

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

Daniel Gusenleitner

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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.

```
require(IrisSpatialFeatures)

## Loading required package: 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"

#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

```
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')
```

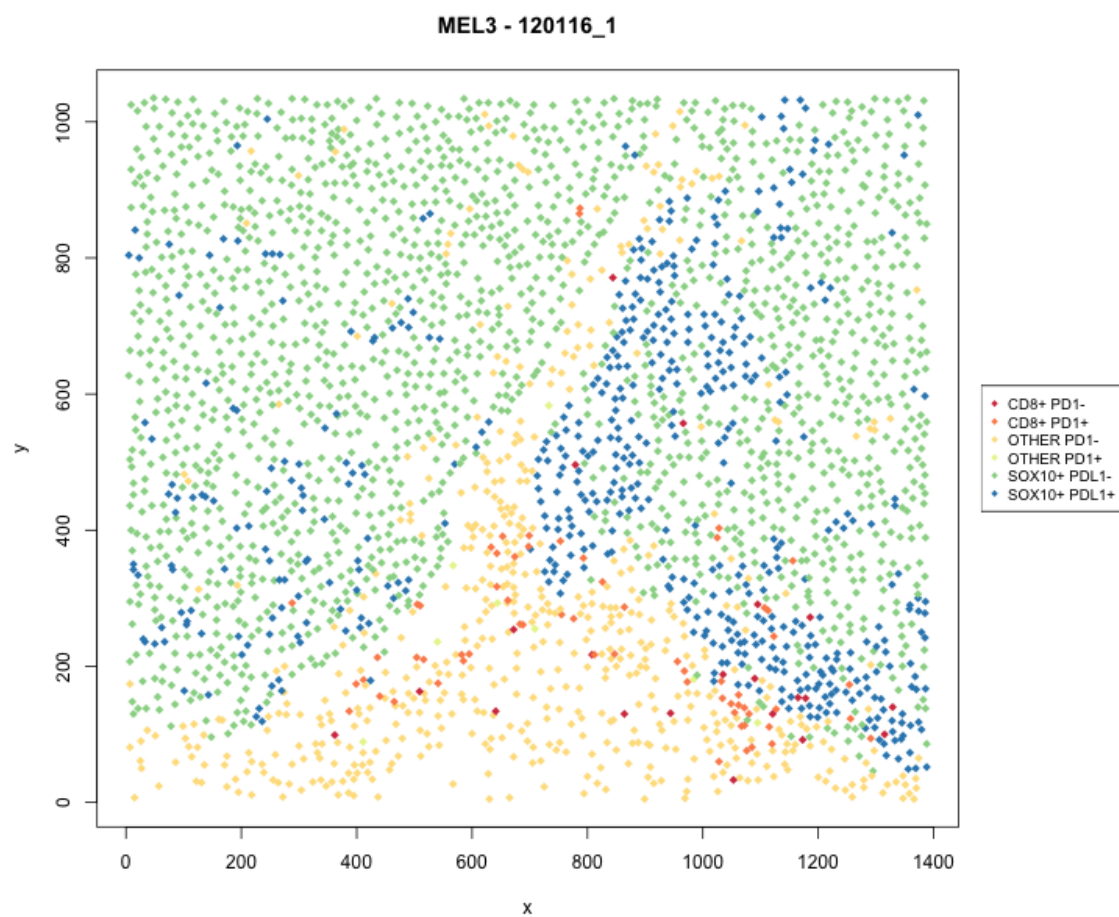


Figure 1: Example of an Overview plot

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

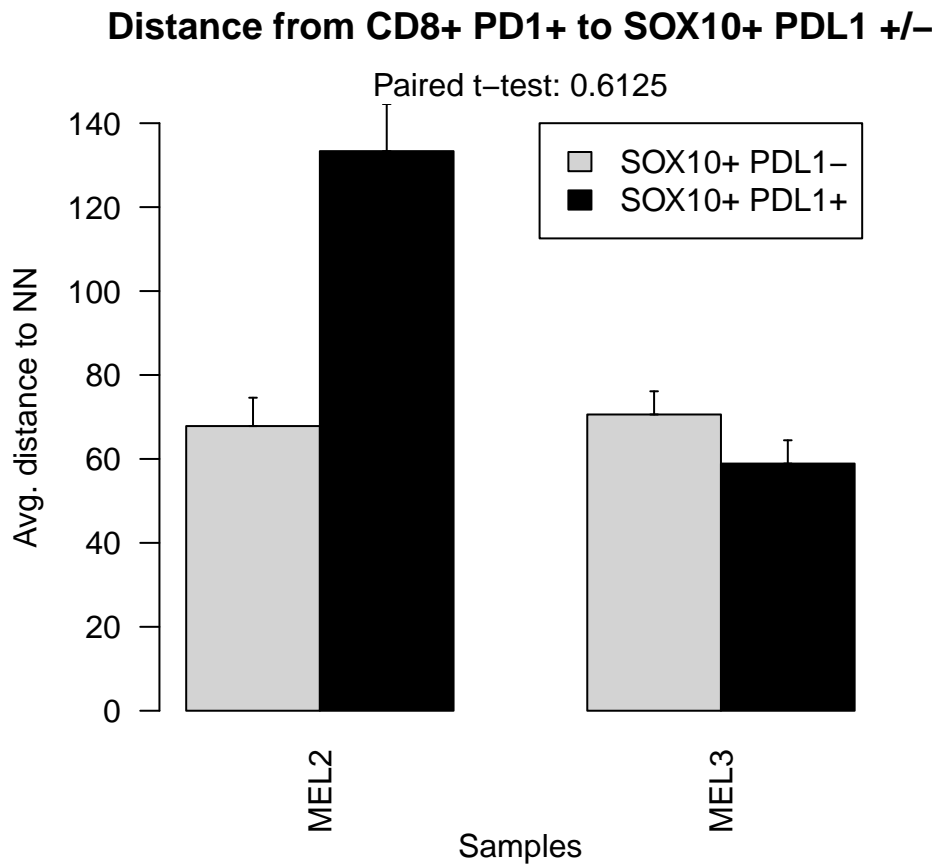
Next step we calculate the average nearest neighbor distances for each cell-type, plot barplots compare different distances and finally generate ray plots that show a visual representation of these distances for each coordinate.

```
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
##
```

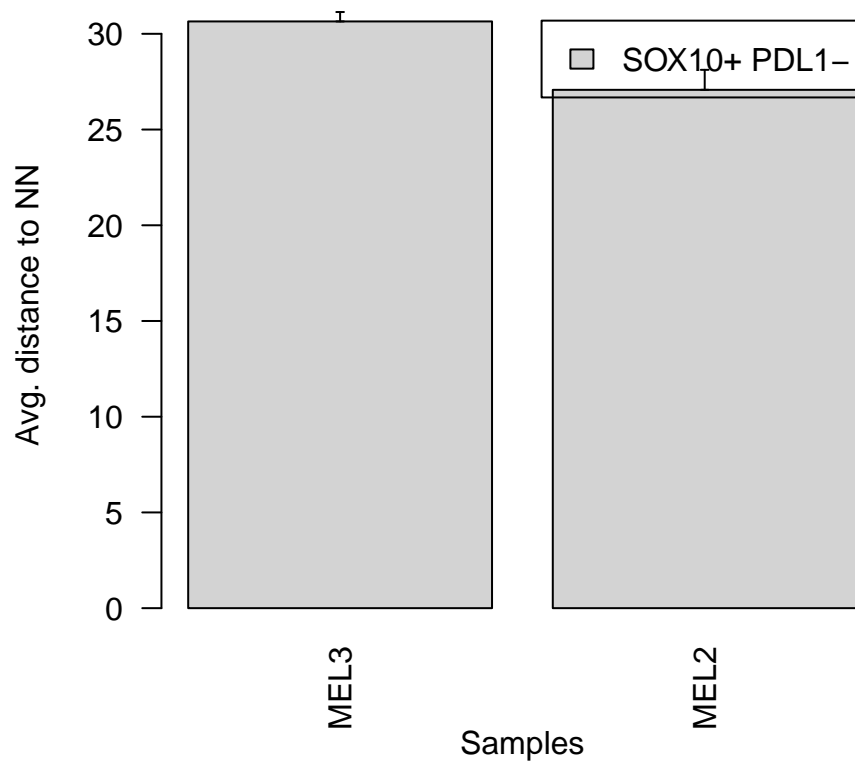
```
## $SE
##           MEL2      MEL3
## CD8+ PD1-  8.8792419 3.4993164
## CD8+ PD1+  2.2356082 2.5140467
## OTHER PD1-  0.9902901 1.5209465
## OTHER PD1+  6.7568488 4.9207914
## SOX10+ PDL1- 1.0443331 0.4908165
## SOX10+ PDL1+ 0.0000000 0.0000000
```

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



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

Distance from SOX10+ PDL1+ to SOX10+ PDL1-



```
#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...
```

```
## 40 solved of 43 issues
```

```
## 24 solved of 24 issues
```

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

```
## 20 solved of 20 issues
```

```
## 18 solved of 22 issues
```

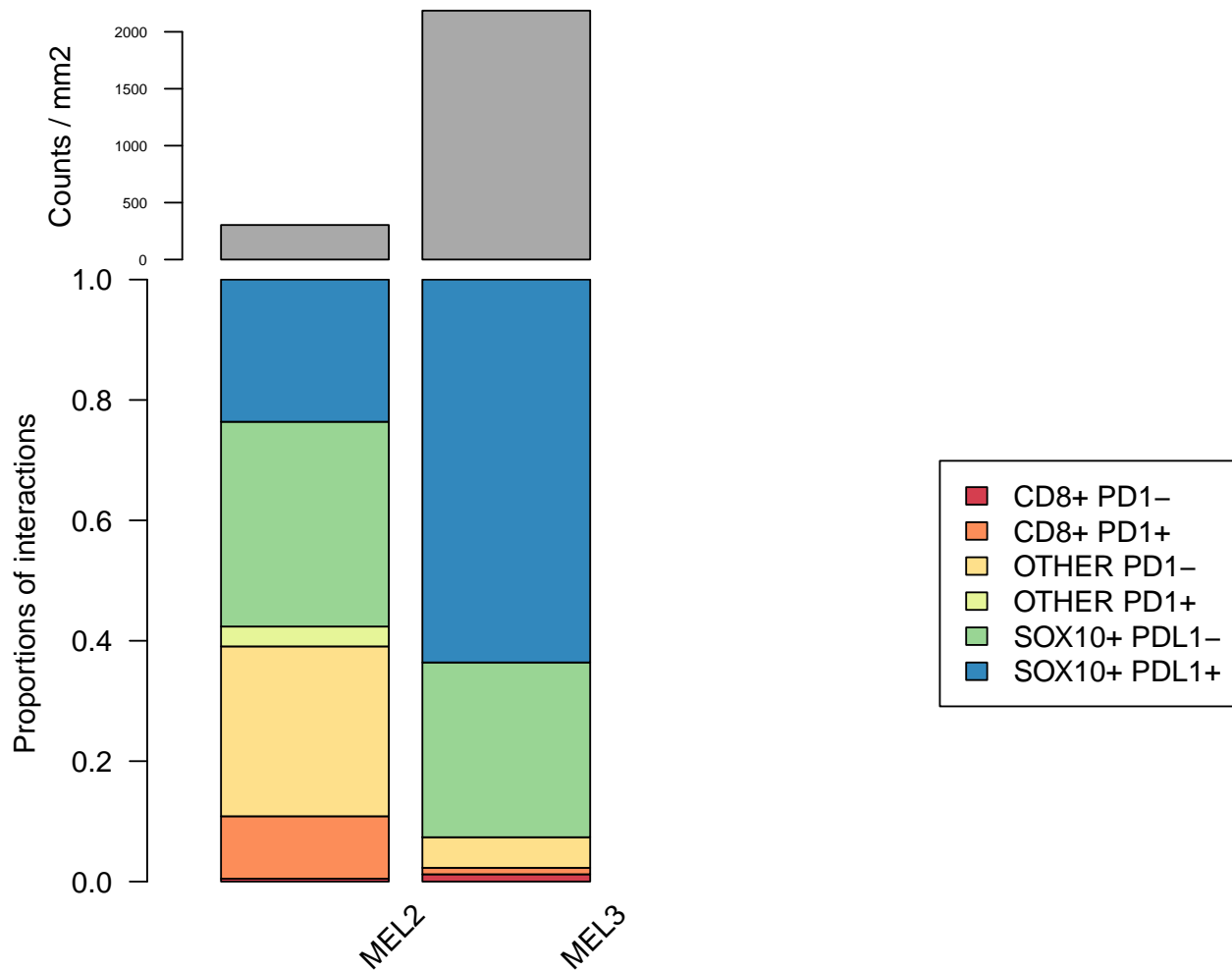
```
get_interactions(dataset, 'SOX10+ PDL1+')
```

```
##           MEL2           MEL3
## CD8+ PD1-  0.00469215 0.01210600
## CD8+ PD1+  0.10379928 0.01075406
## OTHER PD1- 0.28215916 0.05077316
## OTHER PD1+ 0.03300016 0.00000000
## SOX10+ PDL1- 0.34017121 0.29035433
## SOX10+ PDL1+ 0.23617803 0.63601245
```

```
#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-

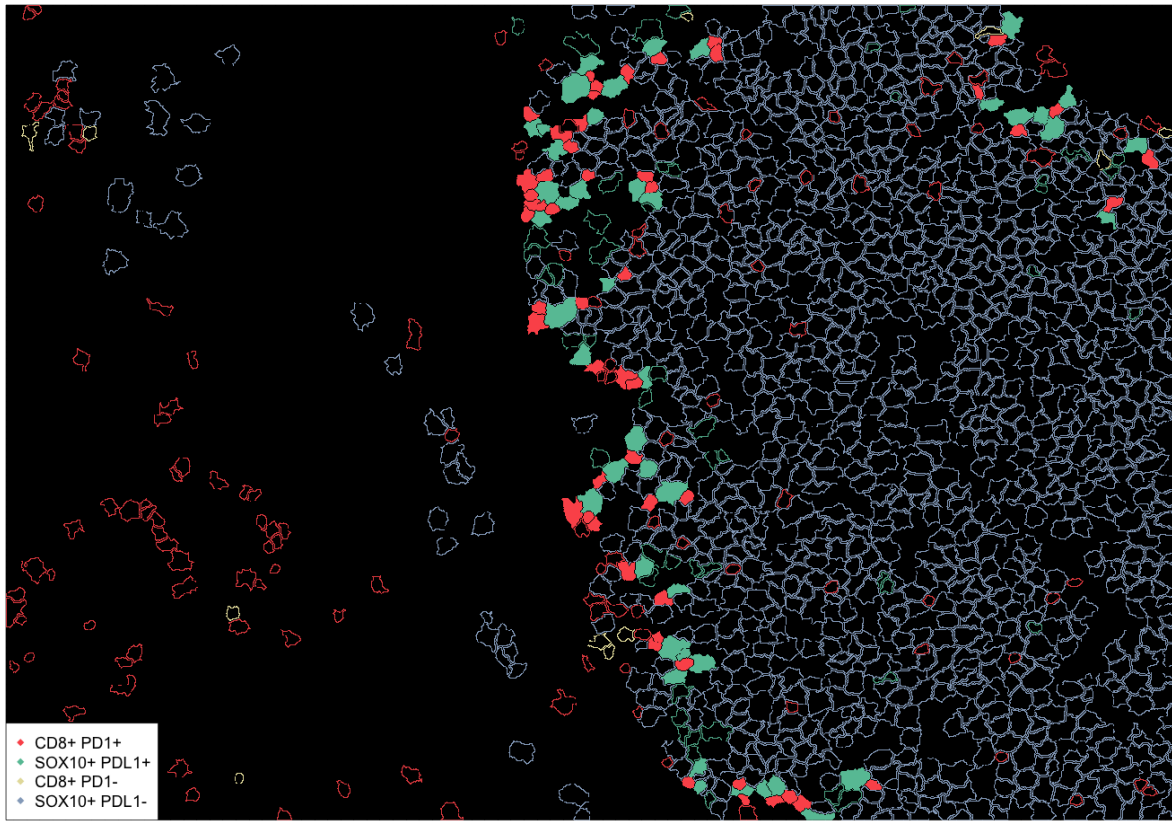


Figure 2: Example of a Interaction map


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

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

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

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

