. In addition, PD1 and PD-L1 expression was scored in each sample and a threshold was determined in inForm that let's us distinguish between PD1+ and PD1- cells as well as PD-L1+/-. PD-L1 is only relevant in melanoma cells, whereas PD1 is relevant in the other two cell types. Since the toy example only shows the area of a very small image, the resulting statistics often contain NA value, because cell types are not present, which is especially the case in ROI analyses. Look at the full example datasets for more realistic examples.

```r
require(IrisSpatialFeatures)
raw_data <- new("ImageSet")
raw_data<- read_raw(raw_data,
                    raw_dir_name=system.file("extdata", package = "IrisSpatialFeatures"),
                    format='Mantra')
```

```
## [1] "Sample: MEL2"
## [1] "Sample: MEL3"
```

```r
#apply all the thresholds PD1 for T and other cells, PD-L1 for macrophages and tumor cells
dataset <- threshold_dataset(raw_data,
                             marker='PD-Ligand-1 (Opal 690)',
                             marker_name='PDL1',
                             base=c('SOX10+'))
dataset <- threshold_dataset(dataset,
                             marker='PD-1 (Opal 540)',
                             marker_name='PD1',
                             base=c('CD8+','OTHER'))
```

### Overview plots

Next we plot all the cell coordinates color coordinated in .pdf format

```r
plot_dir <- file.path(tempdir(),'plots')
if (!file.exists(plot_dir)){
    dir.create(file.path(plot_dir))
}
p <- overview_plot(dataset,outdir=plot_dir,palette=NULL,type='png')
```
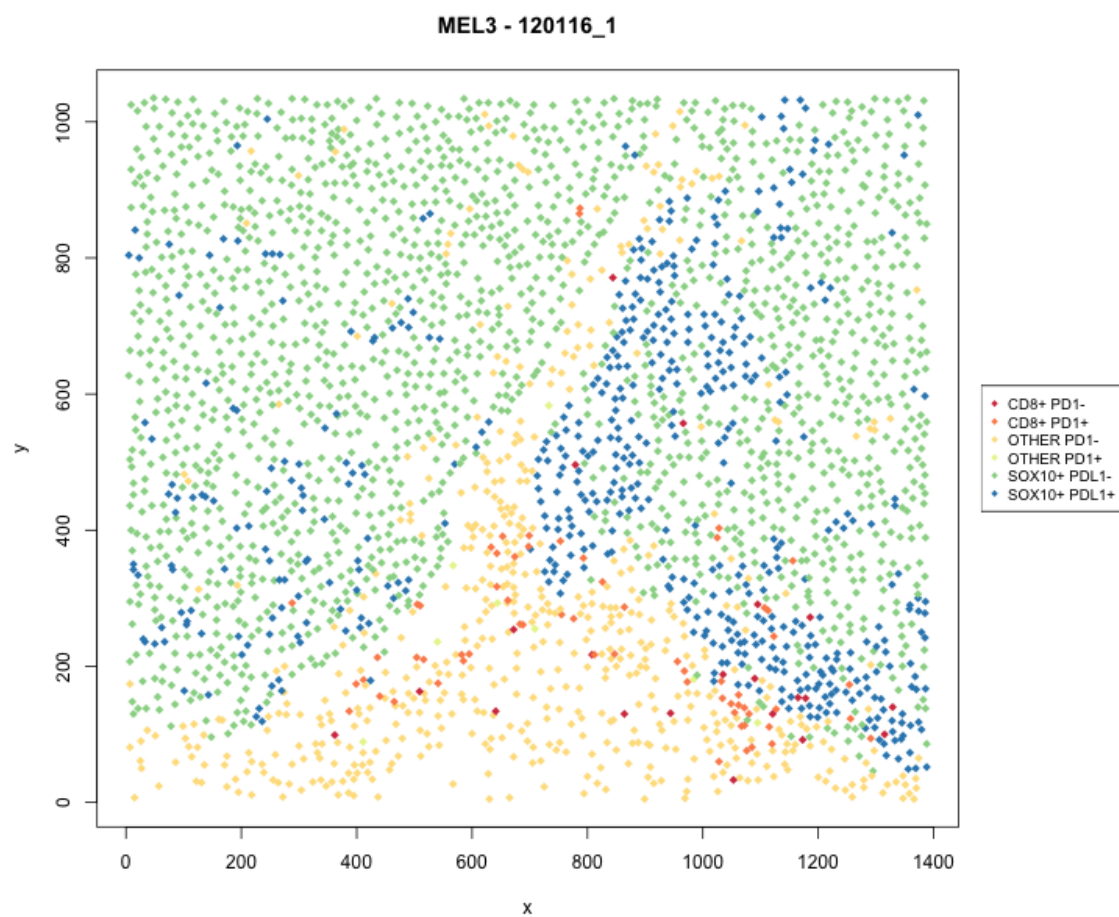
Figure 1: Example of an Overview plot

## Extract counts and ratios

Here we extract counts per mm2 for each marker, both for each coordinate and collapsed across the multiple images per sample.

```
get_counts_per_mm2(dataset)
```

```
##                 MEL2             MEL3
## CD8+ PD1-    "  21 +/-    9.8" "  95 +/-   36.5"
## CD8+ PD1+    " 327 +/- 189.5" " 147 +/-   29.5"
## OTHER PD1-   "2235 +/- 373.4" "1475 +/-   71.6"
## OTHER PD1+   " 157 +/-  44.9" "  14 +/-    8.4"
## SOX10+ PDL1- "3431 +/- 480.1" "3814 +/- 1009.4"
## SOX10+ PDL1+ " 303 +/-  36.5" "2186 +/-  714.6"
```

```
get_counts_per_mm2_noncollapsed(dataset)
```

```
## $MEL2
##           CD8+ PD1- CD8+ PD1+ OTHER PD1- OTHER PD1+ SOX10+ PDL1-
## 080416_2   30.88565  516.6327   2608.434   202.1606     2950.984
## 080416_7   11.23115  137.5815   1861.562   112.3115     3911.247
##           SOX10+ PDL1+
## 080416_2      266.7397
## 080416_7      339.7422
##
## $MEL3
##            CD8+ PD1- CD8+ PD1+ OTHER PD1- OTHER PD1+ SOX10+ PDL1-
## 120116_1   58.96352  176.8905   1547.090  22.462292     4823.777
## 120116_2  131.96596  117.9270   1403.893   5.615573     2804.979
##           SOX10+ PDL1+
## 120116_1      1471.280
## 120116_2      2900.443
```

```
get_count_ratios(dataset,'SOX10+ PDL1-','SOX10+ PDL1+')
```

```
##            MEL2            MEL3            <NA>            <NA>
## "11.06 +/- NA" "11.51 +/- NA" " 3.28 +/- NA" " 0.97 +/- NA"
```
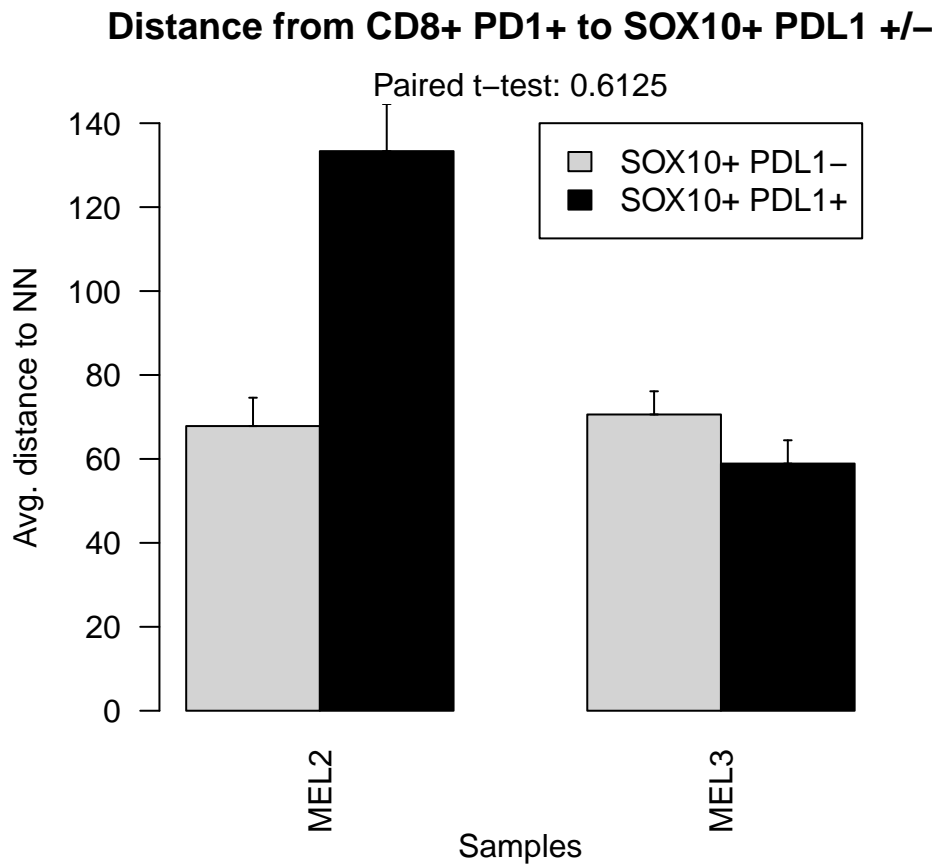
## Nearest neighbor analysis

Next step we calculate the average nearest neighbor distances for each cell-type, plot barplots compare different distances and finally generate ray plots that show a visual representation of these distances for each coordinate.

```
dataset <- extract_nearest_neighbor(dataset,min_num_cells=2)
get_nearest_neighbors(dataset,"SOX10+ PDL1+")
```

```
## $mean
##                    MEL2       MEL3
## CD8+ PD1-     219.95758 177.05966
## CD8+ PD1+      47.26242 140.91845
## OTHER PD1-     28.21915  79.21432
## OTHER PD1+    108.54489 463.99940
## SOX10+ PDL1-   27.07400  30.64719
## SOX10+ PDL1+    0.00000   0.00000
##
```
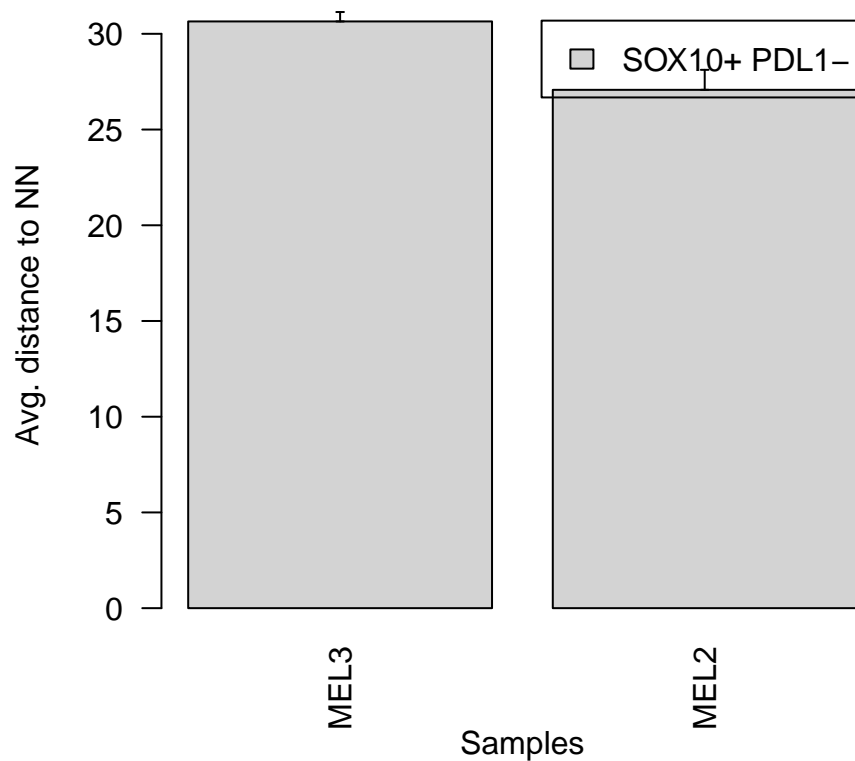
```
## $SE
##                  MEL2      MEL3
## CD8+ PD1-    8.8792419 3.4993164
## CD8+ PD1+    2.2356082 2.5140467
## OTHER PD1-   0.9902901 1.5209465
## OTHER PD1+   6.7568488 4.9207914
## SOX10+ PDL1- 1.0443331 0.4908165
## SOX10+ PDL1+ 0.0000000 0.0000000
```

```
p <- plot_nearest_neighbor(dataset,'CD8+ PD1+','SOX10+ PDL1')
```

**Distance from CD8+ PD1+ to SOX10+ PDL1 +/−**



```
p <- plot_nearest_neighbor(dataset,'SOX10+ PDL1+','SOX10+ PDL1-')
```

# Distance from SOX10+ PDL1+ to SOX10+ PDL1−



```r
#ray plots for
plot_dir <- file.path(tempdir(),'ray_plots')
if (!file.exists(plot_dir)){
    dir.create(file.path(plot_dir))
}
neighbor_ray_plot(dataset,from_type='CD8+ PD1+',to_type='SOX10+ PDL1+',plot_dir=plot_dir,format ='pdf')
```

```
## $MEL2
## $MEL2$`080416_2`
## null device
##            1
##
## $MEL2$`080416_7`
## null device
##            1
##
##
## $MEL3
## $MEL3$`120116_1`
## null device
##            1
##
## $MEL3$`120116_2`
## null device
##            1
```

## Interaction analysis

Here we extract the interactions between different cell types, generate an interaction profile for SOX10+ PDL1+ cells and also generate interaction maps for each coordinate showing the interactions between CD8+ PD1+ cells and SOX10+ PD-L1+ cells.

```
dataset <- extract_interactions(dataset)
```

```
## MEL2  ... processing...
```

```
## 29 solved of 36 issues
## 11 solved of 13 issues
```

```
## MEL3  ... processing...
```

```
## 16 solved of 18 issues
## 15 solved of 19 issues
```
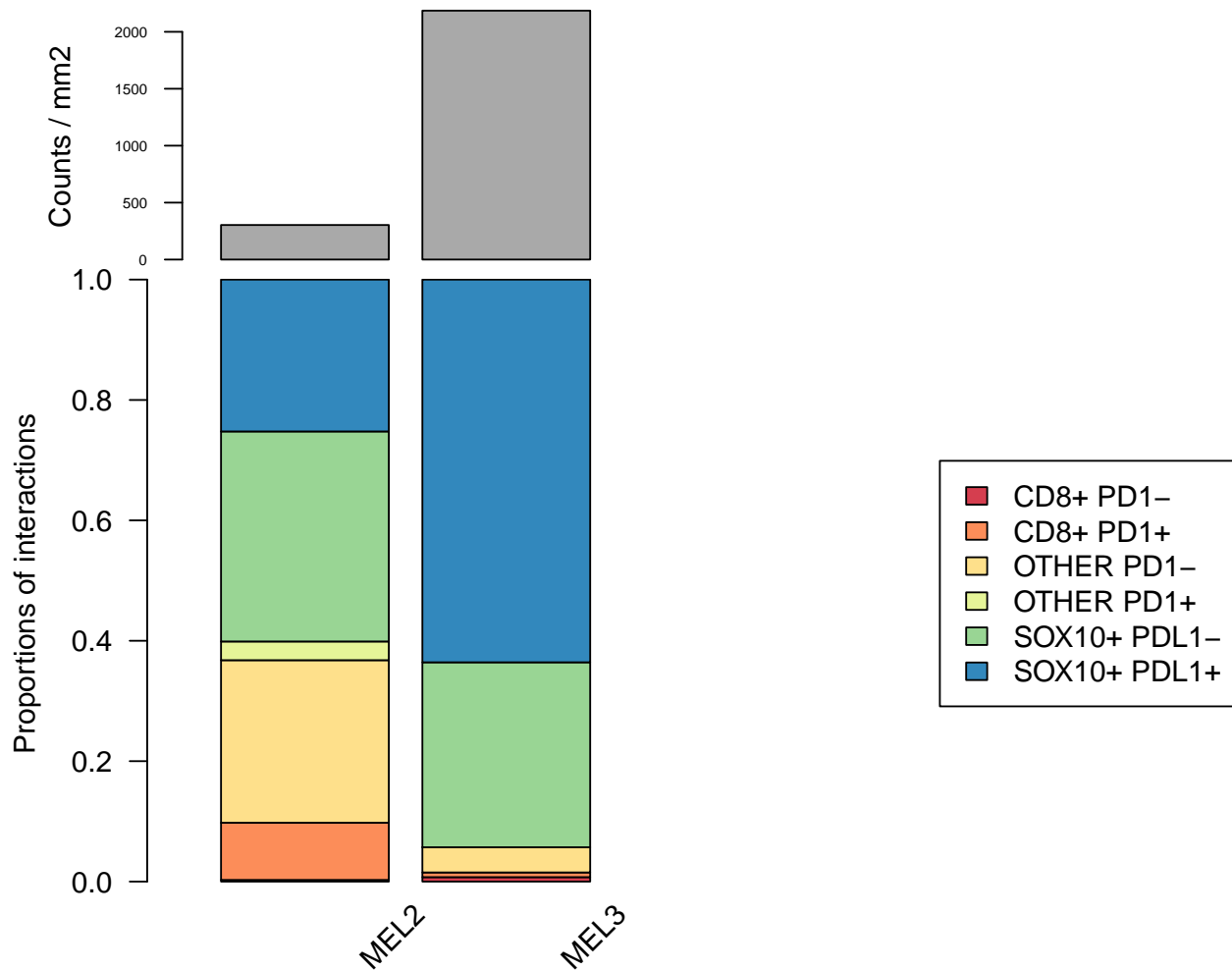
```
get_interactions(dataset,'SOX10+ PDL1+')
```

```
##                   MEL2        MEL3
## CD8+ PD1-    0.002687426 0.007307972
## CD8+ PD1+    0.095003831 0.007819282
## OTHER PD1-   0.270041866 0.041832681
## OTHER PD1+   0.031083077 0.000000000
## SOX10+ PDL1- 0.348876723 0.307243647
## SOX10+ PDL1+ 0.252307077 0.635796418
```

```
#plotting interaction summaries
p <- plot_interactions(dataset,'SOX10+ PDL1+',xlim_fix=4)
```

## Interactions with SOX10+ PDL1+



```
#plotting interaction maps
int_markers <- c('CD8+ PD1+','SOX10+ PDL1+')
int_marker_cols <- c('#fc5858','#66c2a4')
silent_markers <- c('CD8+ PD1-','SOX10+ PDL1-' )
silent_col=c('#fff7bc','#a6bddb')
plot_dir <- file.path(tempdir(),'interaction_maps')
if (!file.exists(plot_dir)){
    dir.create(file.path(plot_dir))
}
p <- interaction_maps(dataset,int_markers,int_marker_cols,silent_markers,
                   silent_col,outdir=plot_dir)
```

```
## Working on sample: MEL2
```

```
## Working on sample: MEL3
```

## Running the proximity analysis

Calculating the number of cells within 25 pixels distance for each cell and then showing the the profile for SOX10+ PDL1-
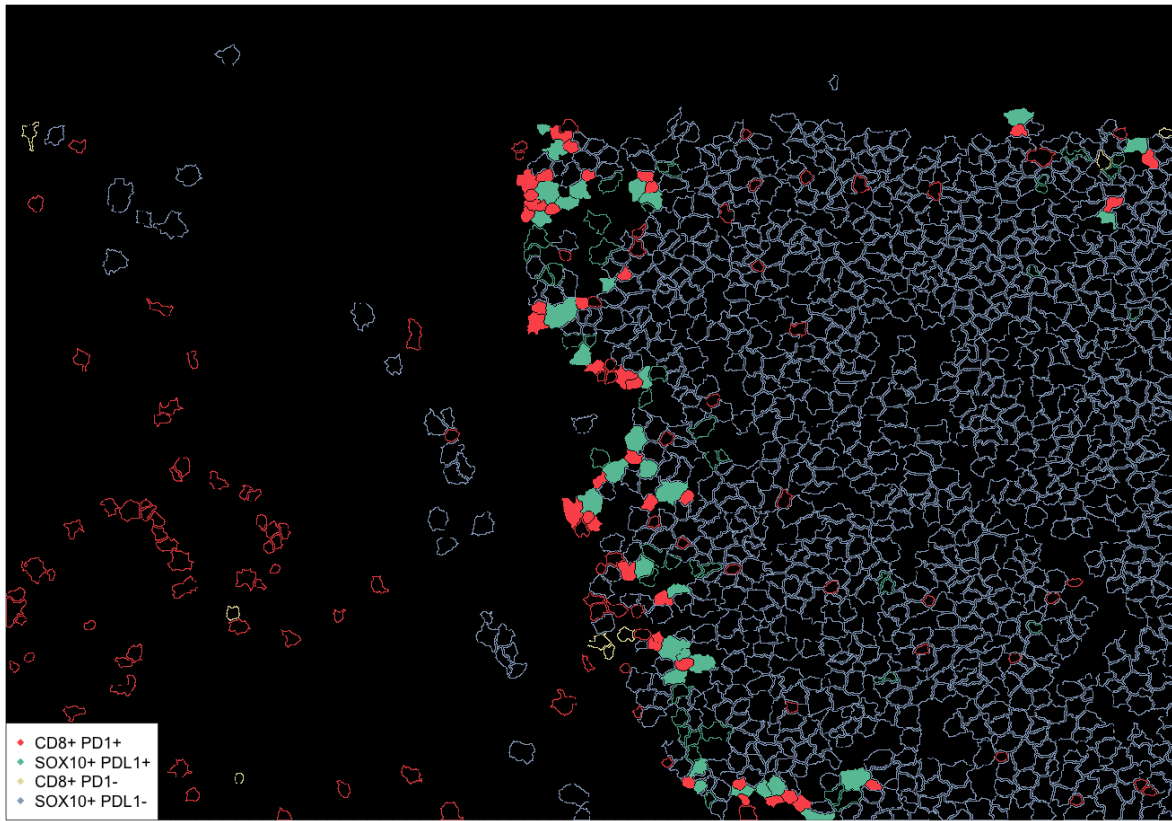
Figure 2: Example of a Interaction map

```
dataset <- extract_proximity(dataset,only_closest=TRUE,radii=25)
```

```
## MEL2  ... processing...
```

```
## MEL3  ... processing...
```

```
p <- plot_proximities(dataset,"SOX10+ PDL1-",xlim_fix=3)
```

**In proximity to SOX10+ PDL1−**