Package 'tlsR'

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Title Detection and Spatial Analysis of Tertiary Lymphoid Structures

Version 0.1.2

Description Fast, reproducible detection and quantitative analysis of tertiary lymphoid structures (TLS) in multiplexed tissue imaging. Implements Independent Component Analysis Trace (ICAT) index, local Ripley's K scanning, automated K Nearest Neighbor (KNN)-based TLS detection, and T-cell clusters identification as described in Amiryousefi et al. (2025) <doi:10.1101/2025.09.21.677465>.

Note TLS, ICAT, KNN are correct technical acronyms.

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Encoding UTF-8

LazyData true

RoxygenNote 7.3.2

Imports fastICA, spatstat.geom, spatstat.explore, dbscan, RANN, grDevices, graphics, stats, utils

Suggests testthat (>= 3.0.0)

Config/testthat/edition 3

Depends R (>= 3.5)

NeedsCompilation no

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`calc_icat` *Calculate ICAT (Immune Cell Arrangement Trace) Index*

Description

Quantifies linear/organized arrangement of cells within a TLS using FastICA.

Arguments

`patientID`	Character. Name of the sample in `ldata`.
`tlsID`	Numeric/integer. TLS identifier.
`ldata`	Named list of data frames (optional; defaults to global `ldata`).

Value

Numeric ICAT value.

Examples

```
data(toy_ldata)
ldata <- detect_TLS("ToySample", k = 30, ldata = toy_ldata)  # First detect TLS
if (max(ldata[["ToySample"]]$tls_id_knn) > 0) {
  icat <- calc_icat("ToySample", tlsID = 1, ldata = ldata)
  icat
}
```

`detect_tic` *Detect Tumor-Infiltrating T-cell Clusters (TIC)*

Description

Uses HDBSCAN approximation on T cells outside TLS regions to find TIC.

Arguments

`sample`	Character. Sample name.
`ldata`	Optional list.

Value

Modified data frame for the sample (invisibly).

Examples

```
data(toy_ldata)
ldata <- detect_TLS("ToySample", k = 30, ldata = toy_ldata)  # Need TLS first
ldata <- detect_tic("ToySample", ldata = ldata)
table(ldata[["ToySample"]]$tcell_cluster_hdbscan)
```

`detect_TLS` *Detect Tertiary Lymphoid Structures using a KNN-density approach*

Description

This function identifies TLS candidates germinated with B cells (BIC) by: 1. Finding regions of high local B-cell density (KNN-based). 2. Requiring a minimum number of neighbouring T cells (T-B cell co-localisation). 3. Applying sensible size and shape filters.

Arguments

`LSP`	Character. Sample name in the global `ldata` list.
`k`	Integer. Number of nearest neighbours to consider (default 30 - works very well on 0.325 um/px imaging).
`bcell_density_threshold`	Numeric. Minimum average 1/k-distance for B cells to be considered "dense" (default 15 um).
`min_B_cells`	Integer. Minimum number of B cells in a candidate TLS (default 50).
`min_T_cells_nearby`	Integer. Minimum T cells within 50 um of the B-cell cluster centre (default 30).
`max_distance_T`	Numeric. Radius (um) to search for surrounding T cells (default 50).
`ldata`	Optional. Named list of data frames. If `NULL`, uses global `ldata`.

Value

The original data frame with two new columns:

`tls_id_knn`	0 = non-TLS, positive integer = TLS cluster ID
`tls_center_x, tls_center_y`	Coordinates of detected TLS centres (only for TLS cells)

Examples

```
data(toy_ldata)
ldata <- detect_TLS("ToySample", k = 30, ldata = toy_ldata)
table(ldata[["ToySample"]]$tls_id_knn)
plot(ldata[["ToySample"]]$x, ldata[["ToySample"]]$y,
     col = ifelse(ldata[["ToySample"]]$tls_id_knn > 0, "red", "gray"),
     pch = 19, cex = 0.5, main = "Detected TLS in toy data")
```

scan_clustering *Scan Tissue for Local Immune Cell Clustering (K-integral)*

Description

Sliding-window Centerel L-Function (CLF) version of the Ripley's K analysis with whole tissue pseudo-plots.

Arguments

ws	Window size in microns.
sample	Character. Sample name in ldata.
phenotype	One of "T cells", "B cells", or "Both".
plot	Logical. Show diagnostic plot?
creep	Integer. Grid density factor.
ldata	Optional list (defaults to global ldata).

Value

List of Lest objects for significant windows.

Examples

```
data(toy_ldata)
  # This one may produce plots and take ~10 sec
  models <- scan_clustering(ws = 500, sample = "ToySample",
                            phenotype = "B cells", plot = FALSE, ldata = toy_ldata)
  length(models)
```

toy_ldata *Toy multiplexed imaging data for examples*

Description

A small synthetic dataset mimicking multiplexed tissue imaging data. It contains one sample named "ToySample" with columns required by tlsR functions.

Usage

```
toy_ldata
```

Format

A named list with one element:

ToySample A data frame with columns:

- x: x-coordinate in microns
- y: y-coordinate in microns
- coarse_phen_vec: Cell phenotype ("B cells", "T cells", or "Other")
- row_index: Integer row index (1 to nrow)
- cflag: Integer flag column (0 for all cells)

Examples

```
data(toy_ldata)
str(toy_ldata[["ToySample"]])
plot(toy_ldata[["ToySample"]]$x, toy_ldata[["ToySample"]]$y,
     col = as.factor(toy_ldata[["ToySample"]]$coarse_phen_vec),
     pch = 19, cex = 0.5, main = "Toy sample cells")
```

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