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.

```
require(IrisSpatialFeatures)
```

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

```
raw_data<- read_raw(path=system.file("extdata", package = "IrisSpatialFeatures"),
                    format='Mantra')
```

```
## [1] "Sample: MEL2"
## [1] "No nuclear map found, skipping .. "
## [1] "No nuclear map found, skipping .. "
## [1] "Sample: MEL3"
## [1] "No nuclear map found, skipping .. "
## [1] "No nuclear map found, skipping .. "
```

```
#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))
```

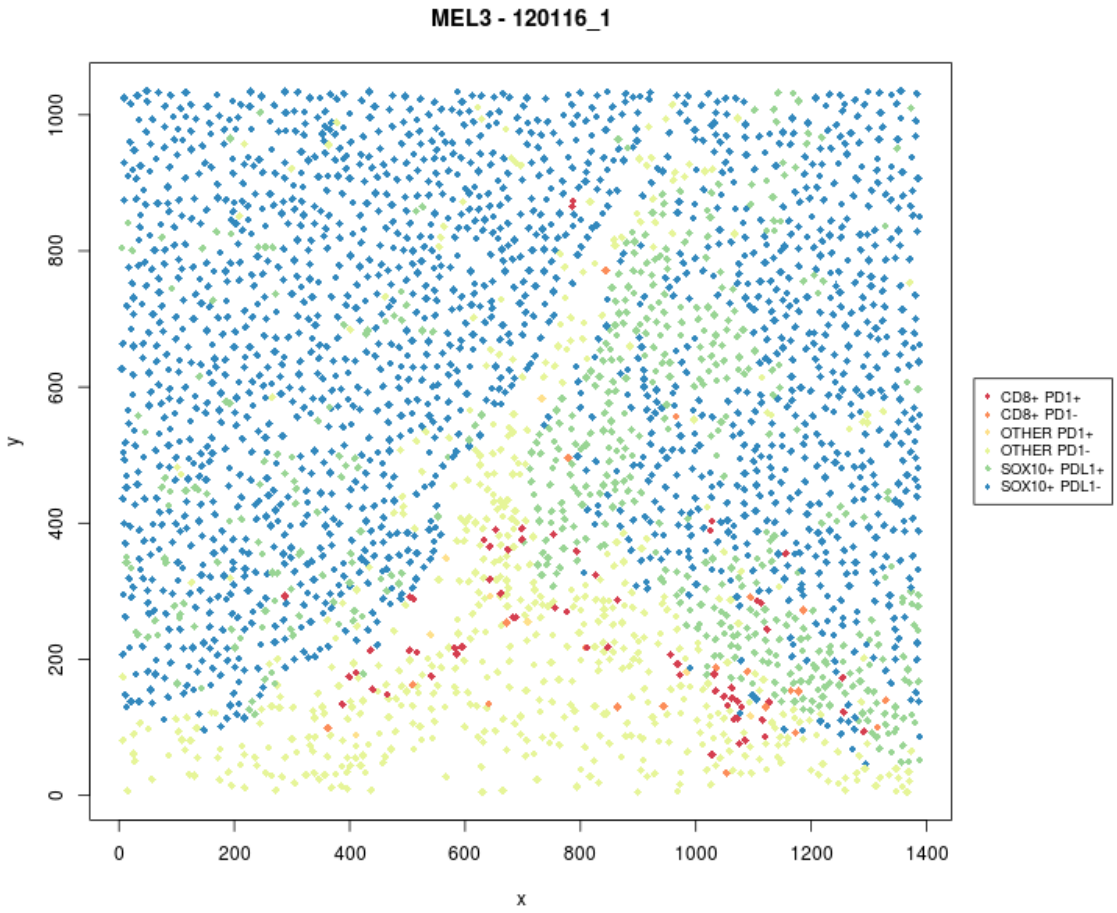


Figure 1: Example of an Overview plot

```
}
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)
```

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

```
get_counts_per_mm2_noncollapsed(dataset)
```

```
## $MEL2
```

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

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

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

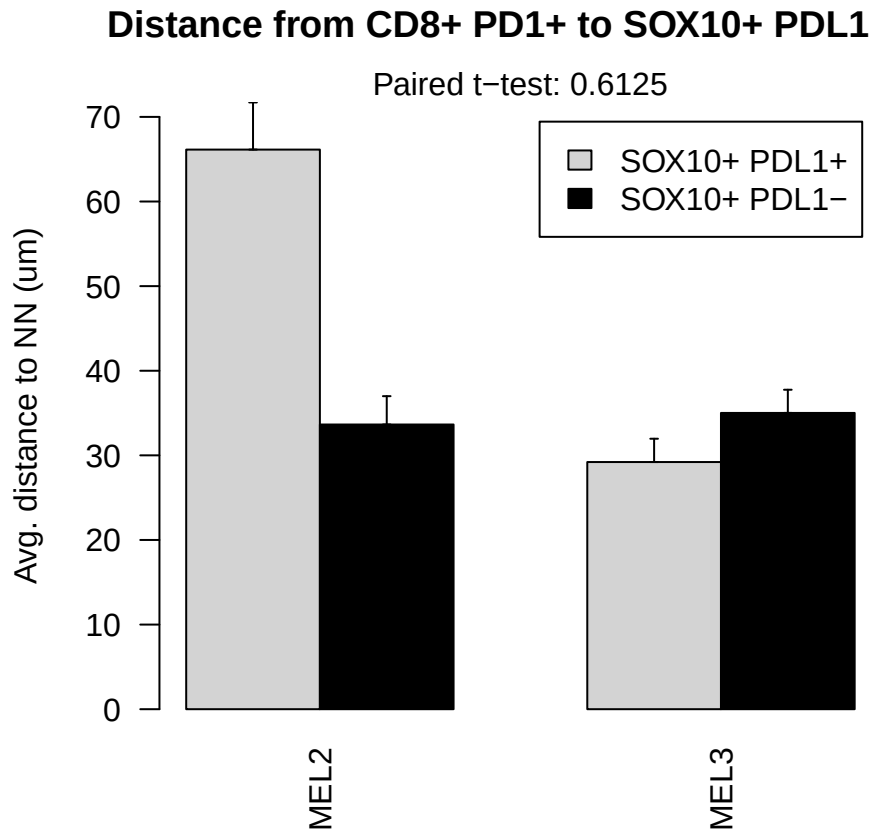
Nearest neighbor analysis

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.

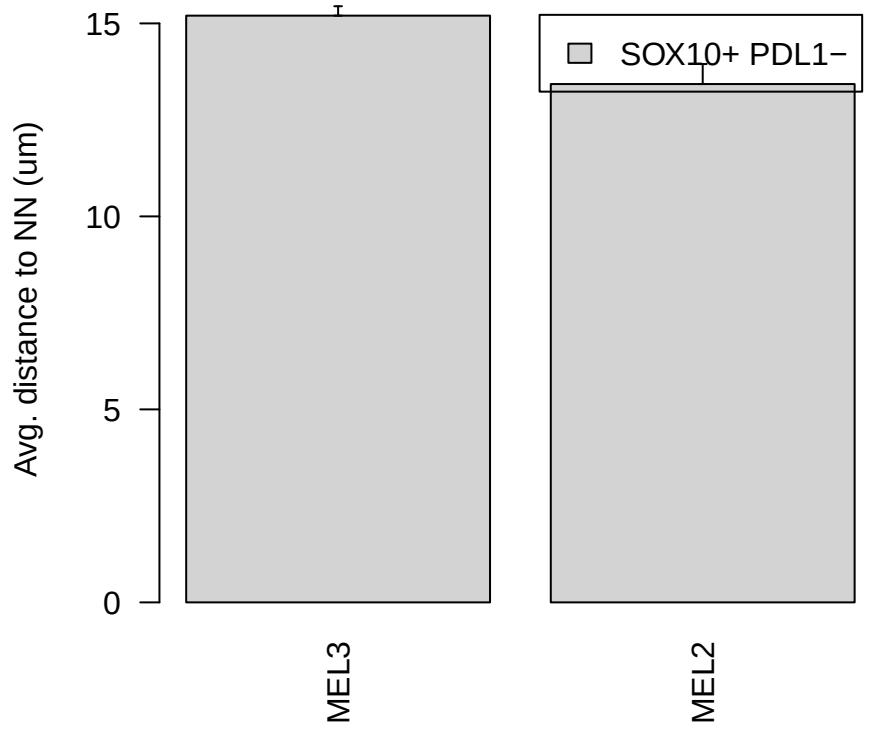
```
dataset <- extract_nearest_neighbor(dataset,min_num_cells=2)
get_nearest_neighbors(dataset,"SOX10+ PDL1+")
```

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

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




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

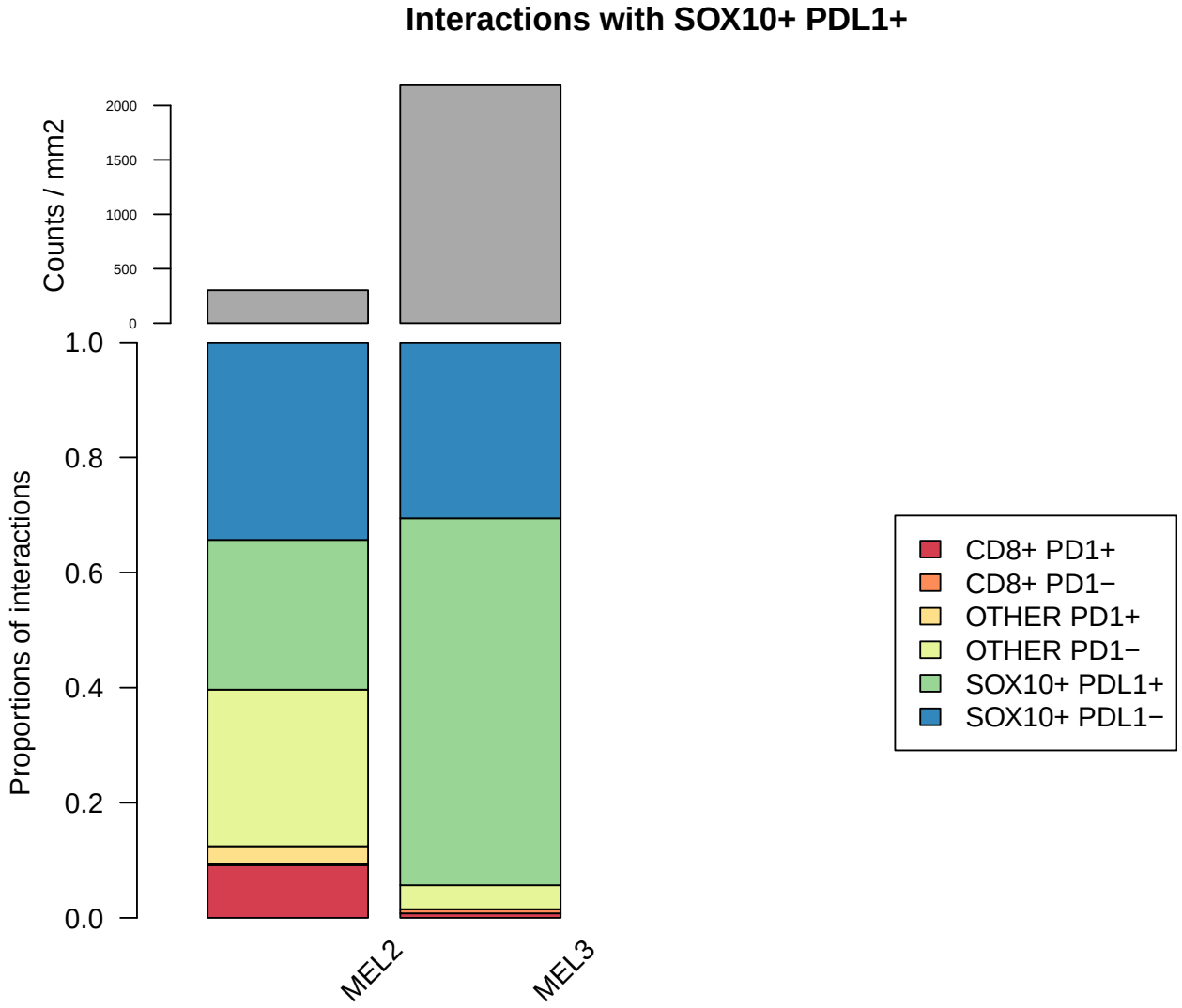
```
dataset <- extract_interactions(dataset)
```

```
## MEL2  ... processing...
## 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+    0.091403815 0.007783132
## CD8+ PD1-    0.002557193 0.007274186
## OTHER PD1+   0.030580652 0.000000000
## OTHER PD1-   0.272013526 0.041639280
## SOX10+ PDL1+ 0.260157447 0.637480209
## SOX10+ PDL1- 0.343287367 0.305823194
```

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




```
#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-

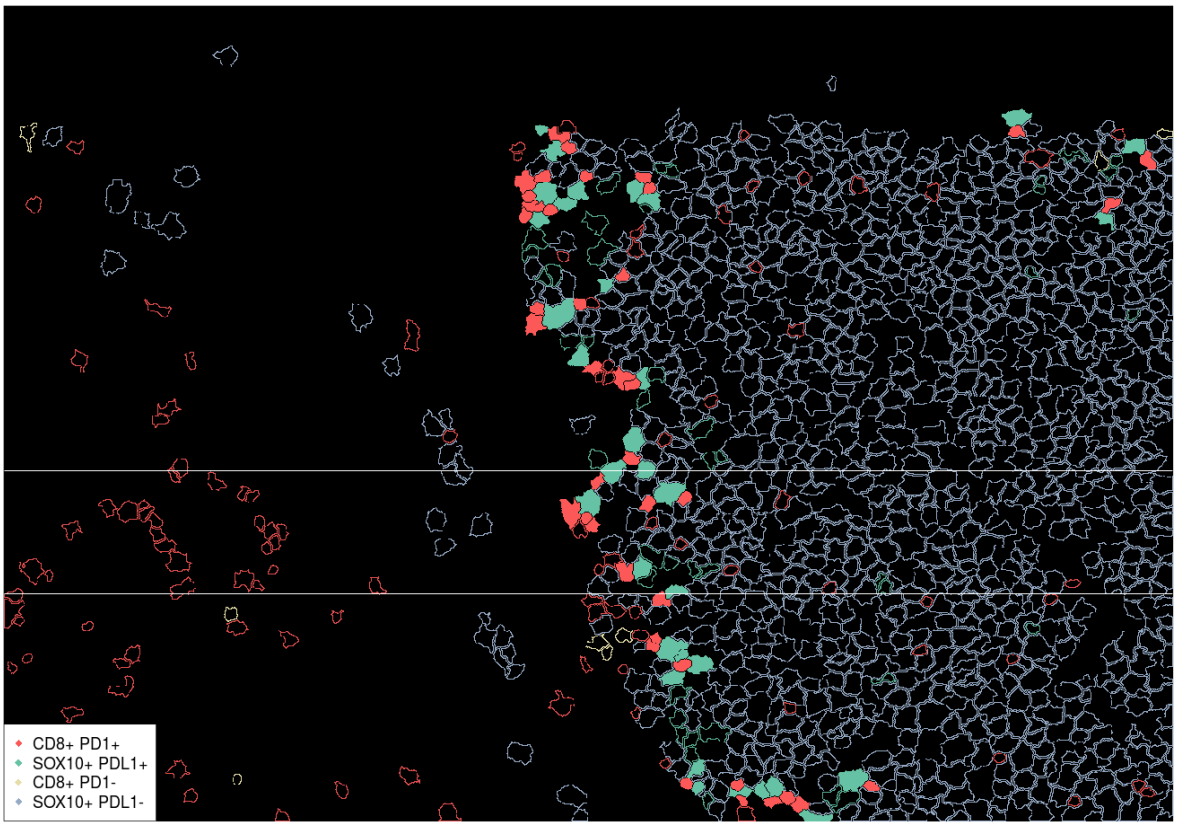


Figure 2: Example of a Interaction map

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

```
## MEL2  ... processing...
## MEL3  ... processing...
```

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