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

Daniel Gusenleitner

2017-07-31

Short example on how to use the IrisSpatialFeatures package

Reading the dataset

This is a toy example, based on 2 sample with 5 coordinates each. The 20x images were acquired on the Mantra system of PerkinElmer, analyzed in inForm and cropped to 200x200 px to reduce the runtime. There are three different phenotypes present in this example, SOX10 or melanoma cell, 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)
```

```
## Loading required package: IrisSpatialFeatures
```

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

```
## [1] "Sample: MEL29822"
## [1] "Sample: MEL33544"
```

```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('./plots')
if (!file.exists(plot_dir)){
    dir.create(file.path(plot_dir))
```
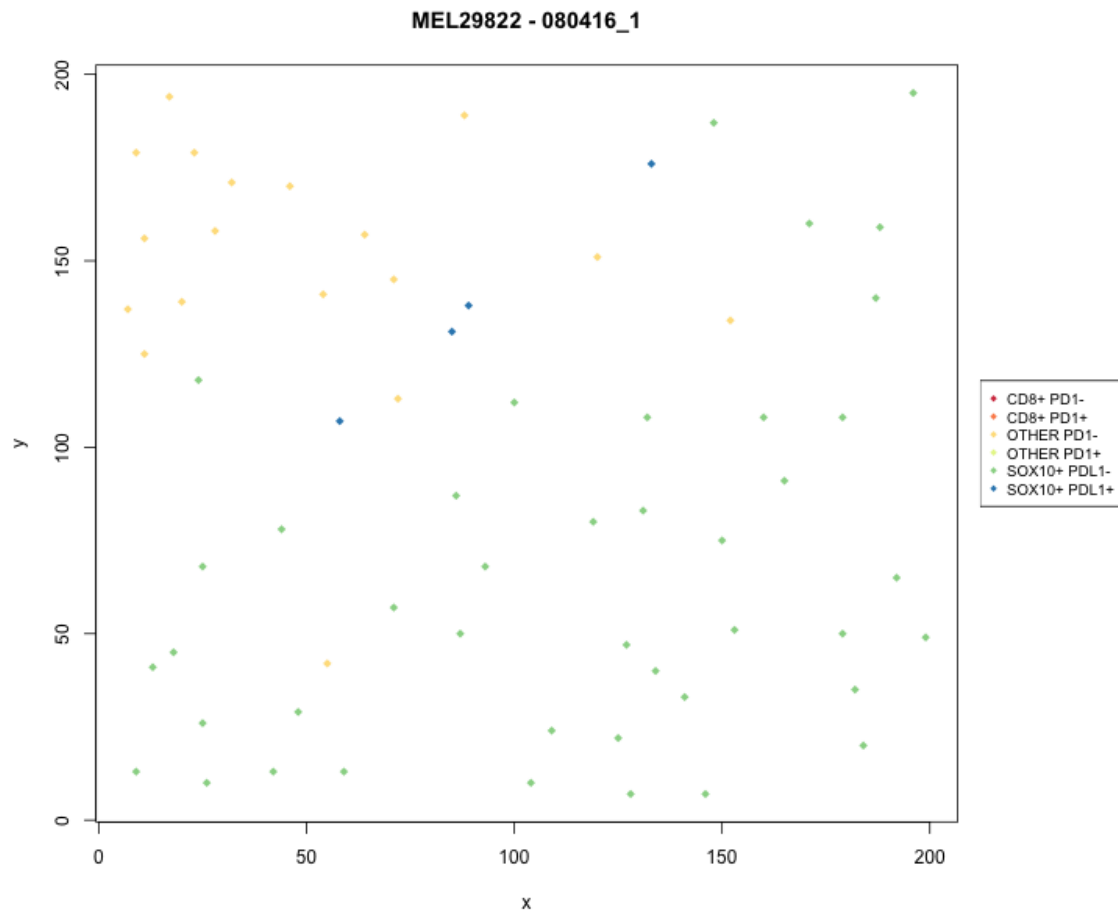
Figure 1: Example of an Overview plot

```
}
p <- overview_plot(dataset,outdir=plot_dir,palette=NULL,type='png')
```

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)
```

```
##                MEL29822        MEL33544
## CD8+ PD1-    " 102 +/- 102" " 203 +/-  129"
## CD8+ PD1+    " 874 +/- 469" " 427 +/-  250"
## OTHER PD1-   "2256 +/- 730" "2175 +/-  522"
## OTHER PD1+   " 467 +/- 418" " 793 +/-  498"
## SOX10+ PDL1- "2195 +/- 781" "3069 +/- 1051"
## SOX10+ PDL1+ " 325 +/-  99" " 264 +/-  181"
```

```
get_counts_per_mm2_noncollapsed(dataset)
```

```
## $MEL29822
```

```
##            CD8+ PD1-  CD8+ PD1+ OTHER PD1- OTHER PD1+ SOX10+ PDL1-
## 080416_1     0.000     0.0000   1829.149     0.0000    4268.0151
## 080416_2     0.000  2540.4852   3150.202  2134.0075     711.3358
## 080416_3     0.000   508.0970   1930.769   203.2388    2642.1046
## 080416_5     0.000   101.6194      0.000     0.0000    3251.8210
## 080416_7   508.097  1219.4329   4369.634     0.0000     101.6194
##            SOX10+ PDL1+
## 080416_1     406.4776
## 080416_2     508.0970
## 080416_3     508.0970
## 080416_5       0.0000
## 080416_7     203.2388
##
## $MEL33544
##            CD8+ PD1-  CD8+ PD1+ OTHER PD1- OTHER PD1+ SOX10+ PDL1-
## 080516_1    0.0000   101.6194   1422.672      0.000    5182.590
## 080516_3    0.0000     0.0000   2642.105      0.000    2845.343
## 080516_4  609.7164   812.9553   1829.149   1625.911    1727.530
## 080516_5  406.4776  1219.4329   3963.157   2337.246       0.000
## 080516_6    0.0000     0.0000   1016.194      0.000    5589.067
##            SOX10+ PDL1+
## 080516_1      0.0000
## 080516_3      0.0000
## 080516_4    914.5747
## 080516_5      0.0000
## 080516_6    406.4776
```

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

```
##       MEL29822       MEL33544           <NA>           <NA>           <NA>
## "10.5 +/- NA" " 1.4 +/- NA" " 5.2 +/- NA" " NaN +/- NA" " 0.5 +/- NA"
##         <NA>           <NA>           <NA>           <NA>           <NA>
## " NaN +/- NA" " NaN +/- NA" " 1.9 +/- NA" " NaN +/- NA" "13.8 +/- 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.

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

```
## $mean
##              MEL29822 MEL33544
## CD8+ PD1-    38.48290 35.57135
## CD8+ PD1+    32.11052 42.33263
## OTHER PD1-   27.62714 23.31080
## OTHER PD1+   66.11071 45.36900
## SOX10+ PDL1- 30.80110 30.67838
## SOX10+ PDL1+  0.00000  0.00000
##
## $SE
##              MEL29822 MEL33544
## CD8+ PD1-    3.401593 3.957848
```
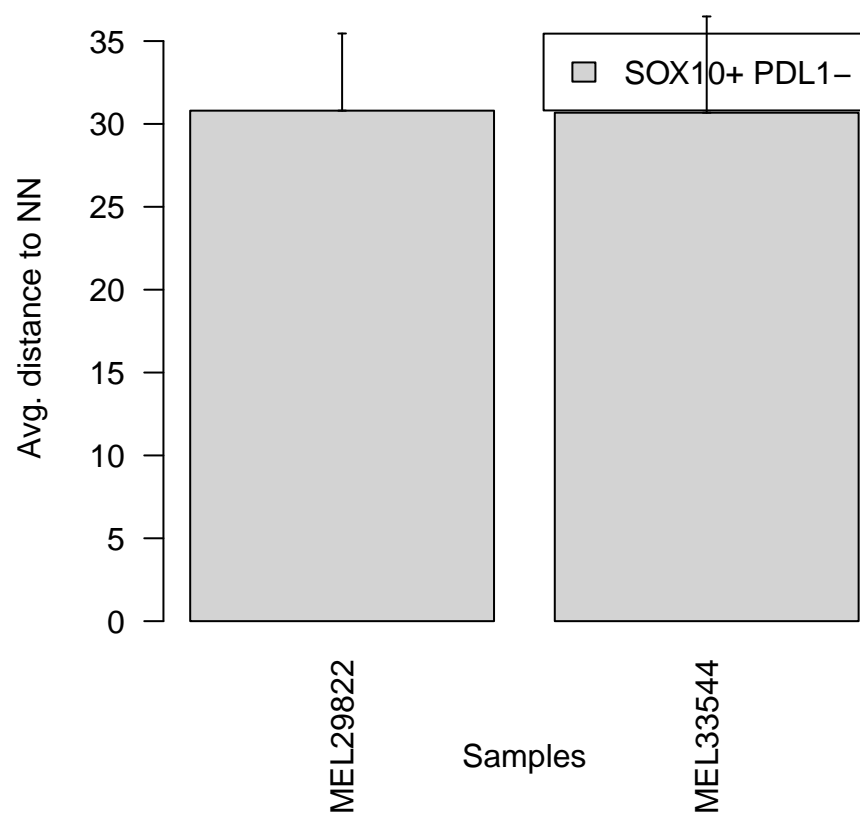
```
## CD8+ PD1+    3.055395 5.012042
## OTHER PD1-   2.127014 2.025604
## OTHER PD1+   5.725620 4.940889
## SOX10+ PDL1- 4.655262 5.805854
## SOX10+ PDL1+ 0.000000 0.000000
p <- plot_nearest_neighbor(dataset,'CD8+ PD1+','SOX10+ PDL1')
```

Distance from CD8+ PD1+ to SOX10+ PDL1 +/–

Paired t–test: 0.464
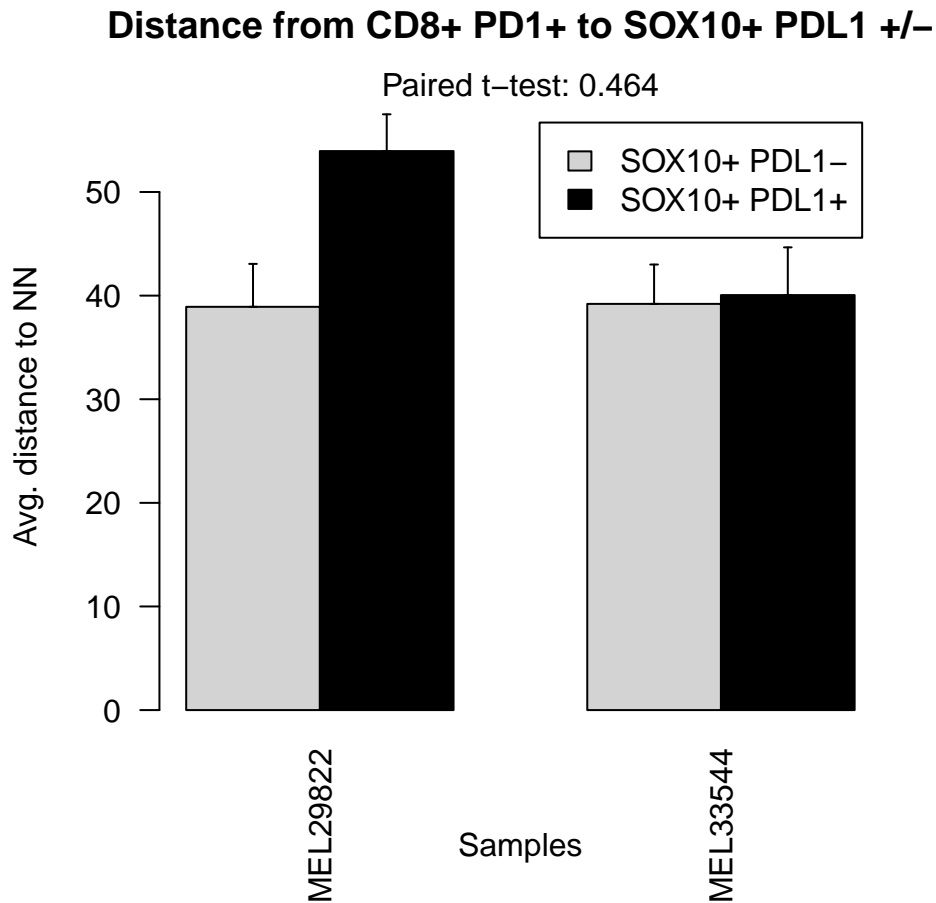

```
p <- plot_nearest_neighbor(dataset,'SOX10+ PDL1+','SOX10+ PDL1-')
```

Distance from SOX10+ PDL1+ to SOX10+ PDL1−


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

```
## $MEL29822
## $MEL29822$`080416_1`
## pdf
##   2
##
## $MEL29822$`080416_2`
## pdf
##   2
##
## $MEL29822$`080416_3`
## pdf
##   2
##
## $MEL29822$`080416_5`
## NULL
##
## $MEL29822$`080416_7`
## pdf
```

```
##    2
##
##
## $MEL33544
## $MEL33544$`080516_1`
## pdf
##    2
##
## $MEL33544$`080516_3`
## pdf
##    2
##
## $MEL33544$`080516_4`
## pdf
##    2
##
## $MEL33544$`080516_5`
## NULL
##
## $MEL33544$`080516_6`
## pdf
##    2
```

Interaction analysis

Here we extract the interactions between different cell types, generate an interaction profile for CD8+ PD1+ 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)
```

```
## MEL29822  ... processing...

## 0 solved of 0 issues
## 2 solved of 2 issues
## 1 solved of 2 issues
## 0 solved of 0 issues
## 0 solved of 0 issues

## MEL33544  ... processing...

## 0 solved of 0 issues
## 0 solved of 0 issues
## 0 solved of 0 issues
## 0 solved of 0 issues
## 0 solved of 0 issues
```

```
get_interactions(dataset,'CD8+ PD1+')
```

```
##              MEL29822   MEL33544
## CD8+ PD1-    0.0000000 0.02777778
## CD8+ PD1+    0.1538462 0.00000000
## OTHER PD1-   0.1538462 0.50000000
## OTHER PD1+   0.2051282 0.02777778
## SOX10+ PDL1- 0.3076923 0.44444444
## SOX10+ PDL1+ 0.1794872 0.00000000
```

```
#plotting interaction summaries
#p <- plot_interactions(dataset,'CD8+ PD1+',xlim_fix=4)

#plotting interaction maps
int_markers <- c('CD8+ PD1+','SOX10+ PDL1+')
int_marker_cols <- c('#dd1c77','#99d8c9')
silent_markers <- c('CD8+ PD1-')
silent_col=c('yellow')
p <- interaction_maps(dataset,int_markers,int_marker_cols,silent_markers,
                      silent_col)
```

```
## Working on sample: MEL29822
```

```
## Working on sample: MEL33544
```

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-

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

```
## MEL29822  ... processing...
```

```
## MEL33544  ... processing...
```

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

In proximity to SOX10+ PDL1−