

June_2

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MBP Feature extraction and analysis

Here I used cropped picture(this is all I have) as an example. There are mainly 2 steps:

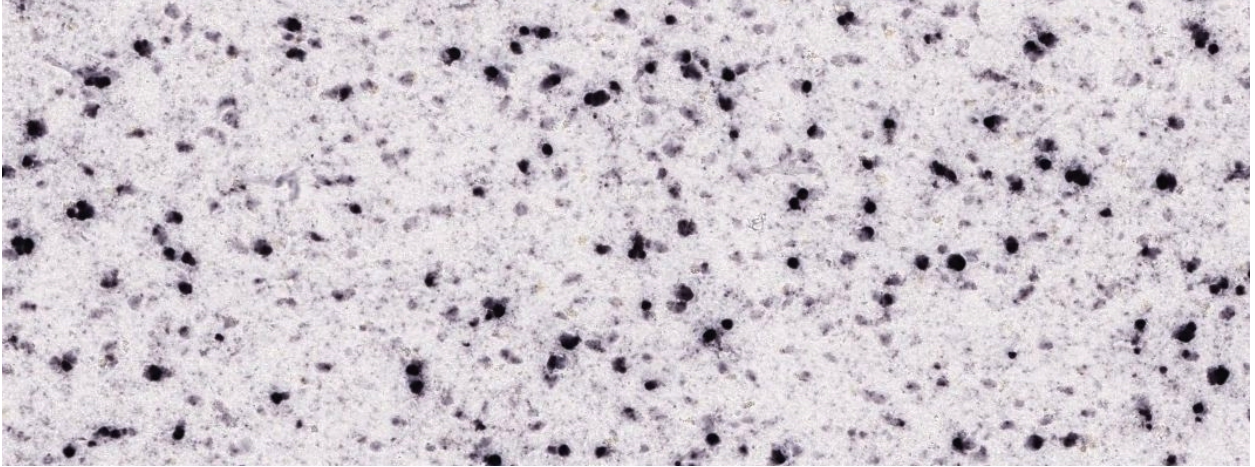
1. Threshold the image, then compute the features;
2. Use the features to compute intensity estimation and other graphs. ## Step1: Feature extraction

Load the package and preview the image.

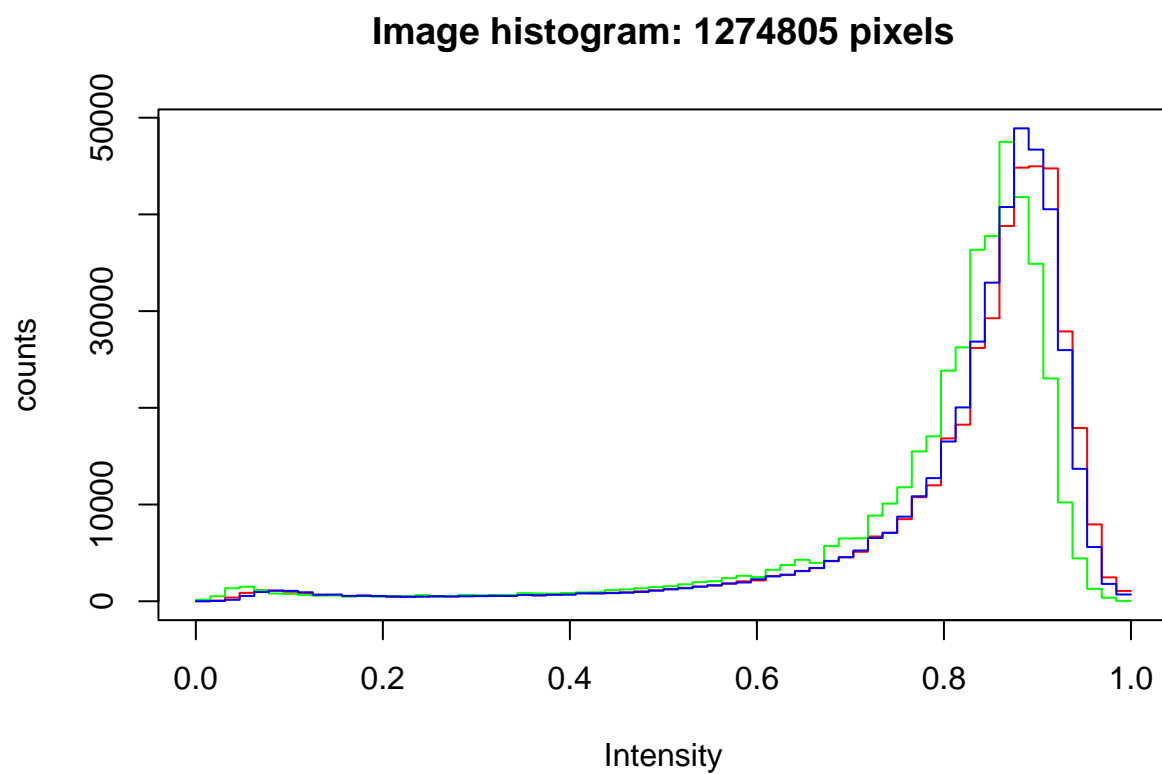
```
library(BiocManager)
```

```
## Bioconductor version '3.14' is out-of-date; the current release version '3.15'  
##   is available with R version '4.2'; see https://bioconductor.org/install
```

```
library(EBImage)  
im=readImage("/Users/hainanxu/Documents/spatial_visual_cortex/data/im3.jpg")  
display(im,method="raster")
```

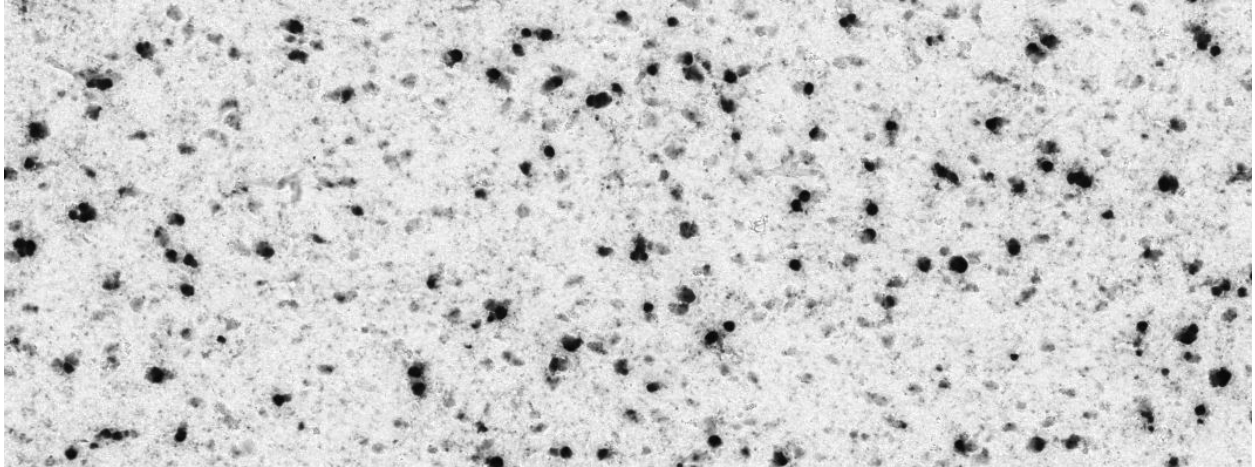


Check the histogram, we can see that there are 3 colors in the histogram, and they are interlaced. To make the segmentation easier, we convert them to greyscale(using the red channel).



Take a look at greyscaled im.

```
display(im,method="raster")
```

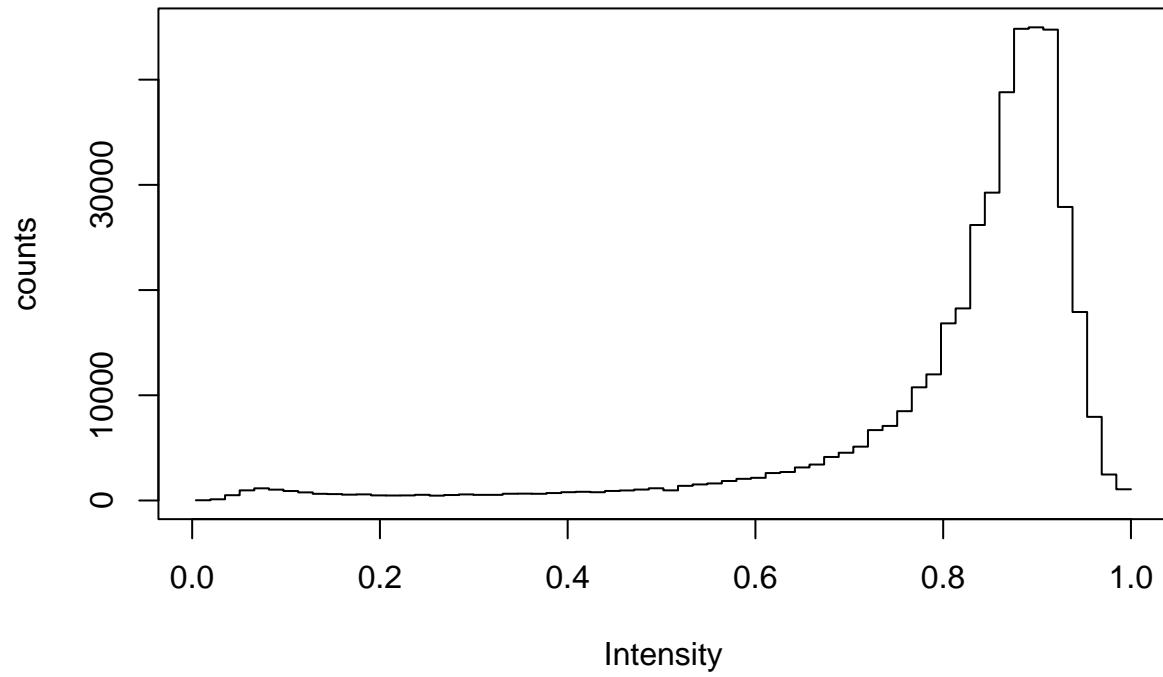


```
#w = makeBrush(size = 30, shape = "gaussian", sigma = 2)
#nucSmooth = filter2(getFrame(r, 1), w)
#display(nucSmooth)
#display(nucSmooth<0.3)
```

After that, we apply a simple method to threshold our greyscale image `im`.

```
hist(im)
```

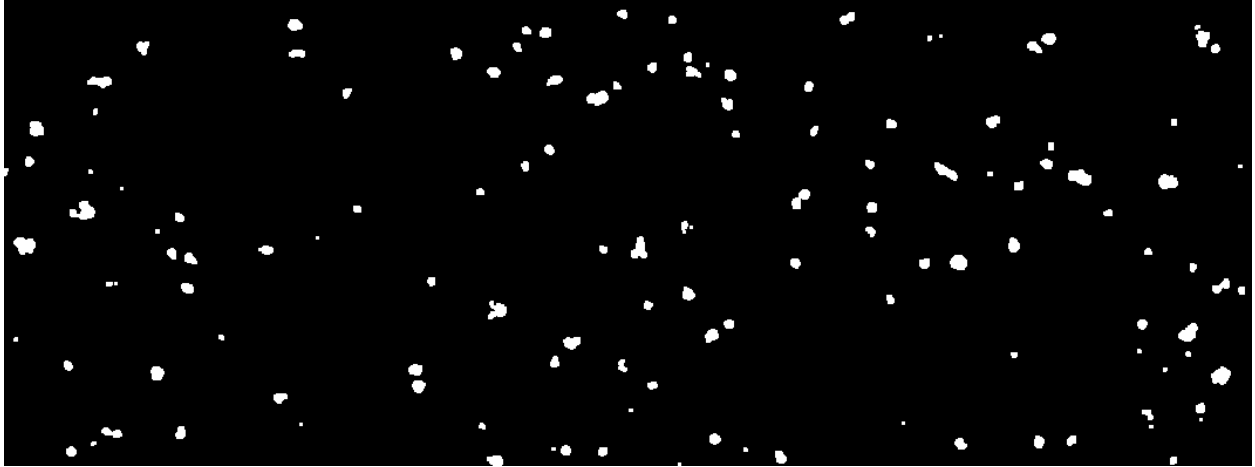
Image histogram: 424935 pixels



```
display(im<0.25)
```



```
rThresh=im<0.25
rOpened = EImage::opening(rThresh,
                          kern = makeBrush(3, shape = "disc"))
display(rOpened)
```



```
rRGB=toRGB(rOpened)
display(rRGB)
writeImage(rRGB, "Ahad_June3.tiff", quality = 100)
```

After thresholding our image, we segment it into different objects.

```
rSeed = bwlabel(rOpened)
display(colorLabels(rSeed),method="raster")
```



Here we computed the features of this slice of image. There are 84 features in total, including shape features and moment features of the MBP cell. We create a new dataframe to store the 3 features that we need right now. The 3 features includes: x coordinate, y coordinate, area of each object. After this, we can convert it to a ppp object.

```
rSeed=bwlabel(r0opened)
table(rSeed)
```

```
## rSeed
##      0      1      2      3      4      5      6      7      8      9     10
## 416219  56    97    44   102    17    47    79   126   101     9
##      11    12    13    14    15    16    17    18    19    20    21
##      19    95    87    50    52    87    83    55    59    12    92
##      22    23    24    25    26    27    28    29    30    31    32
##      85    84    91   146    40    59    55   175    86    22    98
##      33    34    35    36    37    38    39    40    41    42    43
##      62    30   134    48    37    35    61    57    75    50    51
##      44    45    46    47    48    49    50    51    52    53    54
##     156    12    23    15   201    20   171    58     9    37   144
##      55    56    57    58    59    60    61    62    63    64    65
##     216    71    40    40    54    48     9    54    16     9   172
##      66    67    68    69    70    71    72    73    74    75    76
##     206   101    77    42    63    35    79   157    64    71    40
##      77    78    79    80    81    82    83    84    85    86    87
##      47   107    23     9    83    96    39    47   164    49    61
##      88    89    90    91    92    93    94    95    96    97    98
##      63   176   104    22    15   124    15    22    27    62    60
```



```
##      99      100      101      102      103      104      105      106      107      108      109
##      55      98      115      188      15      100      50      82      62      12      57
##     110     111     112     113     114     115     116     117     118     119     120
##       9       9       9      28      12      79     100      76      80      58      78
##     121     122     123     124     125     126     127     128     129     130
##      17      66      64      12      56      15      87     104      39       9
```

```
F1 = computeFeatures(rSeed,im, xname = "r",
                     refnames = "r")
```

Step2: Intensity estimation and other graphs

First, create a new dataframe MBP. We can explore the distributions of the cells by computing the marginal distribution.

```
library(ggplot2)
library(ggExtra)
x_coord<-F1[,1]
y_coord<-F1[,2]
size<-F1[,6]
```

```
View(F1)
```

```
MBP<-data.frame(x=x_coord,
                y=y_coord,
                size=size
                )
```

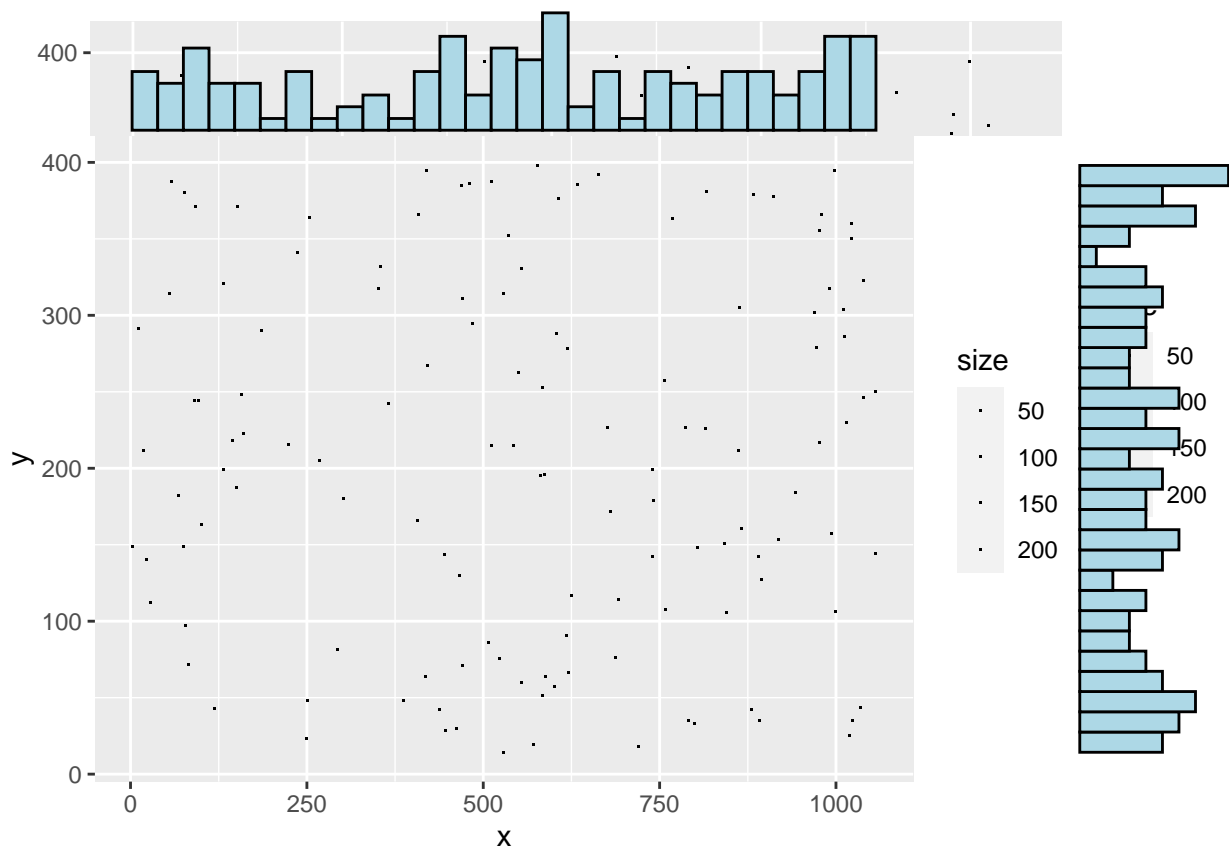
```
range(size)
```

```
## [1]  9 216
```

```
head(MBP)
```

```
##           x           y size
## 1  528.4286  14.30357   56
## 2  720.3608  18.02062   97
## 3  570.8182  19.18182   44
## 4  248.9020  23.55882  102
## 5 1018.9412  25.41176   17
## 6  446.3404  28.36170   47
```

```
a<-ggplot(MBP,
          aes(x = x, y = y,size=size)) + geom_point(shape = ".",aes(size=size))
a
ggMarginal(a,type="histogram",fill = "lightblue")
```



Secondly, convert MBP as a point process object. Here each circle indicates a cell, and the size of the cell is corresponding to the area each cell as occupied in pixel.

```
library("spatstat")
```

```
## Loading required package: spatstat.data
```

```
## Loading required package: spatstat.geom
```

```
## spatstat.geom 2.4-0
```

```
##
```

```
## Attaching package: 'spatstat.geom'
```

```
## The following objects are masked from 'package:EBImage':
```

```
##
```

```
##      affine, closing, distmap, opening, rotate
```

```
## Loading required package: spatstat.random
```

```
## spatstat.random 2.2-0
```

```
## Loading required package: spatstat.core
```

```
## Loading required package: nlme
```

```
## Loading required package: rpart

## spatstat.core 2.4-4

##
## Attaching package: 'spatstat.core'

## The following object is masked from 'package:BiocManager':
##
##      valid

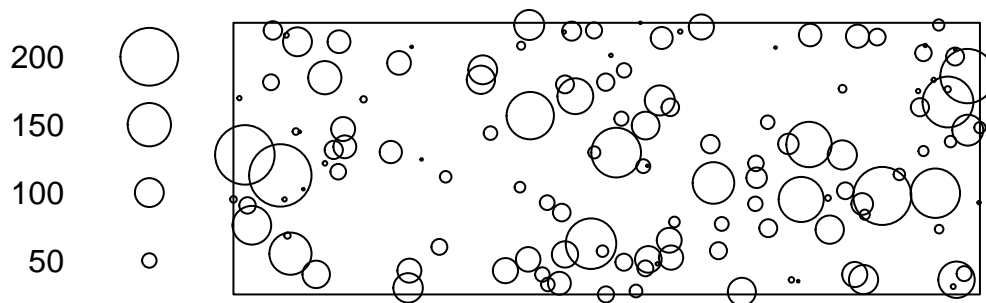
## Loading required package: spatstat.linnet

## spatstat.linnet 2.3-2

##
## spatstat 2.3-4      (nickname: 'Watch this space')
## For an introduction to spatstat, type 'beginner'

ln = with(MBP,
  ppp(x = x, y = y, marks = size, xrange = range(x), yrange = range(y)))
plot(ln)
```

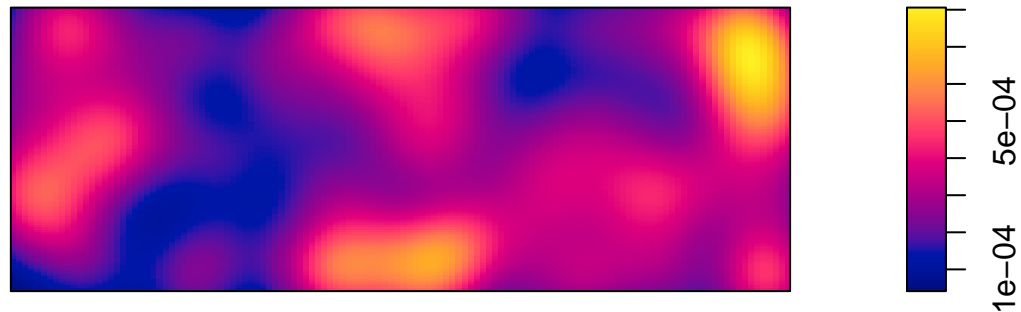
ln



Thirdly, we can explore the first order effect: estimate the intensity of this particular region.

```
d = density(subset(ln), edge=TRUE, diggle=TRUE)
plot(d)
```

d



Fourthly, we can explore the second order effect: randomly pick a point, what is the distance to it's nearest neighbor?

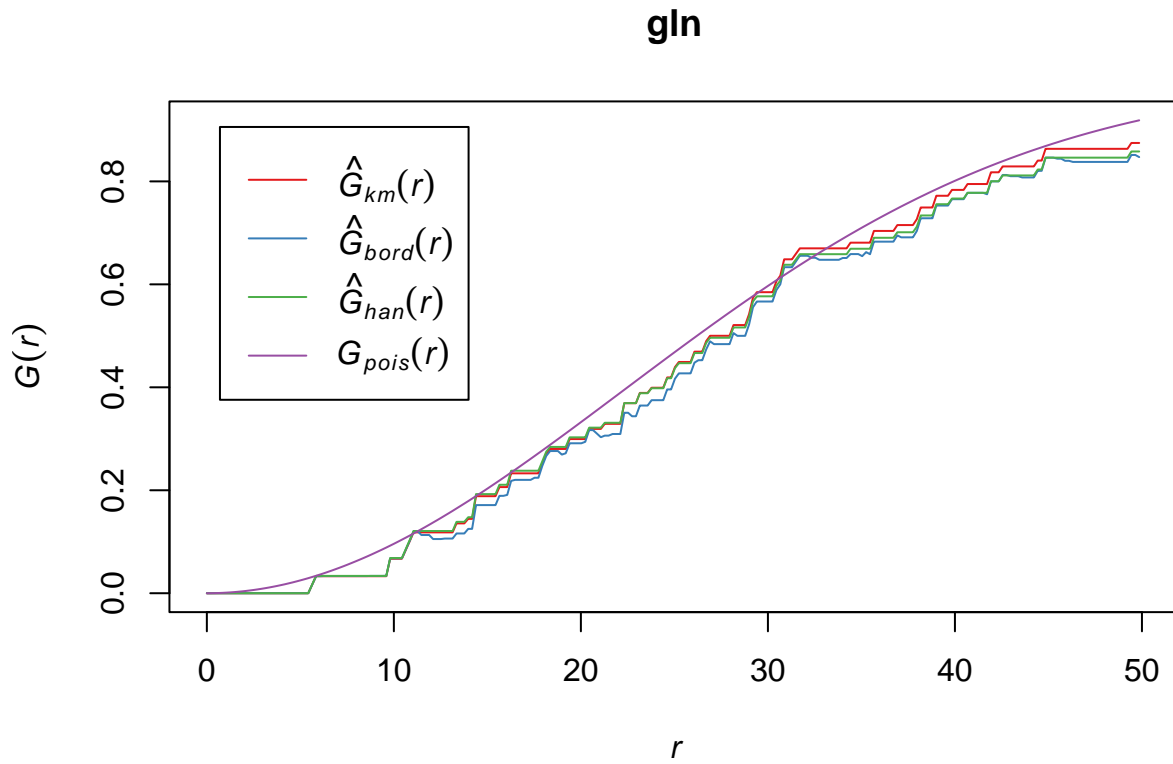
G function: the cumulative distribution function of the distance. Here the purple line indicates the poisson process. The second graph is the zoomed in version of the first graph.

```
gln = Gest(ln)
gln
```

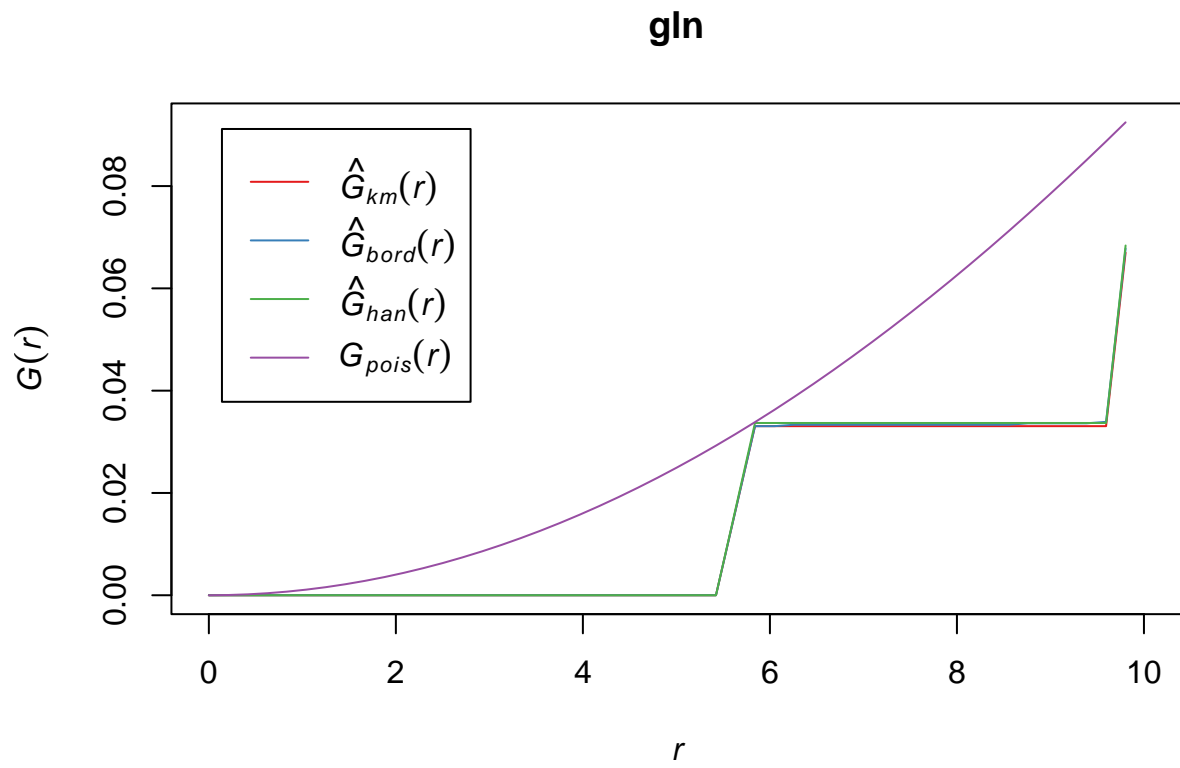
```
## Function value object (class 'fv')
## for the function r -> G(r)
## .....
##      Math.label      Description
## r      r              distance argument r
## theo   G[pois](r)     theoretical Poisson G(r)
## han    hat(G)[han](r) Hanisch estimate of G(r)
## rs     hat(G)[bord](r) border corrected estimate of G(r)
## km     hat(G)[km](r)  Kaplan-Meier estimate of G(r)
## hazard hat(h)[km](r) Kaplan-Meier estimate of hazard function h(r)
## theohaz h[pois](r)    theoretical Poisson hazard function h(r)
## .....
## Default plot formula: .~r
## where "." stands for 'km', 'rs', 'han', 'theo'
```

```
## Recommended range of argument r: [0, 50.895]
## Available range of argument r: [0, 106.8]
```

```
library("RColorBrewer")
plot(gln, xlim = c(0, 50), lty = 1, col = brewer.pal(4, "Set1"))
```

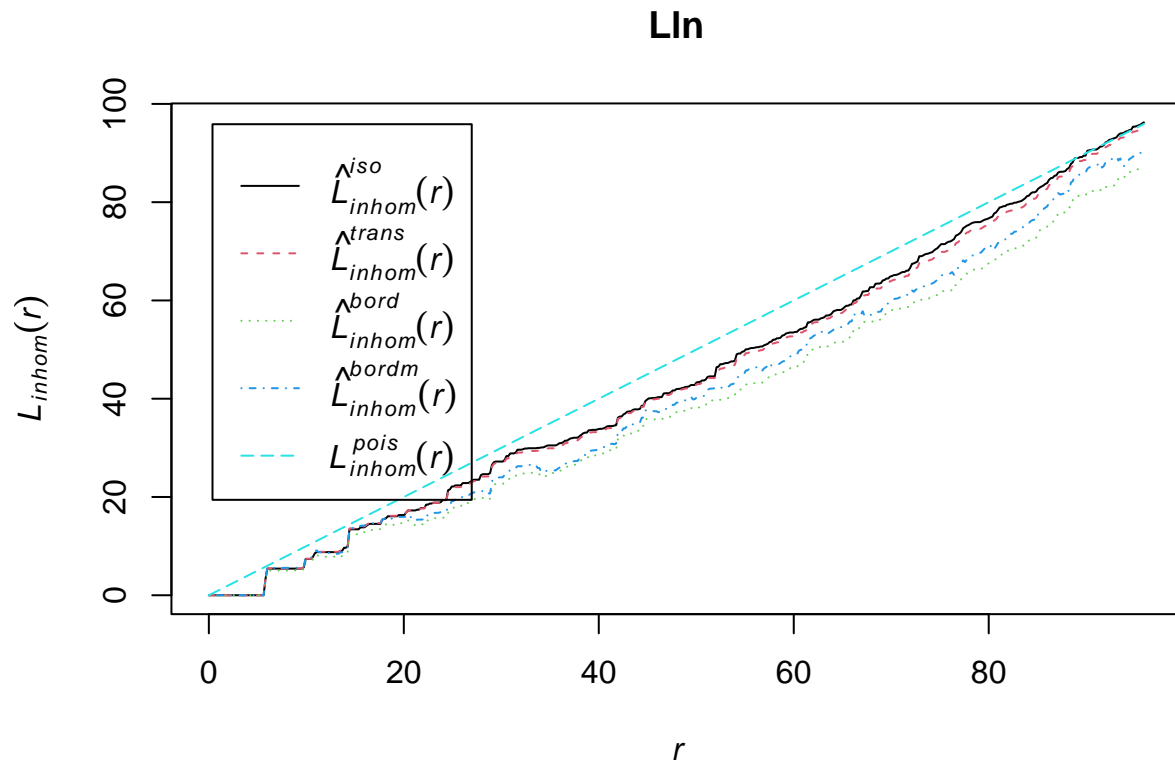


```
plot(gln, xlim = c(0, 10), lty = 1, col = brewer.pal(4, "Set1"))
```



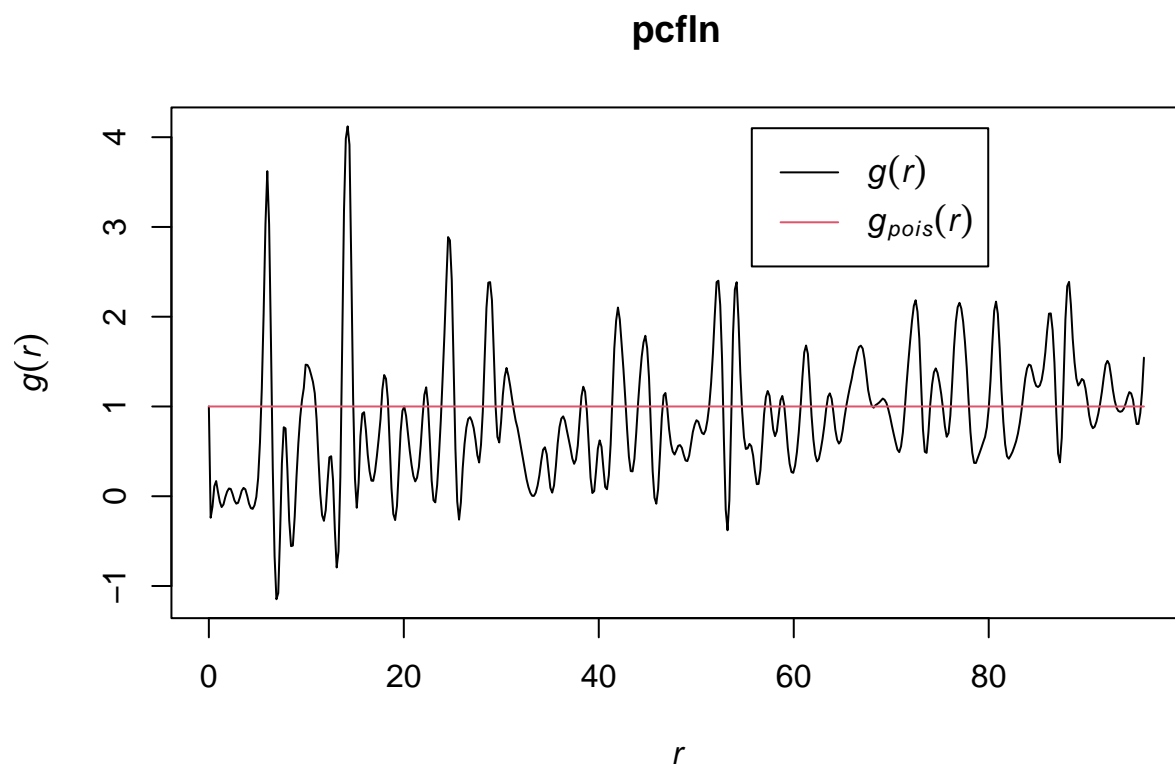
Ripley's K function: for estimation and visualization, transfer K function as L function.

```
Lln = Linhom(ln)
plot(Lln)
```



Paired correlation function:

```
pcfln = pcf(Kinhom(ln))
plot(pcfln, lty = 1)
```



```
plot(pcfln, lty = 1, xlim = c(0, 100))
```


pcfln

