Spatial statistics: Object-based colocalization

The data

IAC and BOB are proteins in the eastern spruce budworm (*Choristoneura fumiferana*) epidermis

You hypothesize that the spatial interaction between IAC and BOB changes with temperature and season.

Extract coordinates

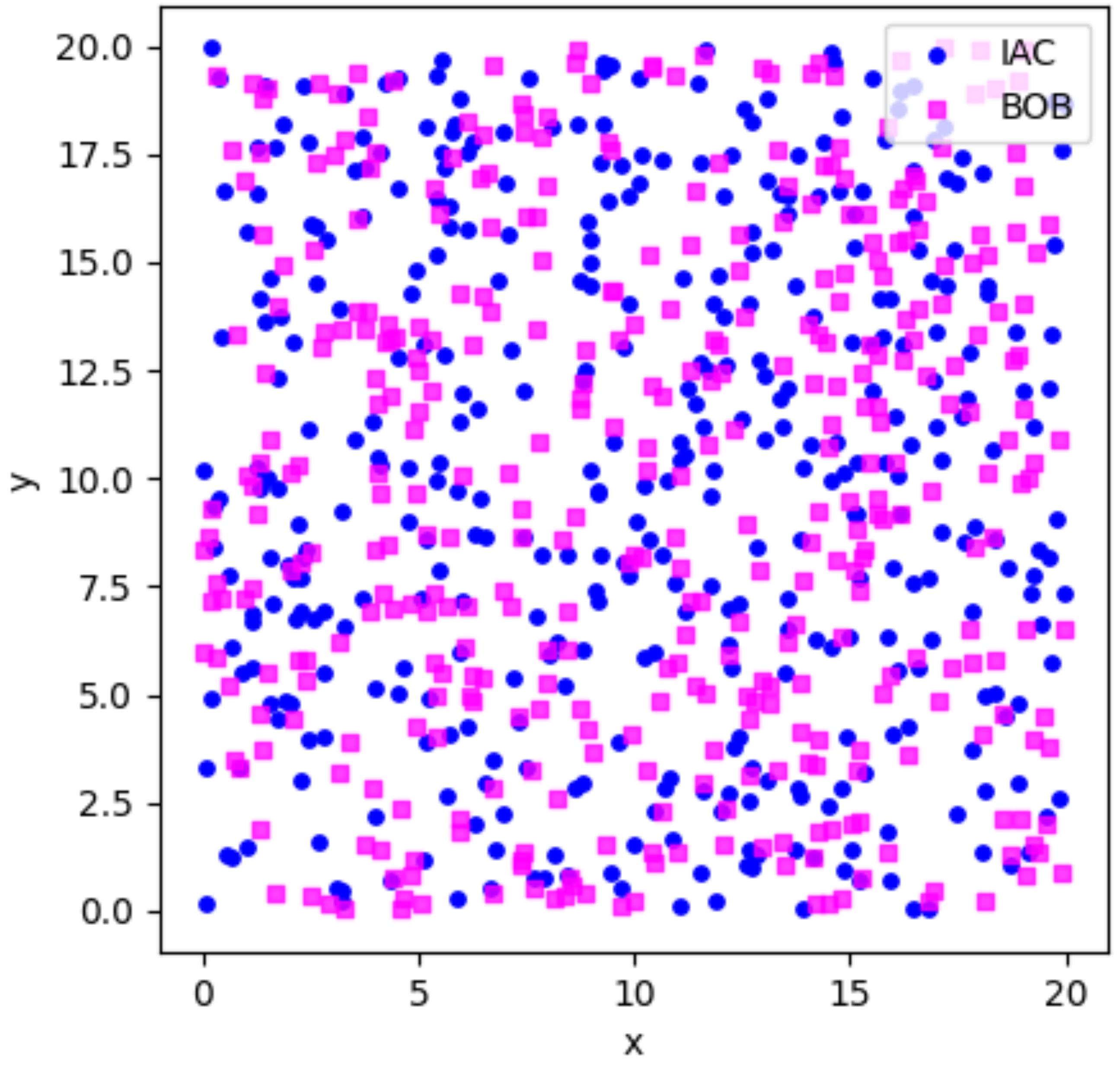
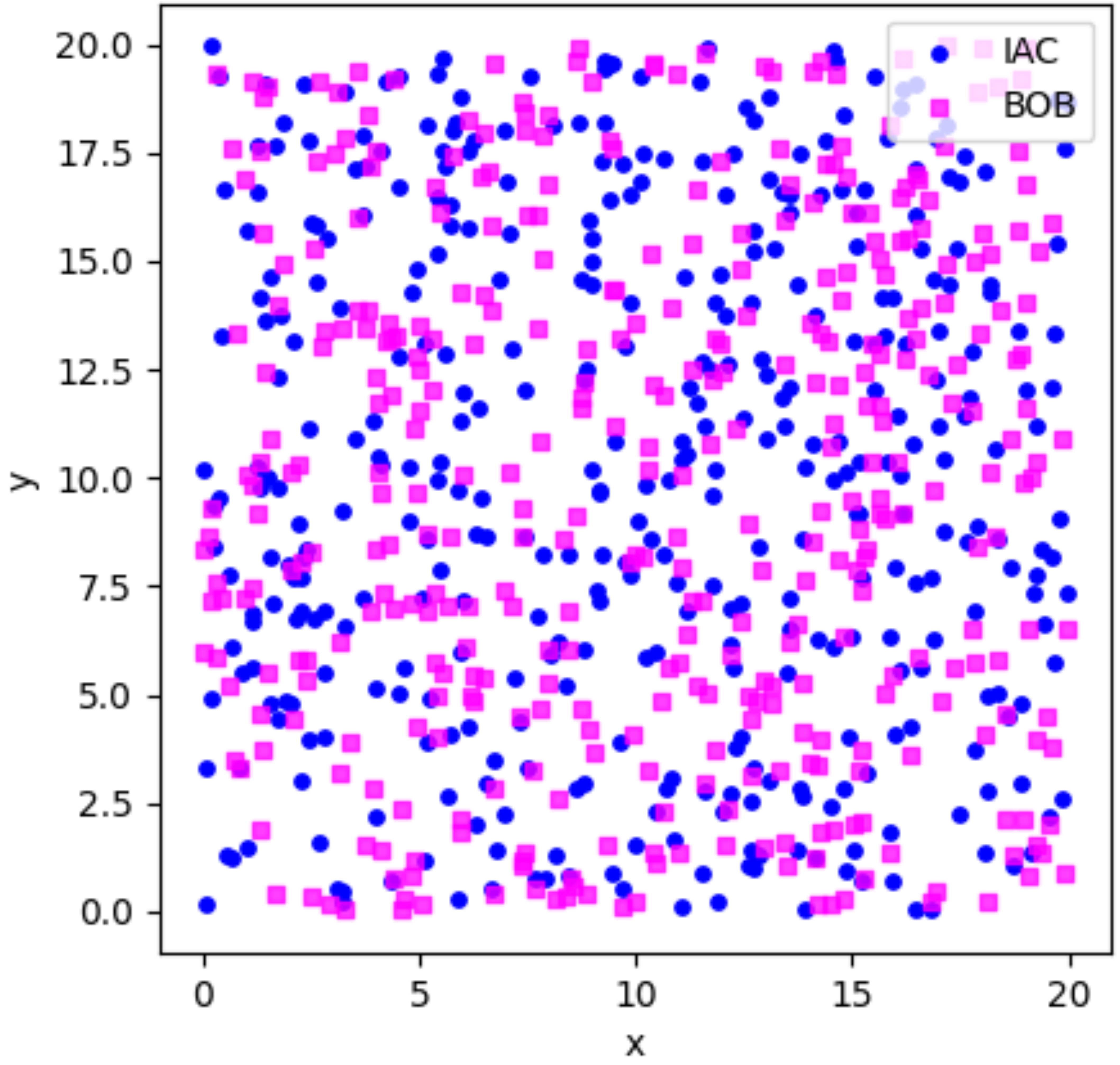


Image: https://commons.wikimedia.org/wiki/File:Choristoneura_fumiferana_larva.jpg; public domain

The data



IAC

x	y
1.6542	4.98028
2.64547	4.95783
6.90183	17.5005
6.98563	17.8339
7.24287	17.1596
6.57113	17.3483
6.41189	16.8281
6.62601	17.4194
6.21376	17.4324
6.74328	16.7232
7.0172	17.5627
6.52185	16.5556
5.93571	17.4
6.28006	17.0457
...	...

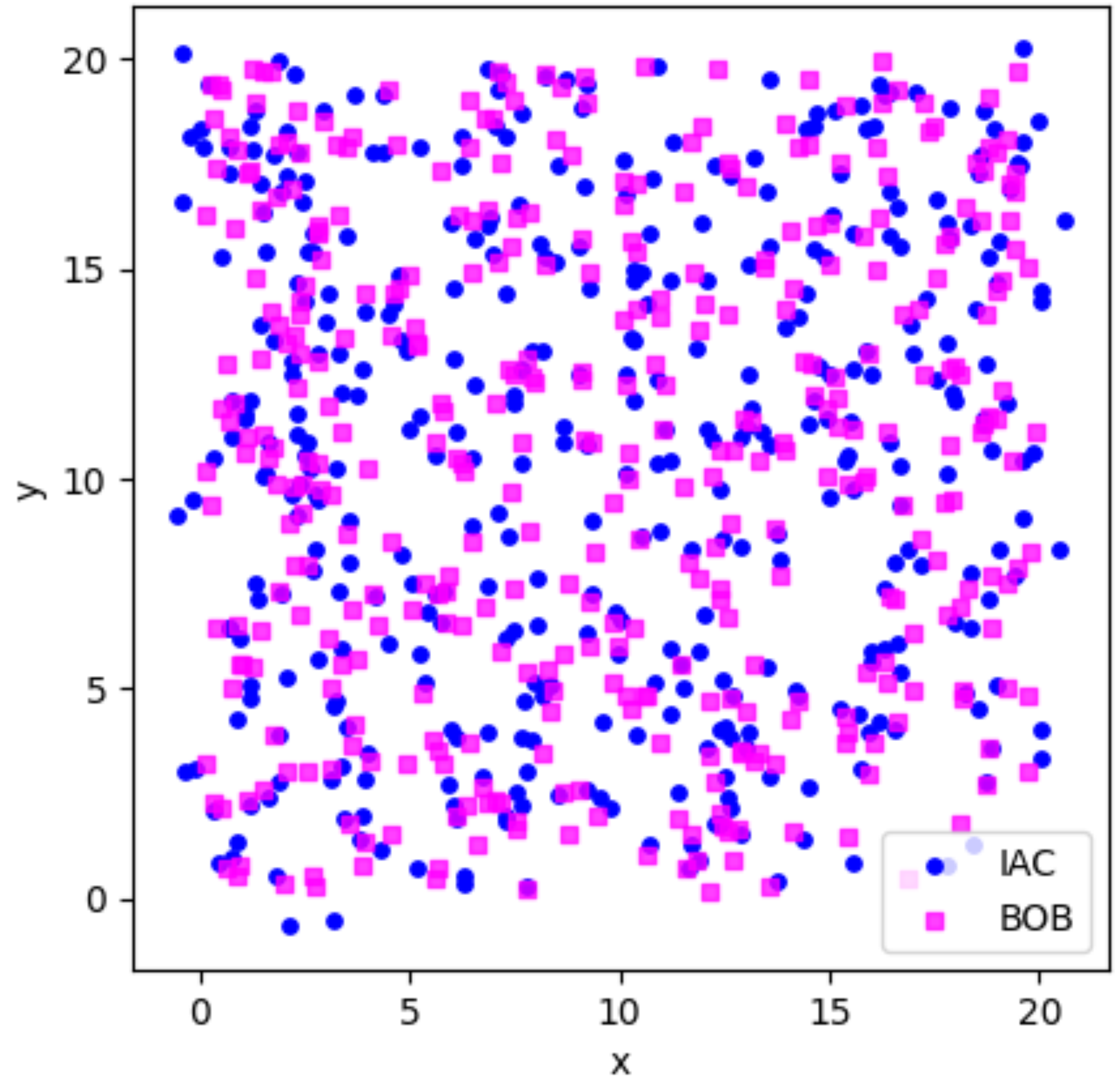
BOB

x	y
2.59176	11.6148
2.35522	12.7033
3.60981	12.9357
2.91734	12.0081
2.46703	12.7667
2.60448	11.849
2.36841	12.6463
1.24649	11.4218
3.67557	4.29607
2.63406	4.7991
2.77047	4.19997
2.90153	4.83014
2.45598	4.98462
4.02456	4.89246
...	...

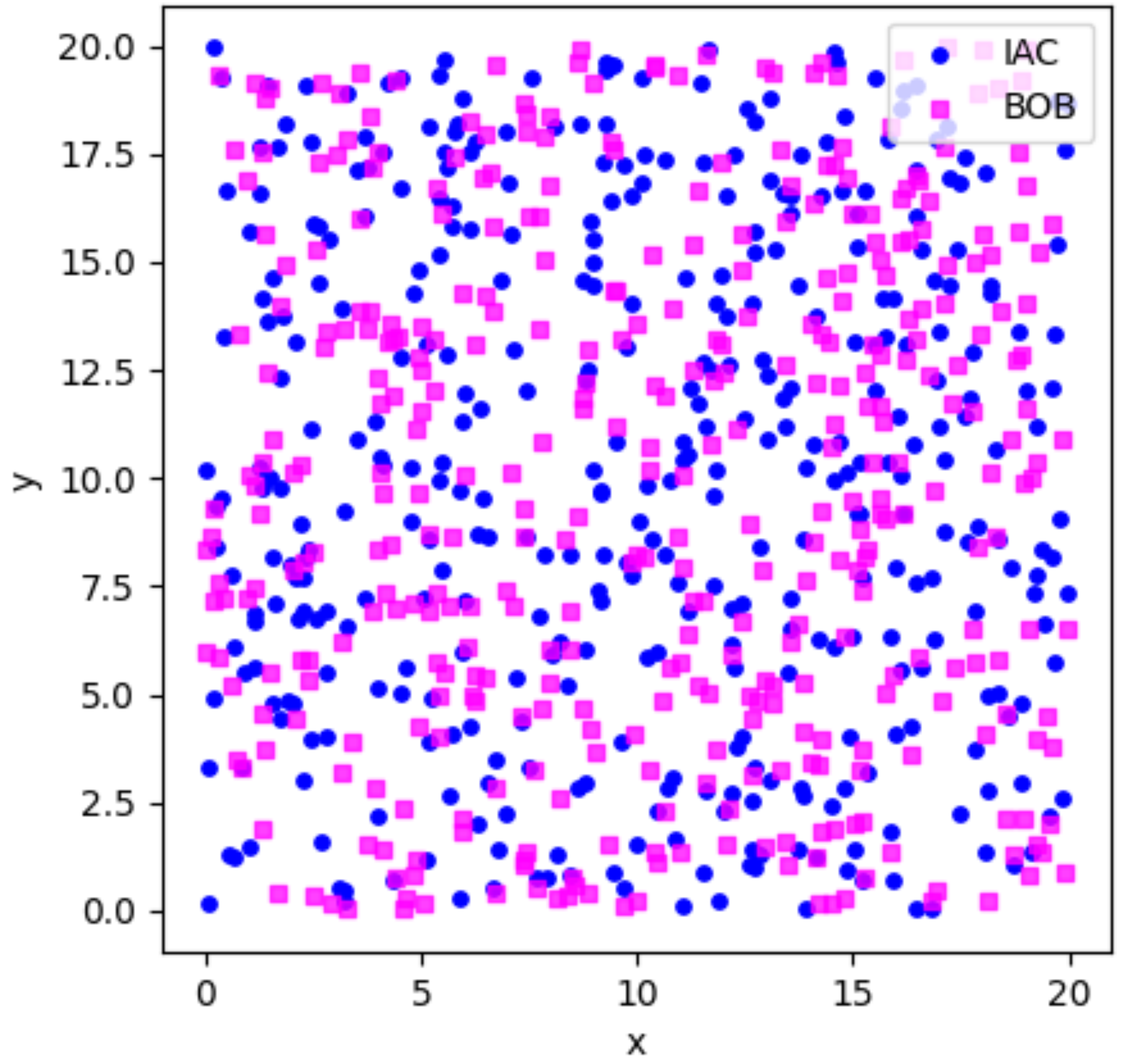
The data — Fall epidermis samples

The data — Fall epidermis samples

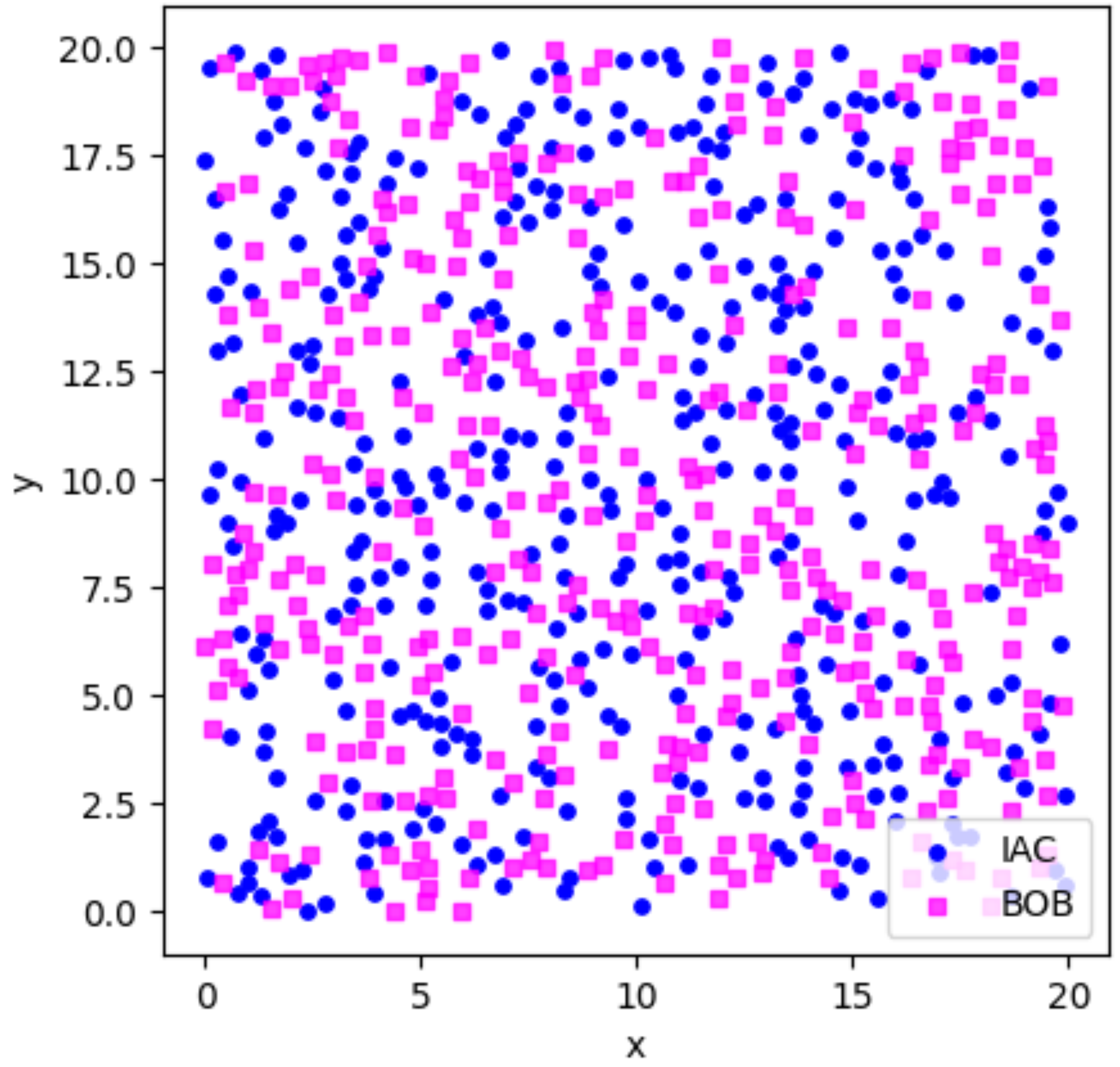
Cold



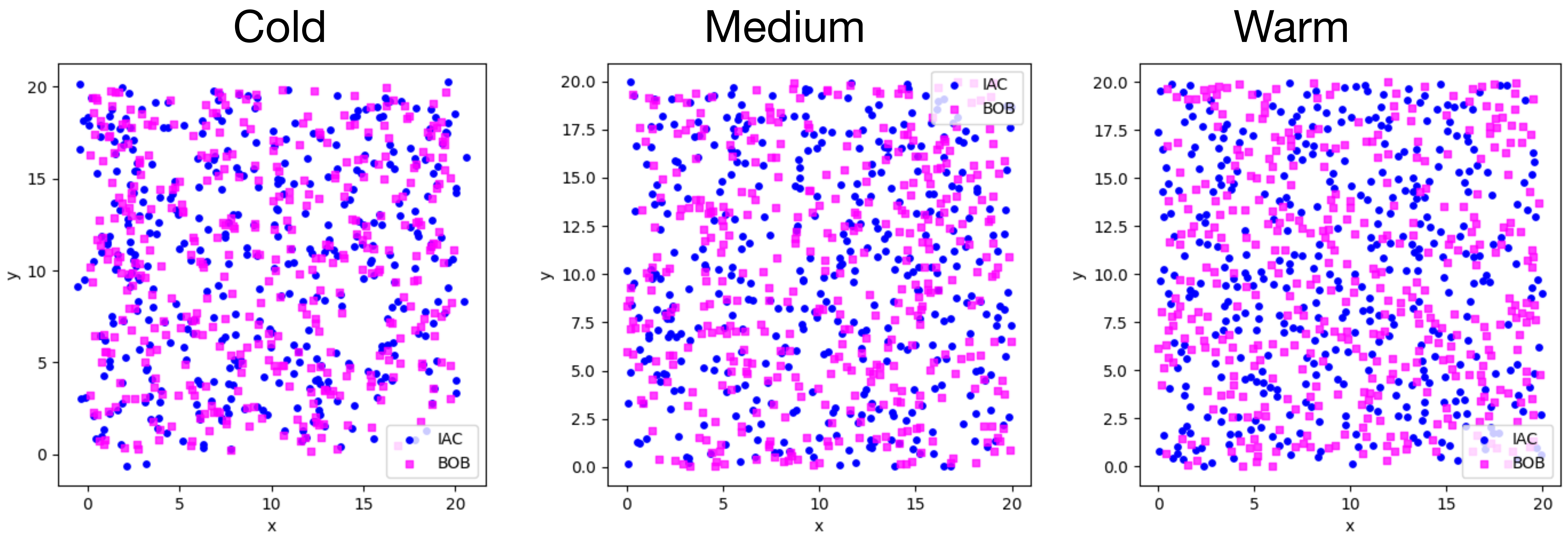
Medium



Warm



The data — Fall epidermis samples



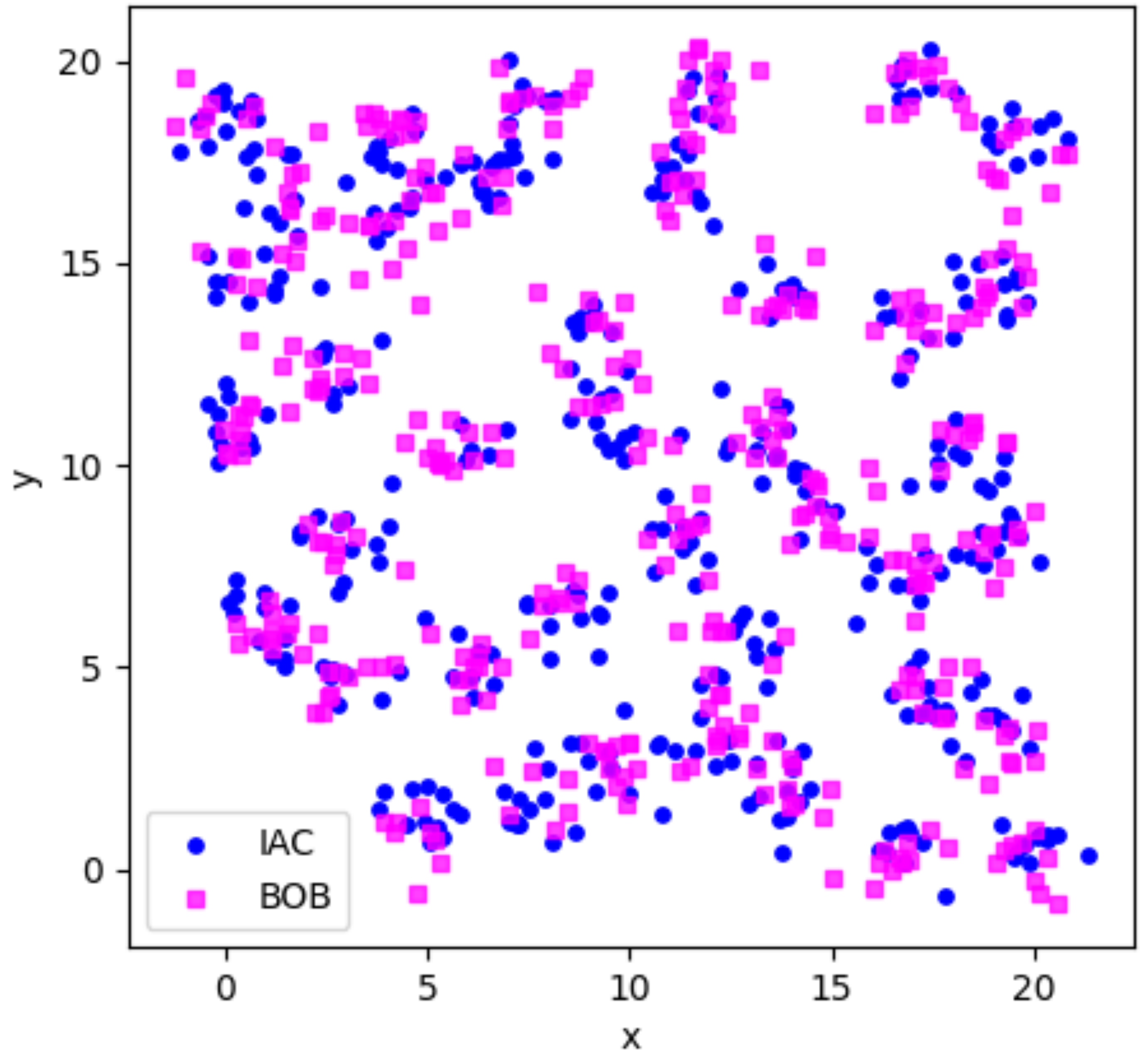
~~Do IAC and BOB attract or repulse each other depending on temperature?~~

Is there an association between attraction and repulsion and temperature?

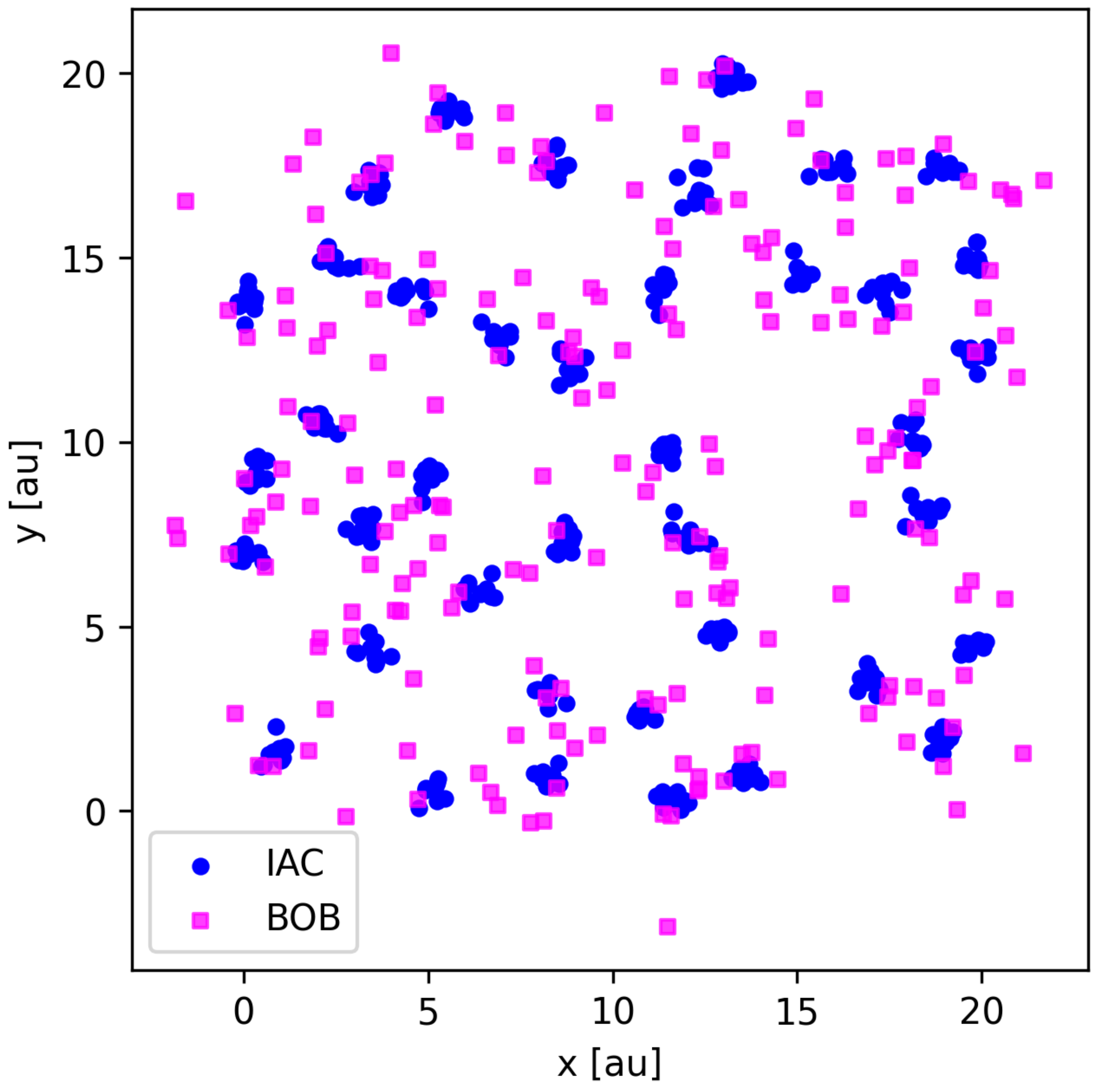
The data — Winter epidermis samples

The data — Winter epidermis samples

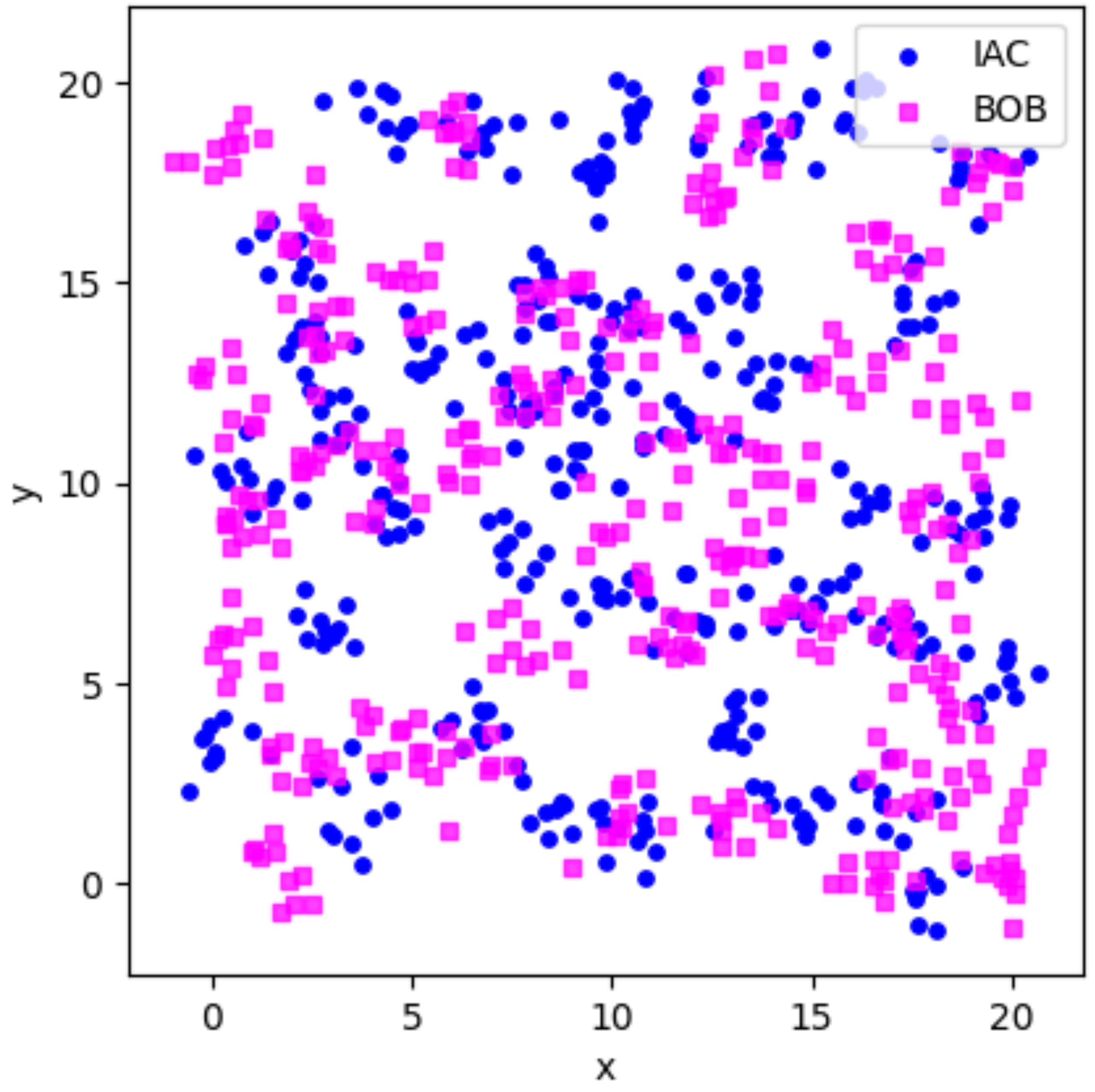
Cold



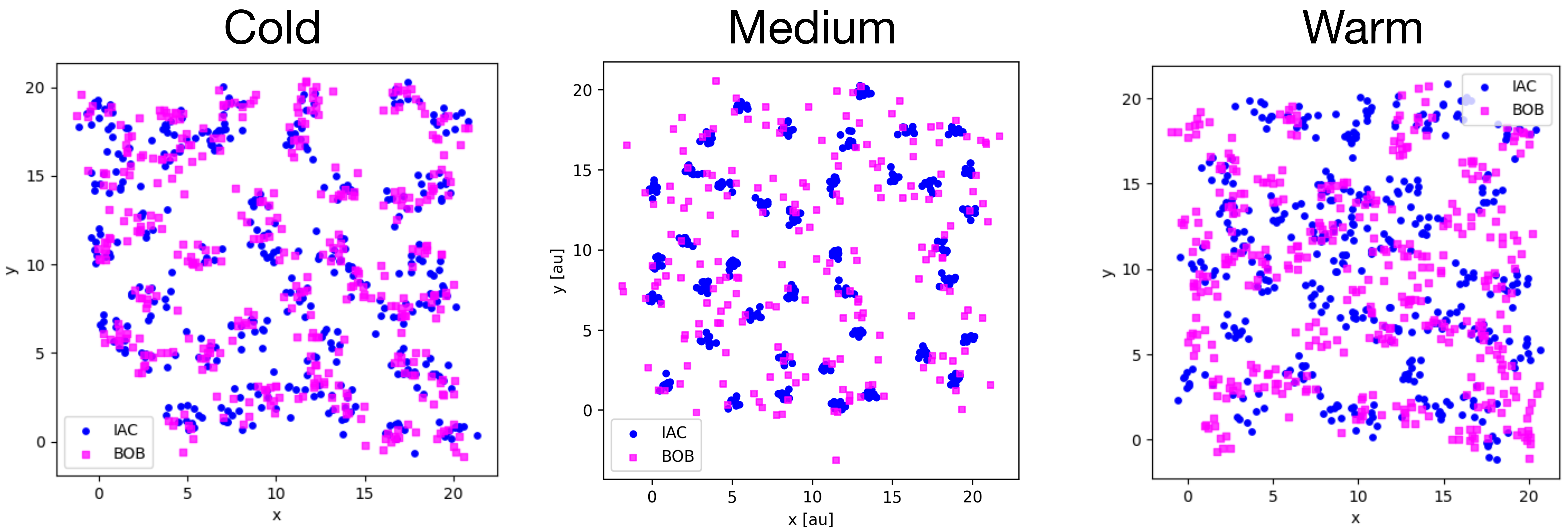
Medium



Warm



The data — Winter epidermis samples



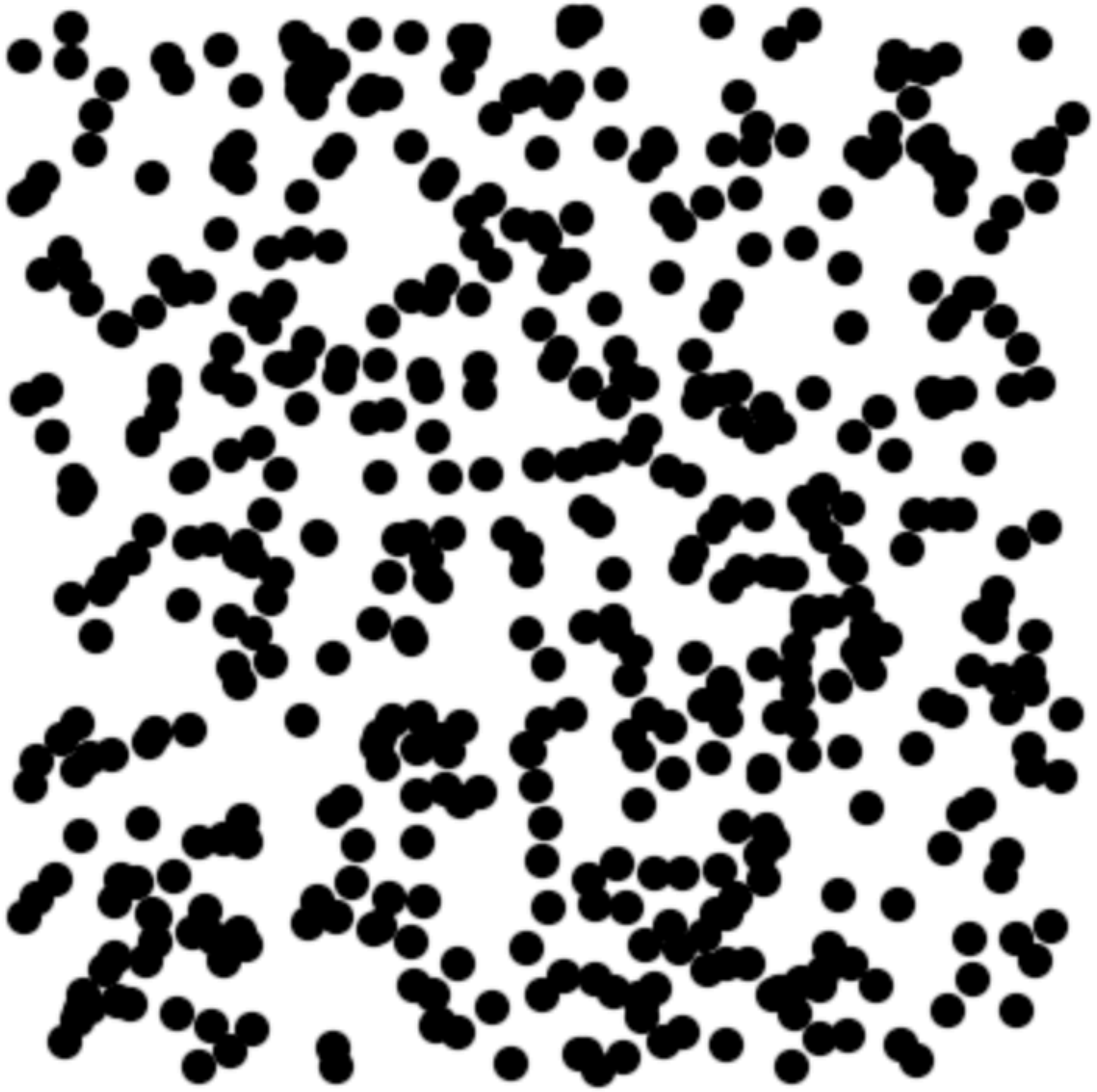
~~Do IAC and BOB attract or repulse each other depending on temperature?~~

Is there an association between attraction and repulsion and temperature?

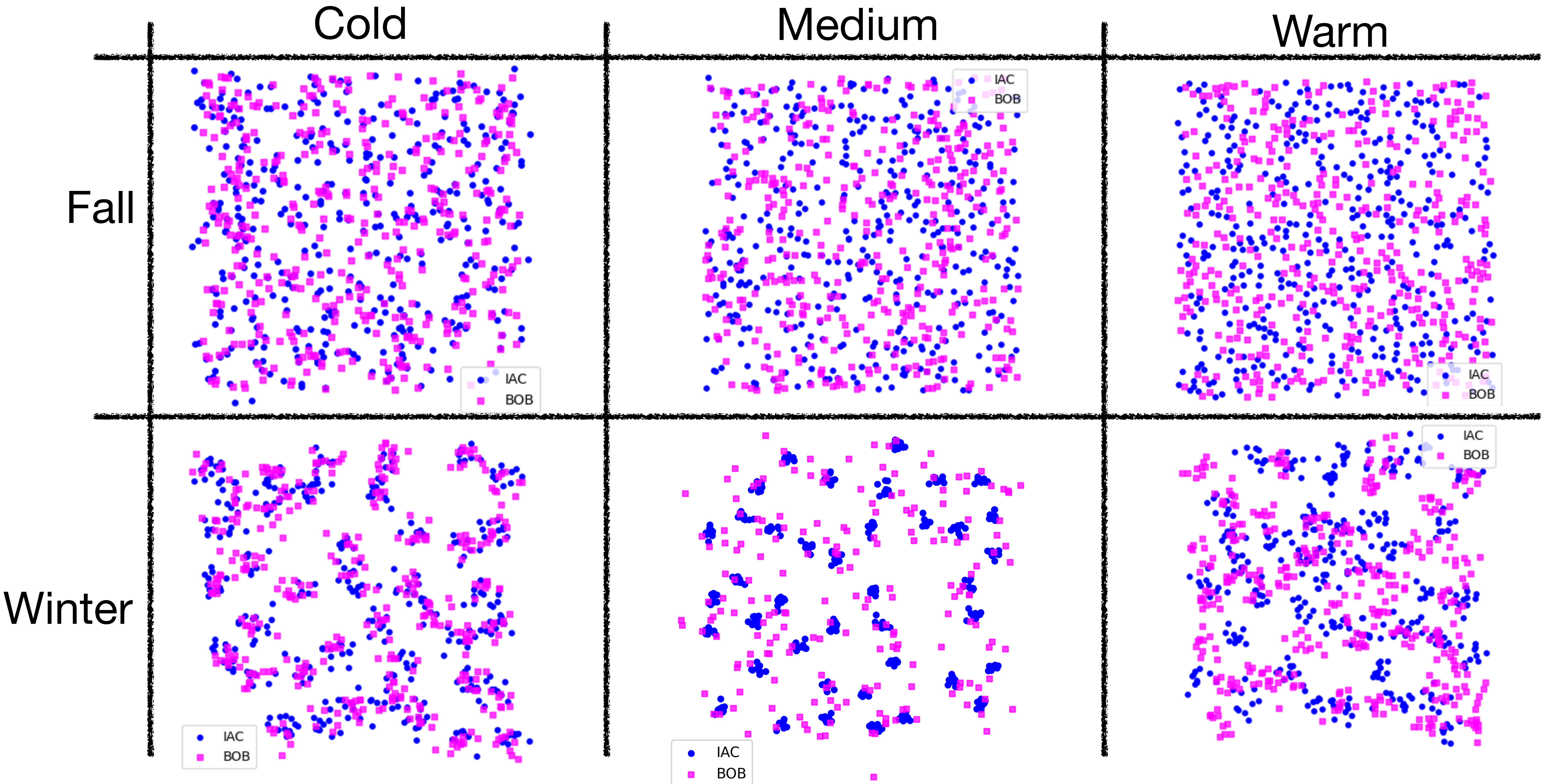
-> 0. Load the data, 1. plot the data

-> 0. Load the data, 1. plot the data

Q: Do you see patterns?

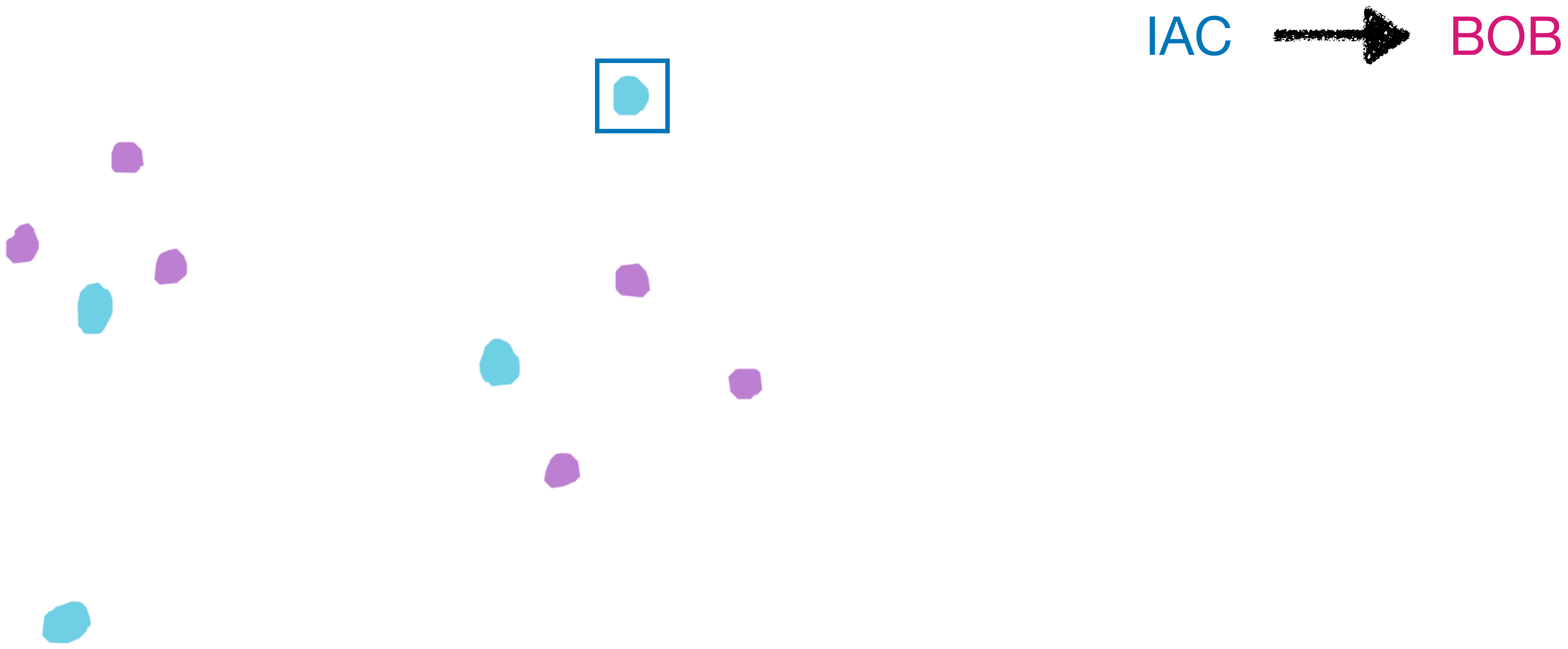


How would you analyze the data?

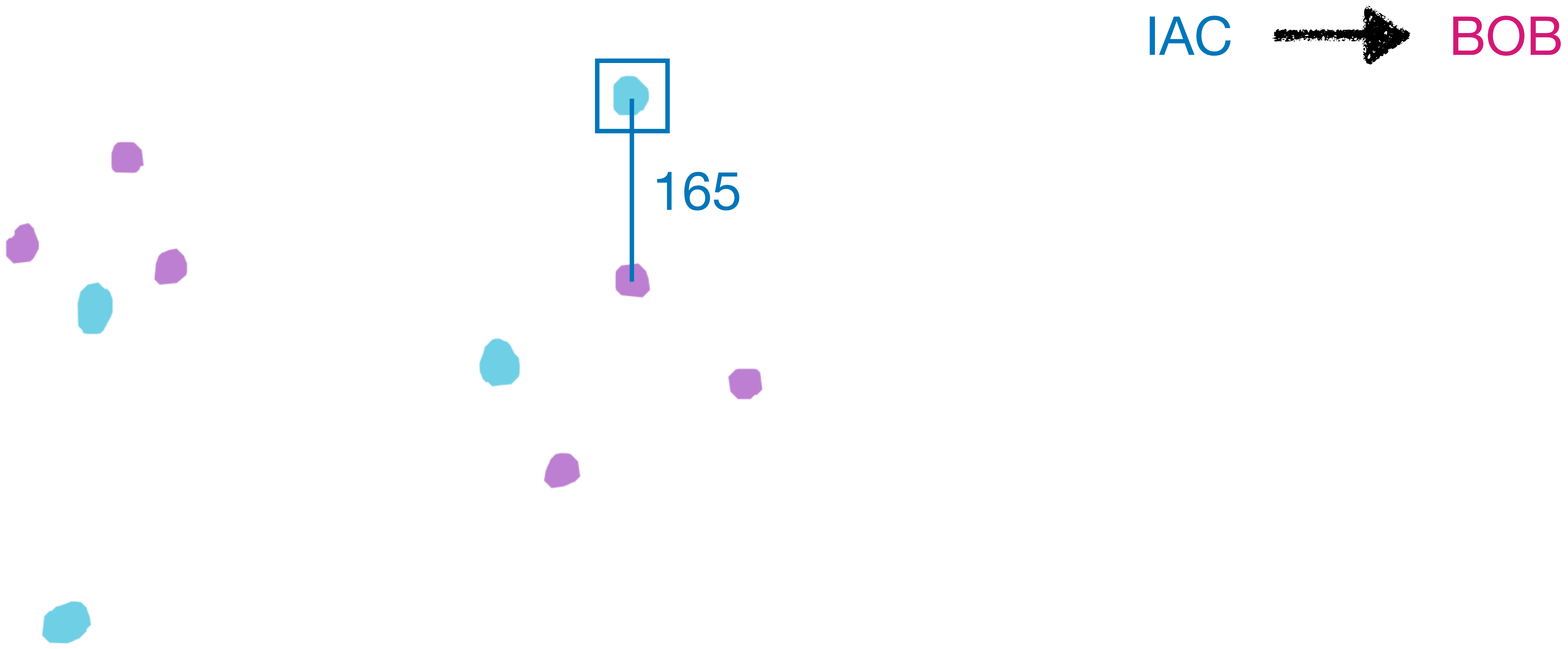


Mean distance to nearest neighbor

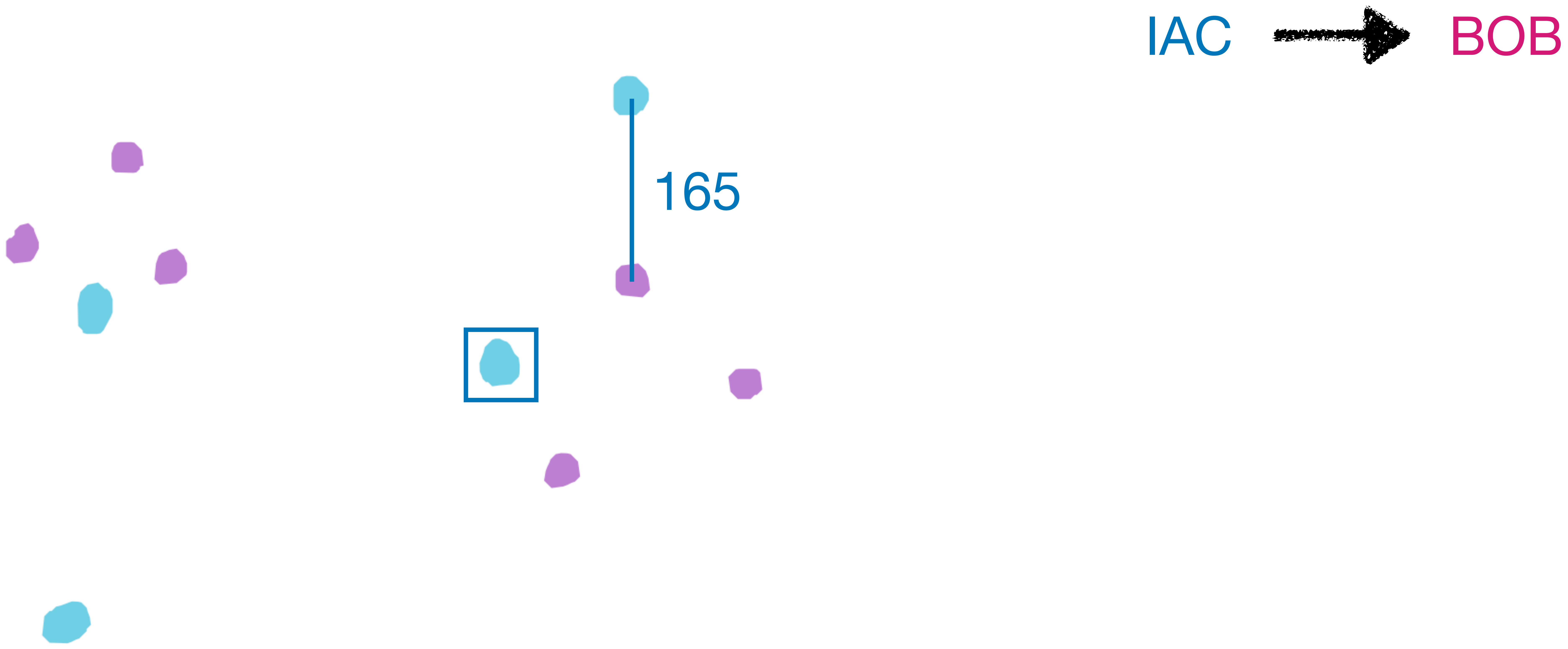
Mean distance to nearest neighbor



Mean distance to nearest neighbor

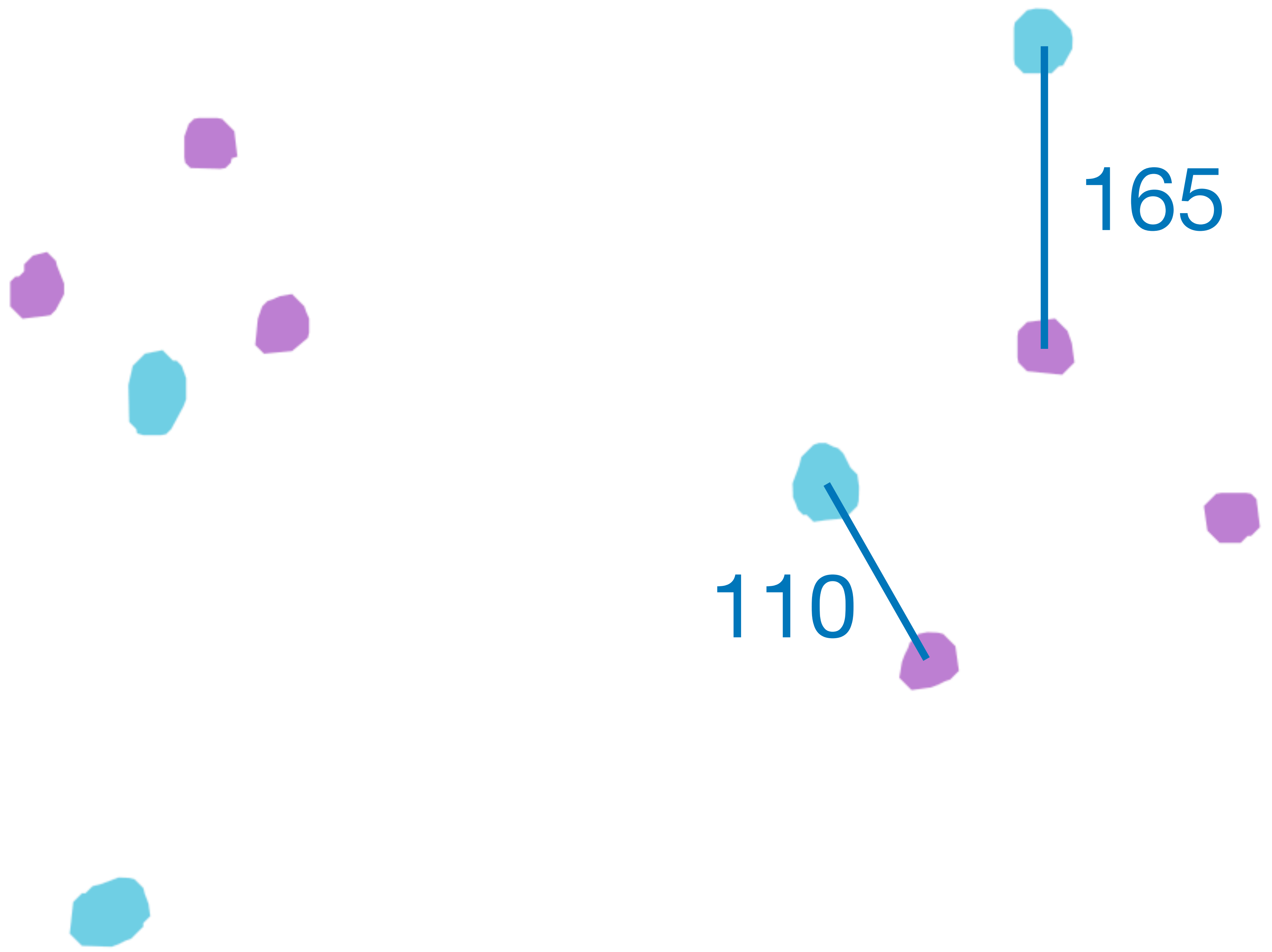


Mean distance to nearest neighbor

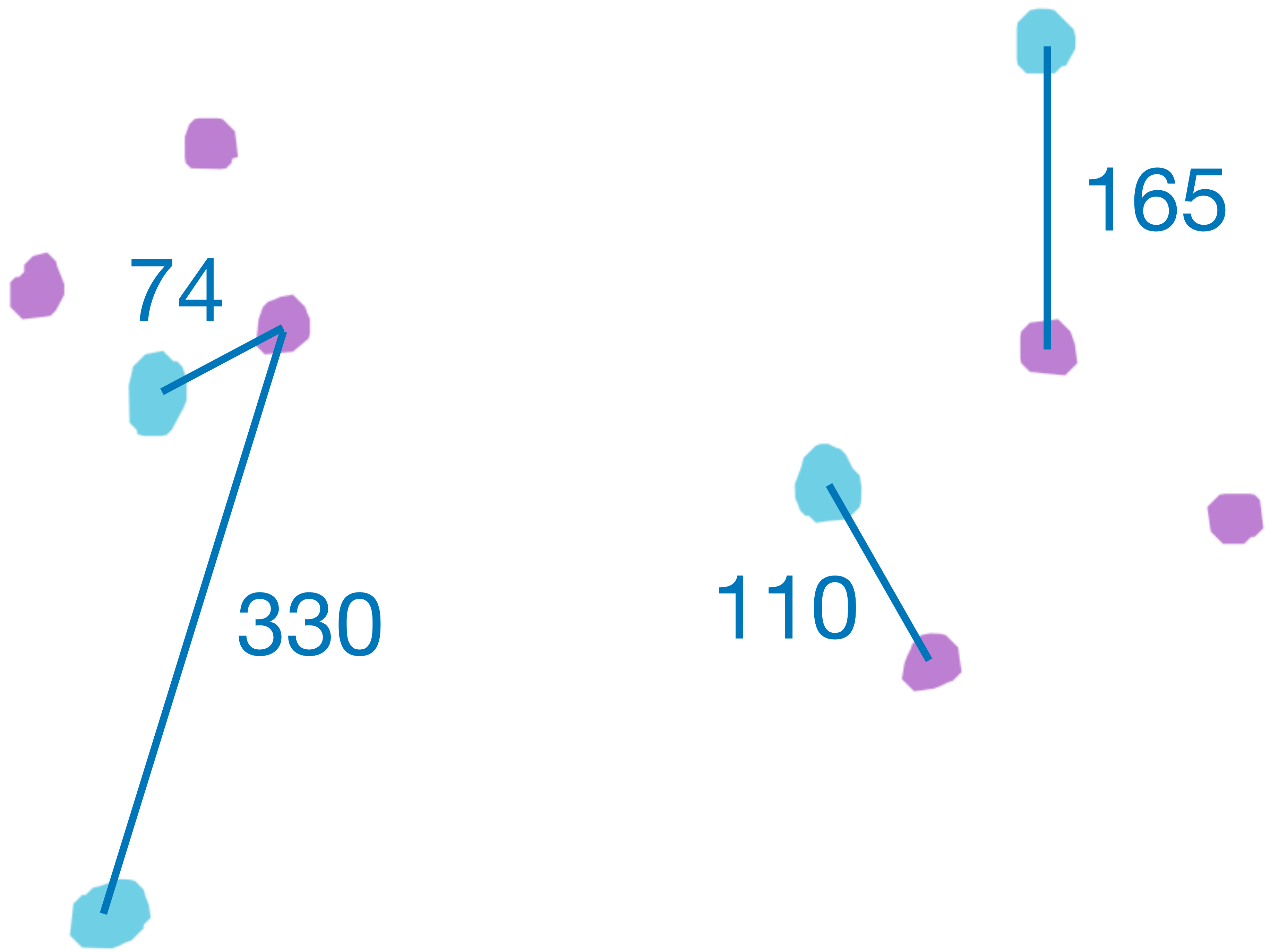


Mean distance to nearest neighbor

IAC → BOB



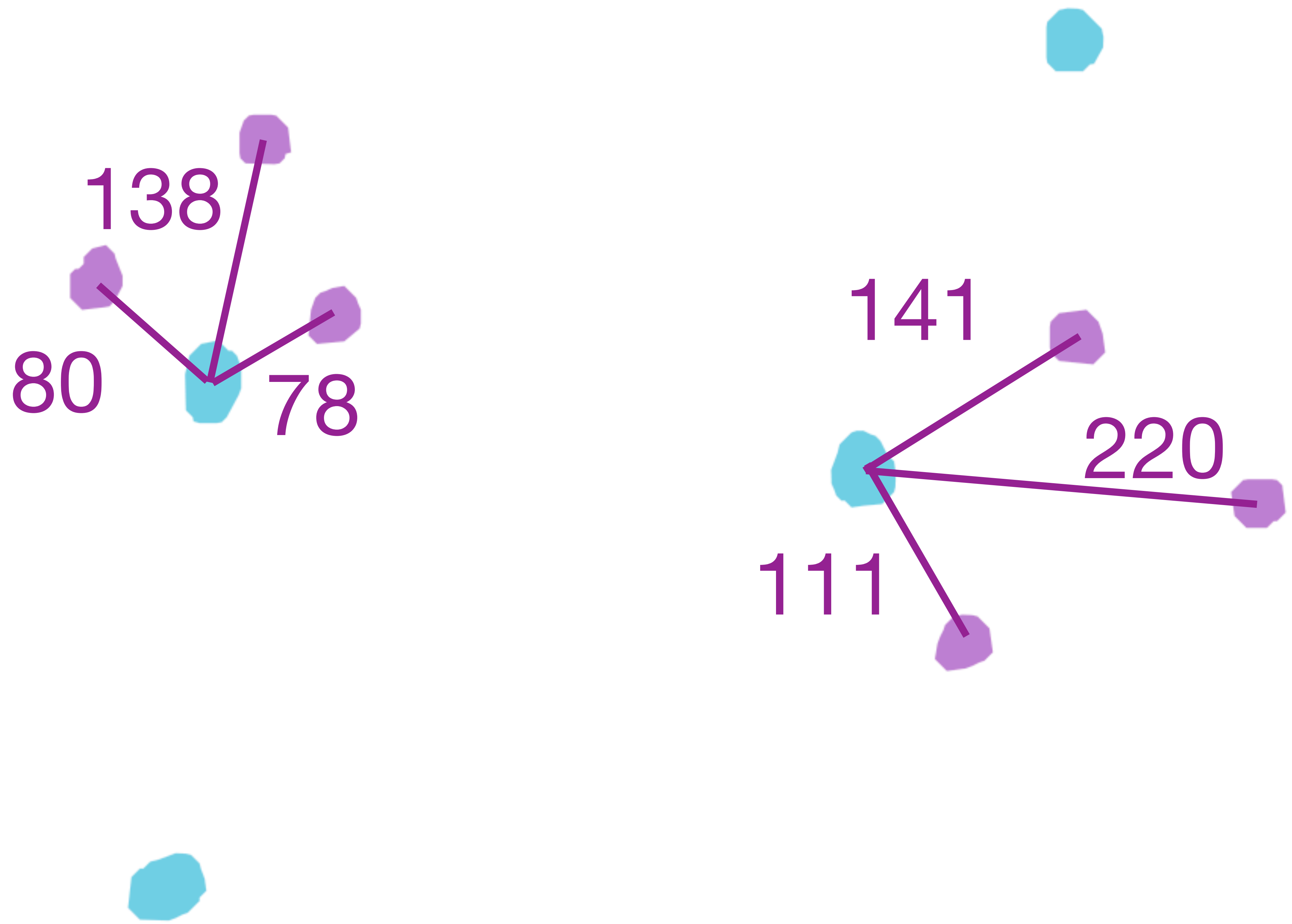
Mean distance to nearest neighbor



IAC → BOB

$$\frac{74 + 330 + 110 + 165}{4} = 169.75$$

Mean distance to nearest neighbor



IAC → BOB

$$\frac{74 + 330 + 110 + 165}{4} = 169.75$$

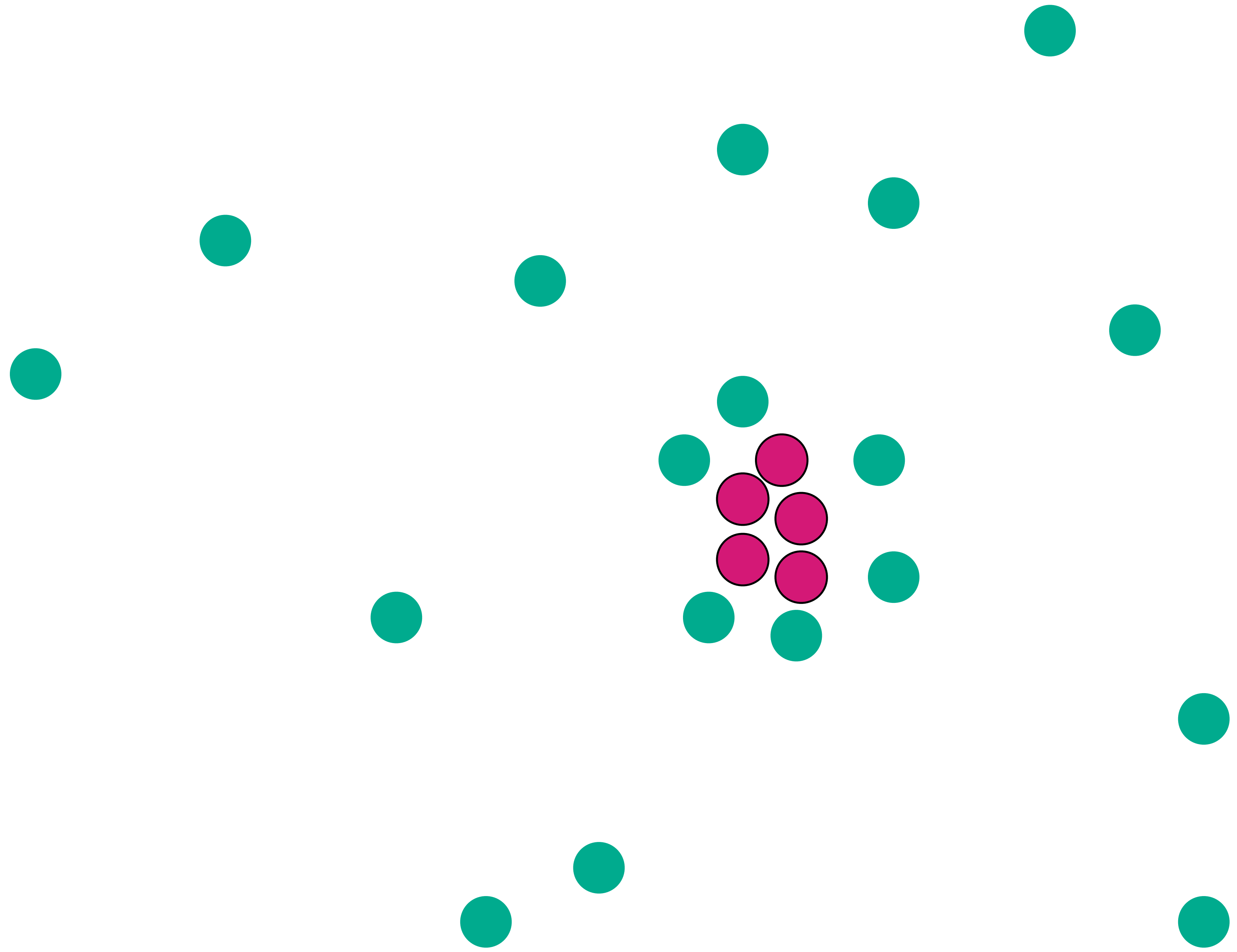
BOB → IAC

$$\frac{80+138+78+111+141+220}{6} = 121$$

Mean distance to nearest neighbor

 = Small mean distance

 = Large mean distance



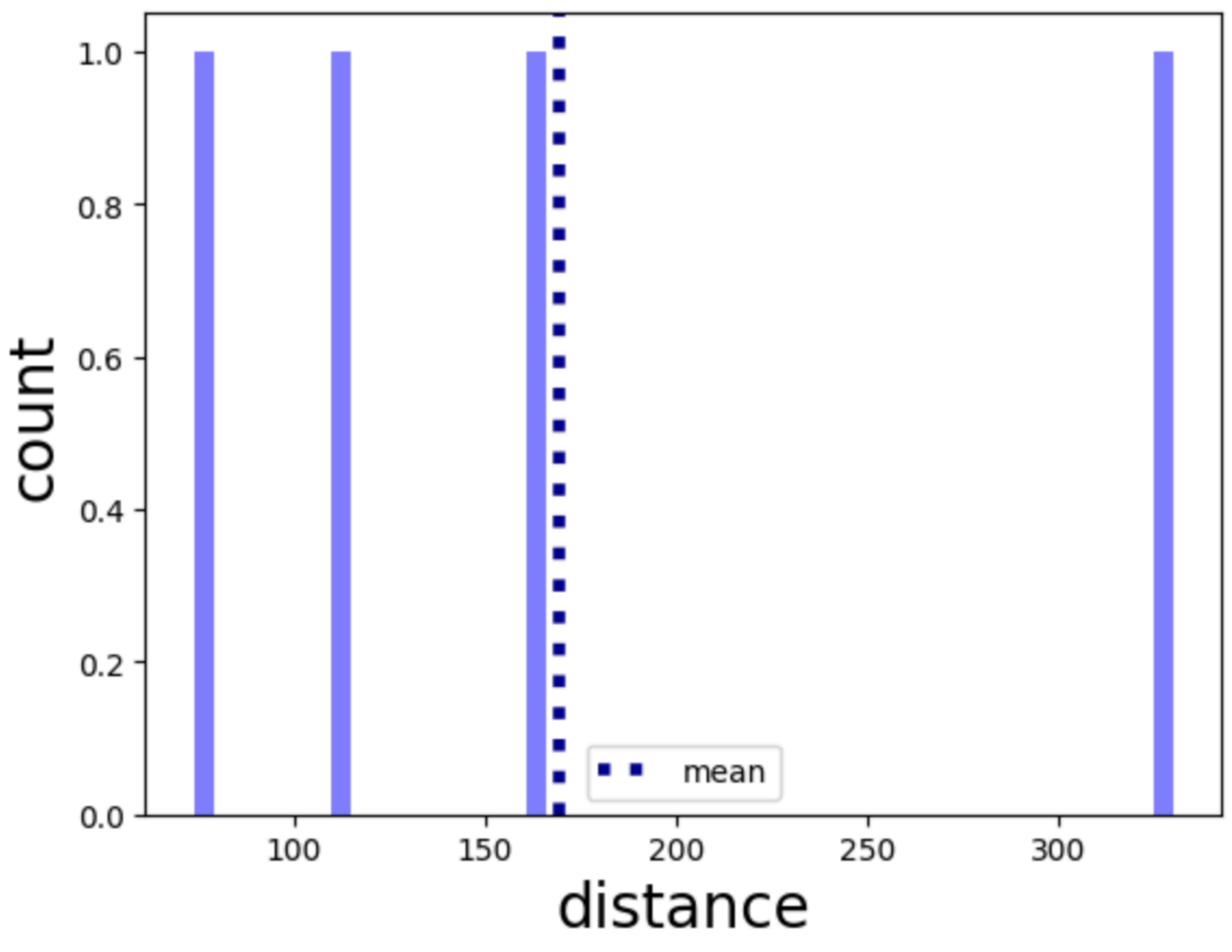
Mean distance to nearest neighbor

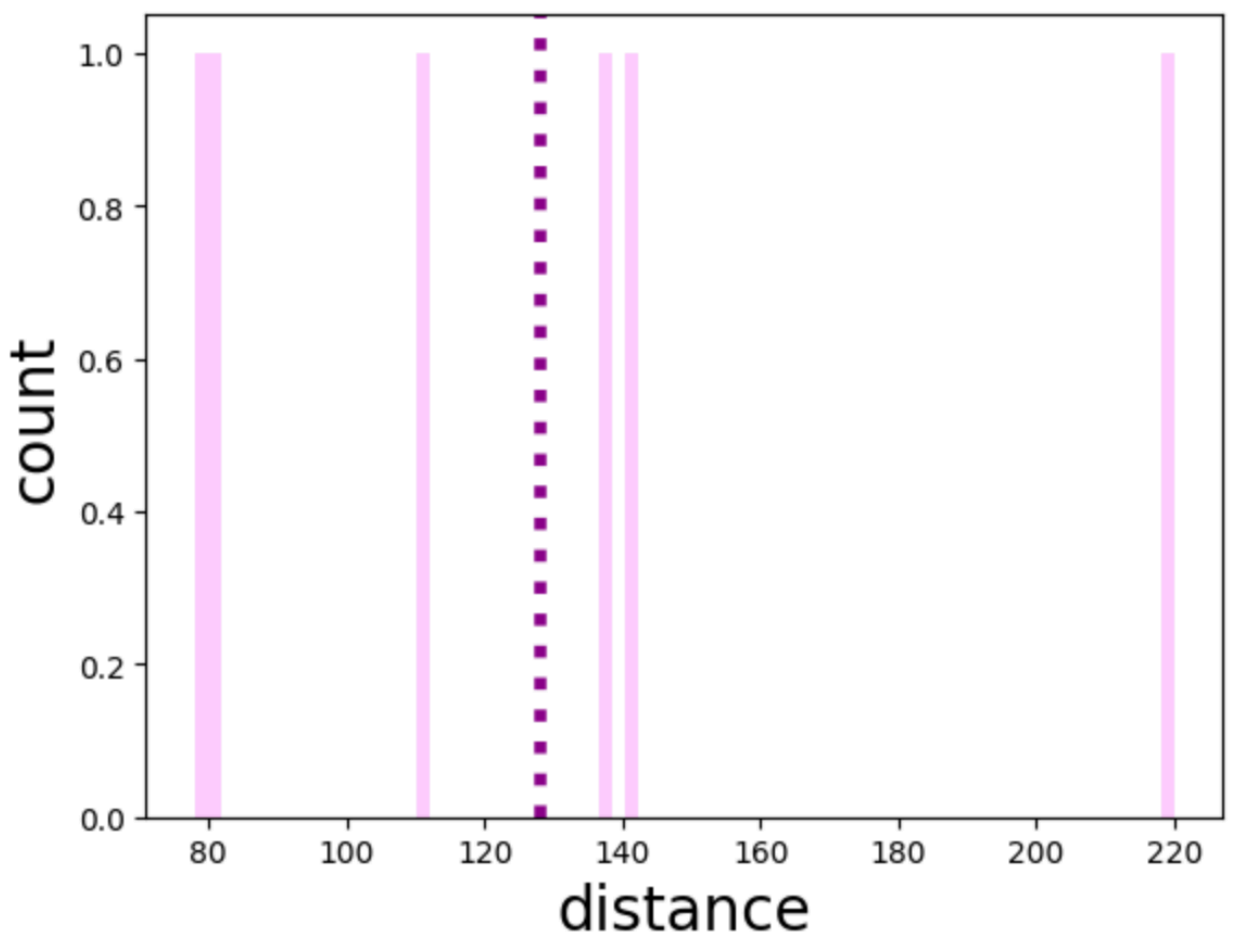
IAC → BOB

Distances	Mean
74, 330, 110, 165	169.75

BOB → IAC

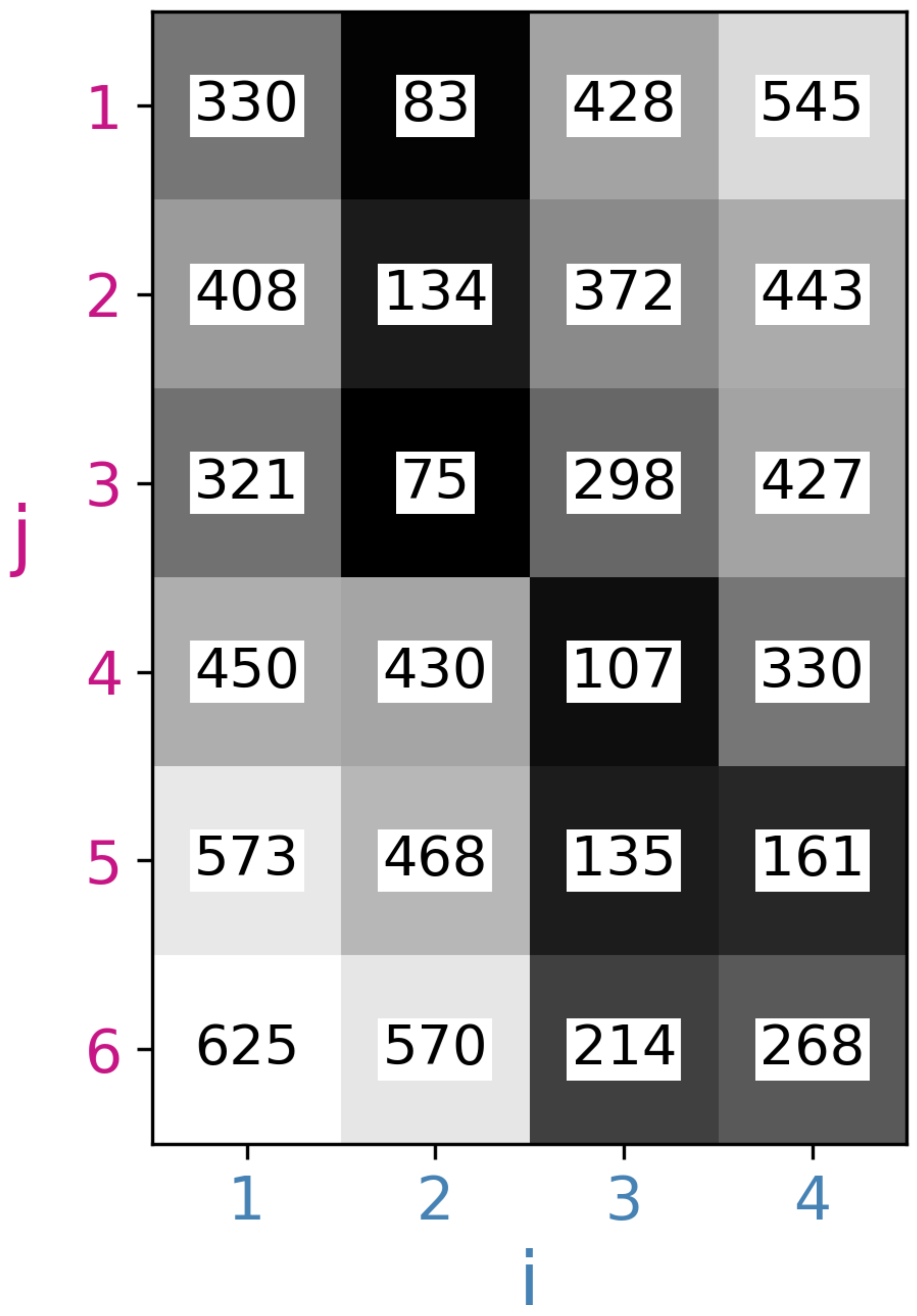
Distances	Mean
80, 138, 78, 111, 141, 220	121

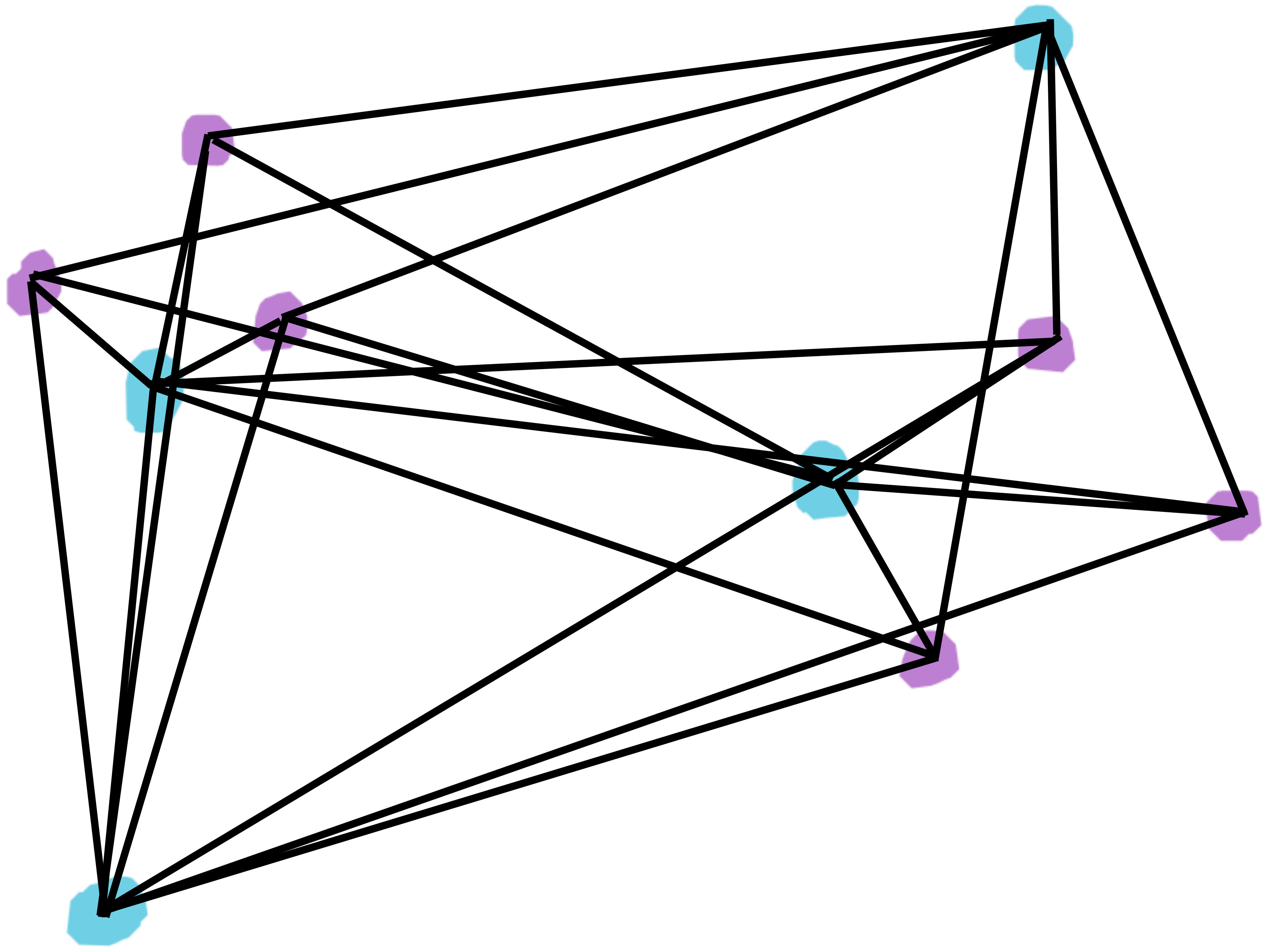




Exercise: Code along: Mean nearest neighbor distance

dist_matrix





Exercise: Code along: Mean nearest neighbor distance

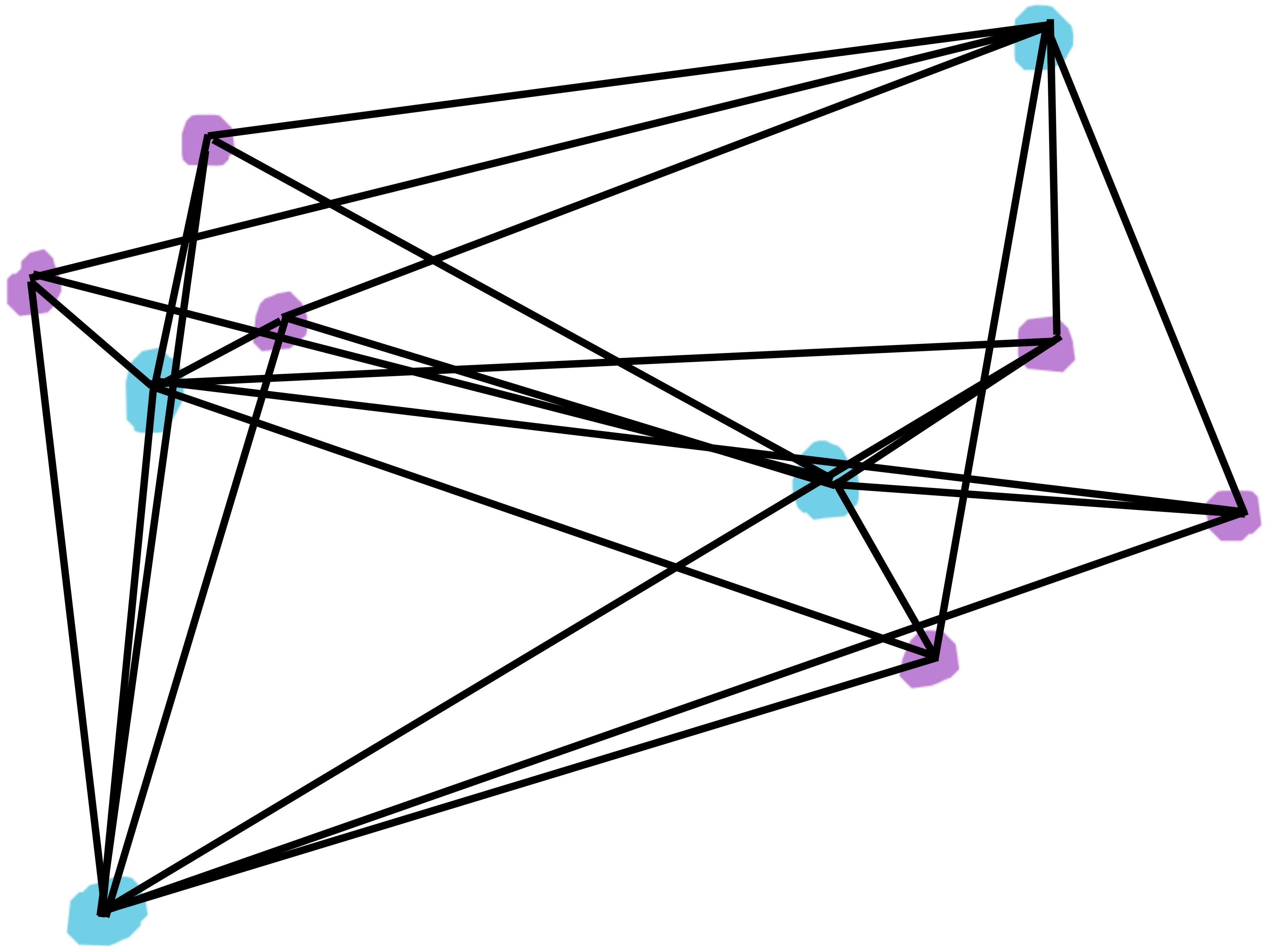
`dist_matrix`

j \ i	1	2	3	4
1	330	83	428	545
2	408	134	372	443
3	321	75	298	427
4	450	430	107	330
5	573	468	135	161
6	625	570	214	268

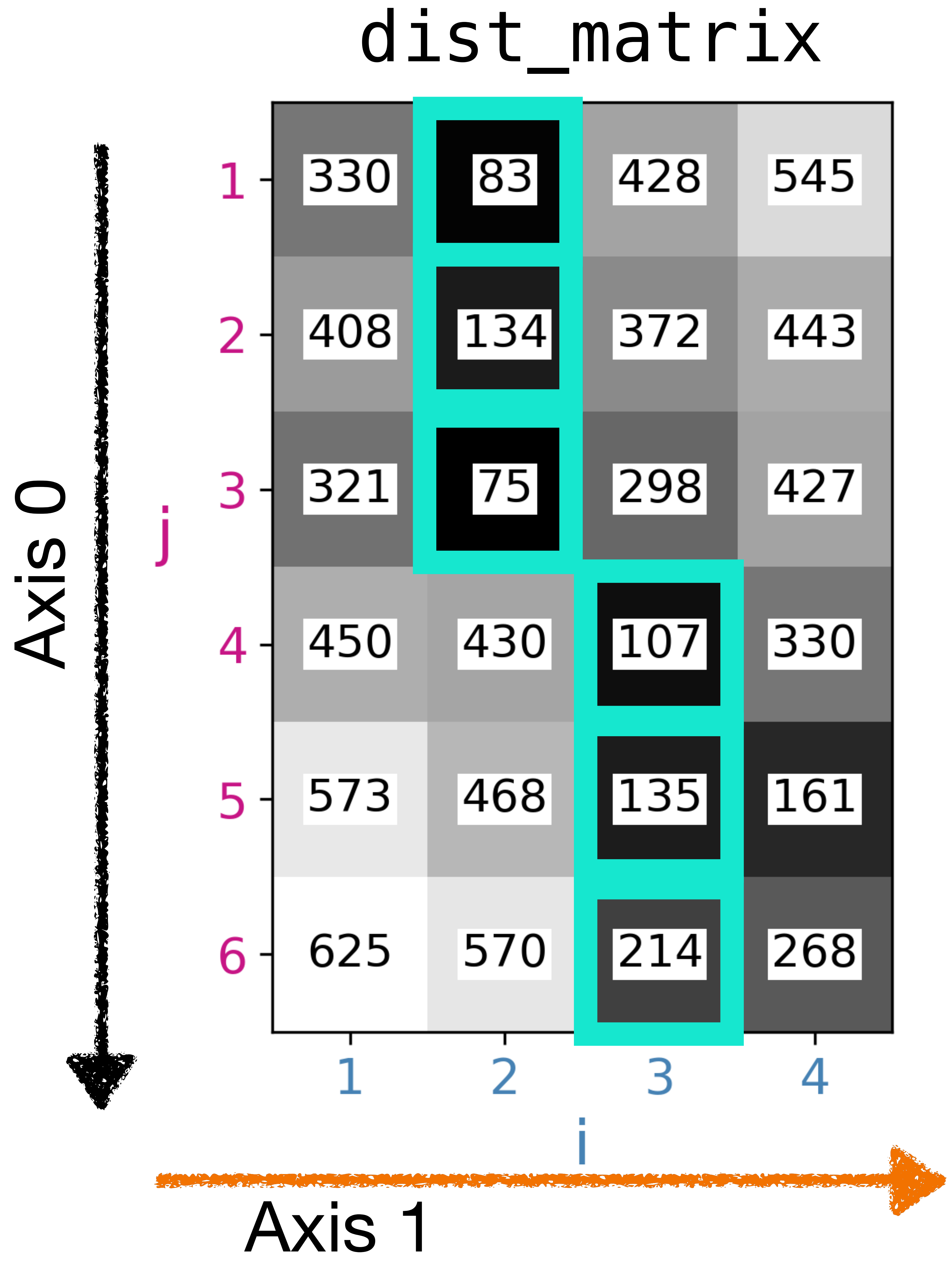
Axis 0 (j, down the rows)

Axis 1 (i, across the columns)

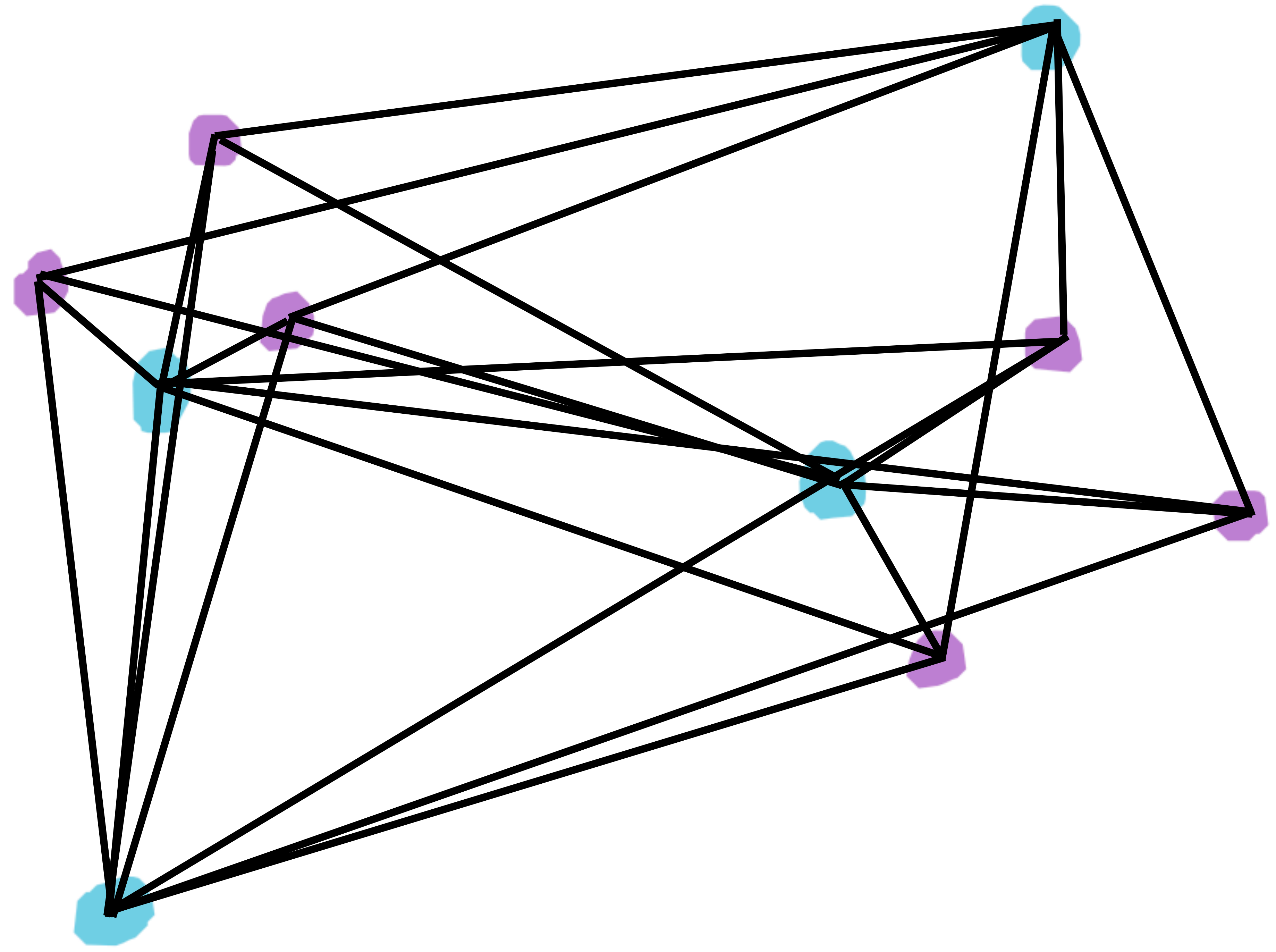
```
np.min(dist_matrix, axis = 1)
```



Exercise: Code along: Mean nearest neighbor distance




```
np.min(dist_matrix, axis = 1)
```



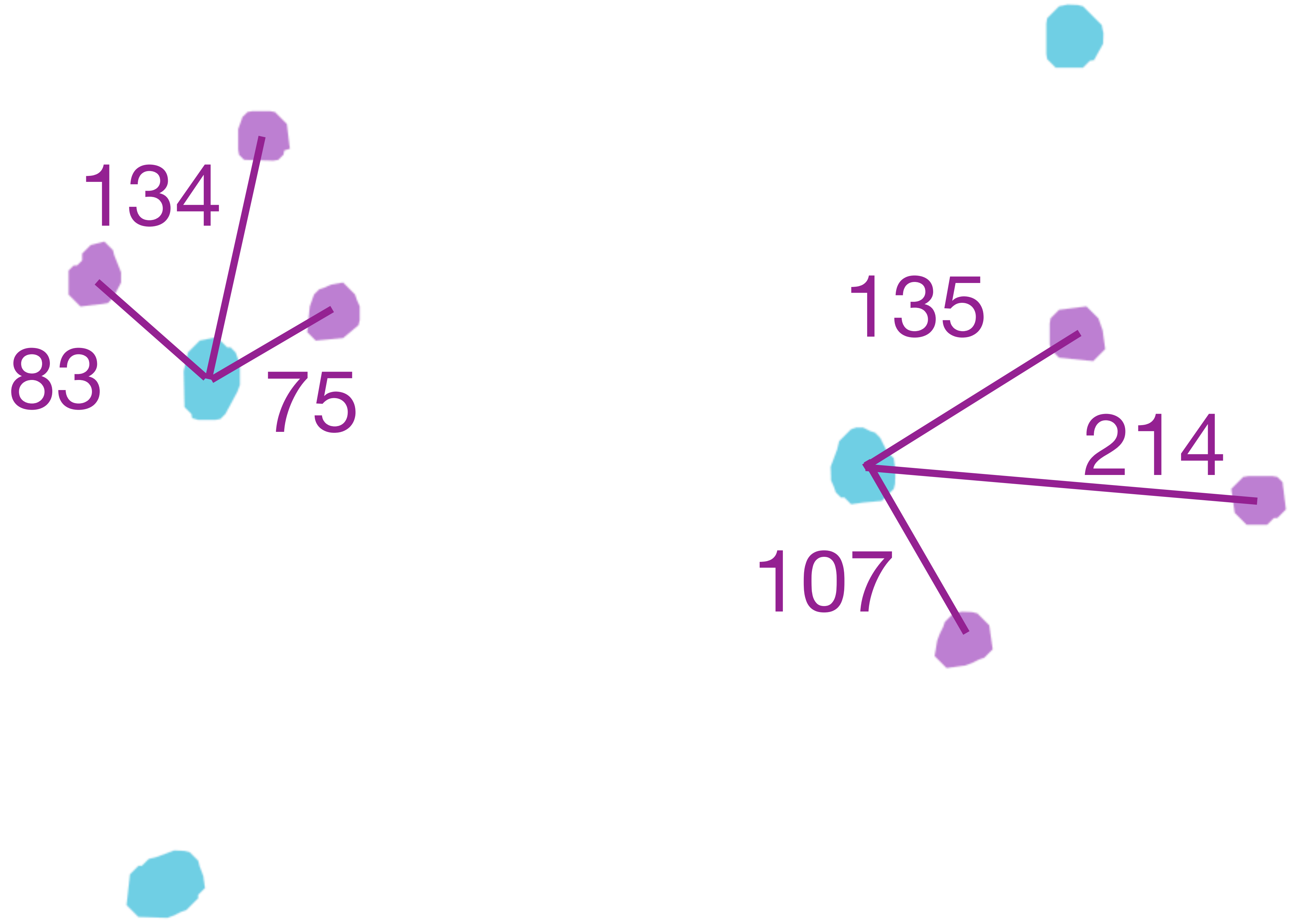
Exercise: Code along: Mean nearest neighbor distance

`dist_matrix`

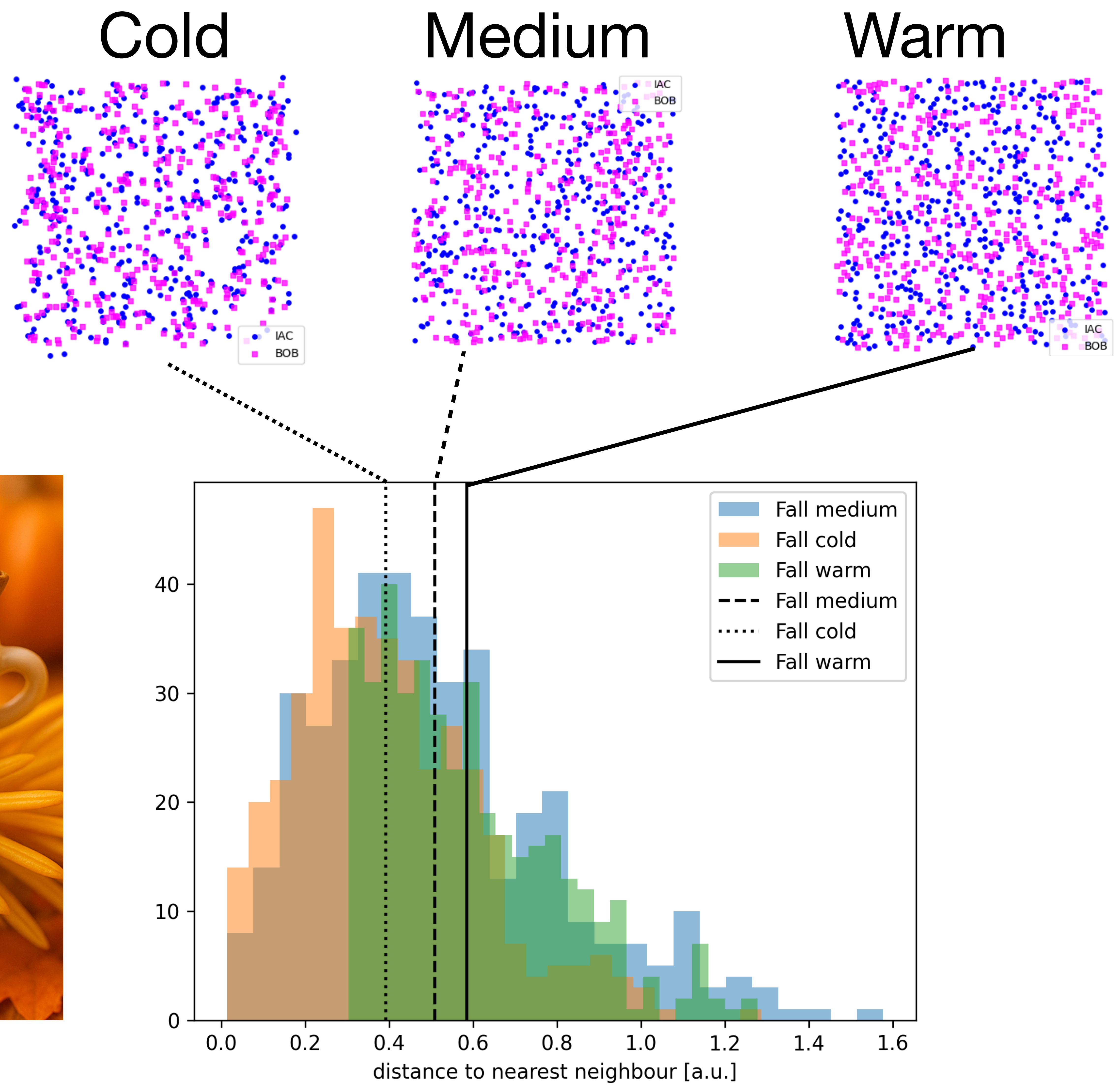
j \ i	1	2	3	4
1	330	83	428	545
2	408	134	372	443
3	321	75	298	427
4	450	430	107	330
5	573	468	135	161
6	625	570	214	268

Axis 0 (j, rows) — Axis 1 (i, columns)

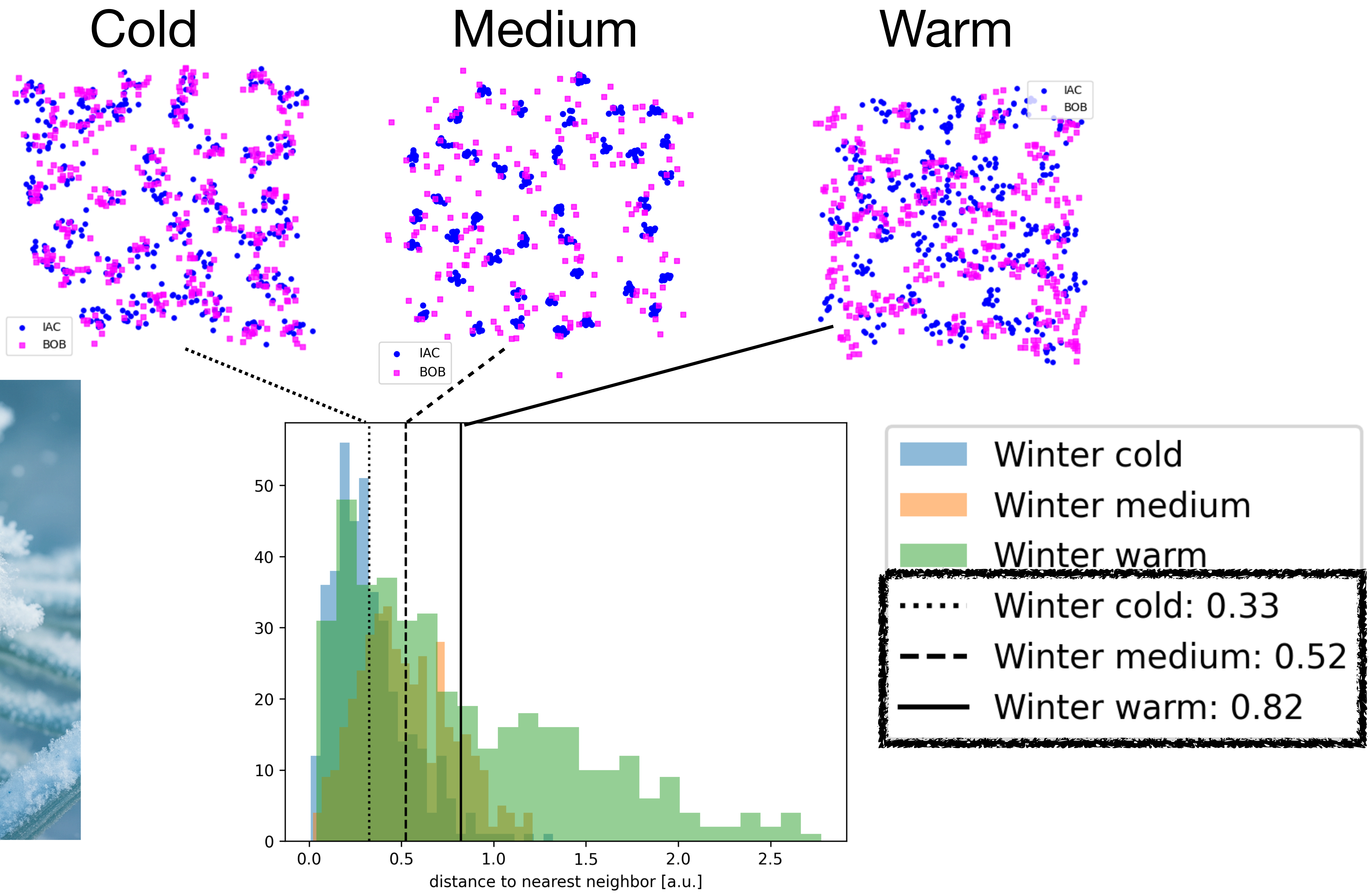
```
np.min(dist_matrix, axis = 1)
```



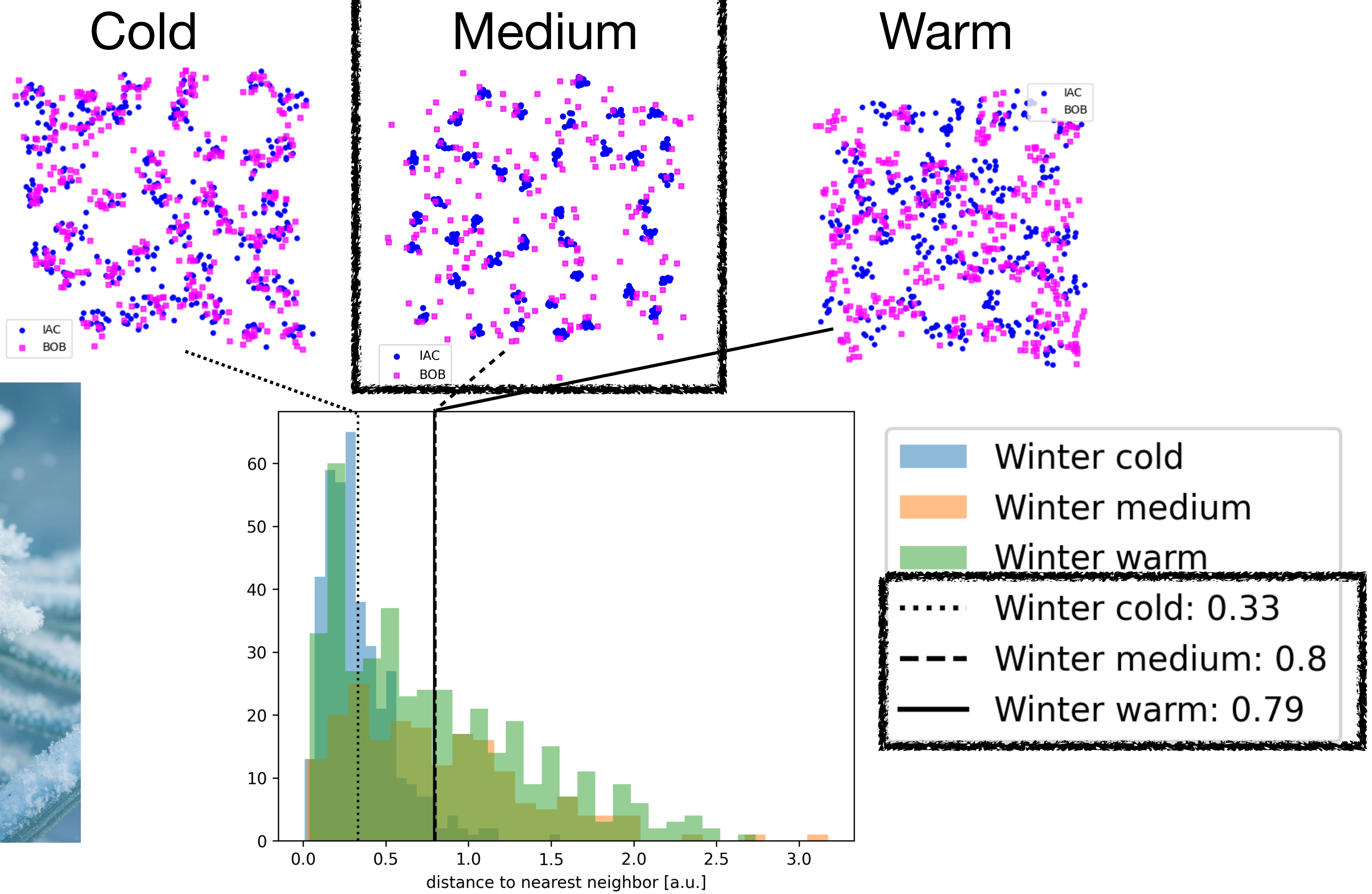
Results: Mean distance IAC -> BOB



Results: Mean distance IAC -> BOB



Results: Mean distance BOB -> IAC



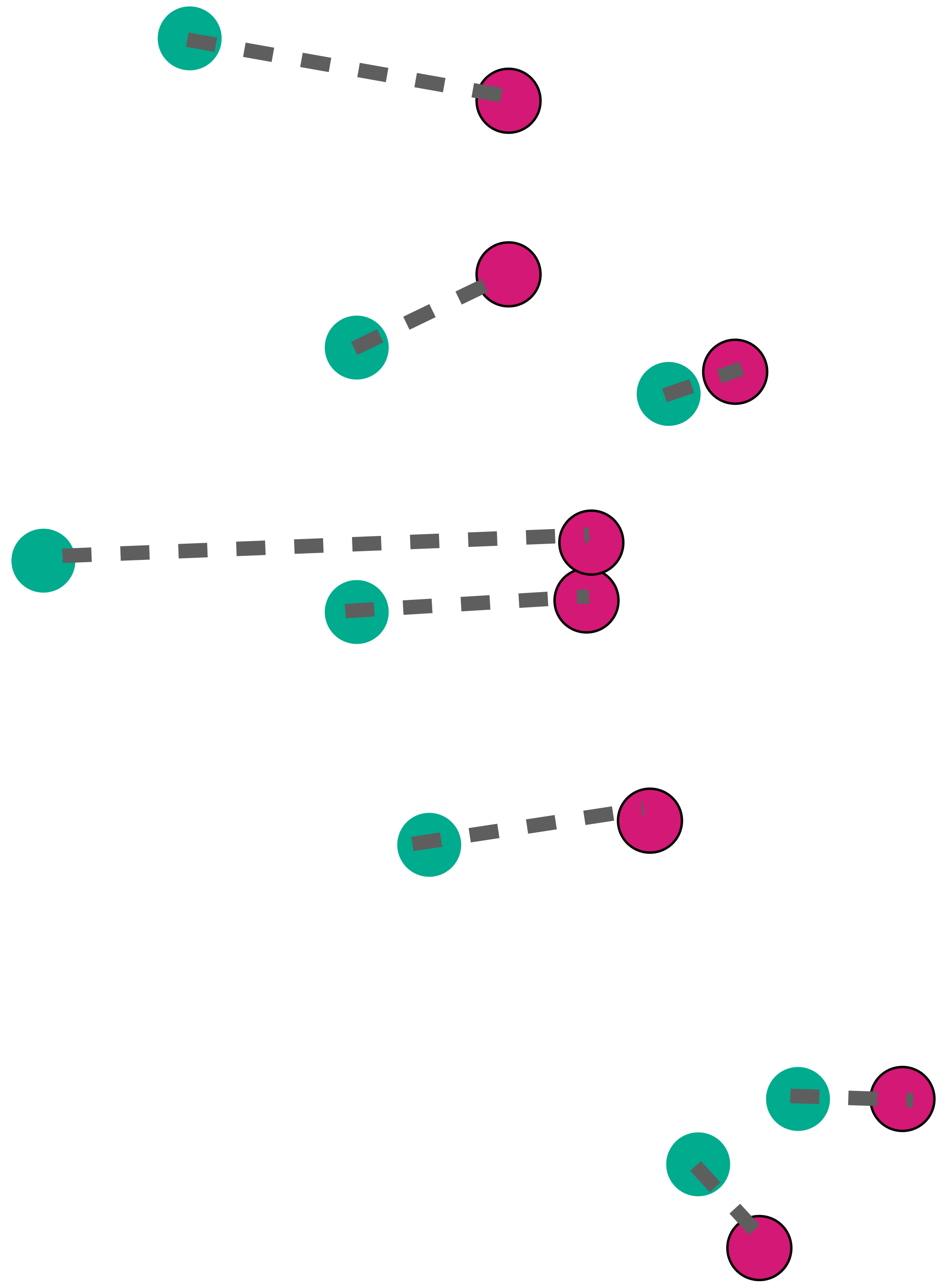
-> Results mean nearest neighbor distance + exercise

Mean distance to nearest neighbor

- Asymmetric: BOB → IAC ≠ IAC → BOB
- Returns: One number
- Range: Short

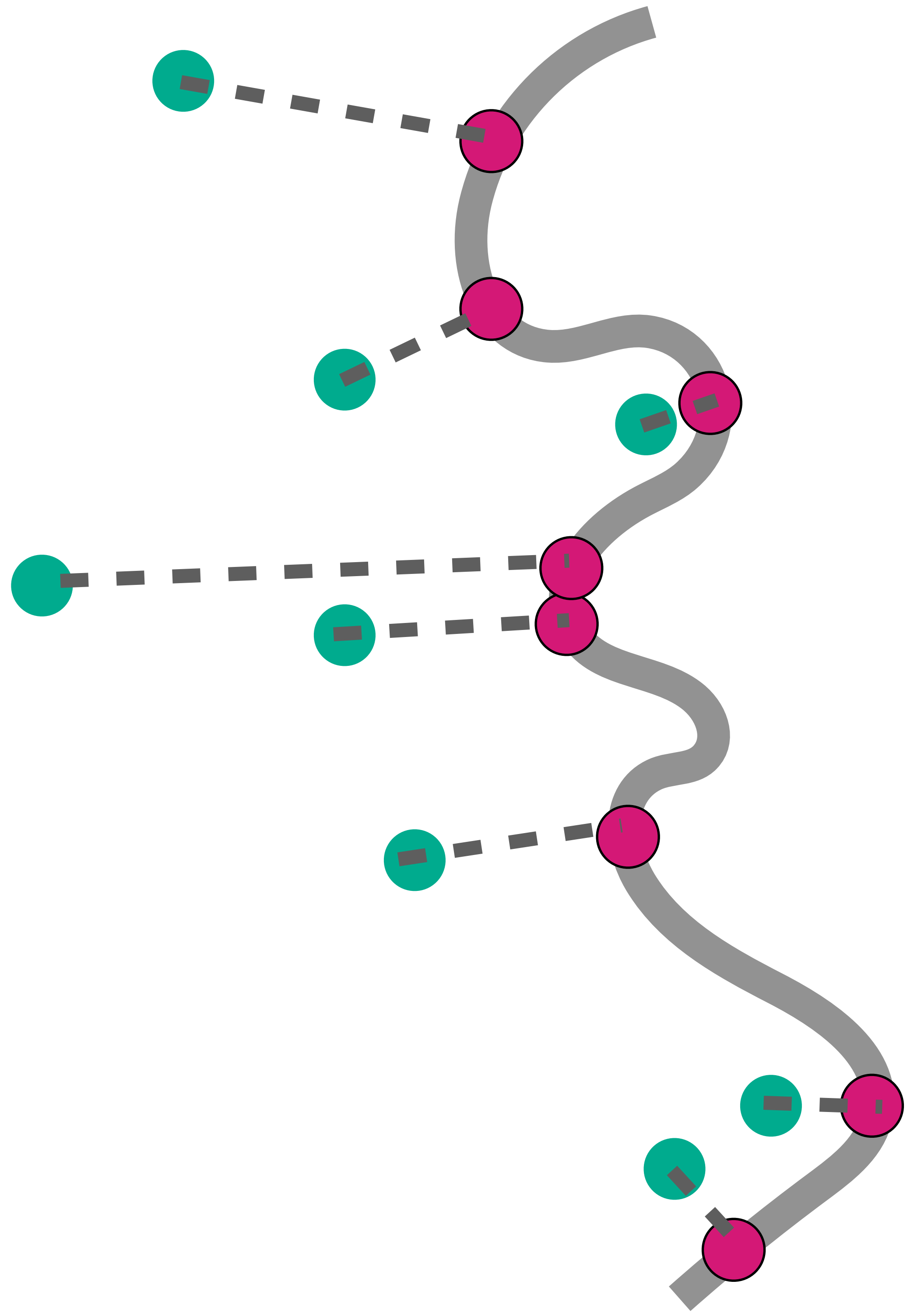
Beyond the mean distance to nearest neighbor

- Similar concepts hold true beyond the realm of just points



Beyond the mean distance to nearest neighbor

- Similar concepts hold true beyond the realm of just points



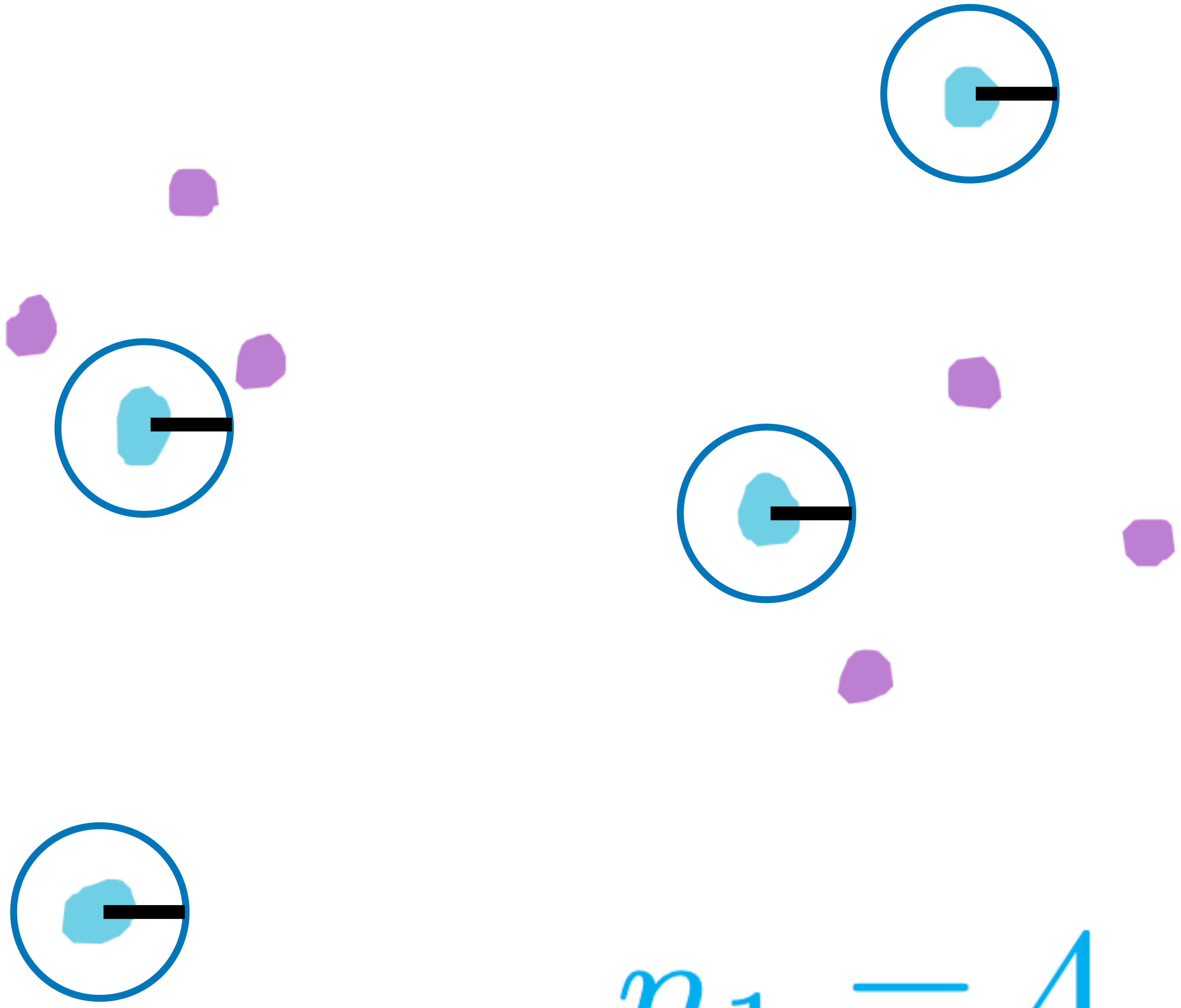
Nearest neighbor function

Mean dist: 1

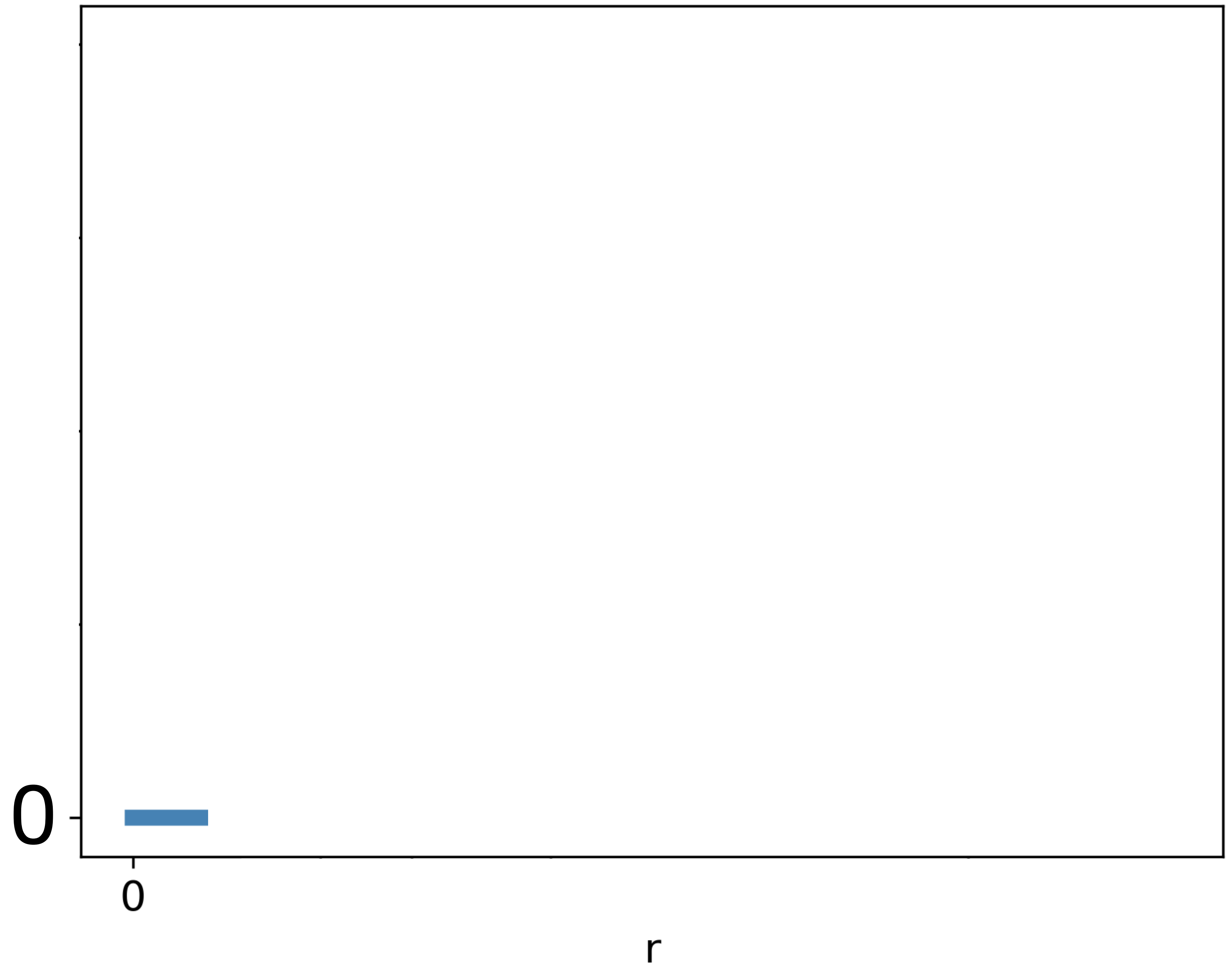
Mean dist: 1

Nearest neighbor function

$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

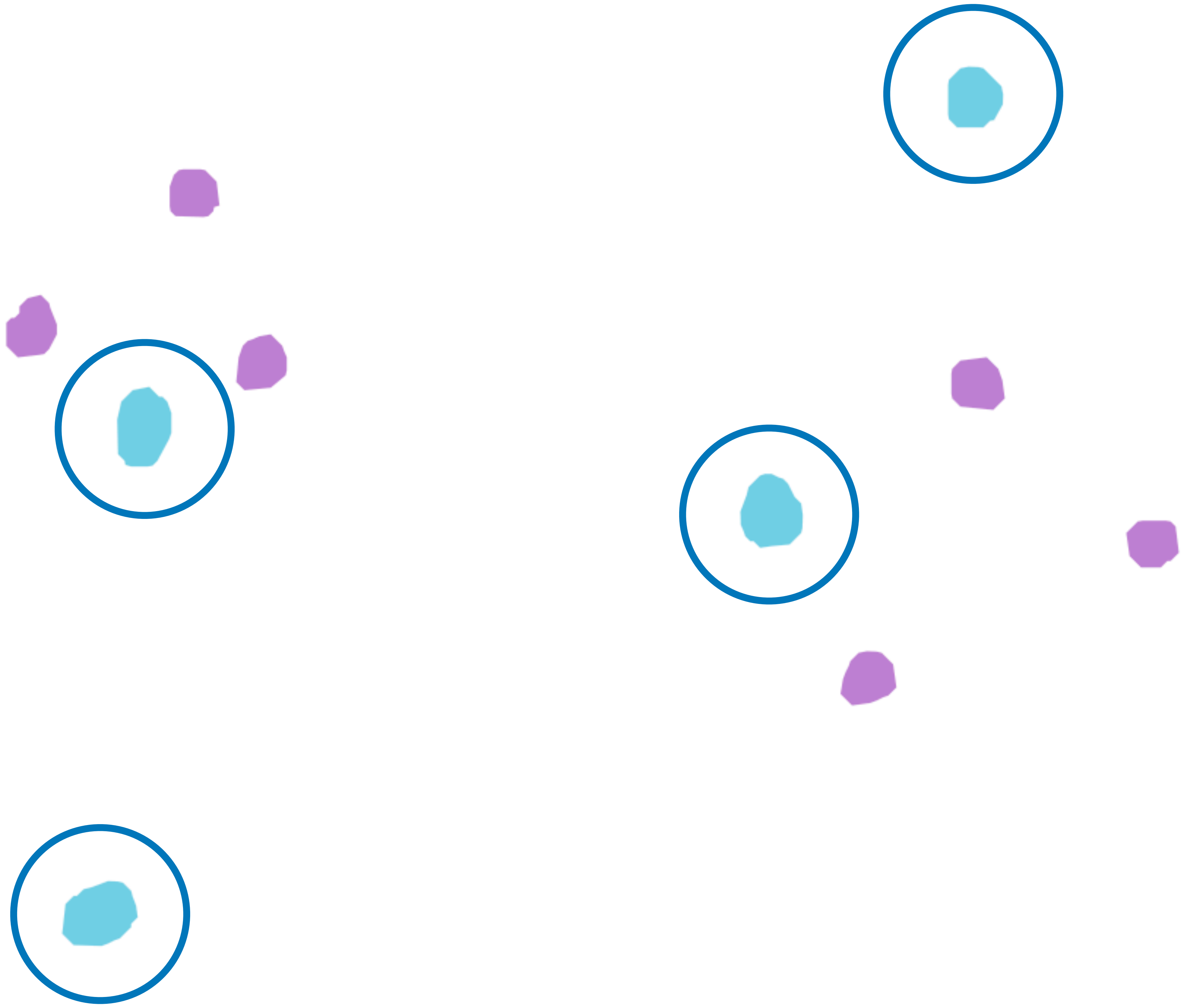


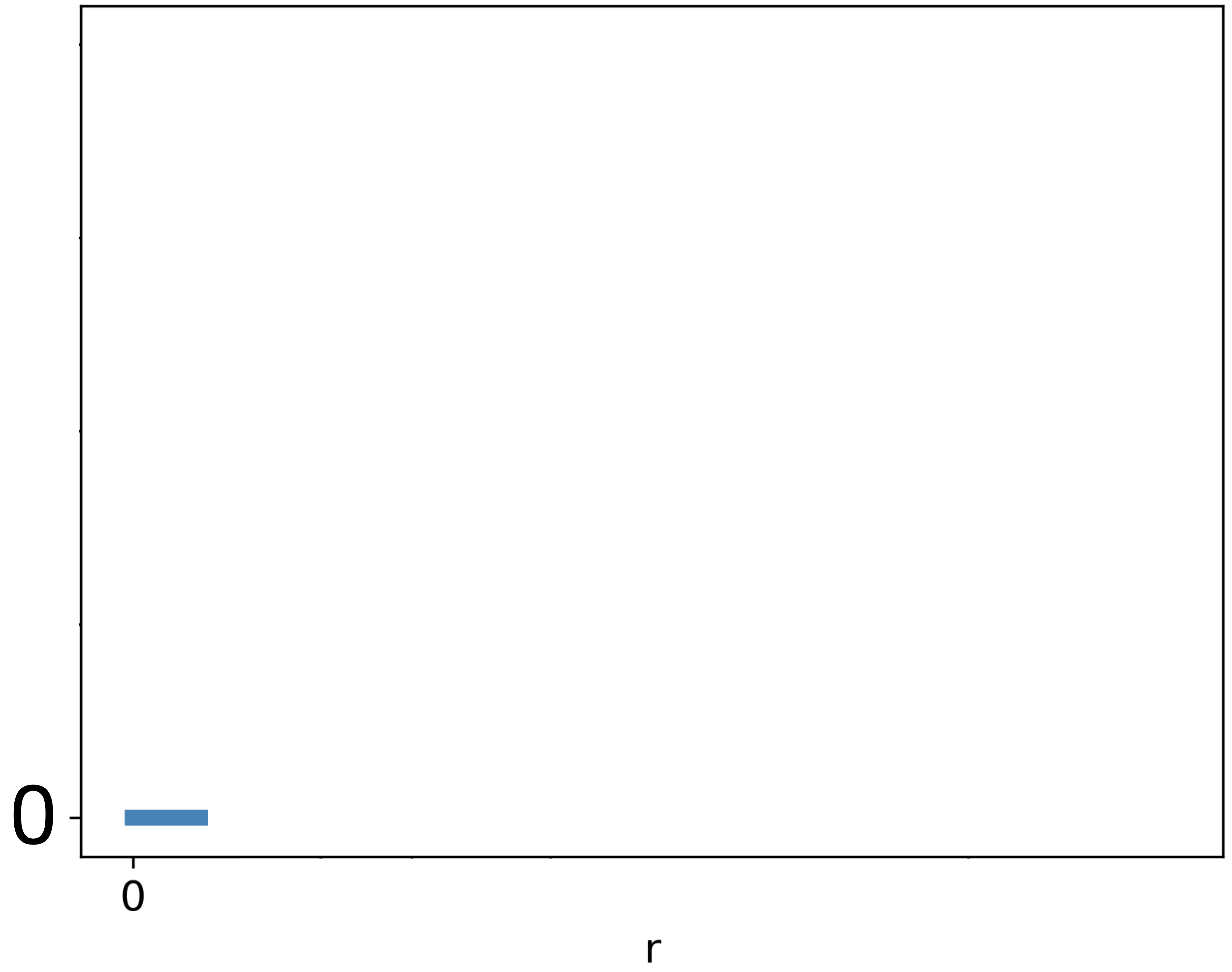
$$n_1 = 4$$



Nearest neighbor function

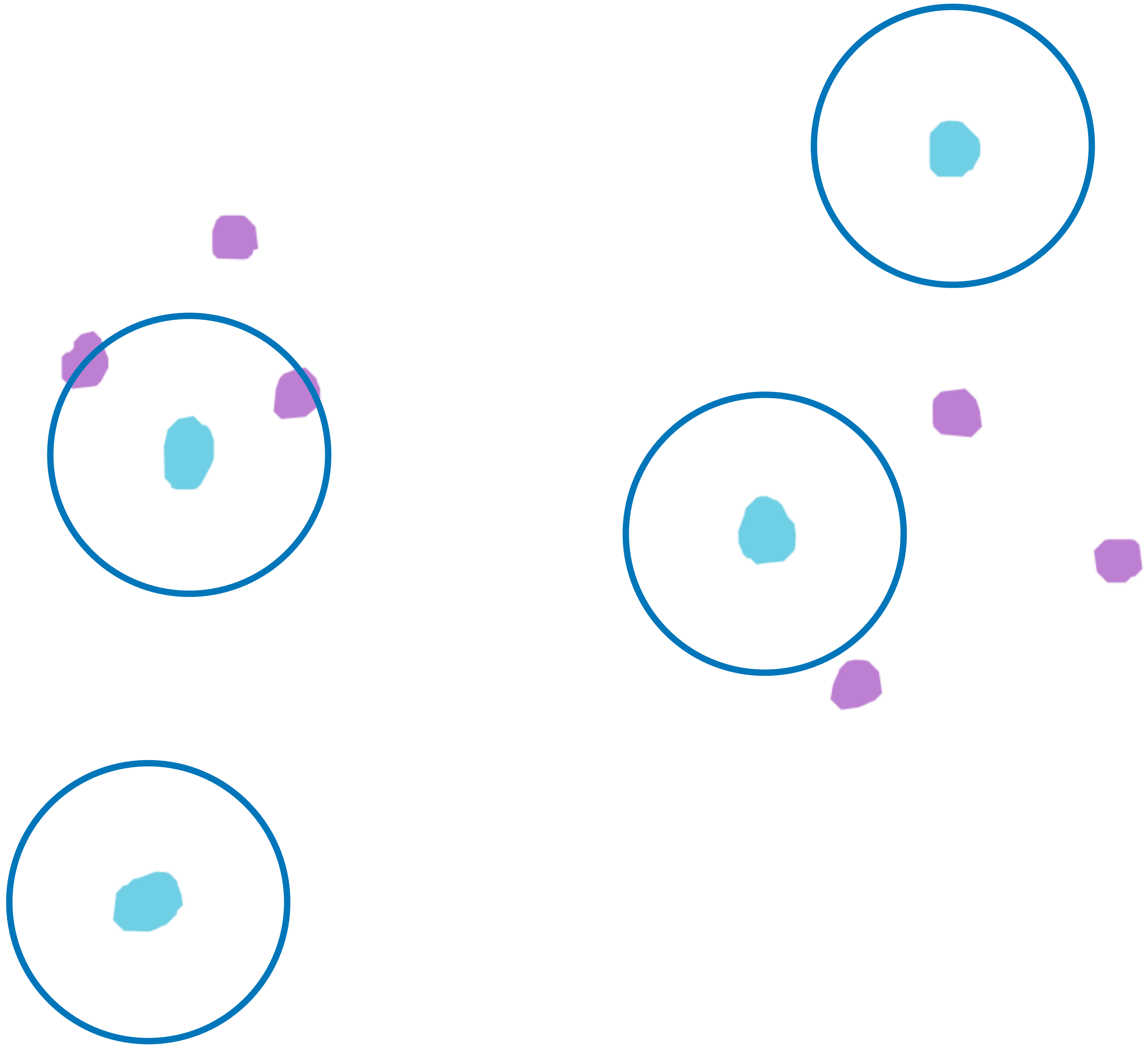
$$S(r) = \frac{1}{n_1}\sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

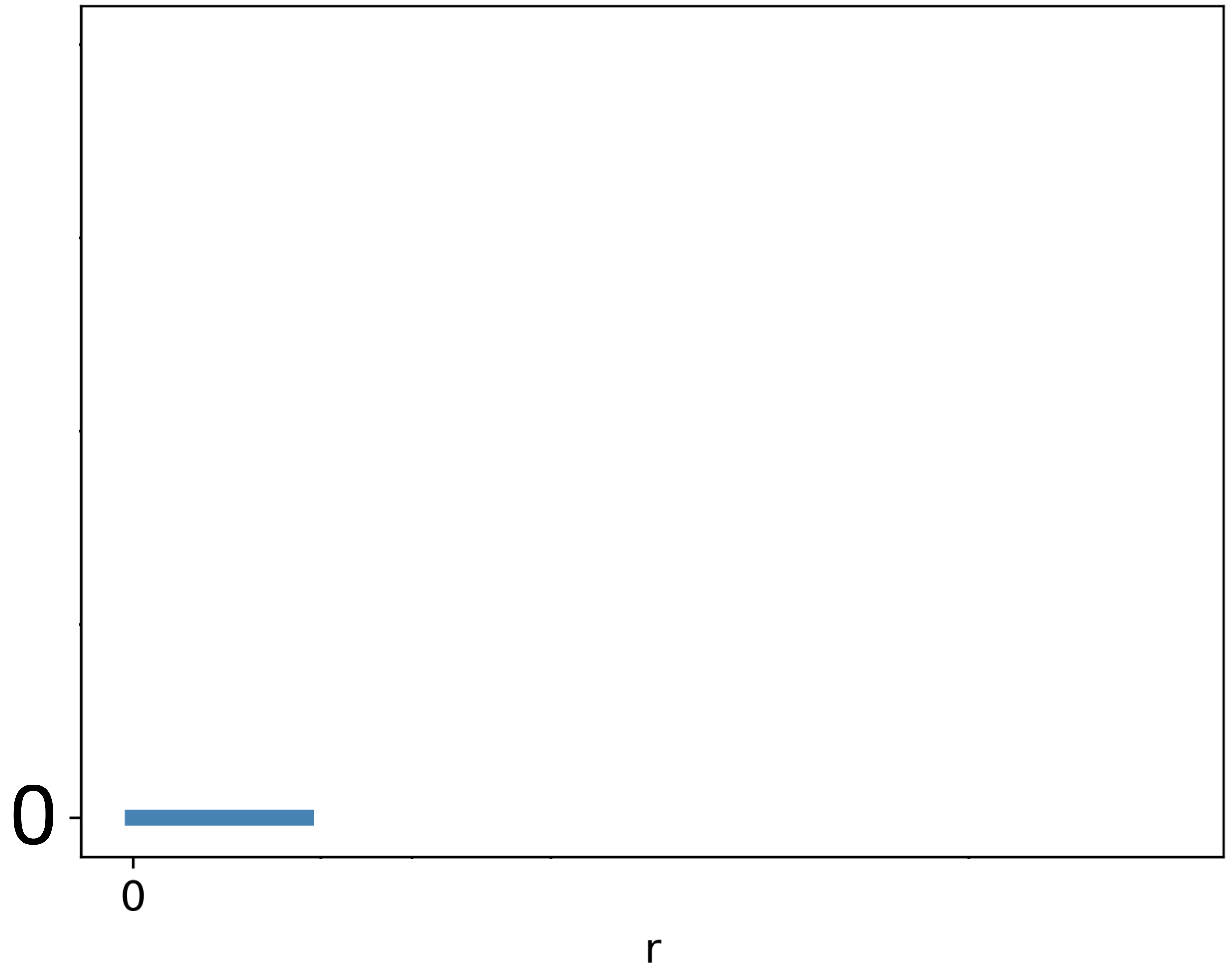




Nearest neighbor function

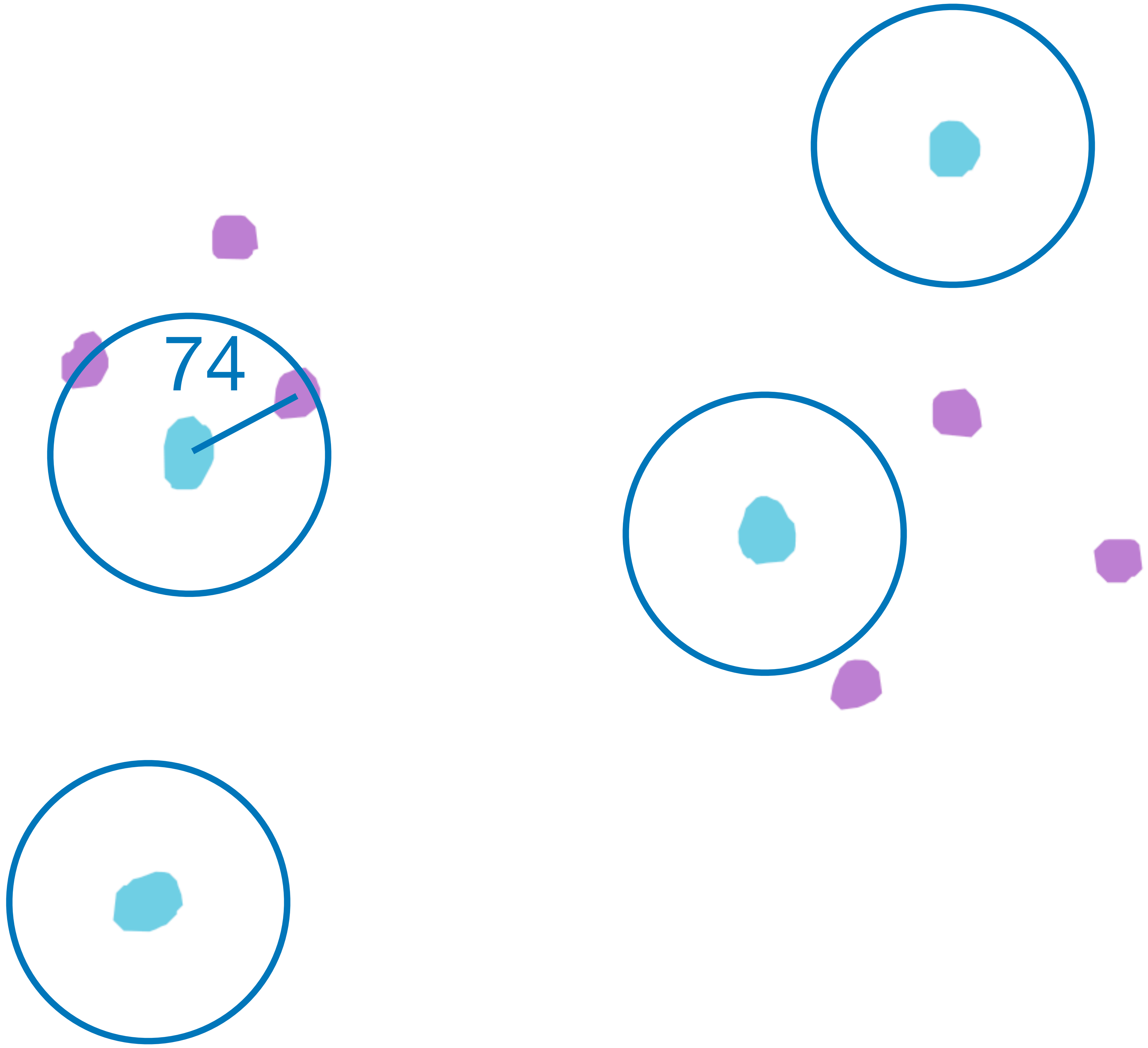
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

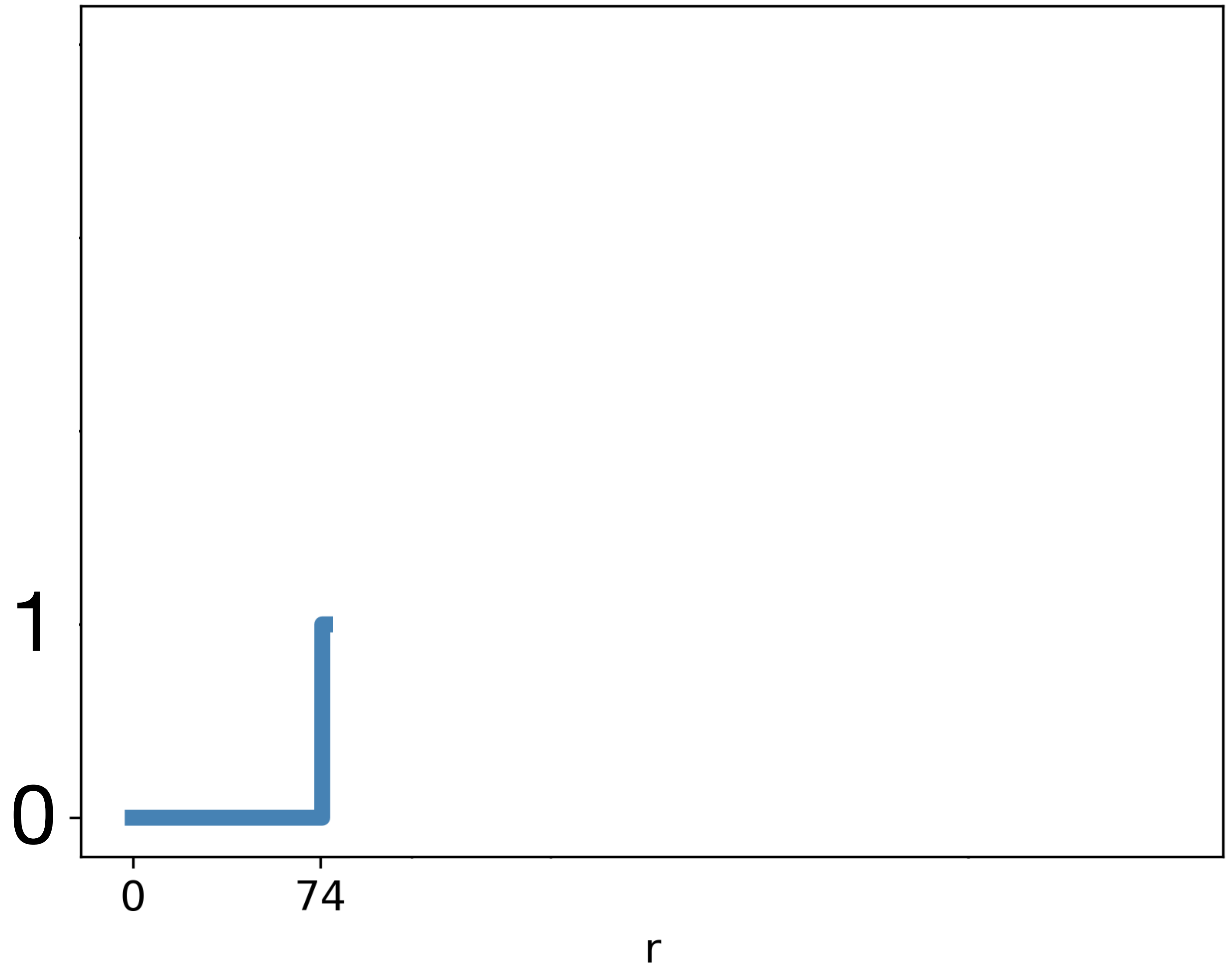




Nearest neighbor function

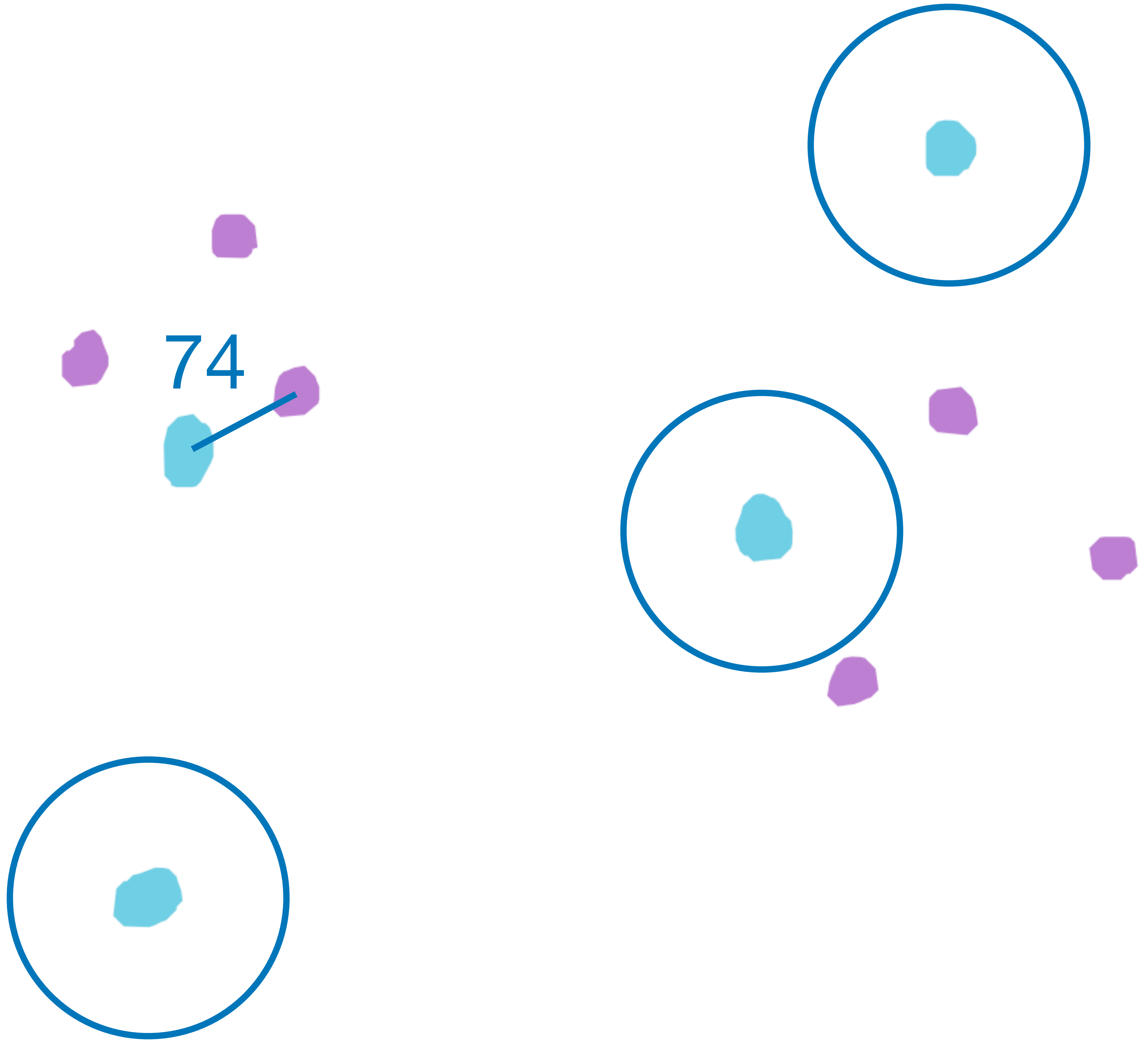
$$S(r) = \frac{1}{n_1}\sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

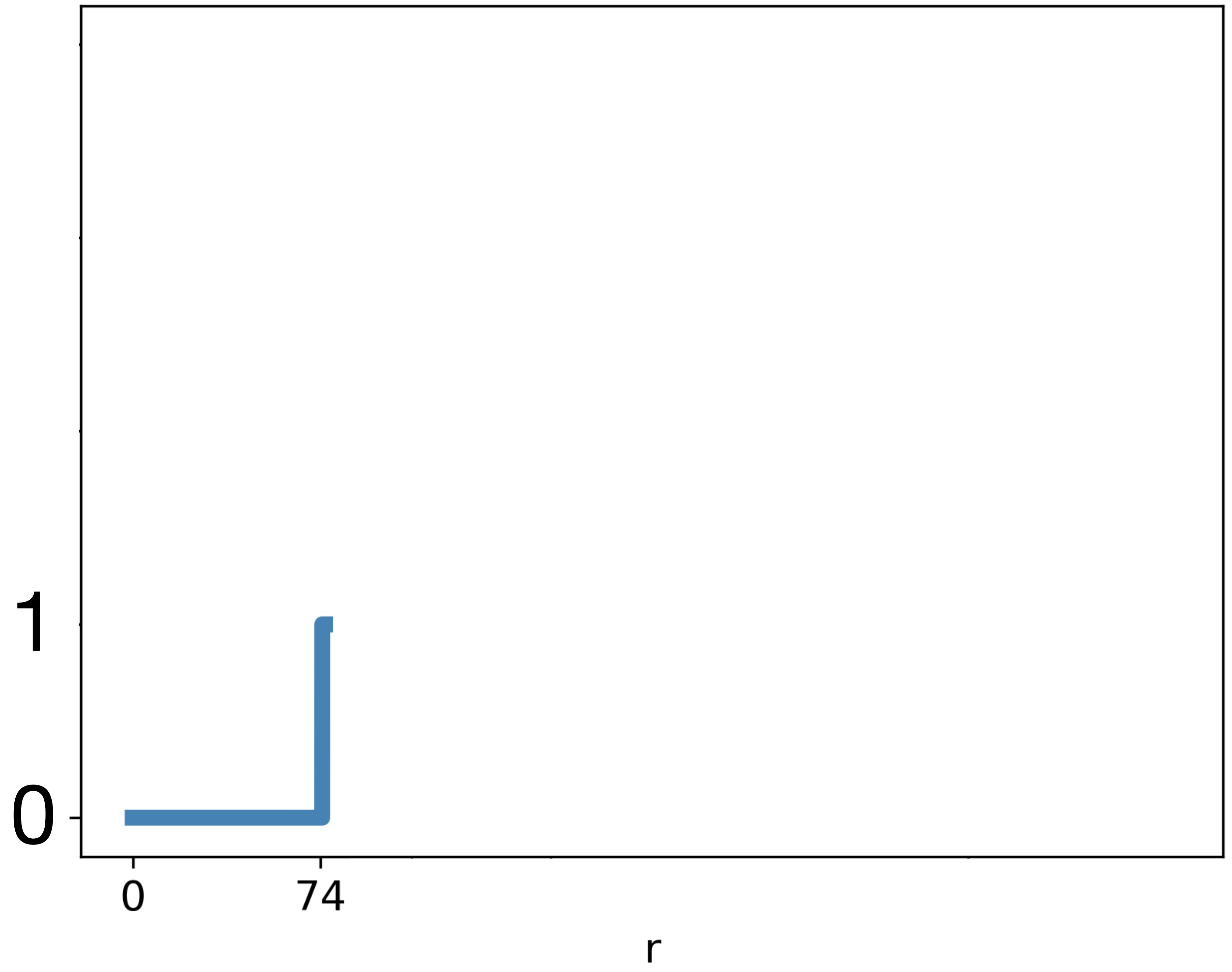




Nearest neighbor function

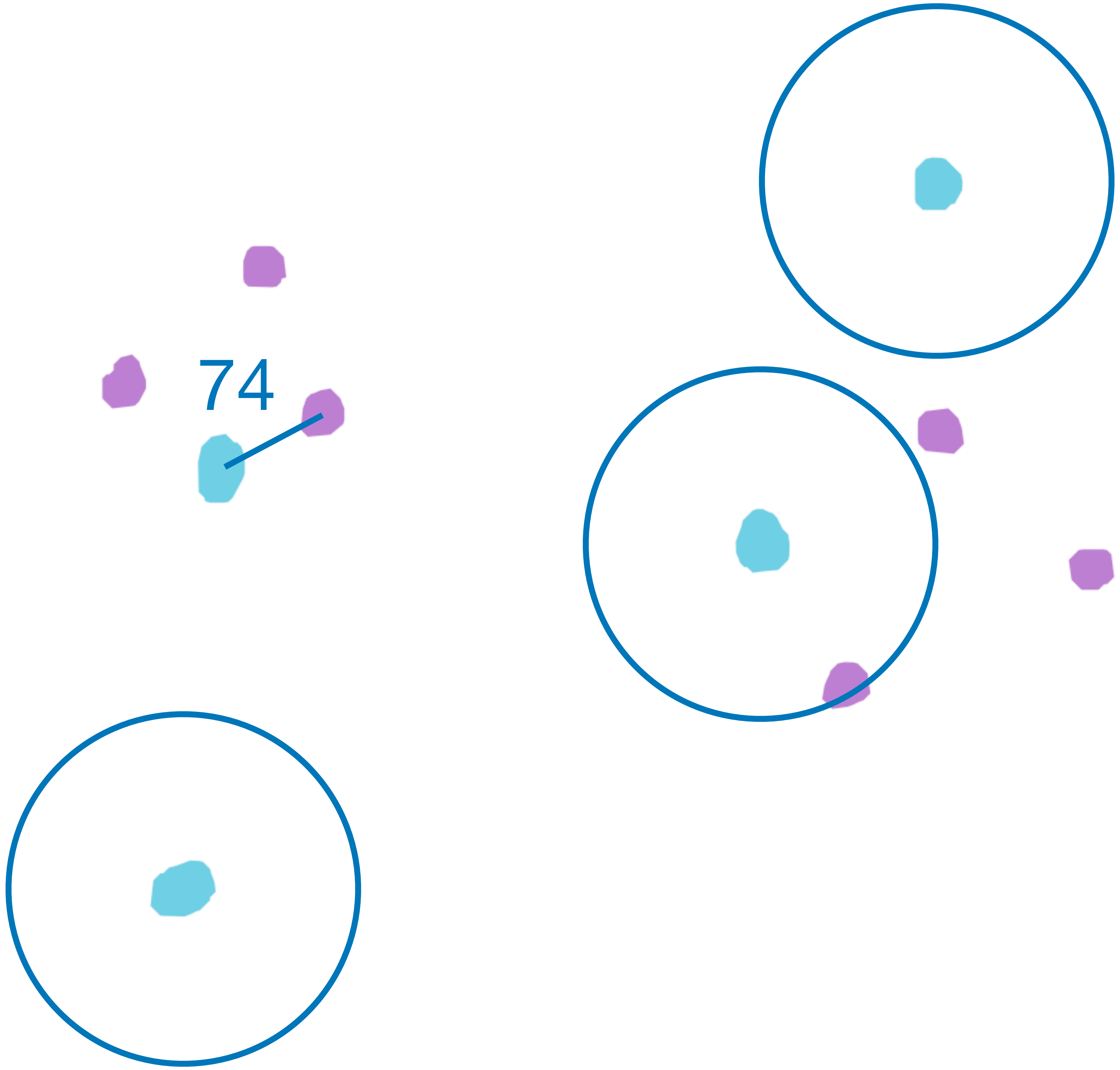
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

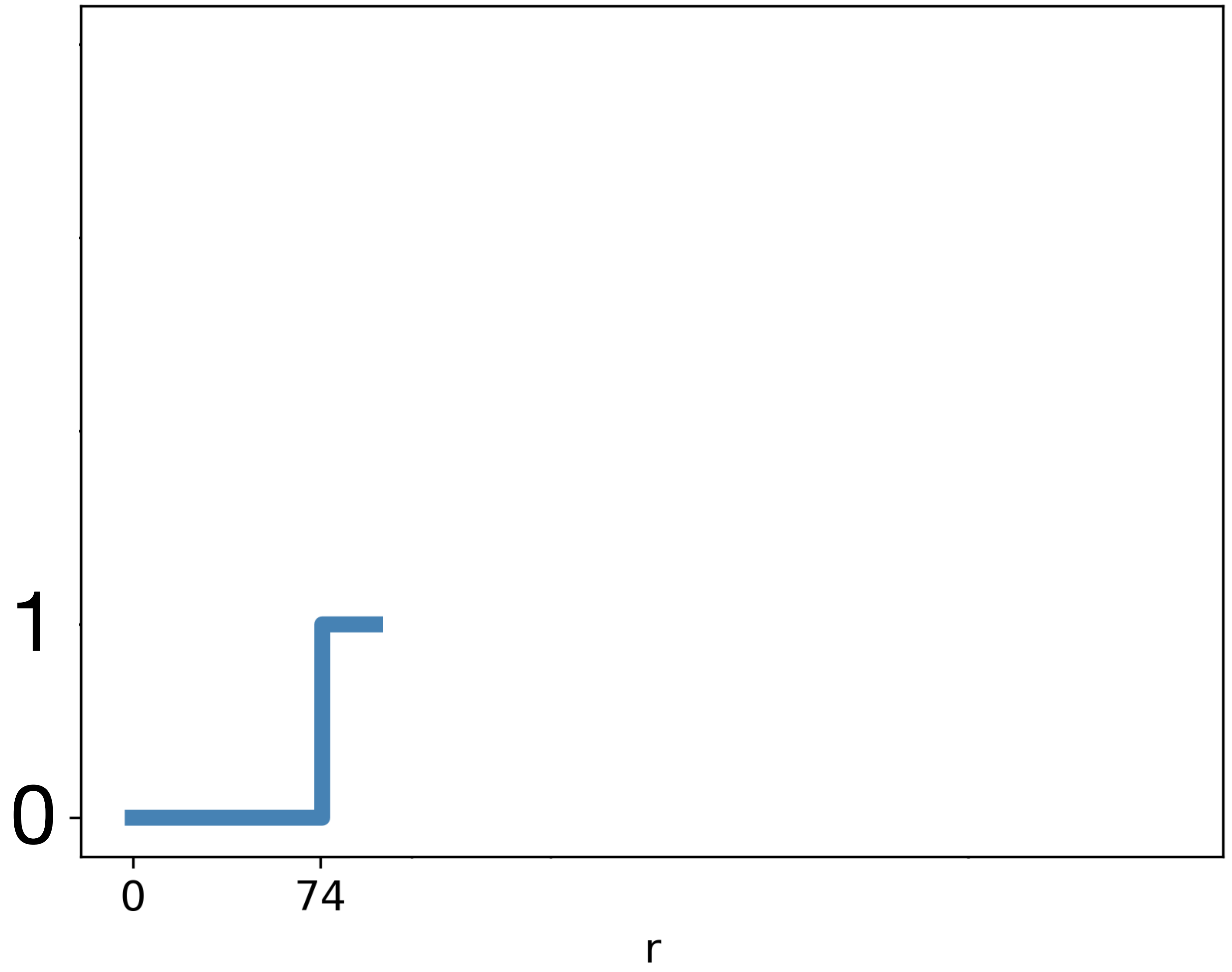




Nearest neighbor function

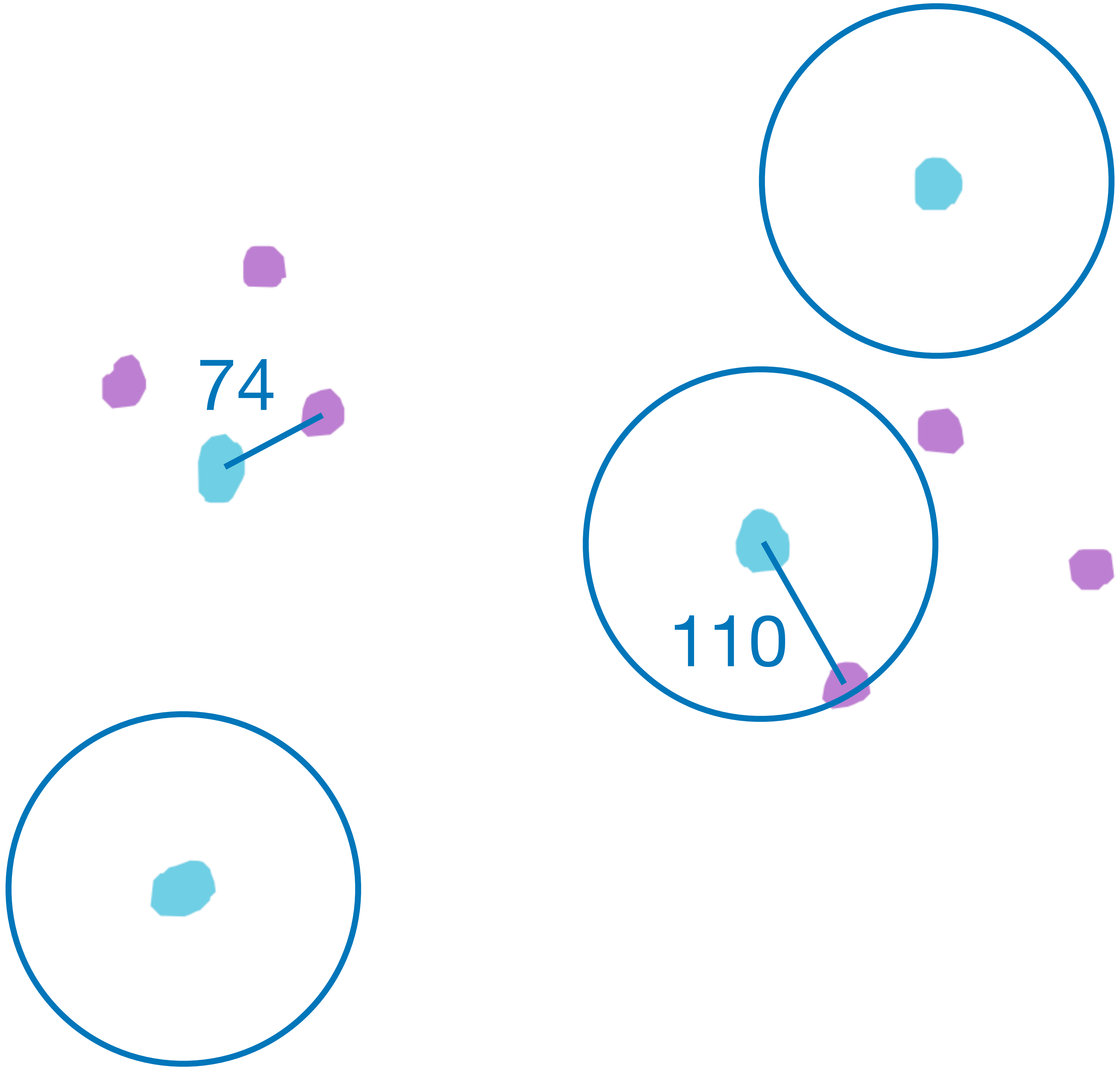
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

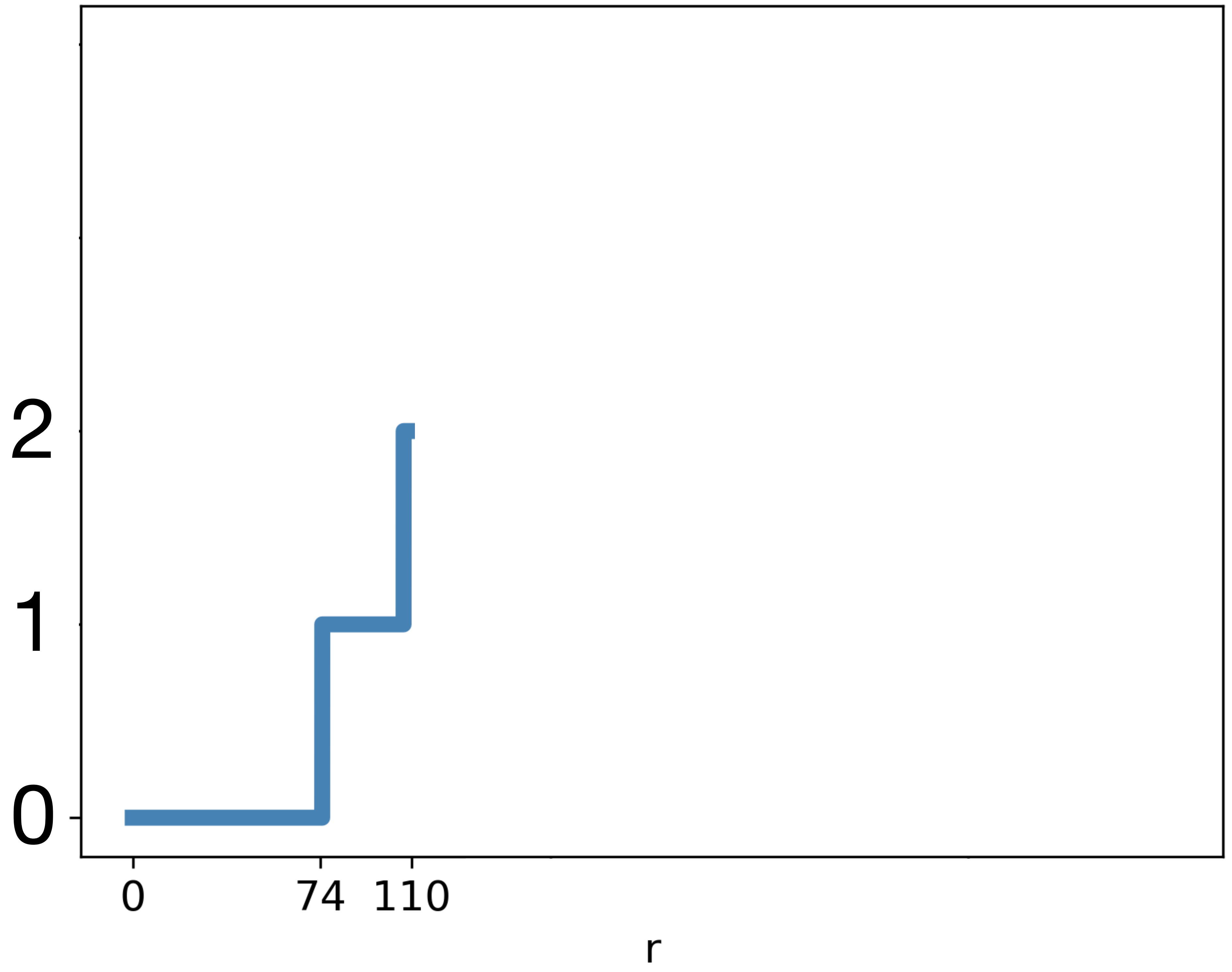




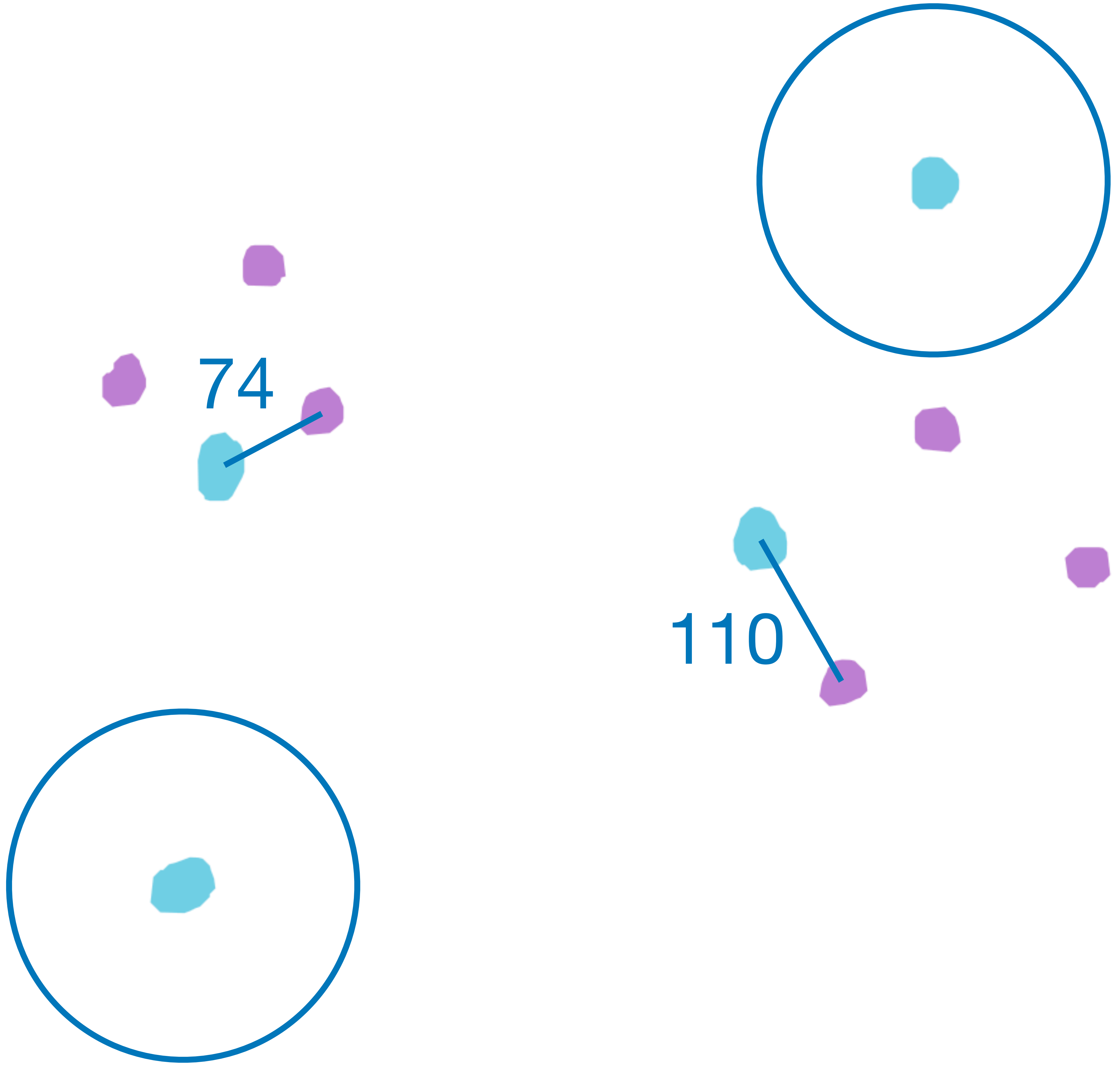
Nearest neighbor function

$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

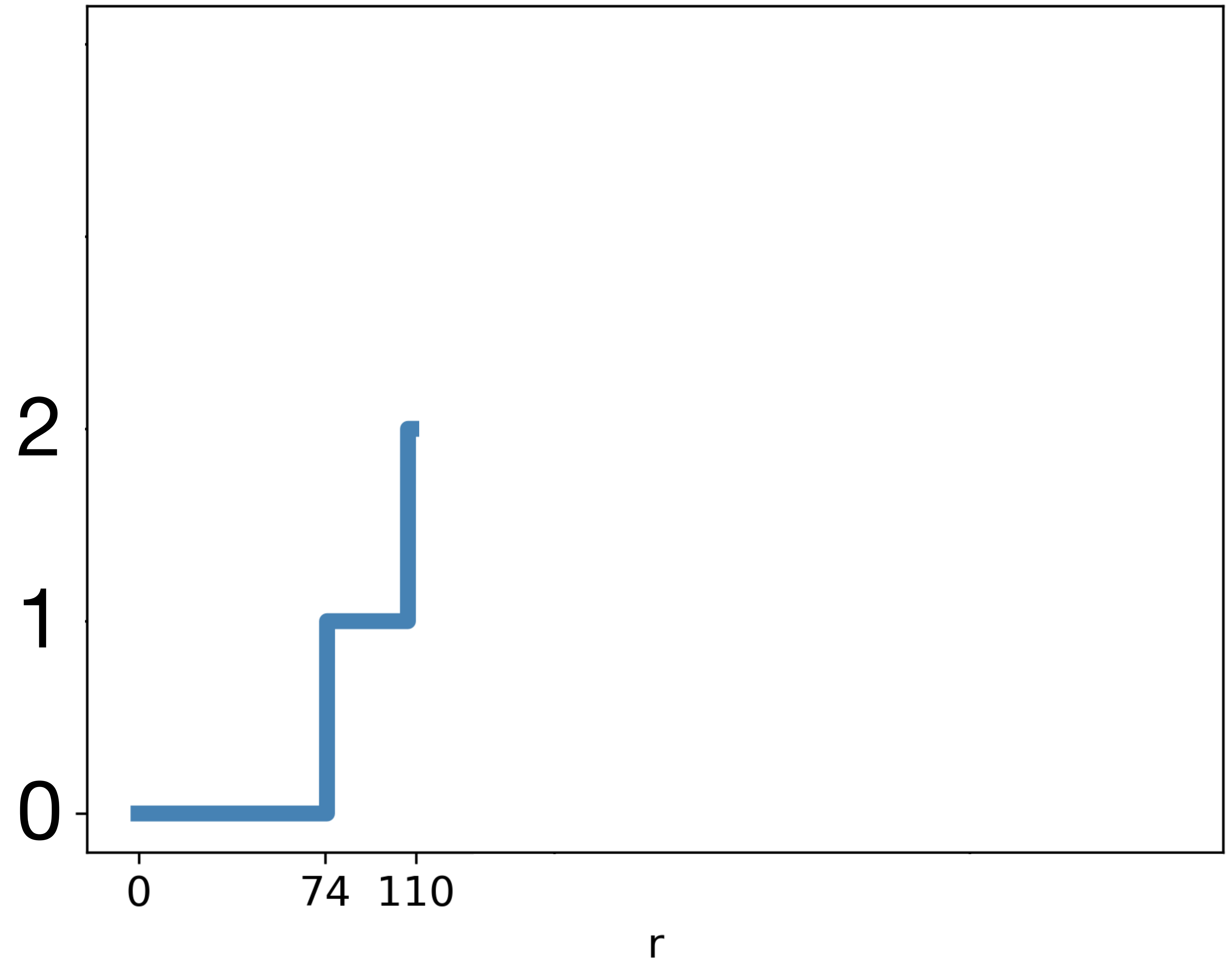




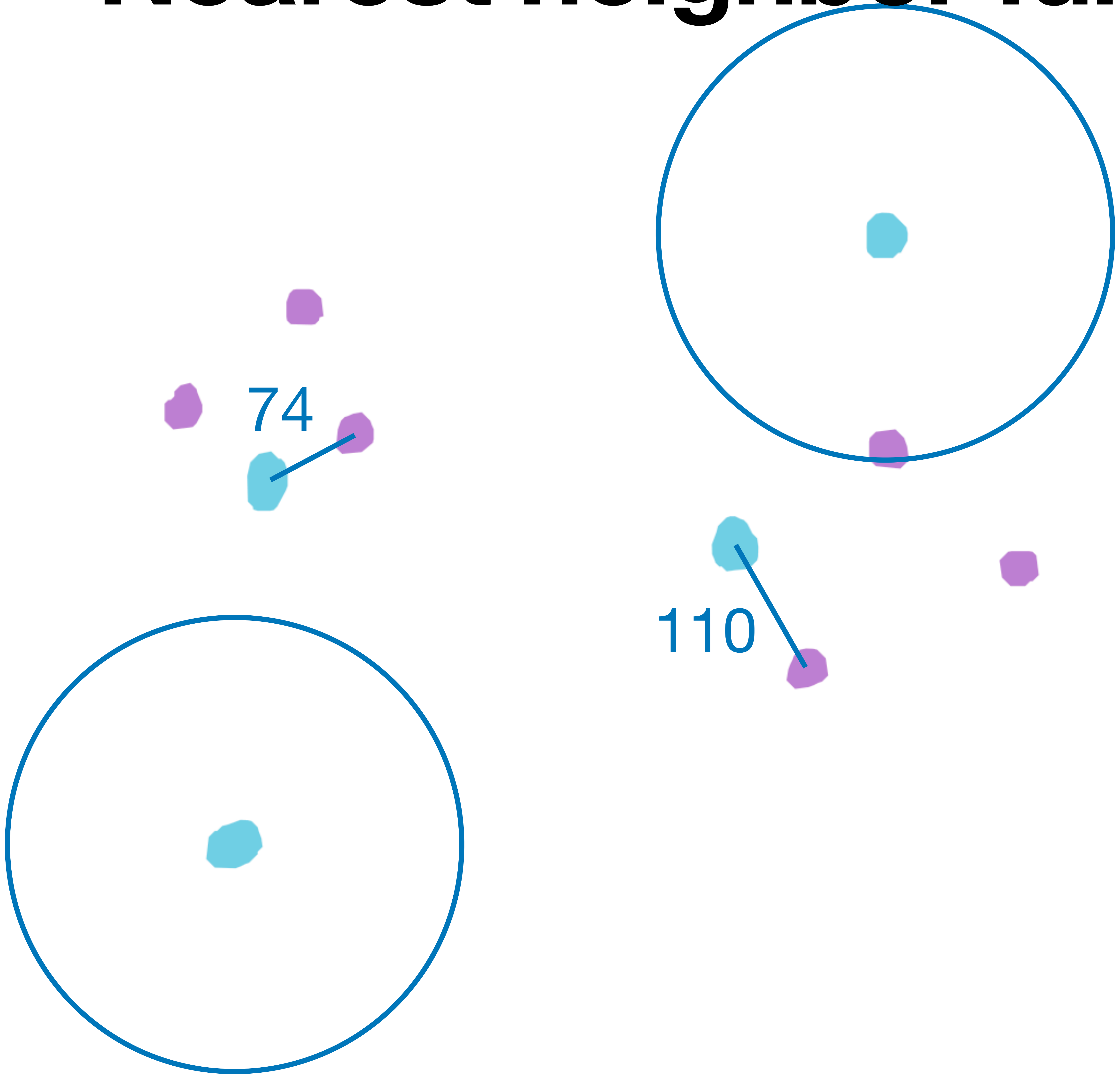
Nearest neighbor function



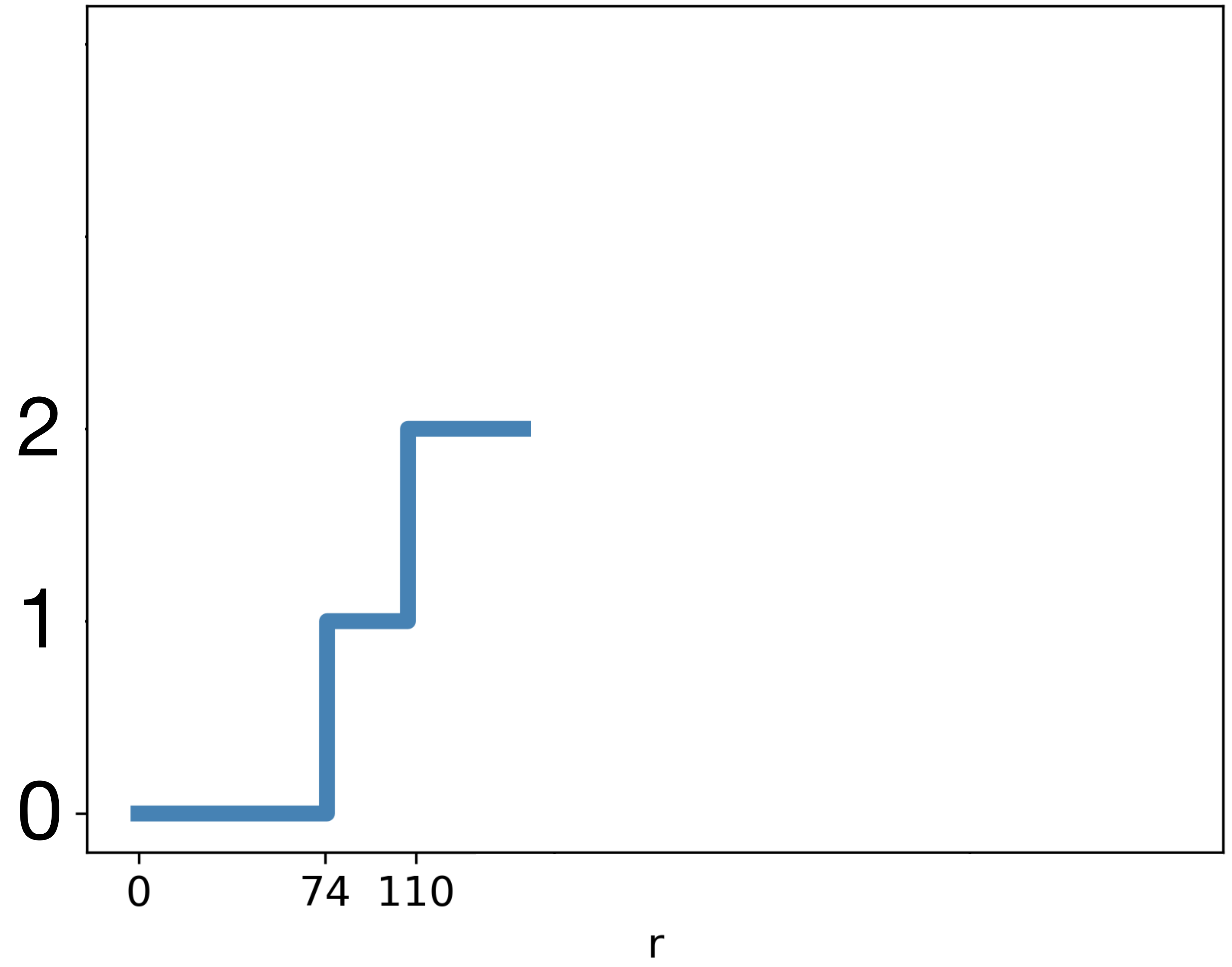
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$



Nearest neighbor function

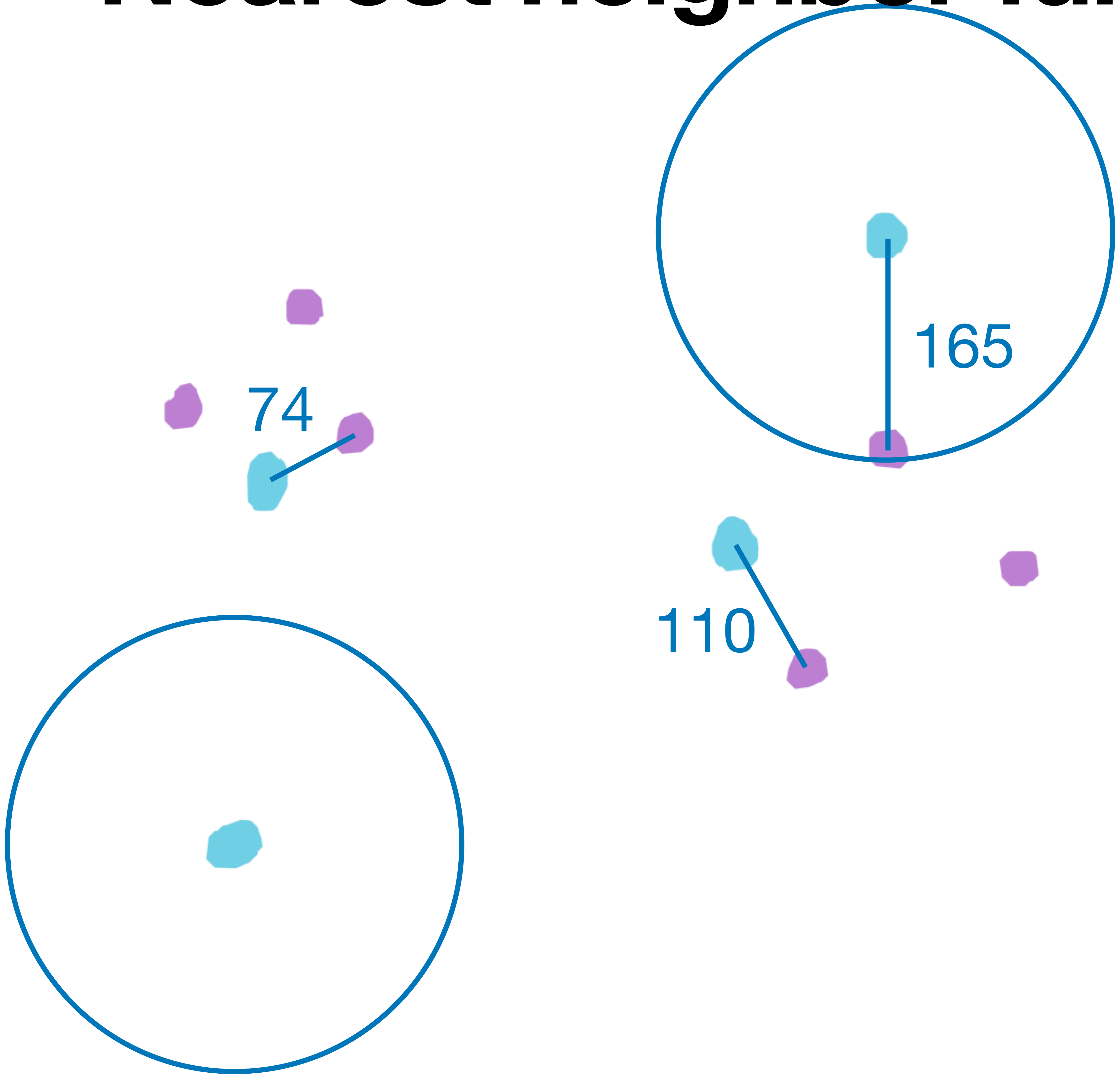


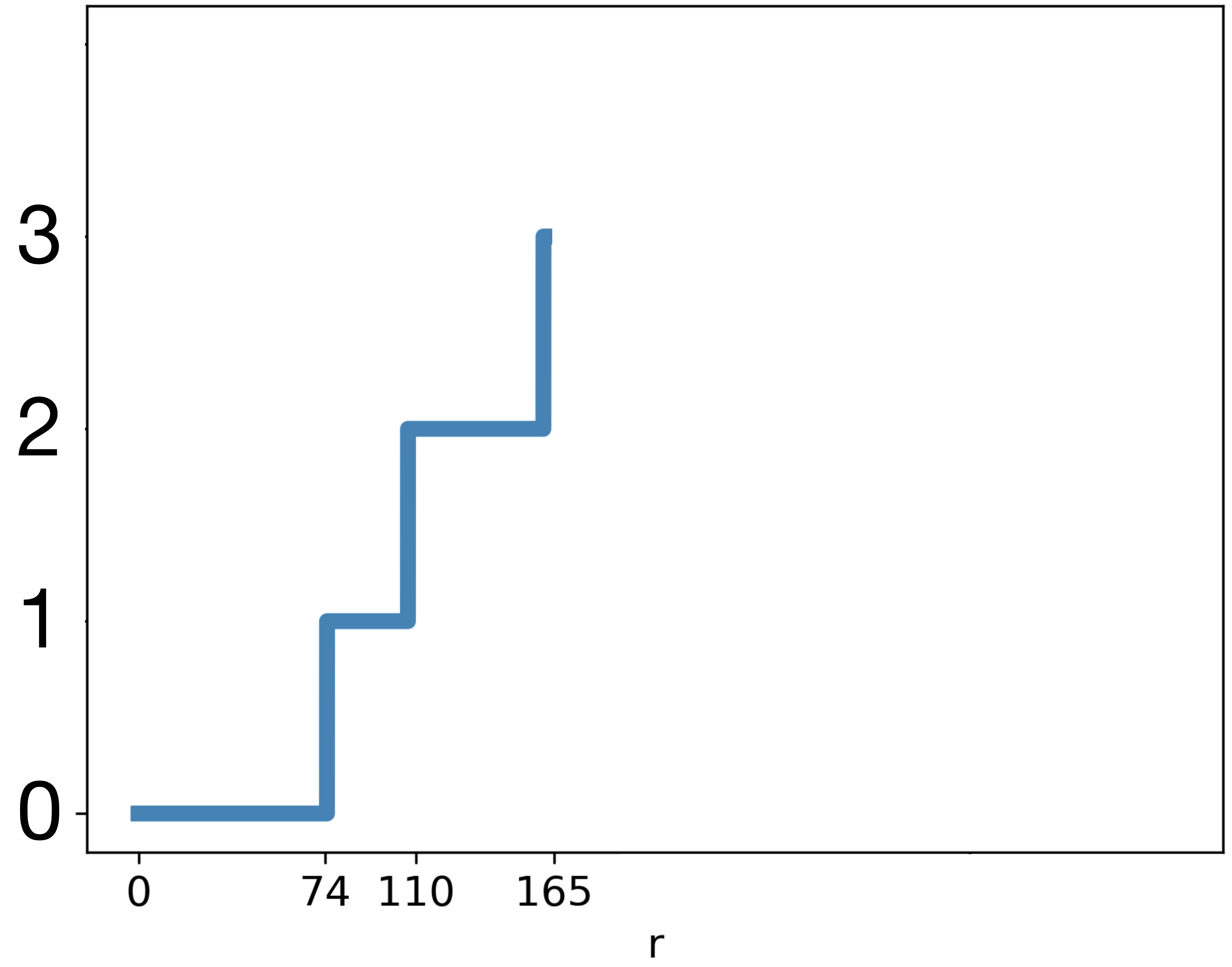
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$



Nearest neighbor function

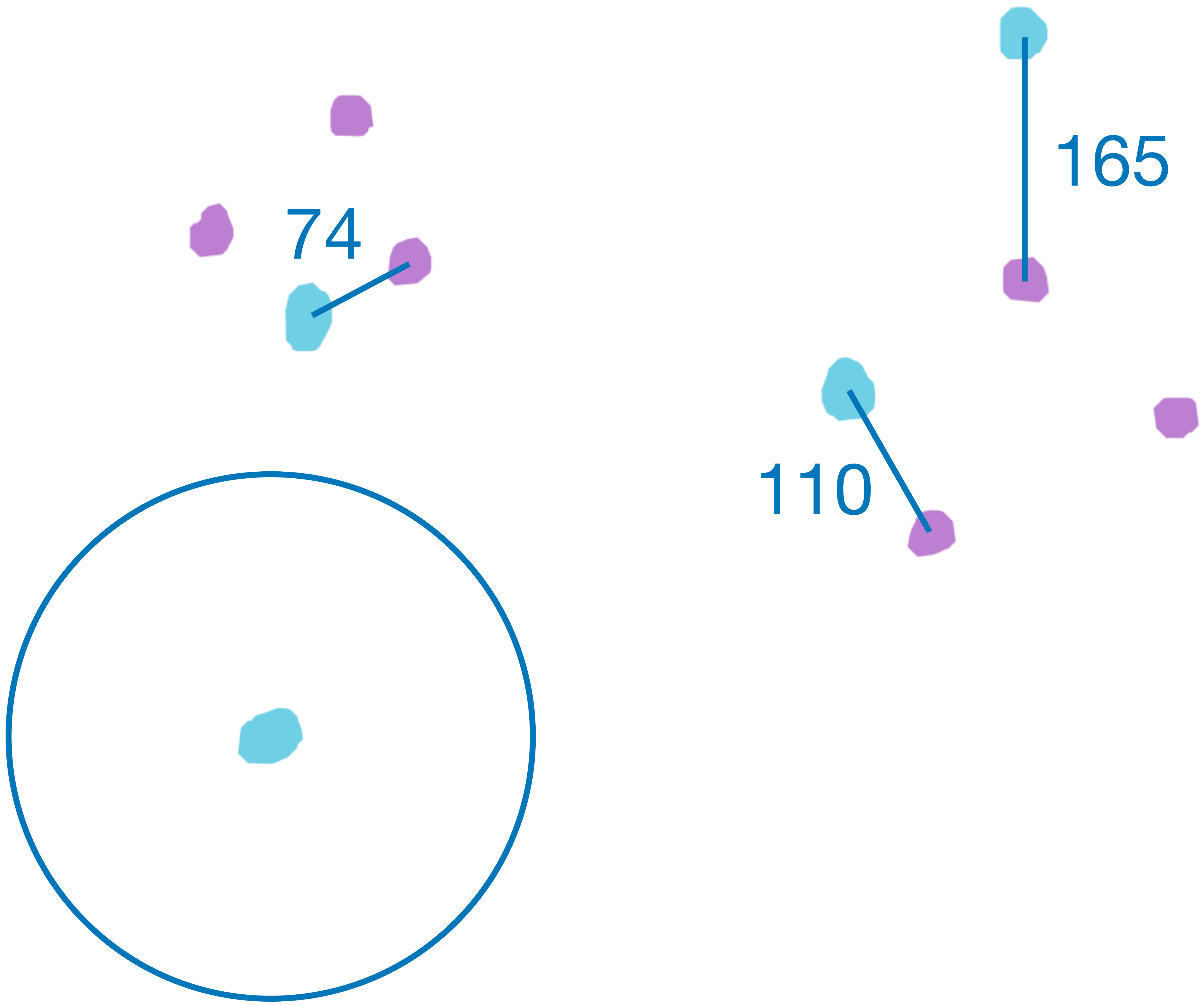
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

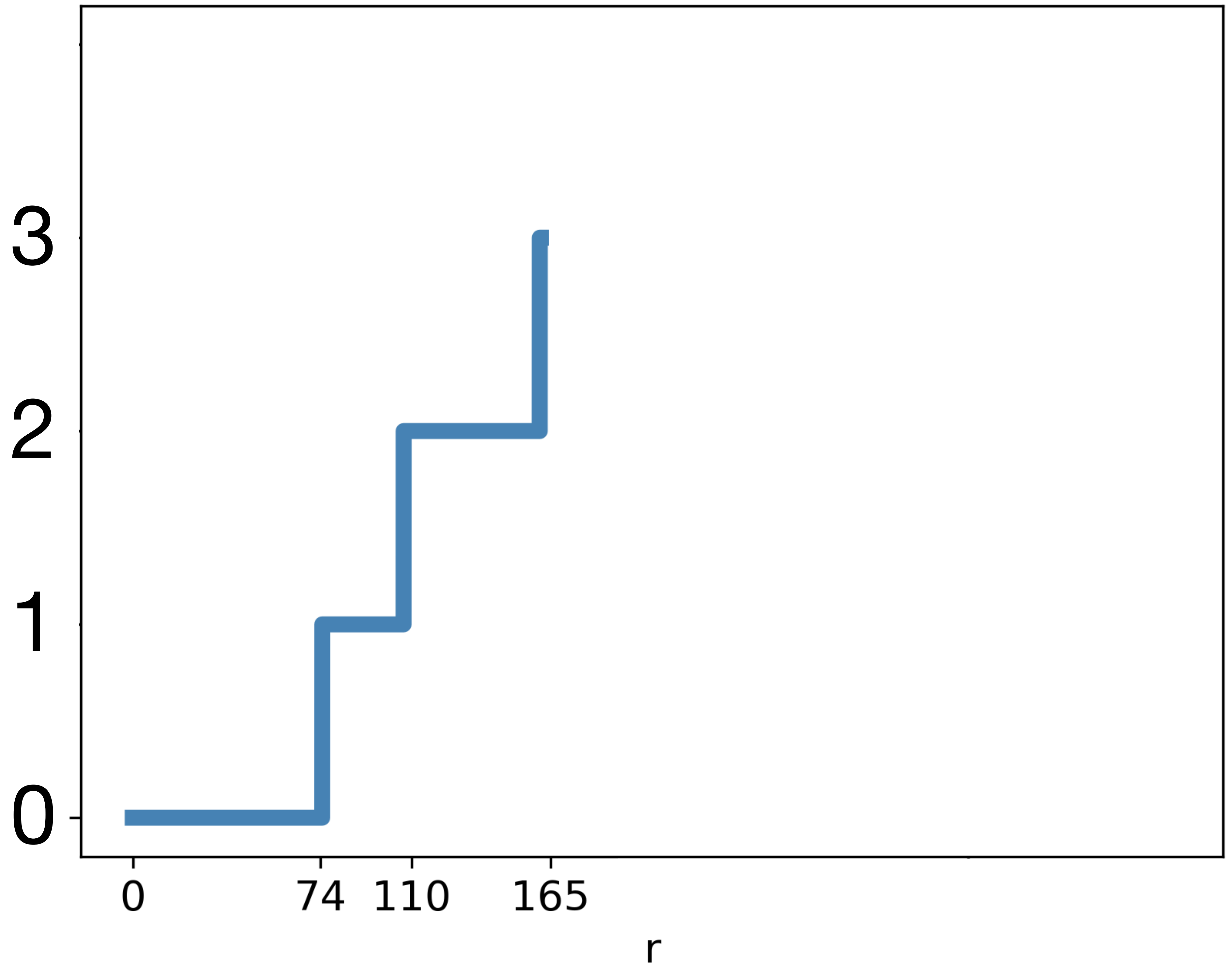




Nearest neighbor function

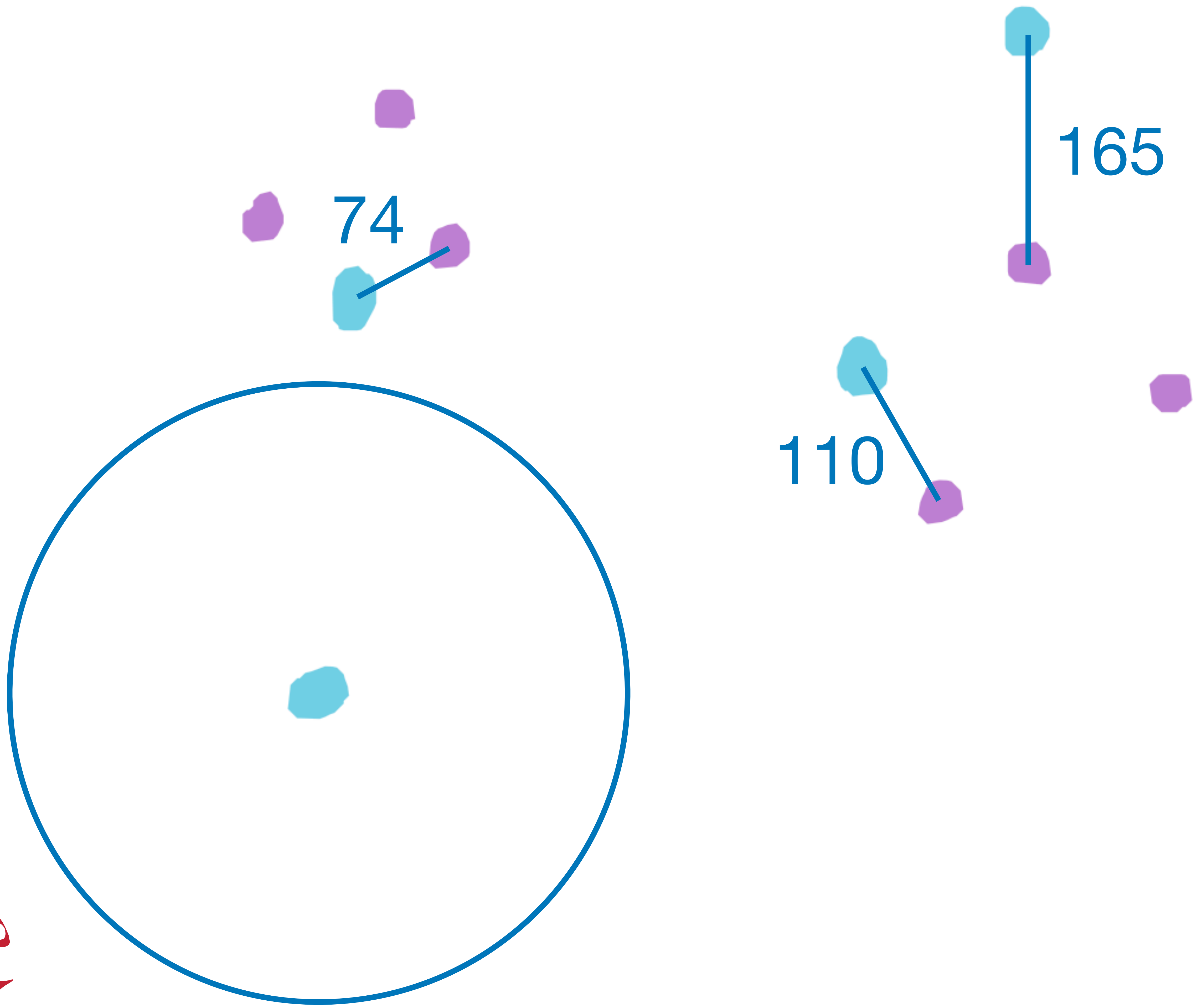
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

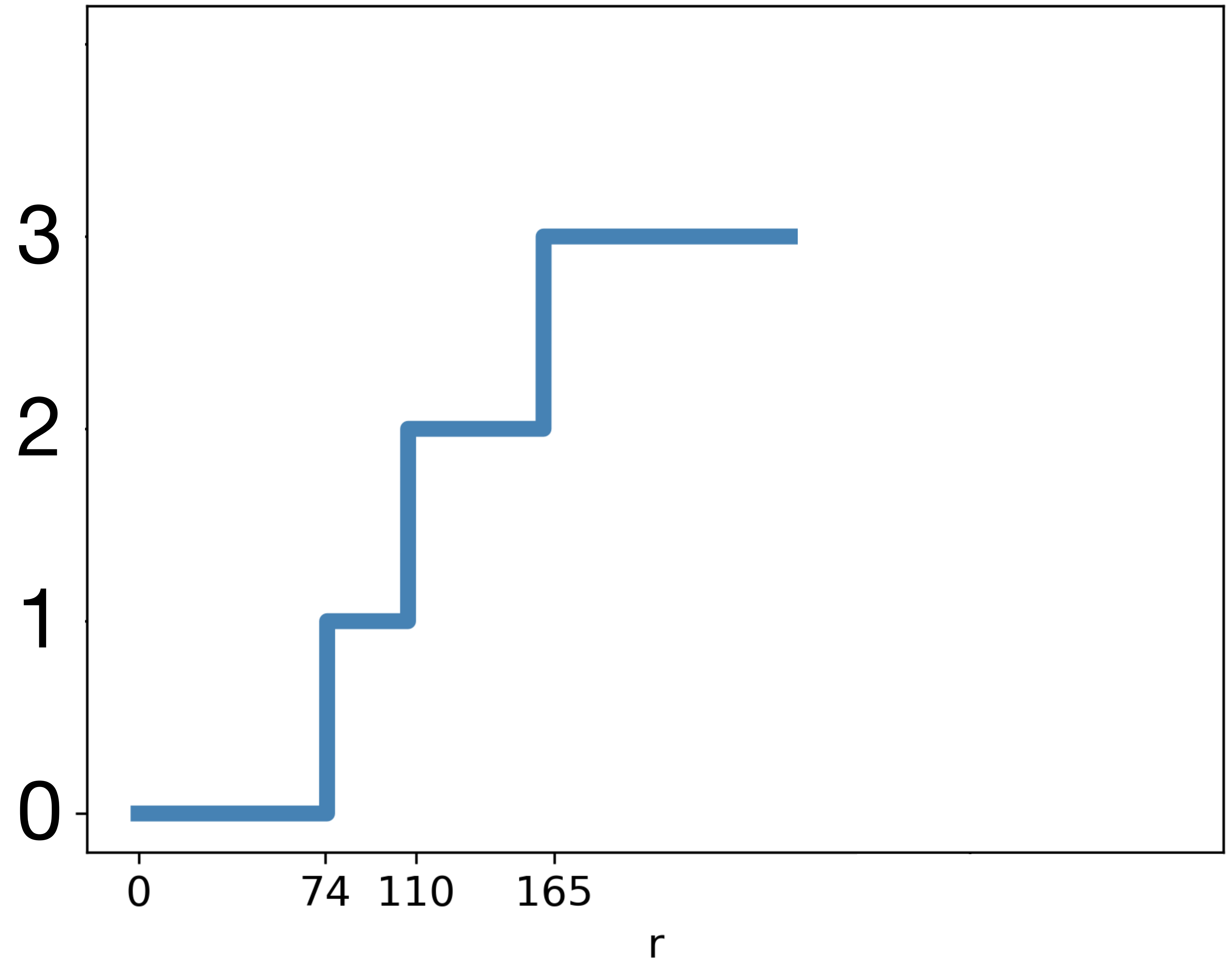




Nearest neighbor function

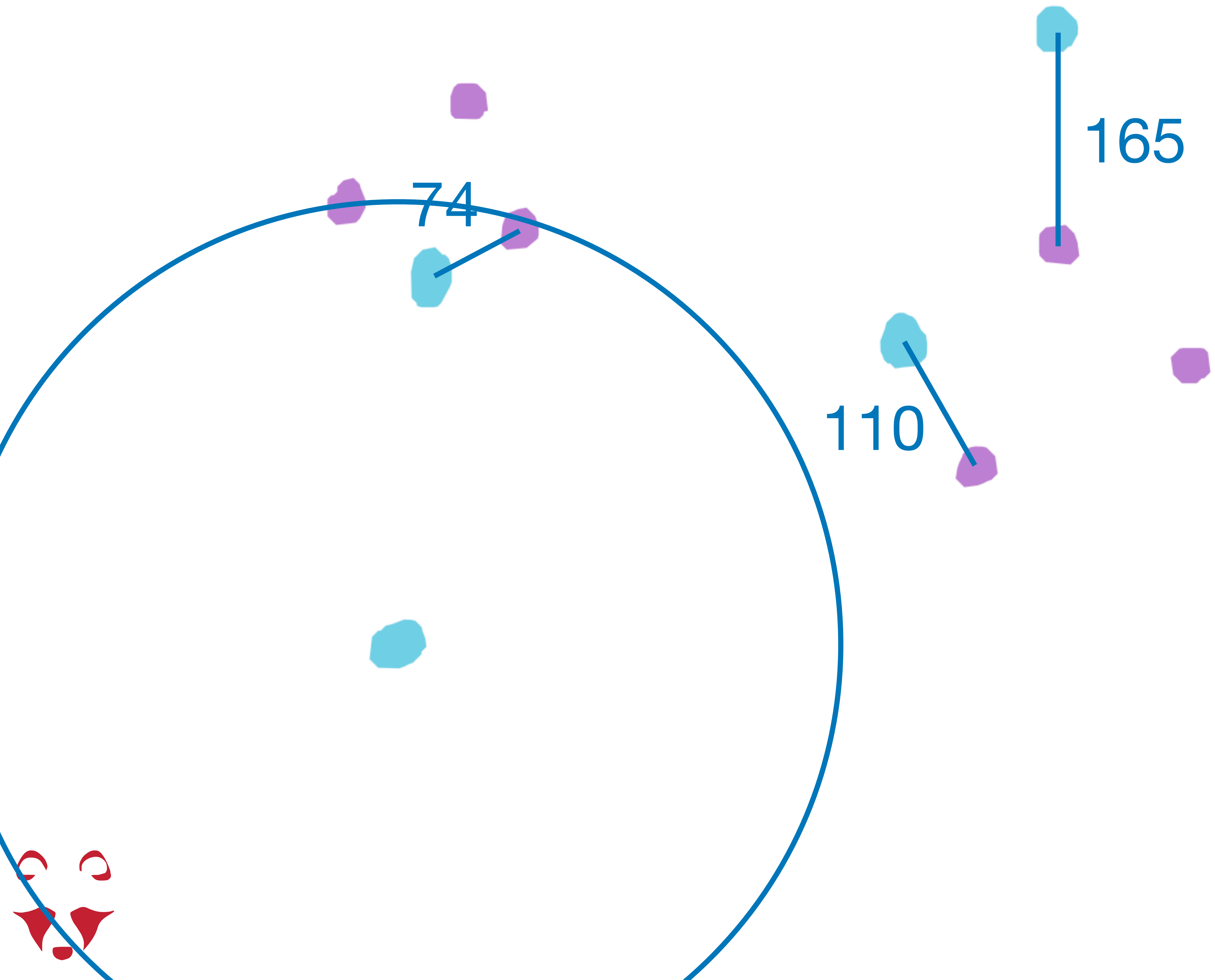
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

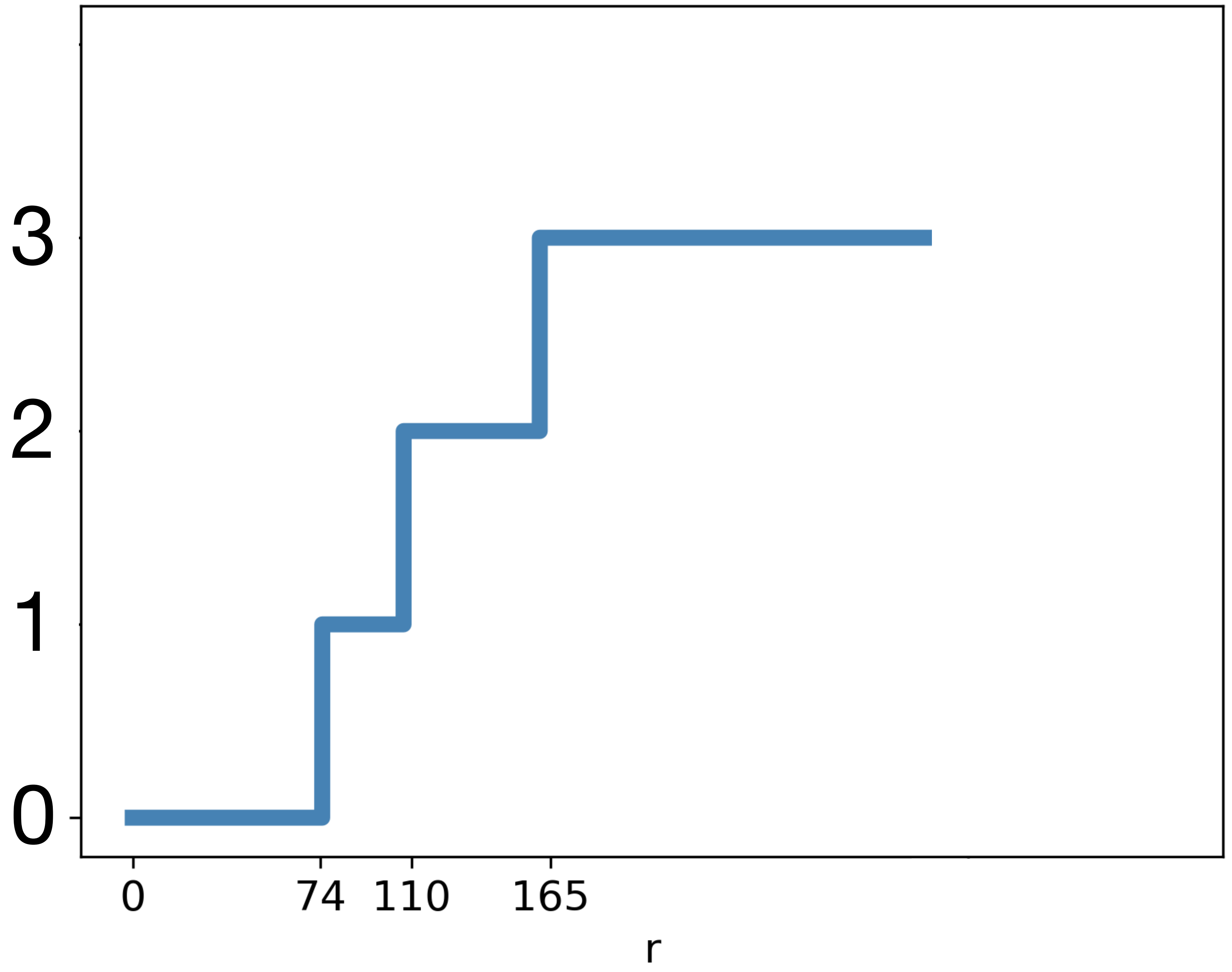




Nearest neighbor function

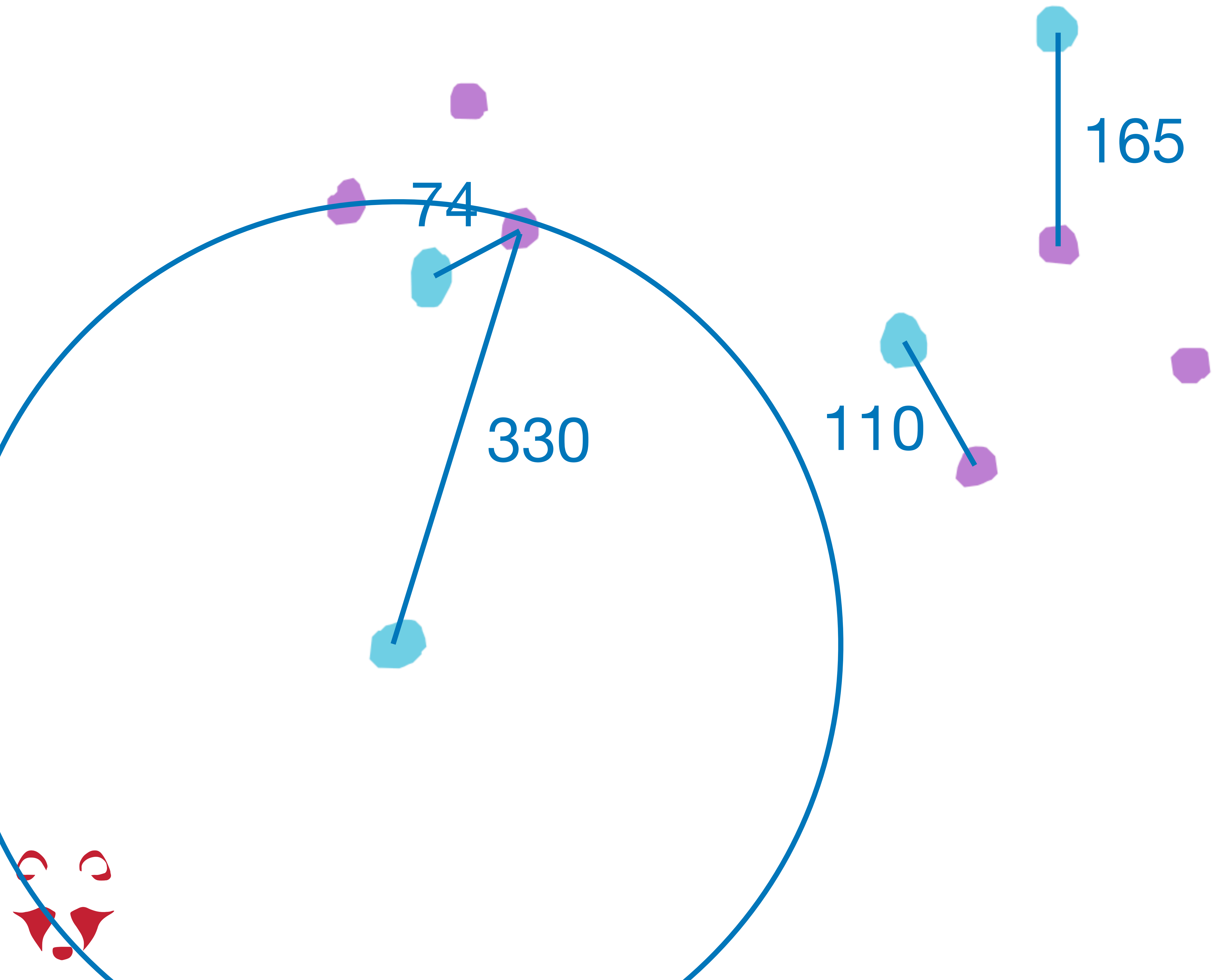
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

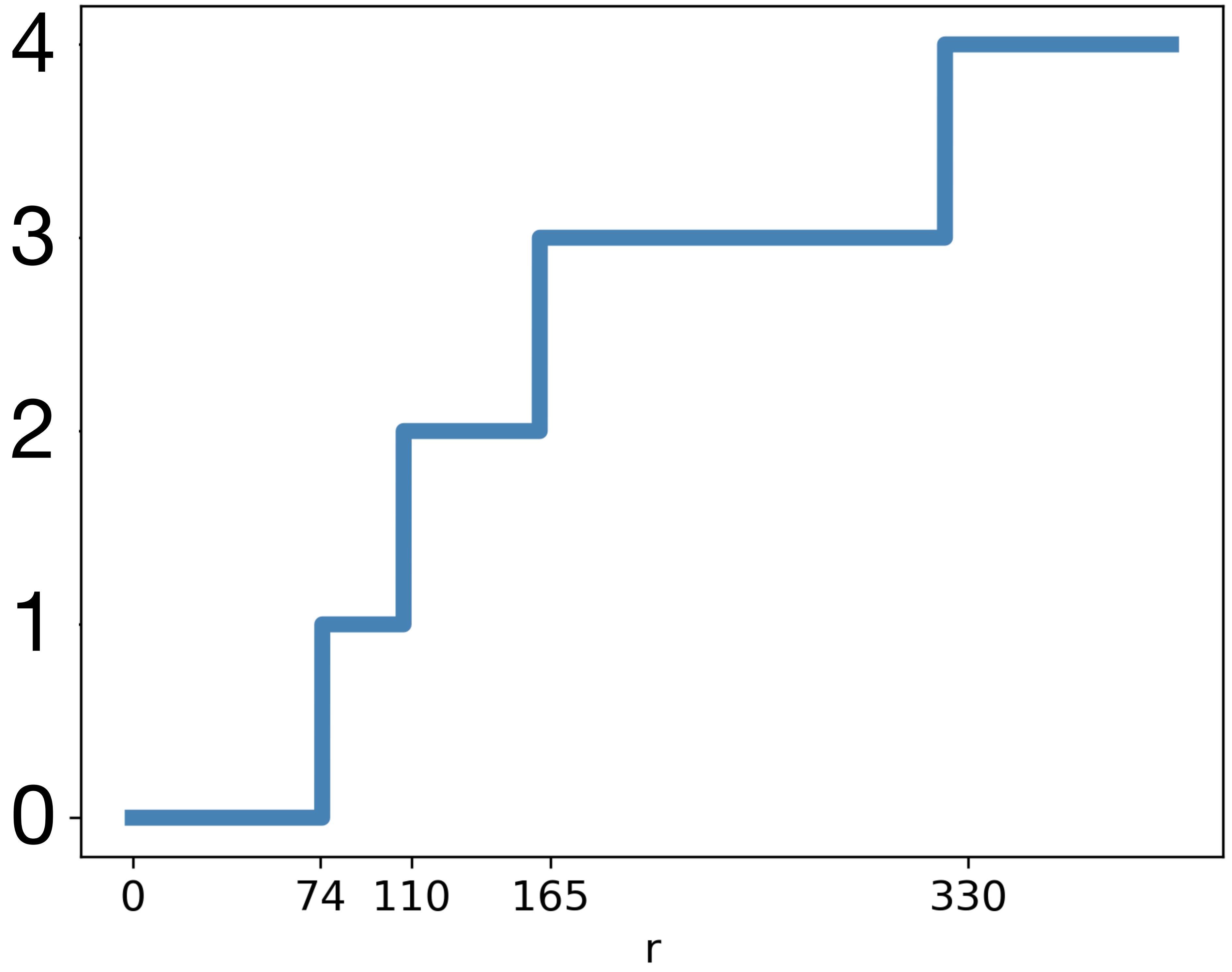




Nearest neighbor function

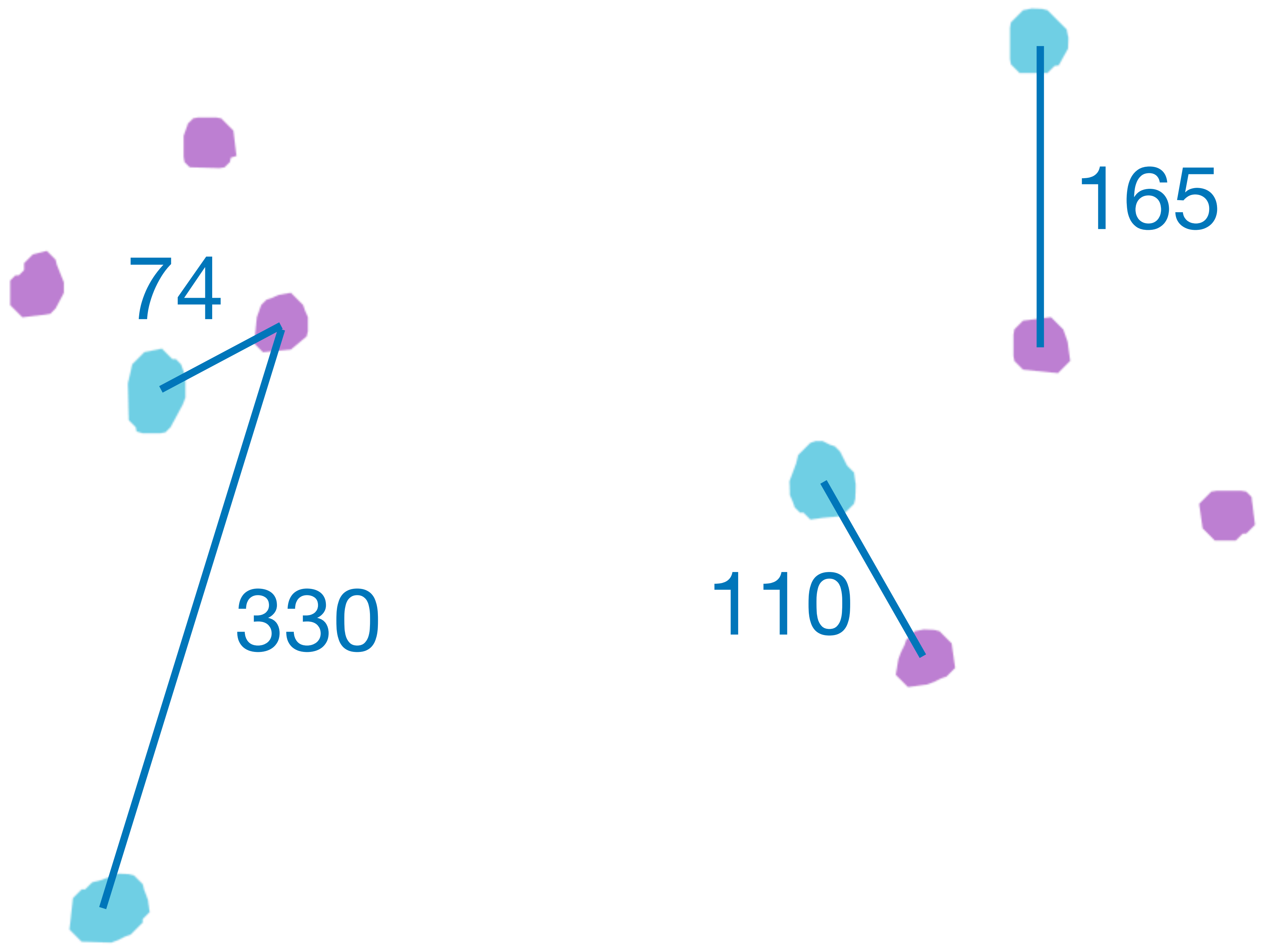
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

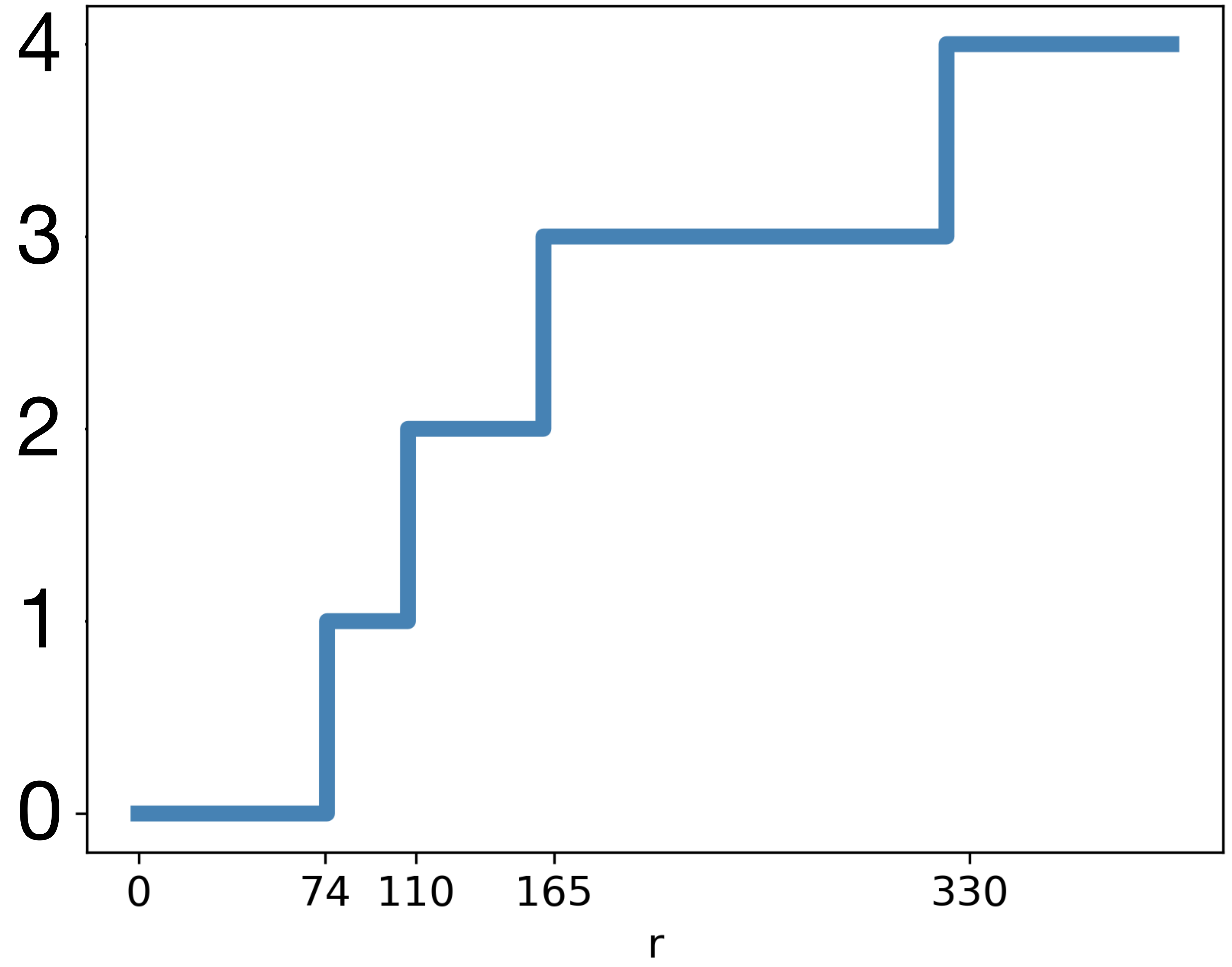




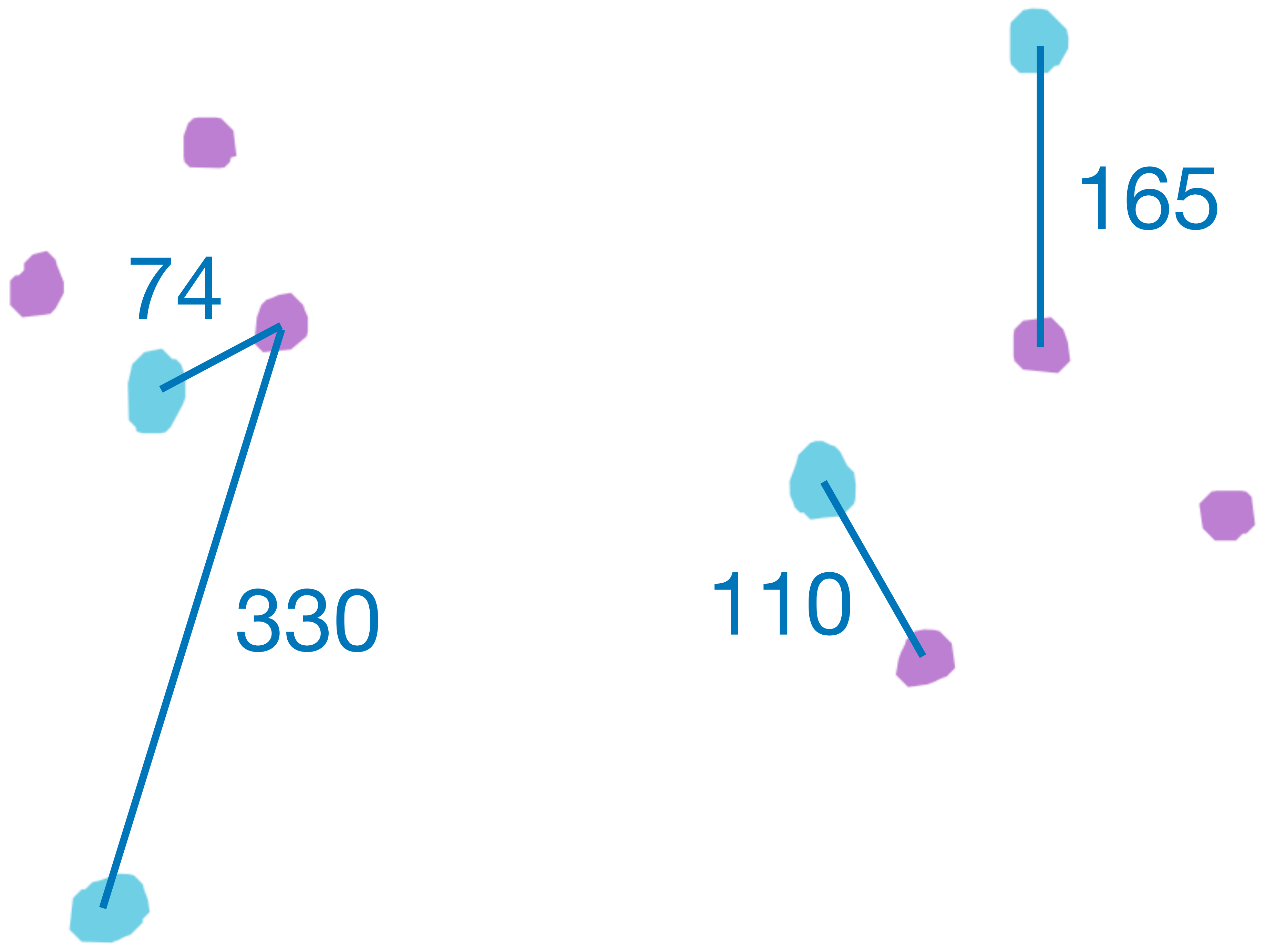
Nearest neighbor function

$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

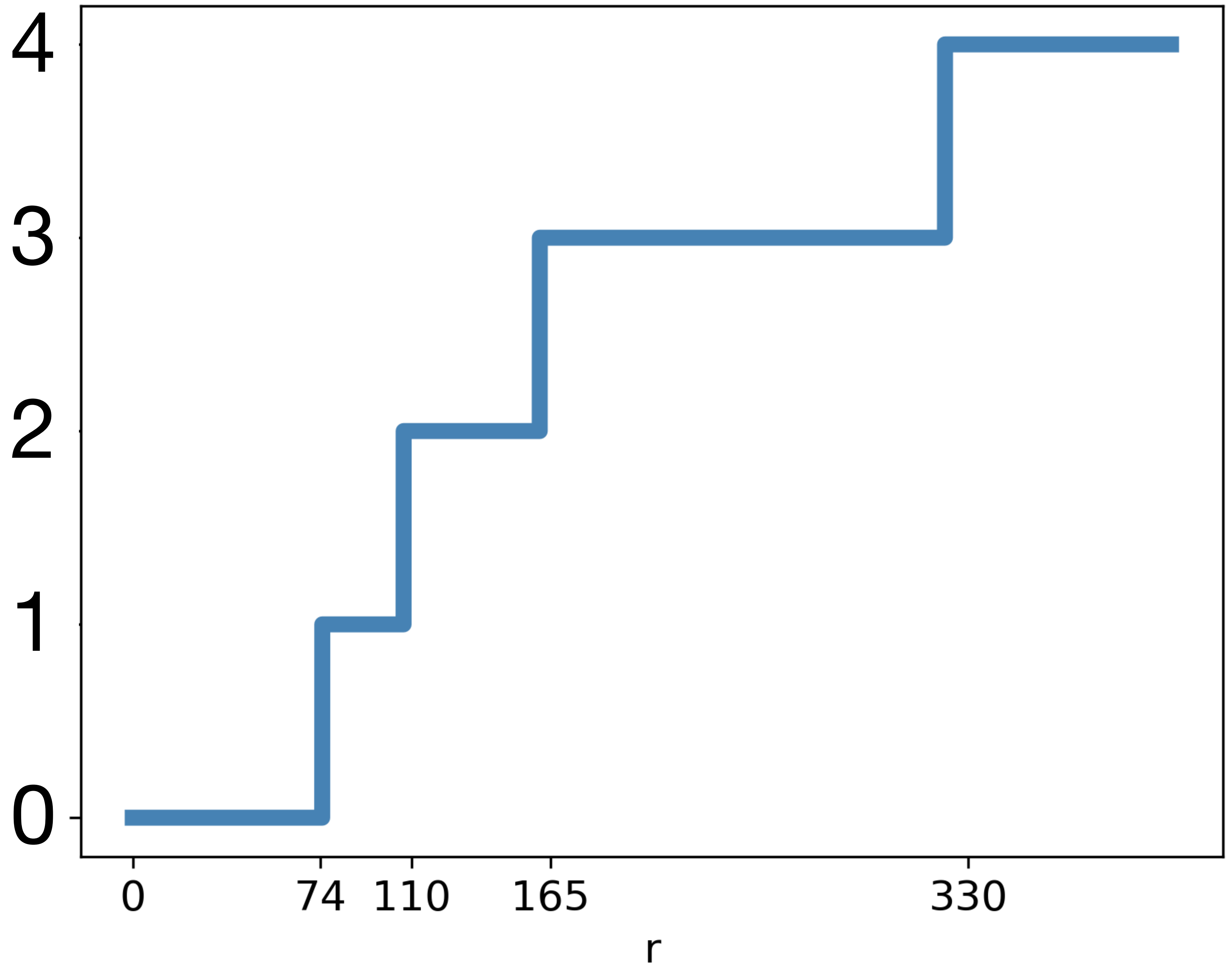




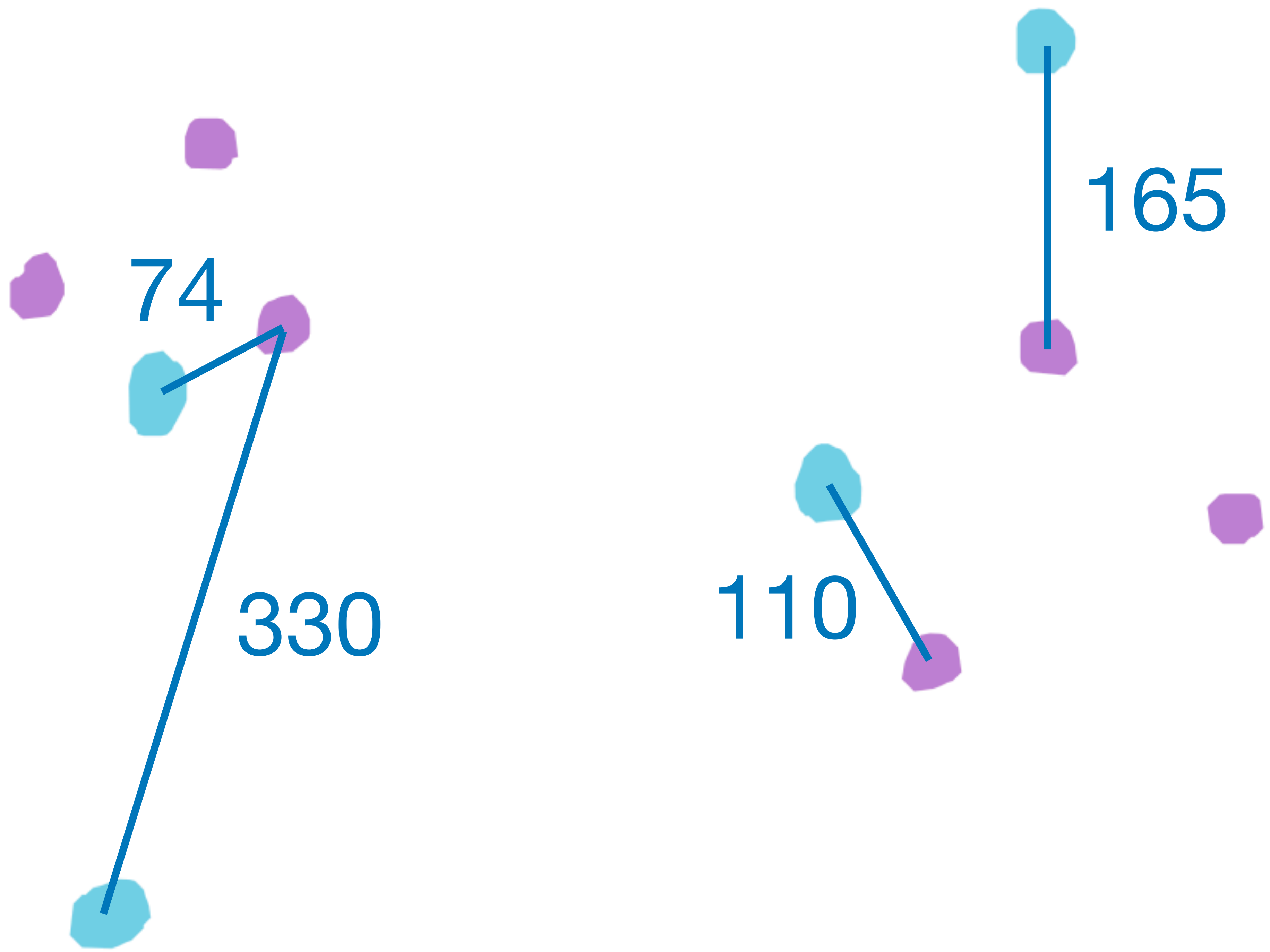
Nearest neighbor function



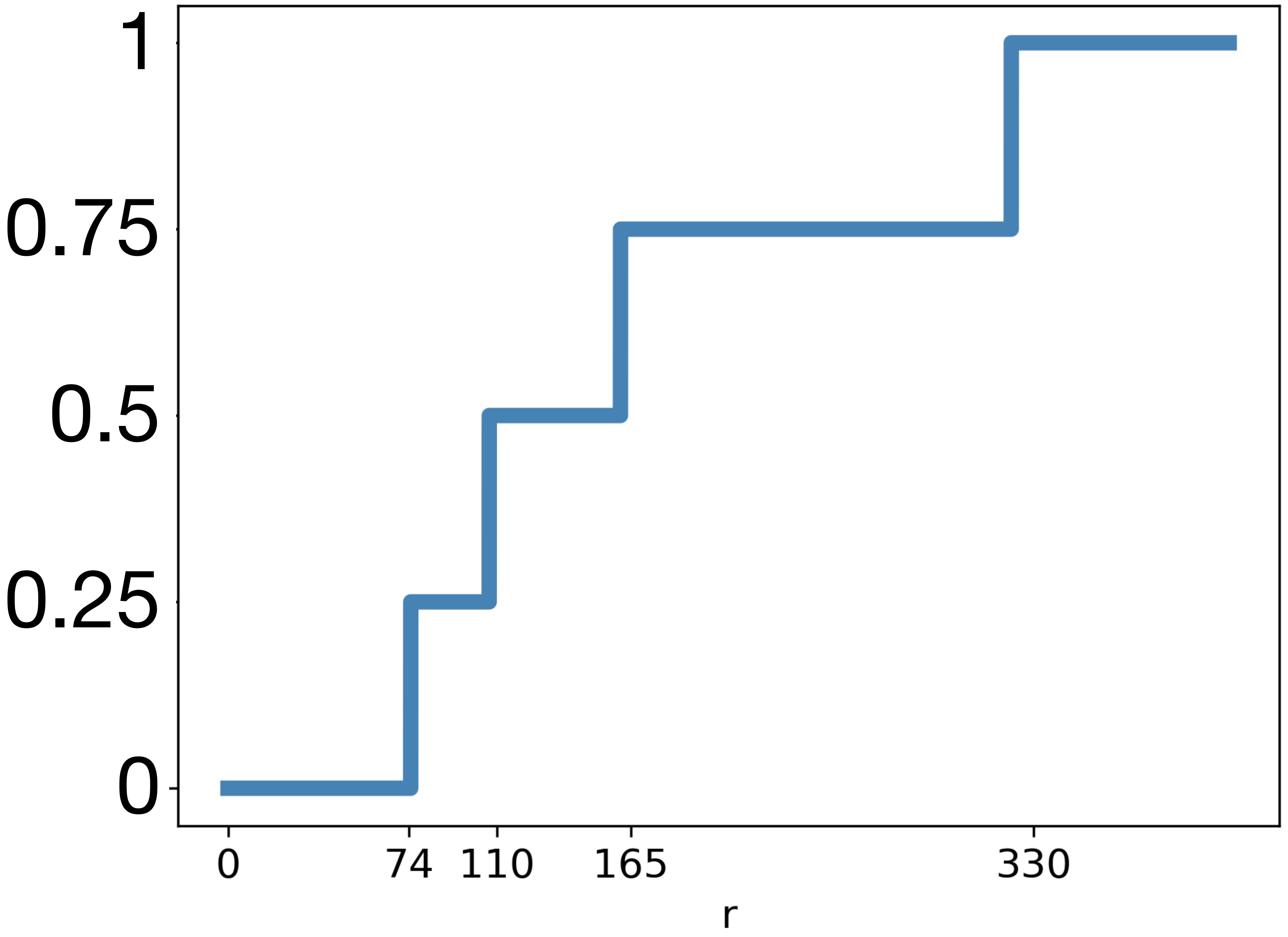
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$



Nearest neighbor function

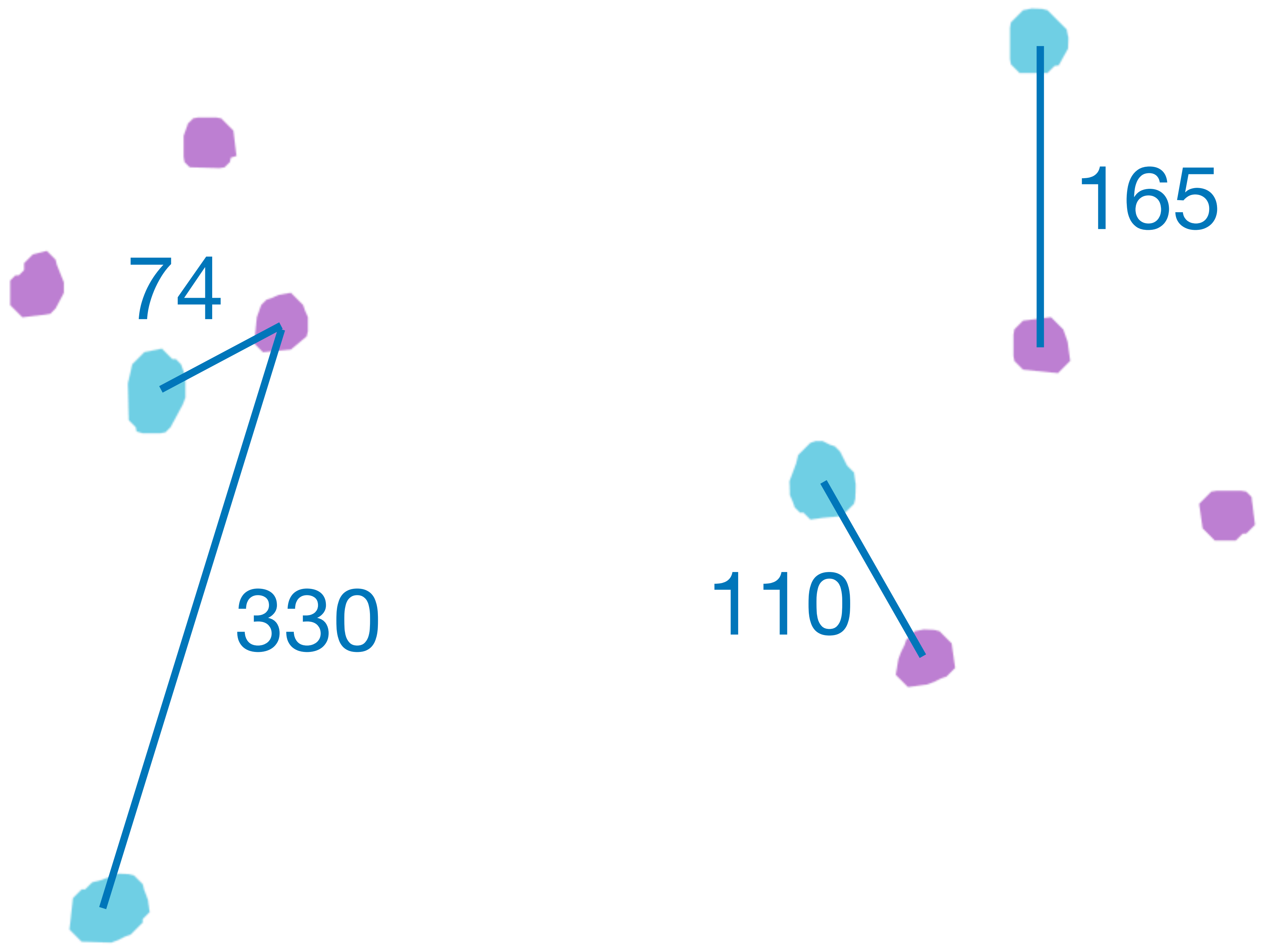


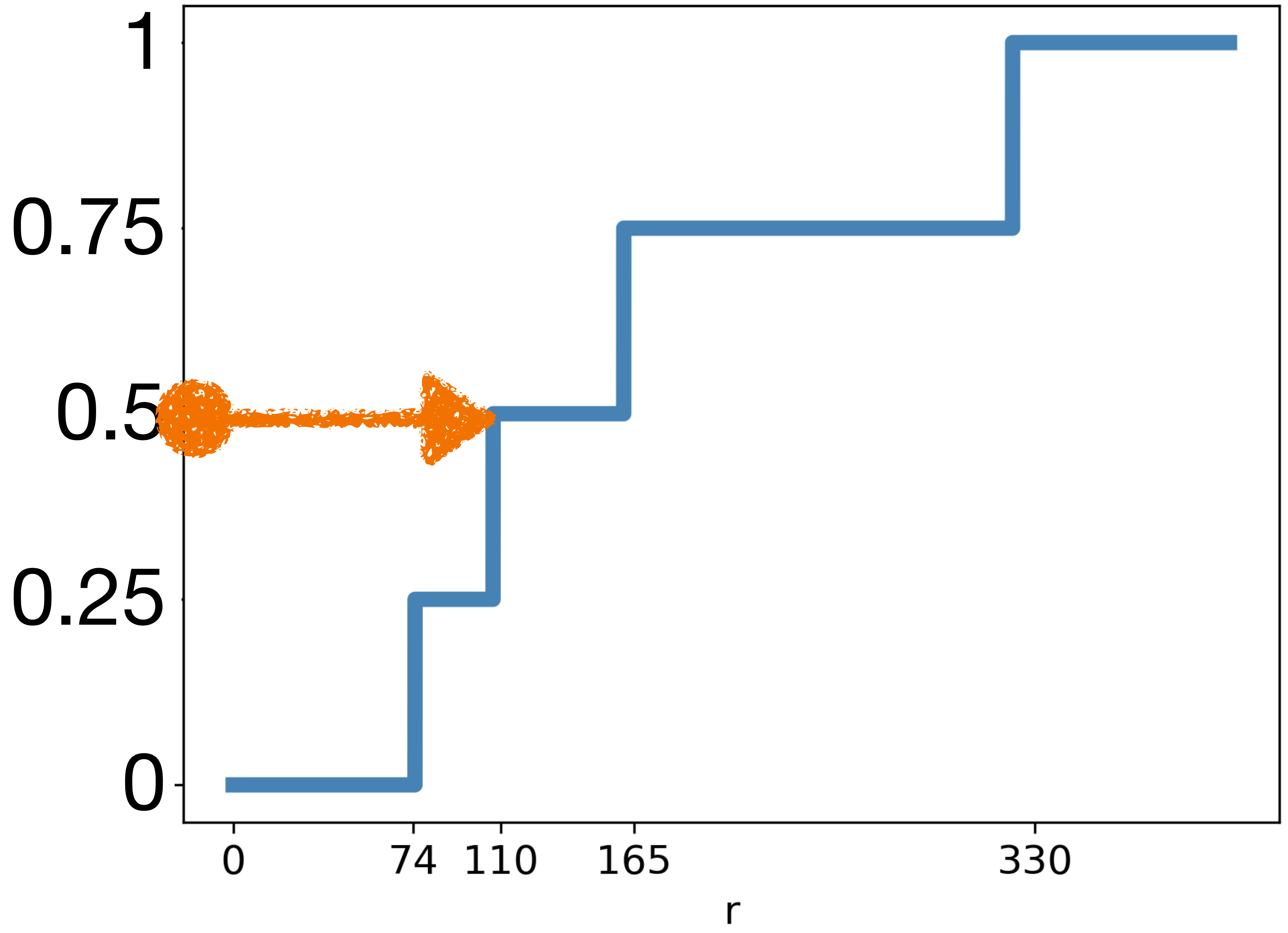
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$



Nearest neighbor function

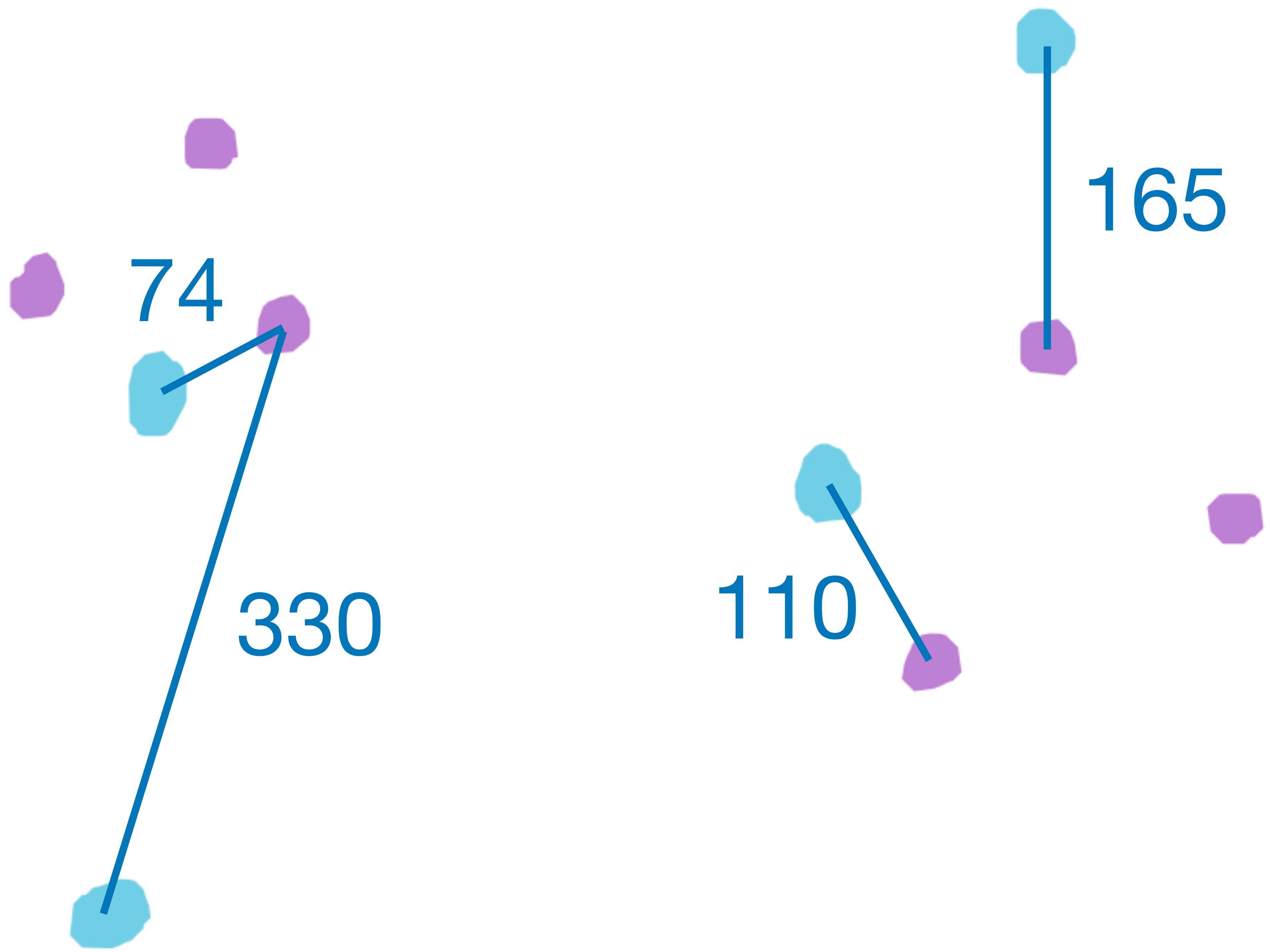
$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

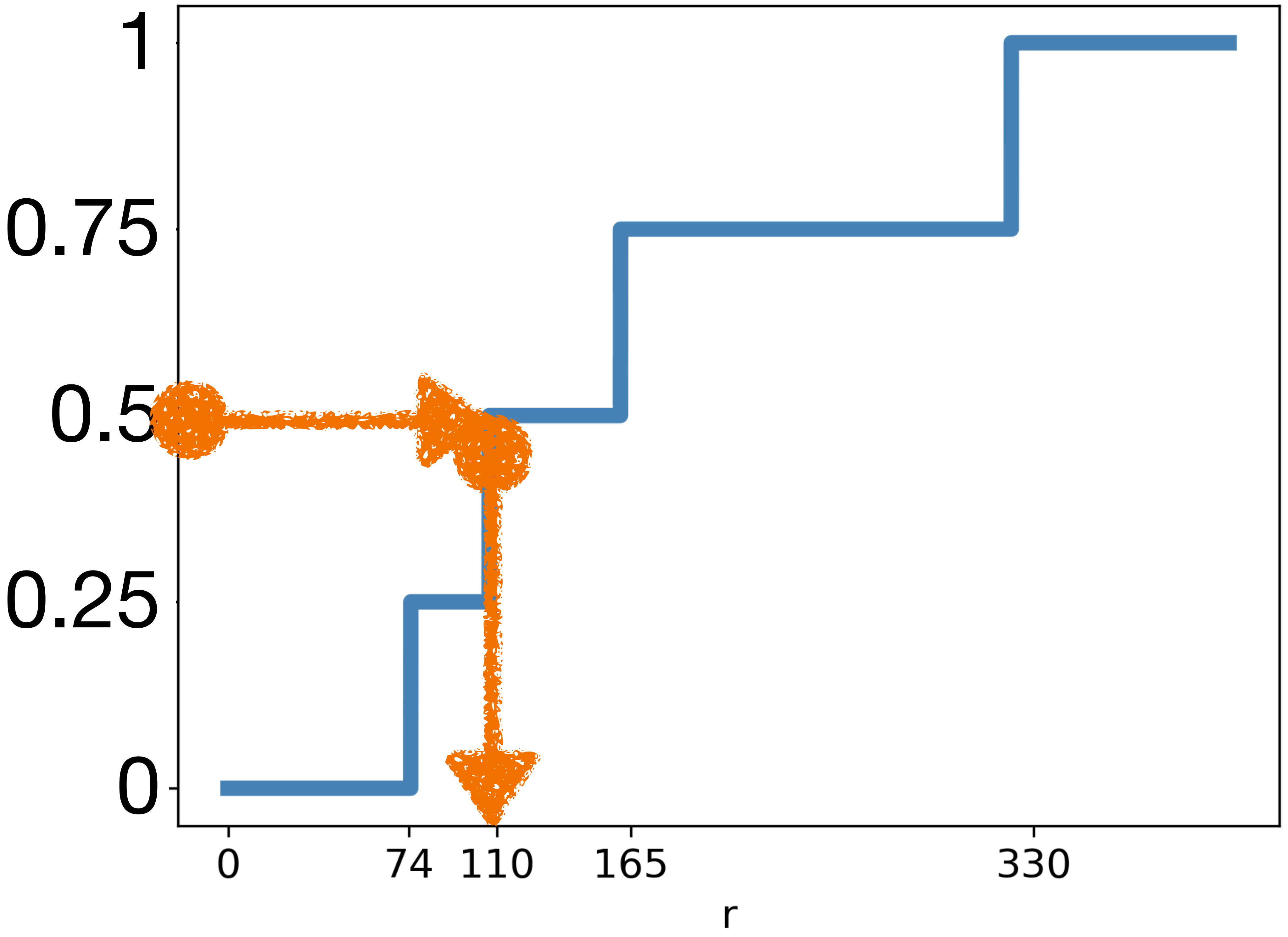




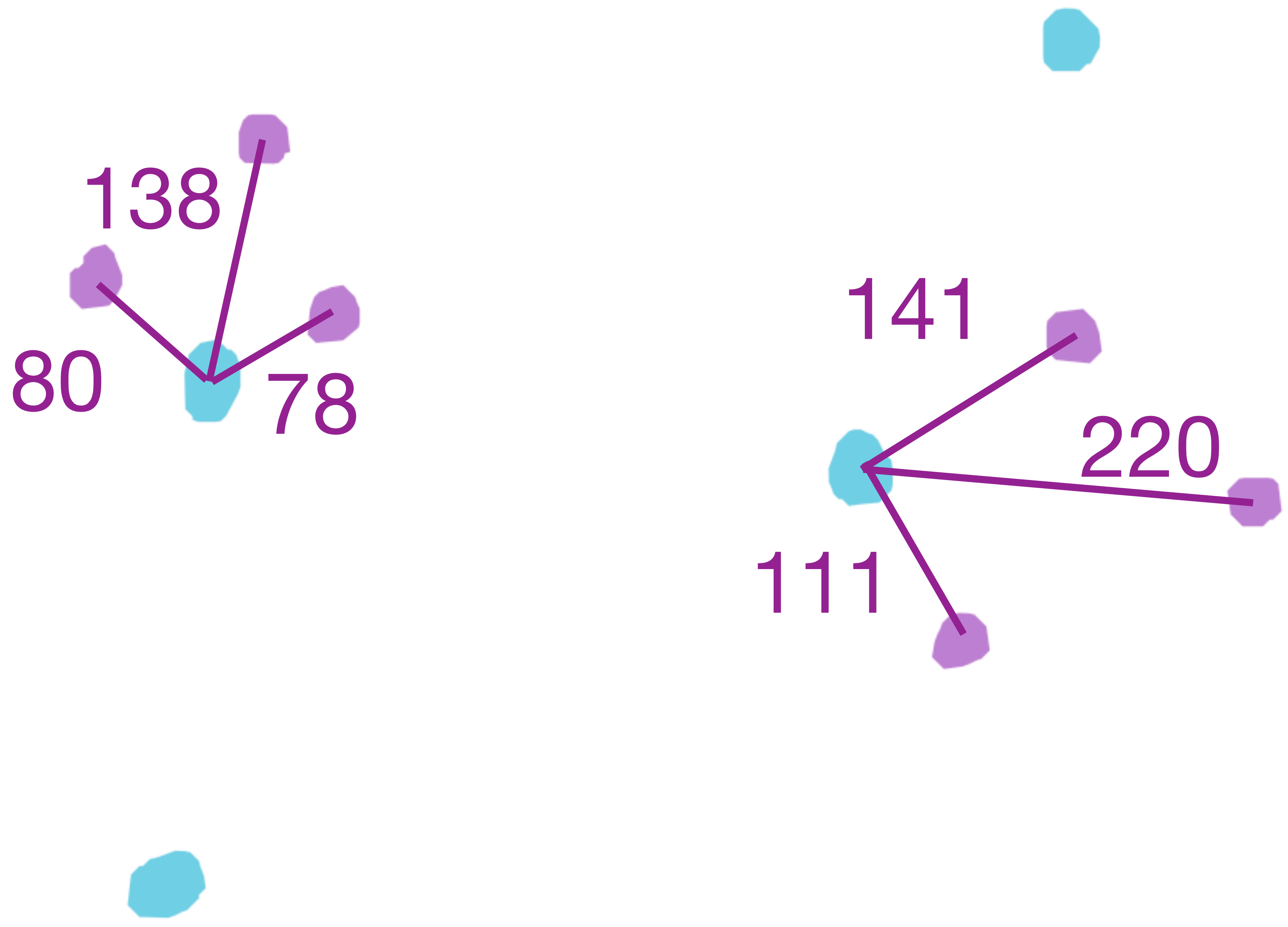
Nearest neighbor function

$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$



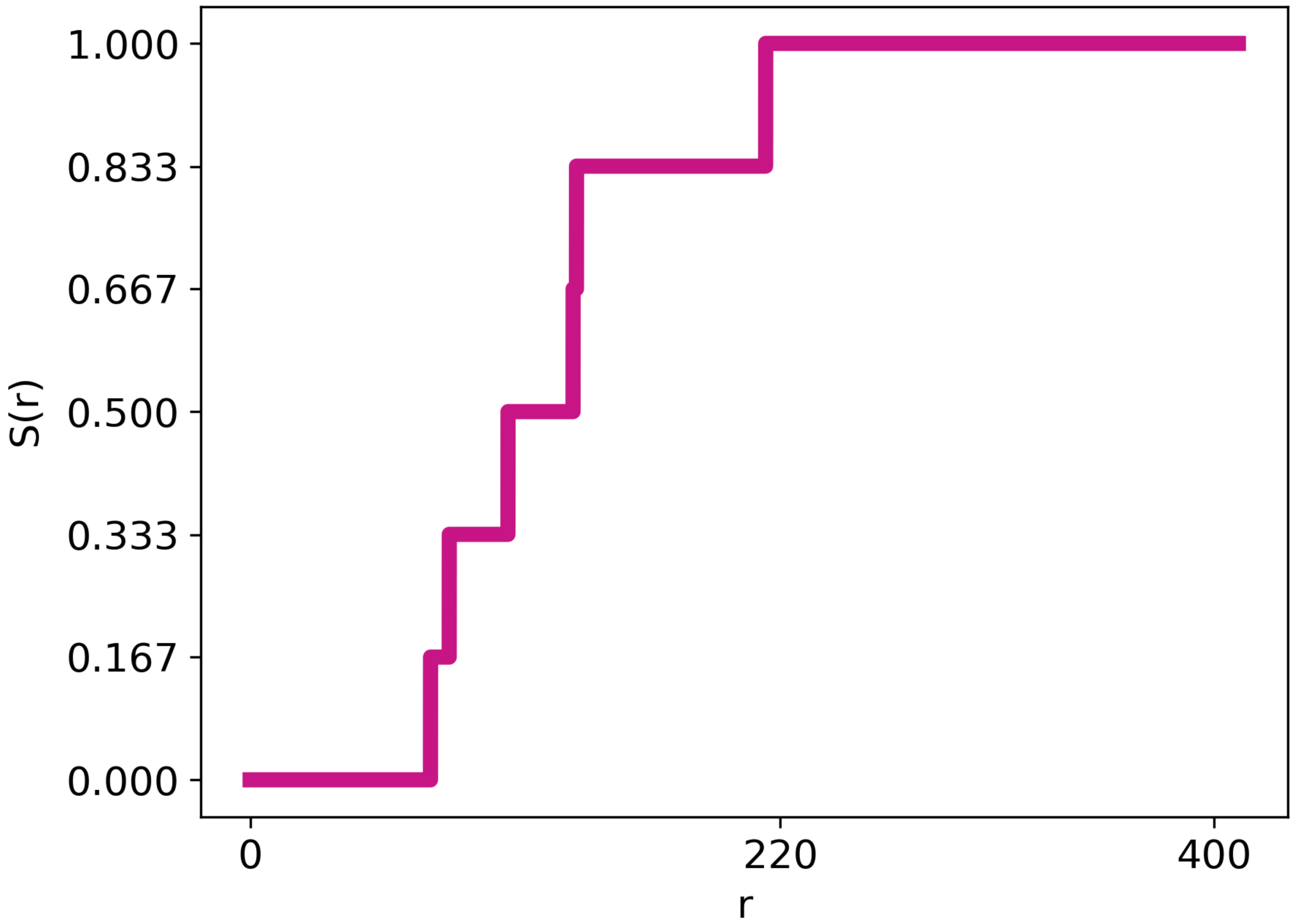


Nearest neighbor function



$$n_1 = 6$$

$$S(r) = \frac{1}{n_1} \sum_{i=1}^{n_1} \mathbf{1}(d_i < r)$$

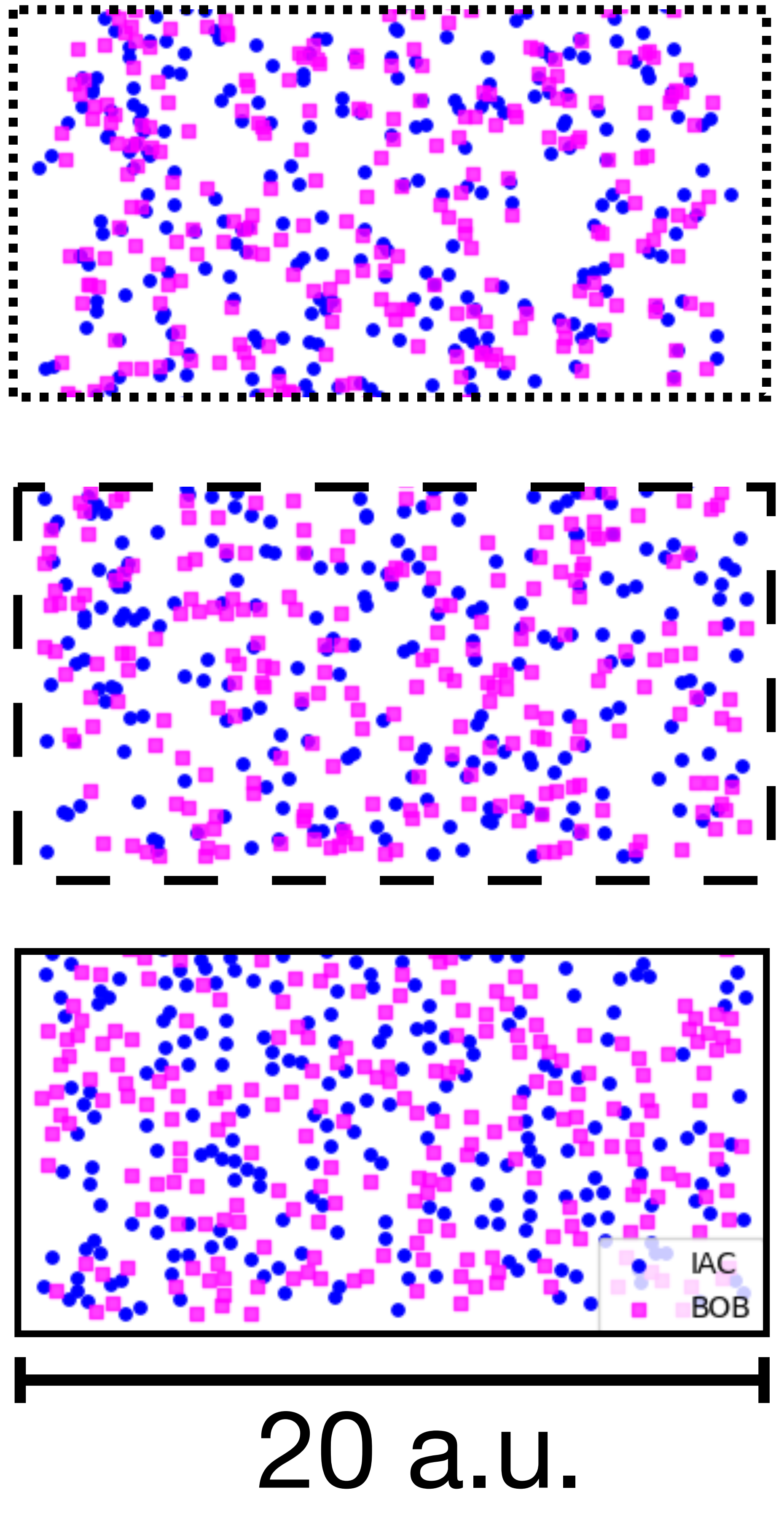


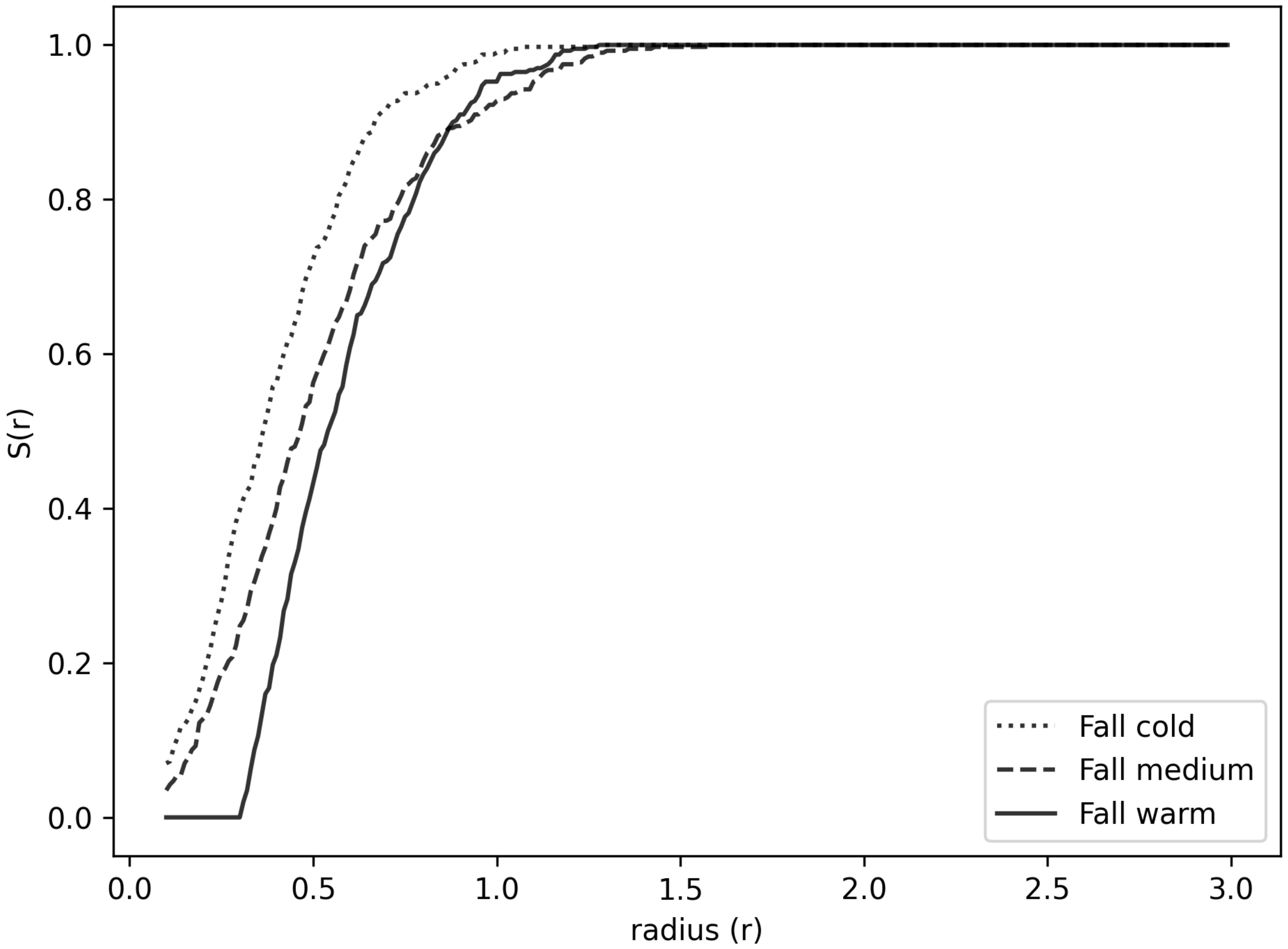
Results: Nearest neighbor function

Fall — cold

Fall — medium

Fall — warm



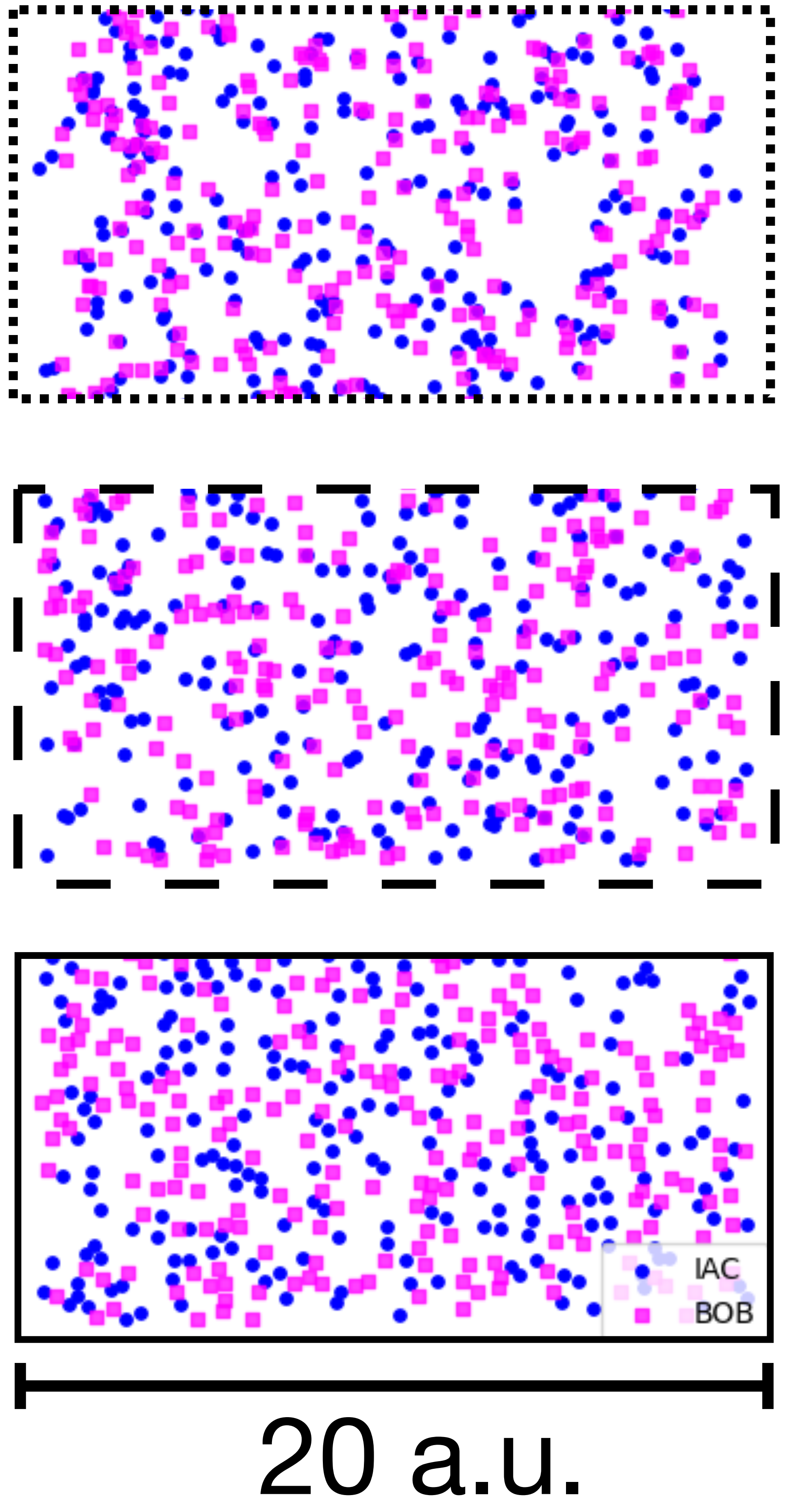


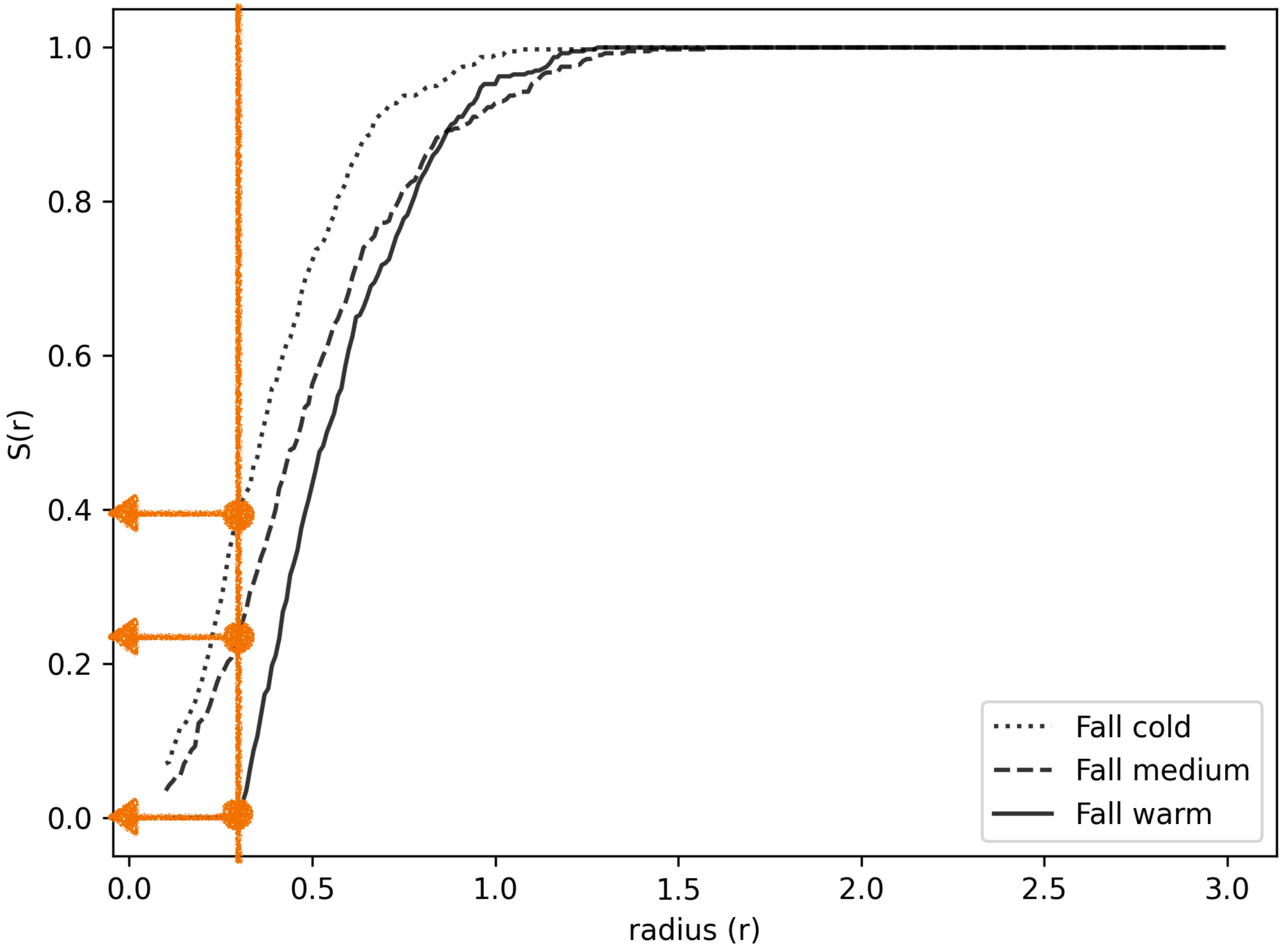
Results: Nearest neighbor function

Fall — cold

Fall — medium

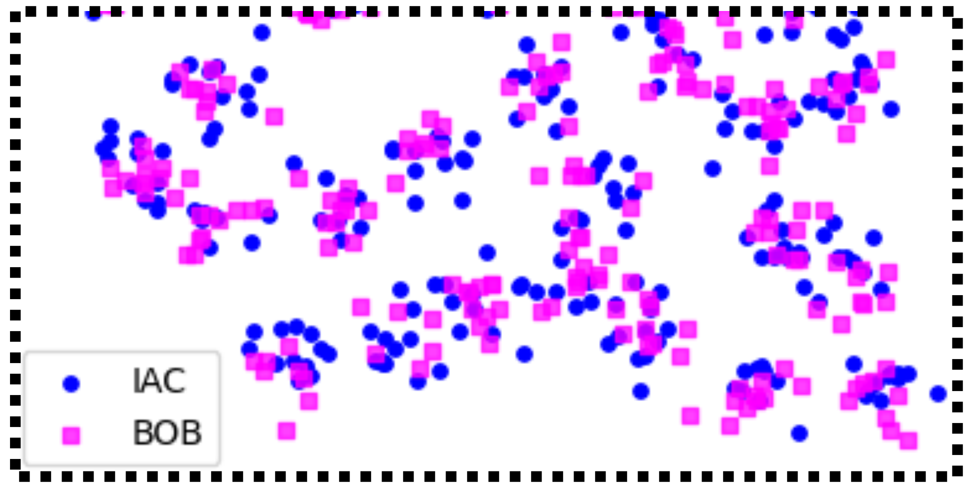
Fall — warm



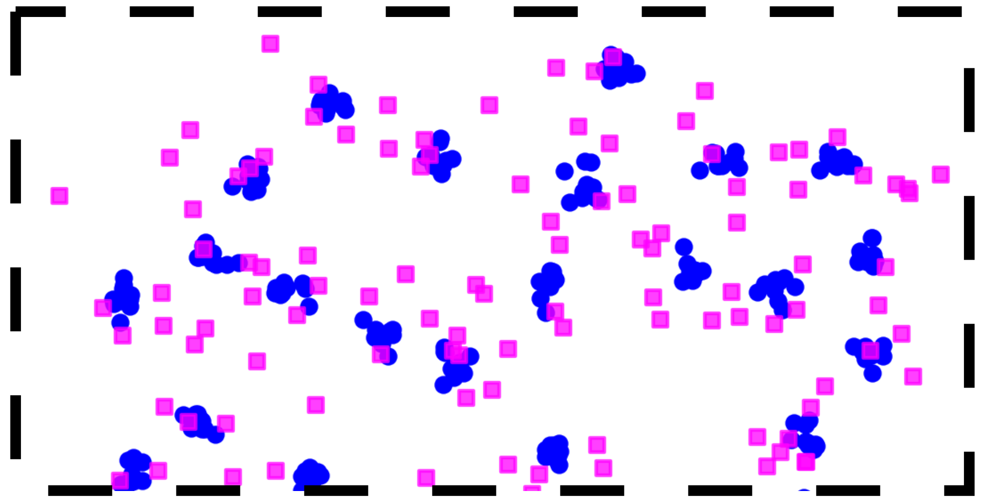


Results: Nearest neighbor function

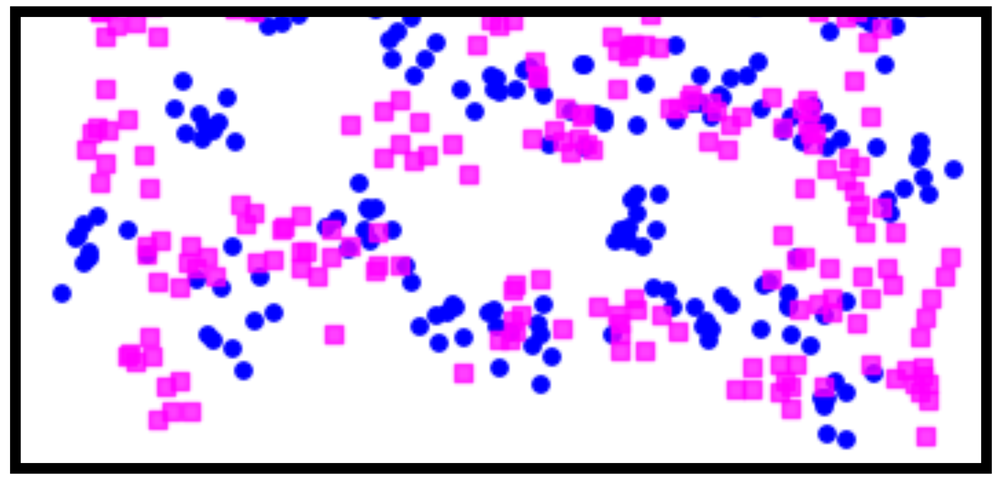
Winter — cold



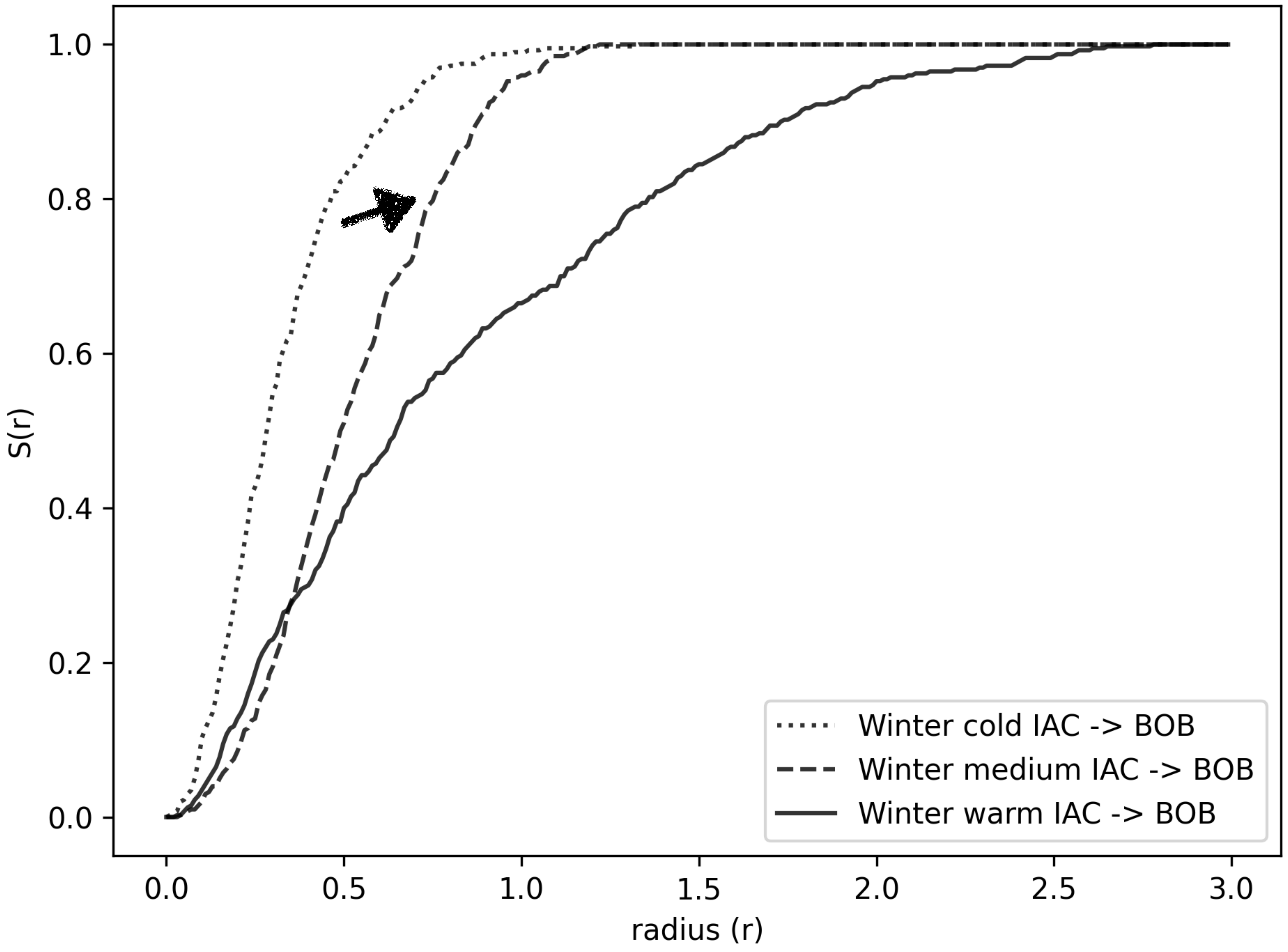
Winter — medium



Winter — warm

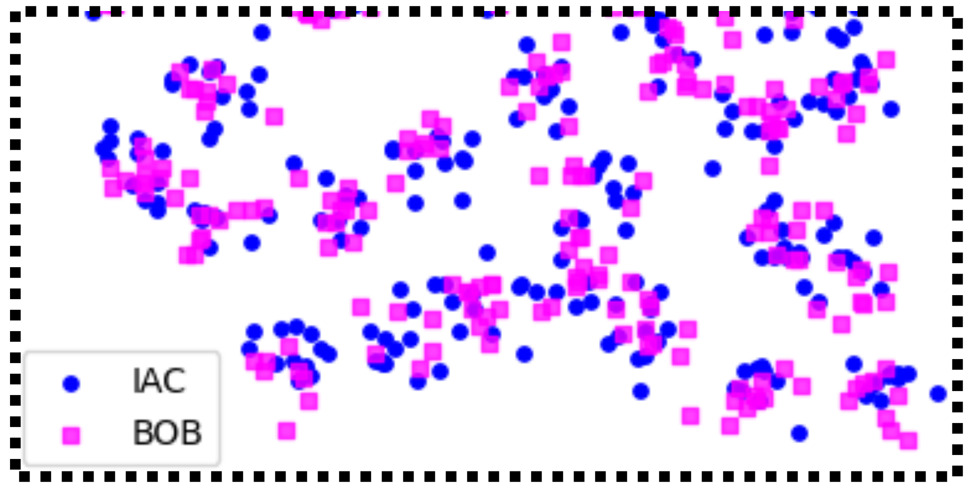


IAC → BOB

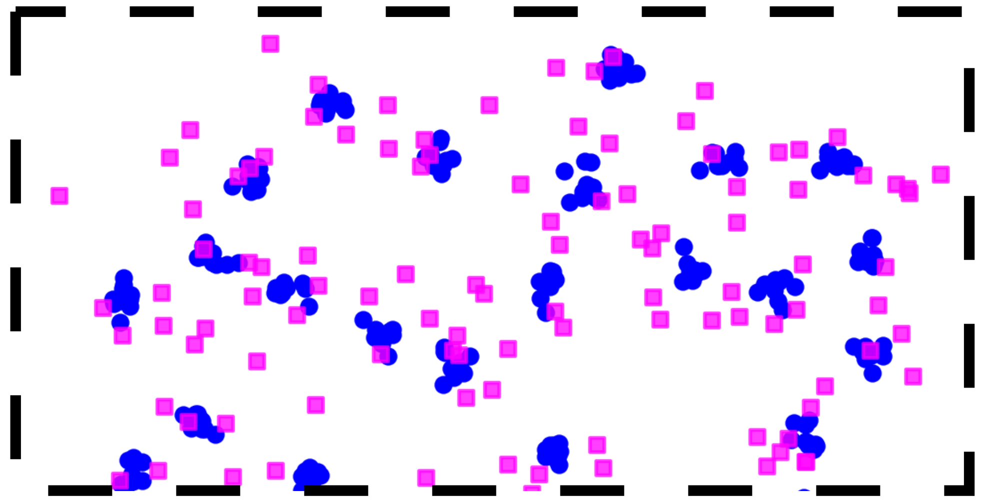


Results: Nearest neighbor function

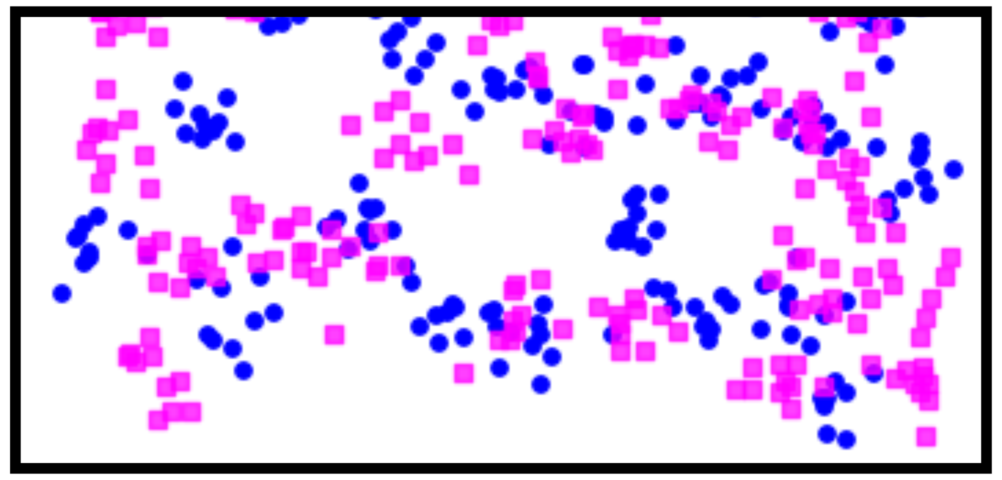
Winter — cold



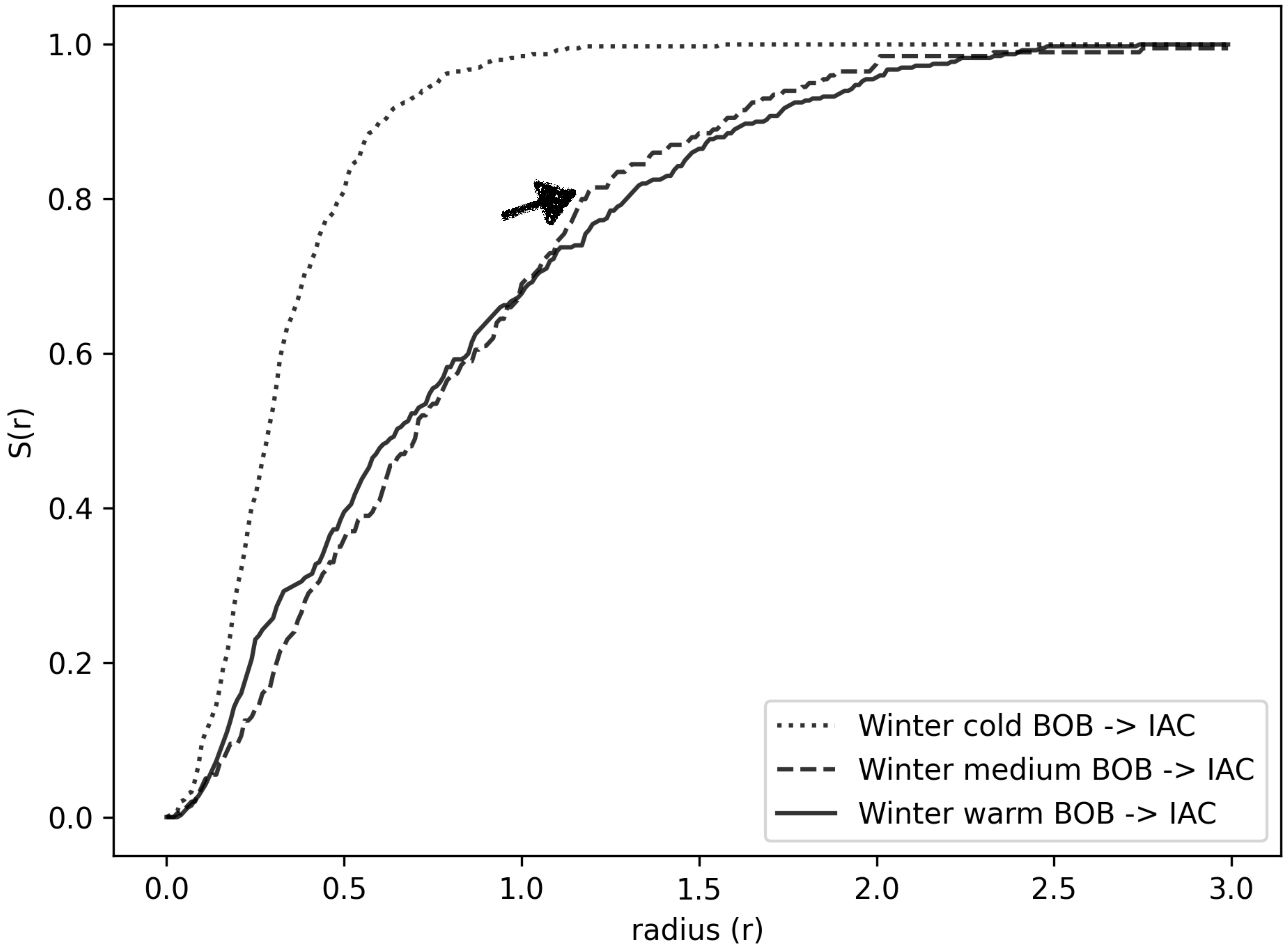
Winter — medium



Winter — warm



BOB ⟶ IAC



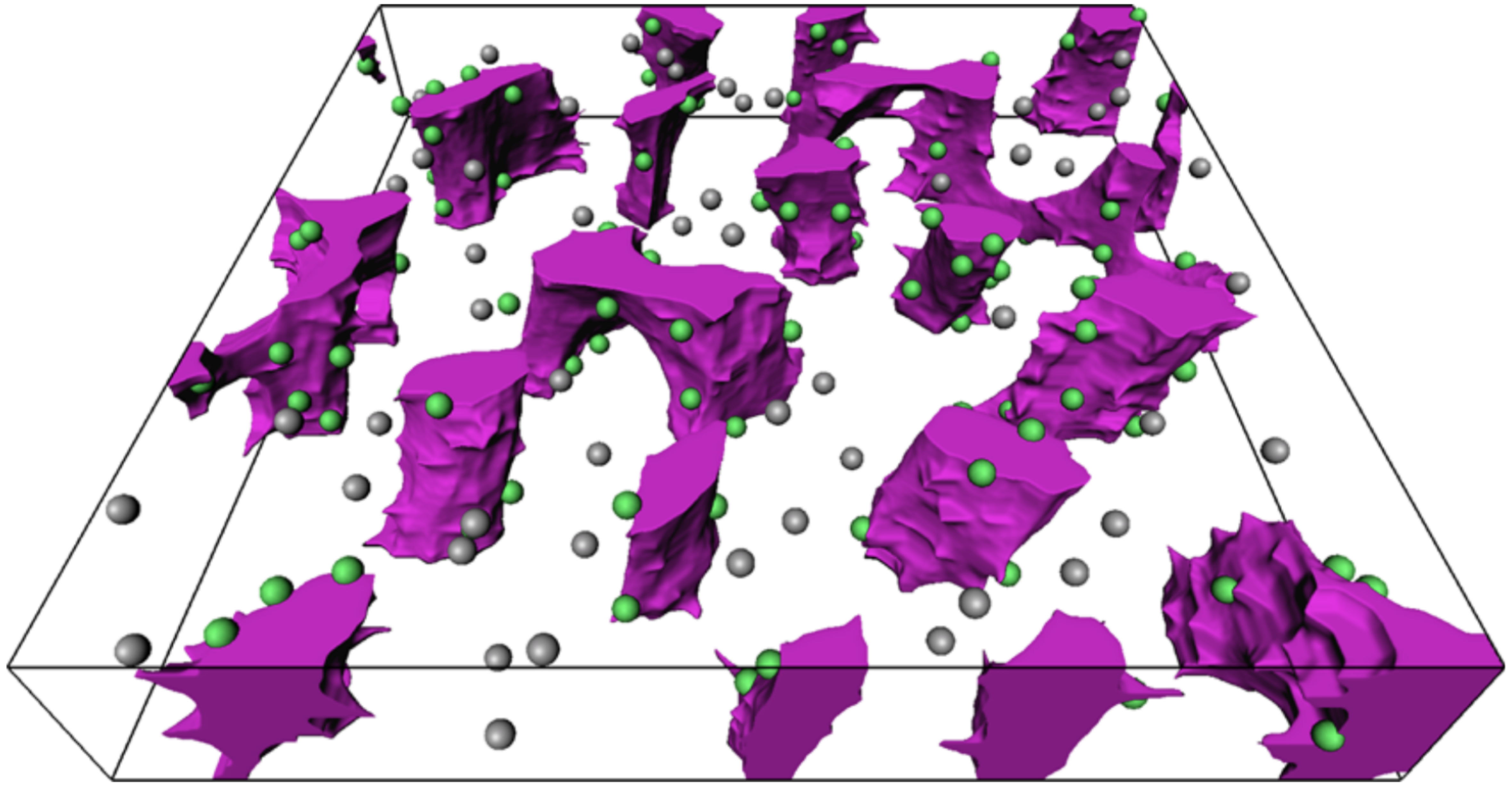
-> Notebook

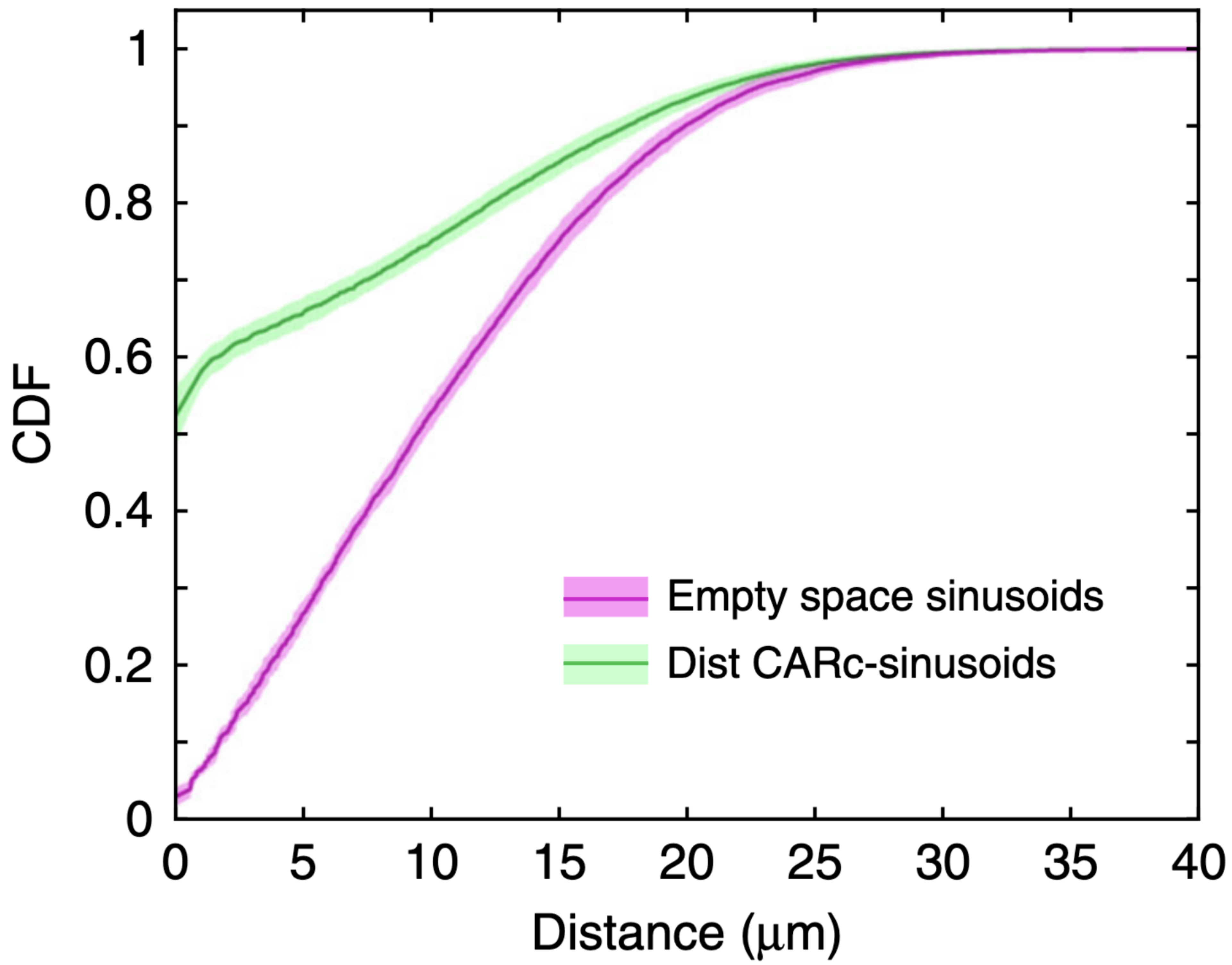
-> Notebook

Nearest neighbor function

- Asymmetric: BOB → IAC ≠ IAC → BOB
- Returns: A number for each radius
- Range: Short

Beyond the nearest neighbor function

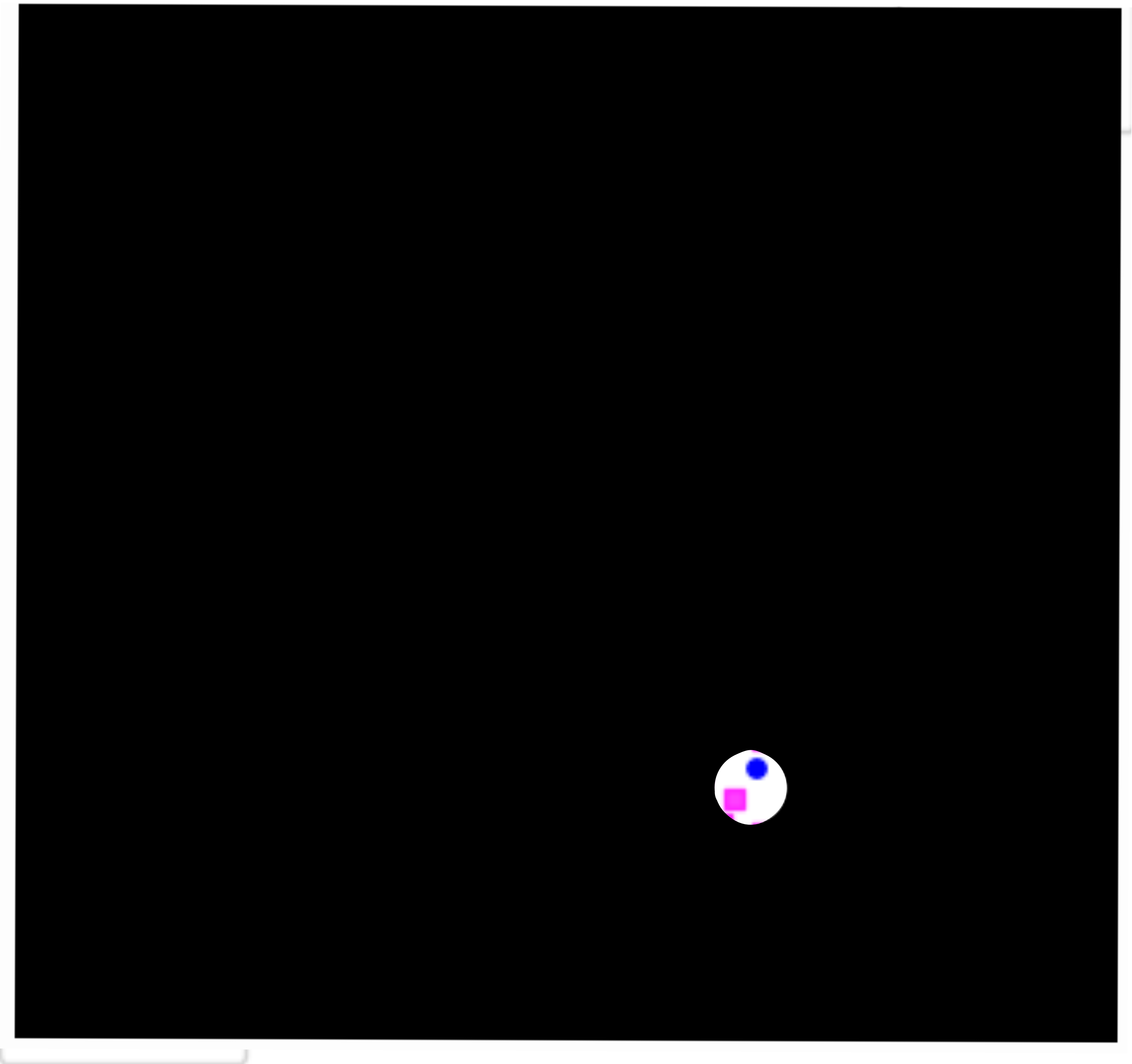




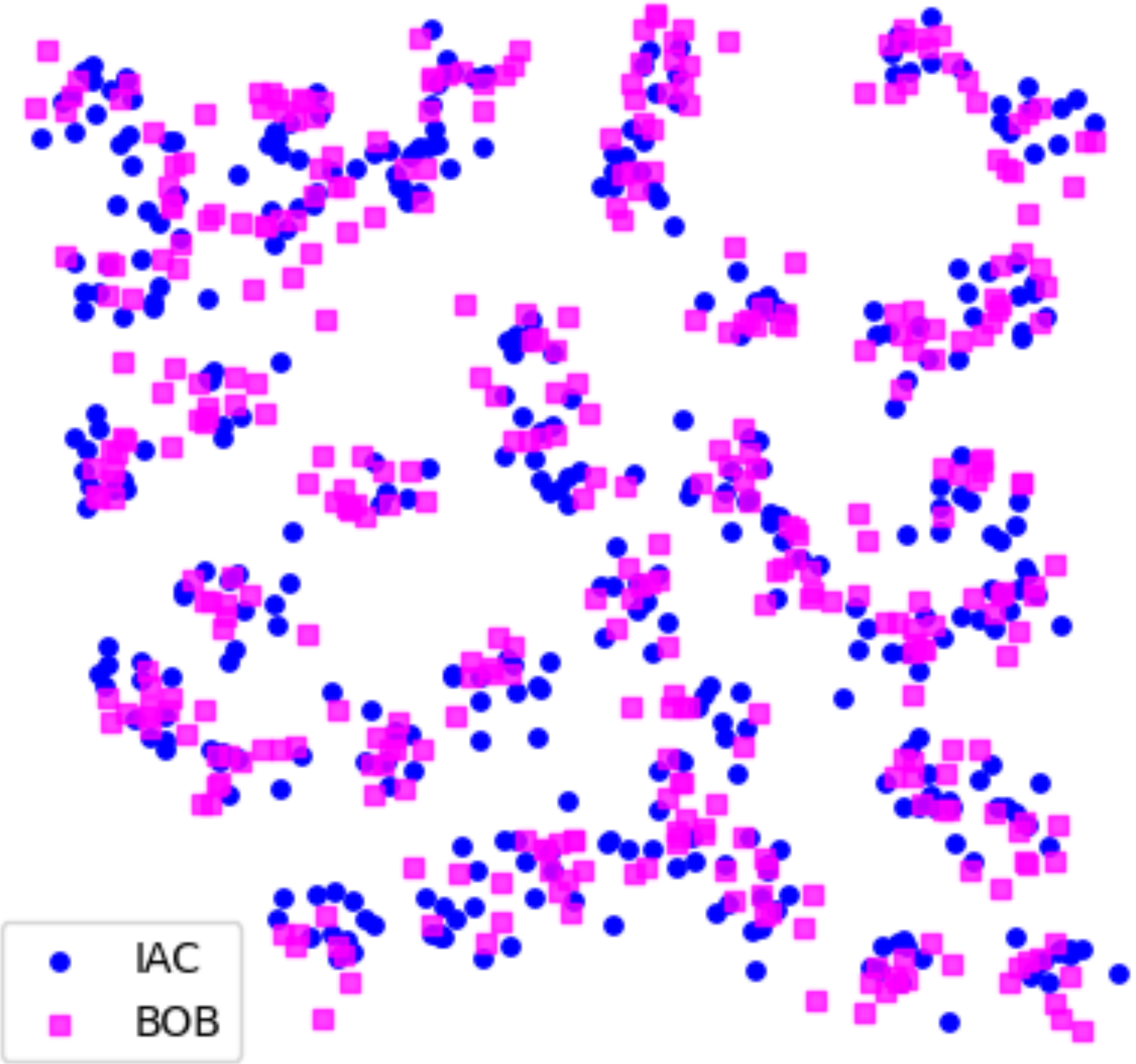
CARcs Sinusoidal vessel wall

Modified from: Gomariz, A., Helbling, P.M., Isringhausen, S. *et al.* Quantitative spatial analysis of haematopoiesis-regulating stromal cells in the bone marrow microenvironment by 3D microscopy. *Nat Commun* **9**, 2532 (2018). https://doi.org/10.1038/s41467-018-04770-z

Ripley’s K function

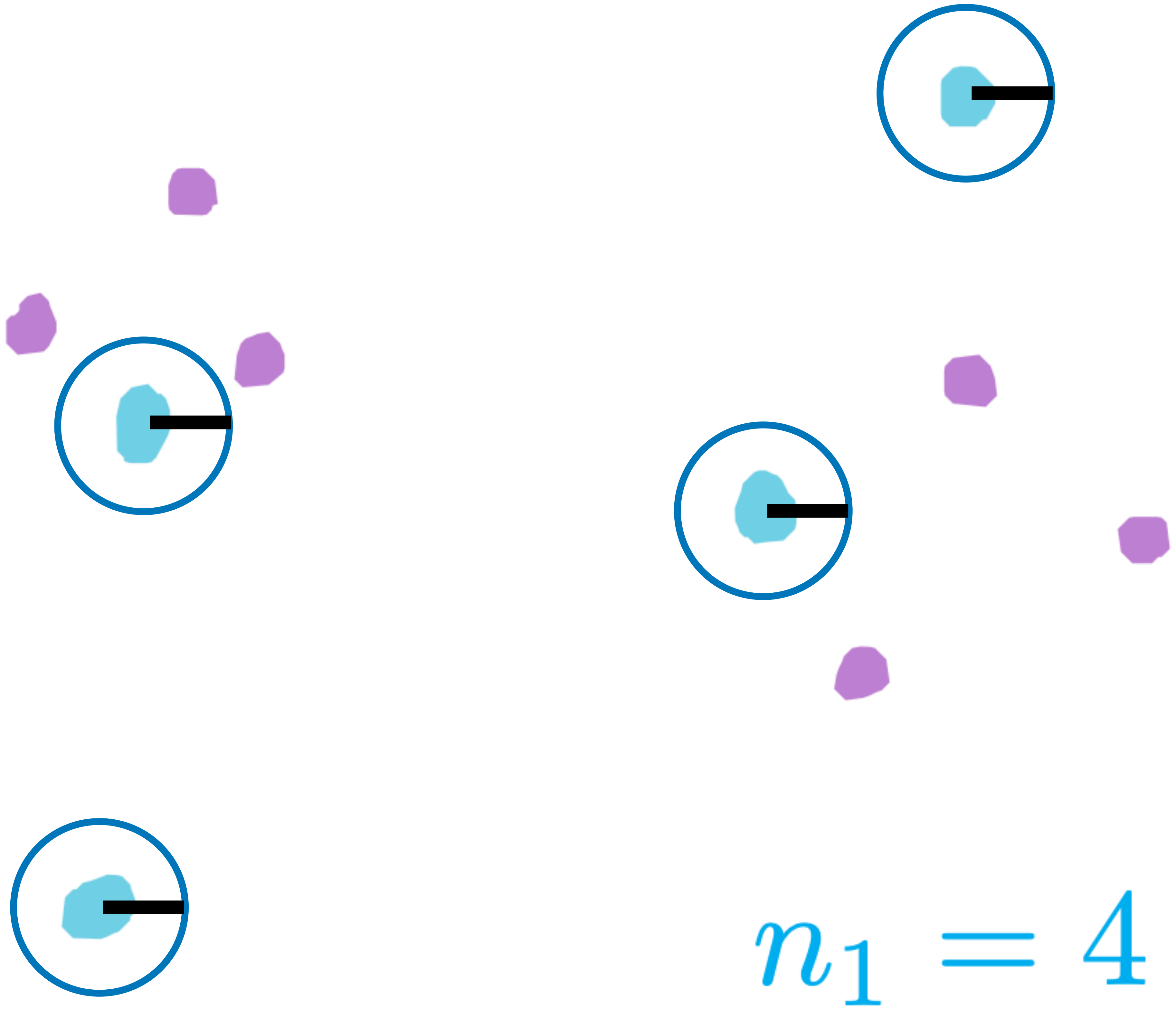


Ripley's K function



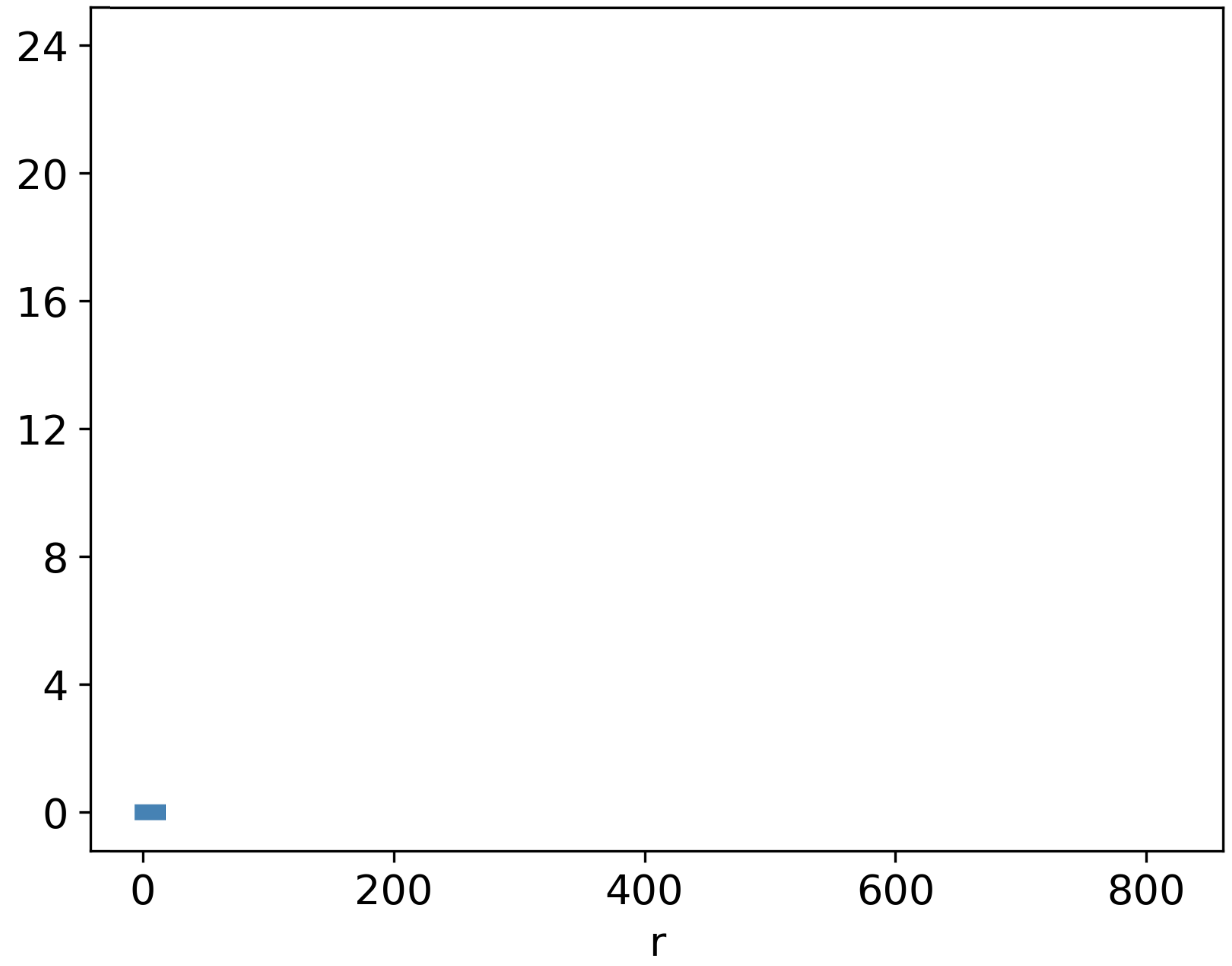
Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r)\, b(i, j, r)$$



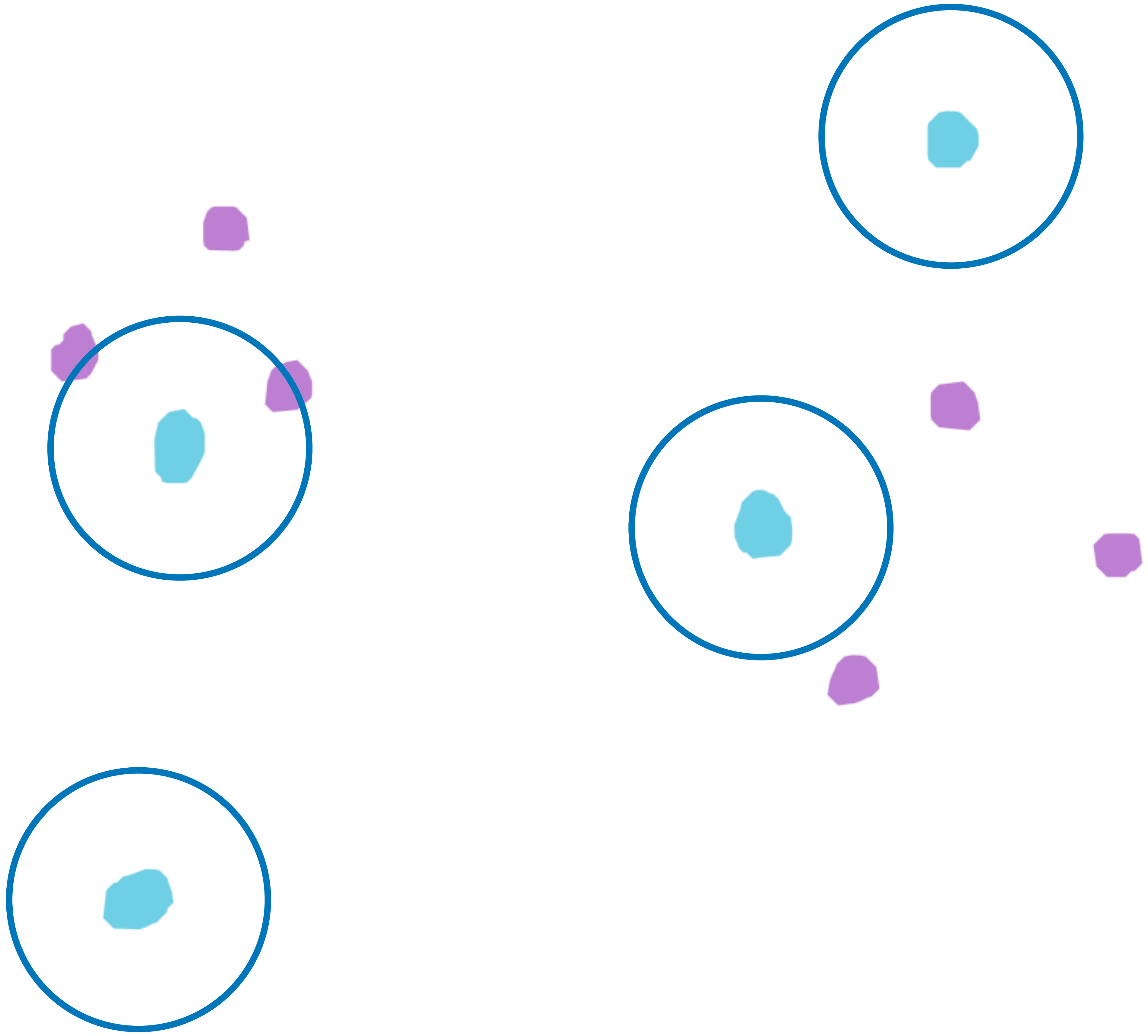
$n_1 = 4$

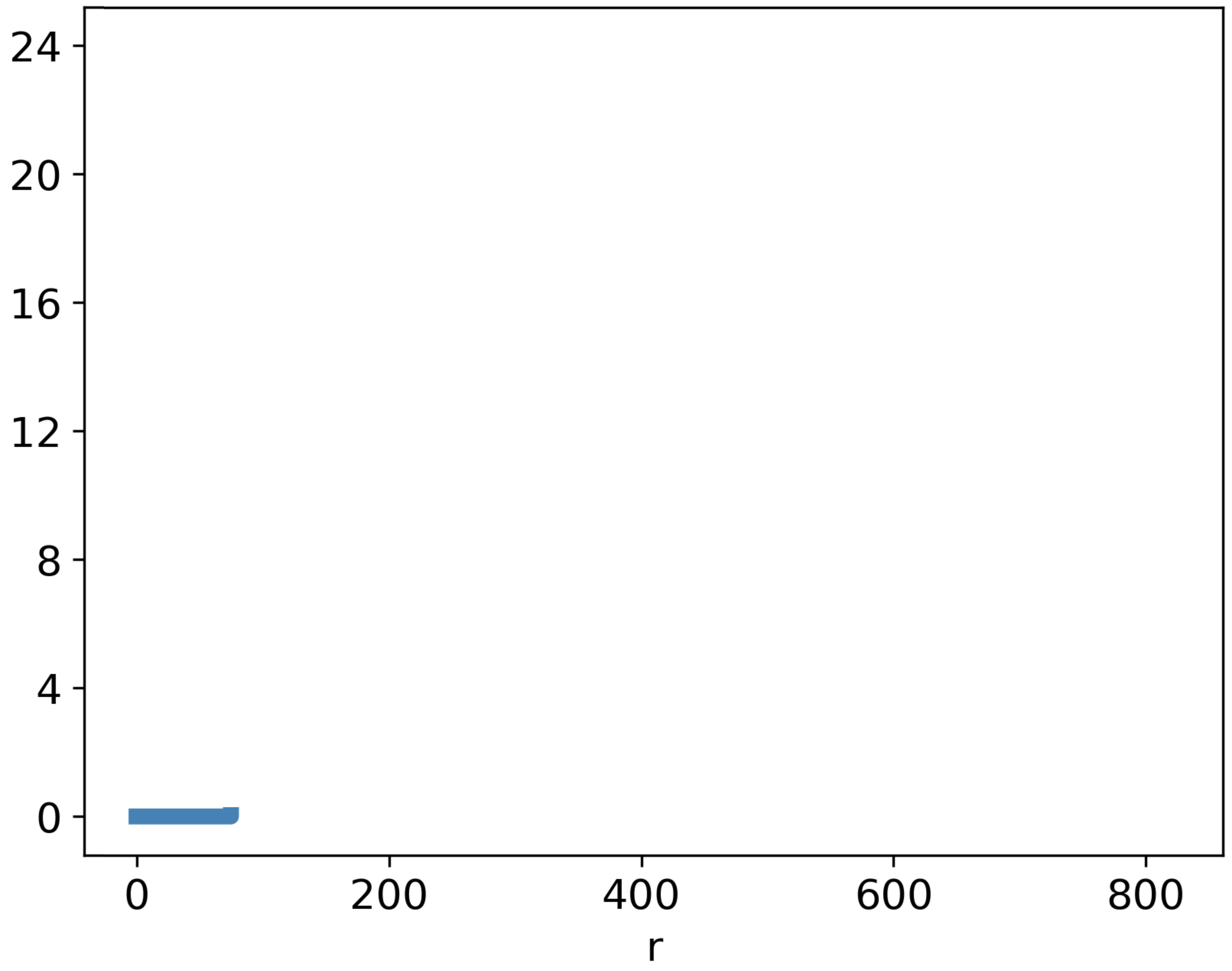
$n_2 = 6$



Ripley's K function

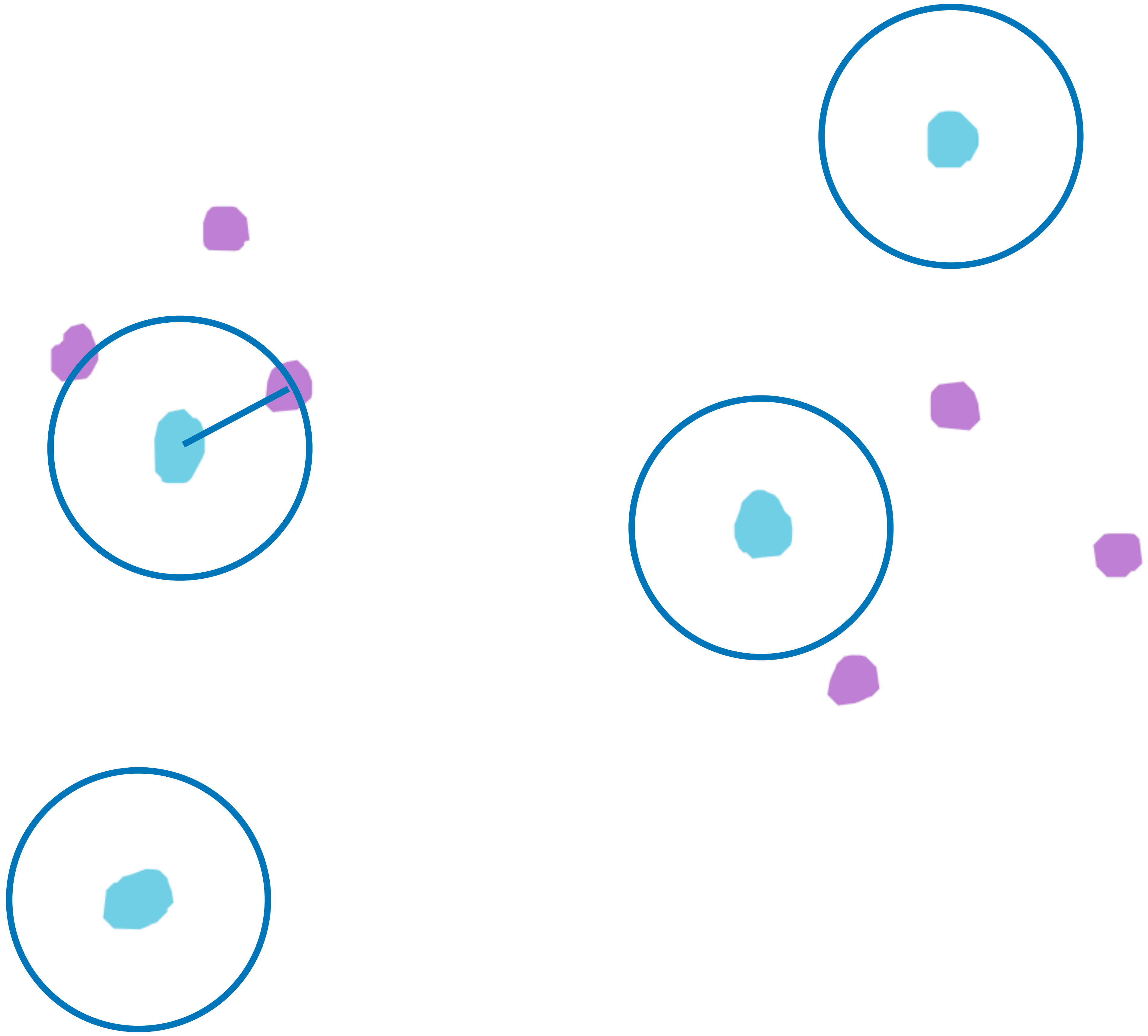
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$

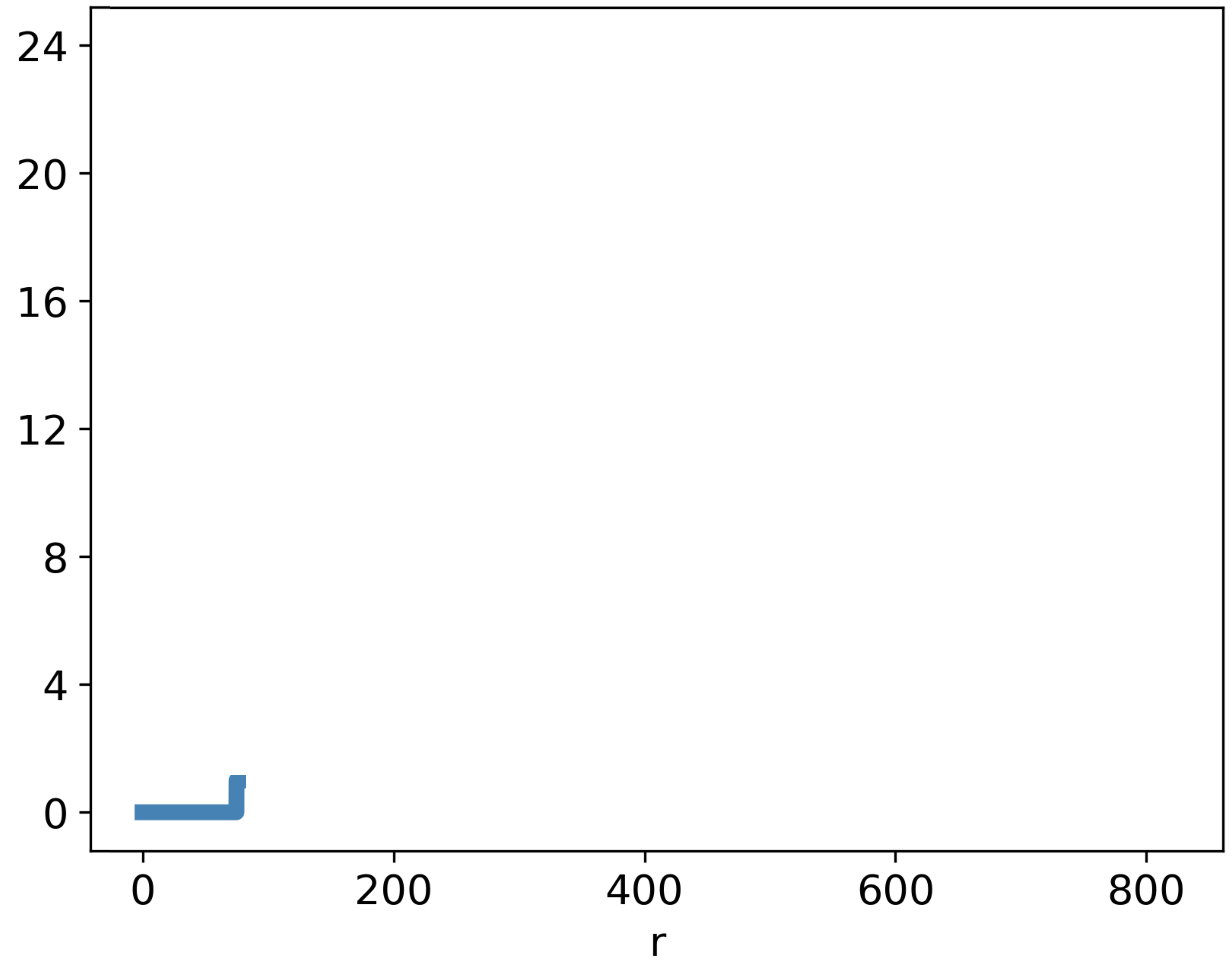




Ripley's K function

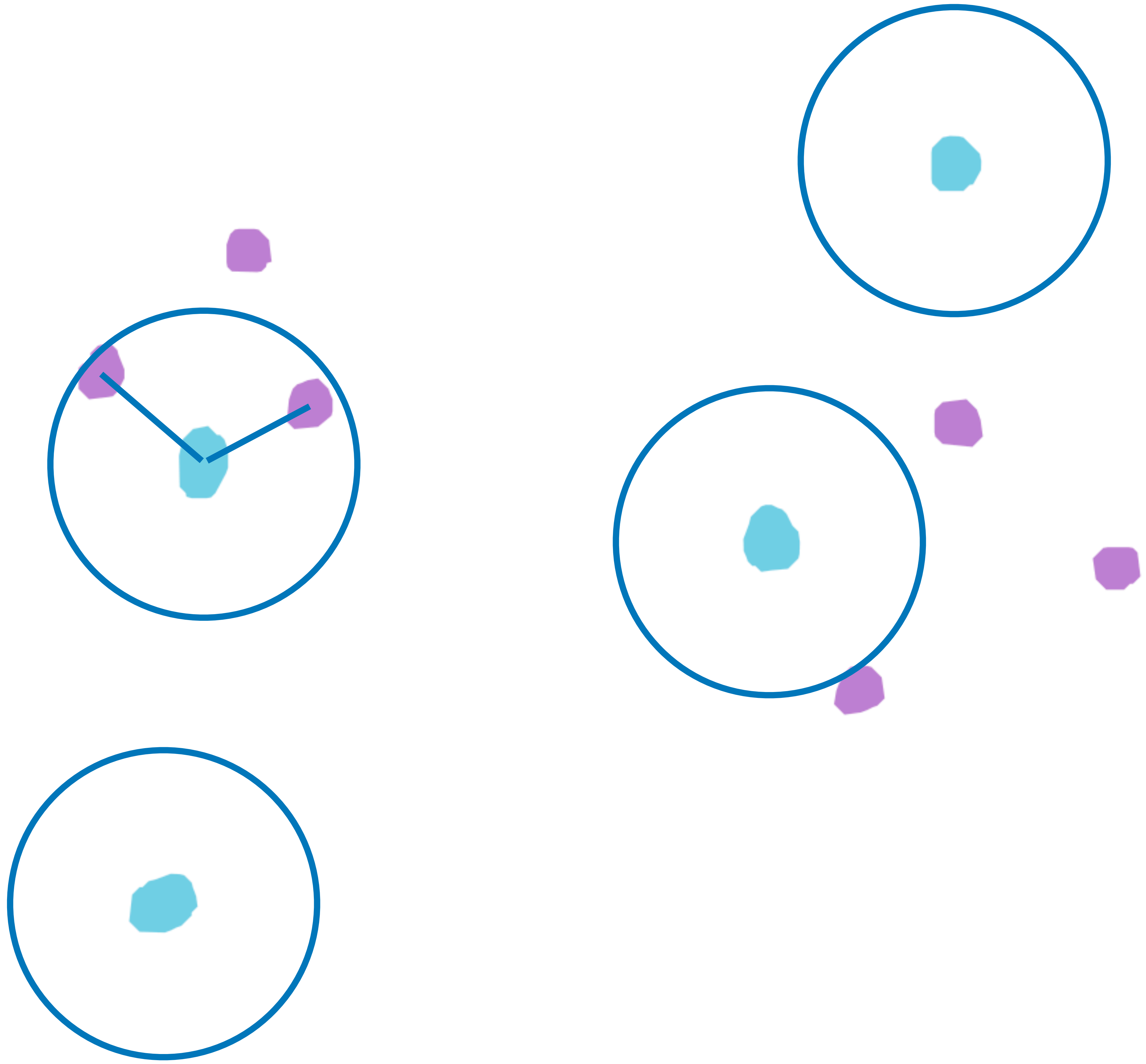
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r)\, b(i, j, r)$$

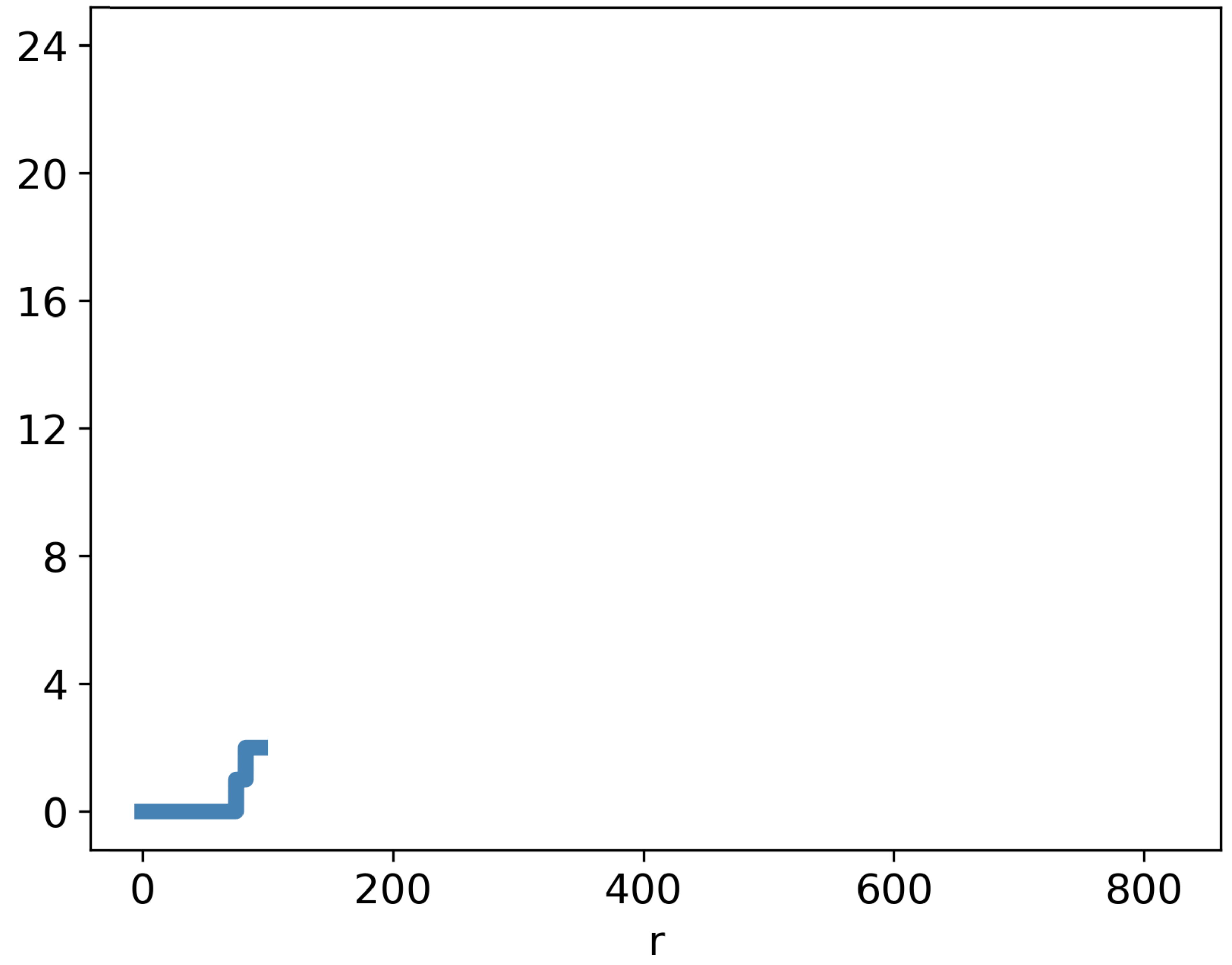




Ripley's K function

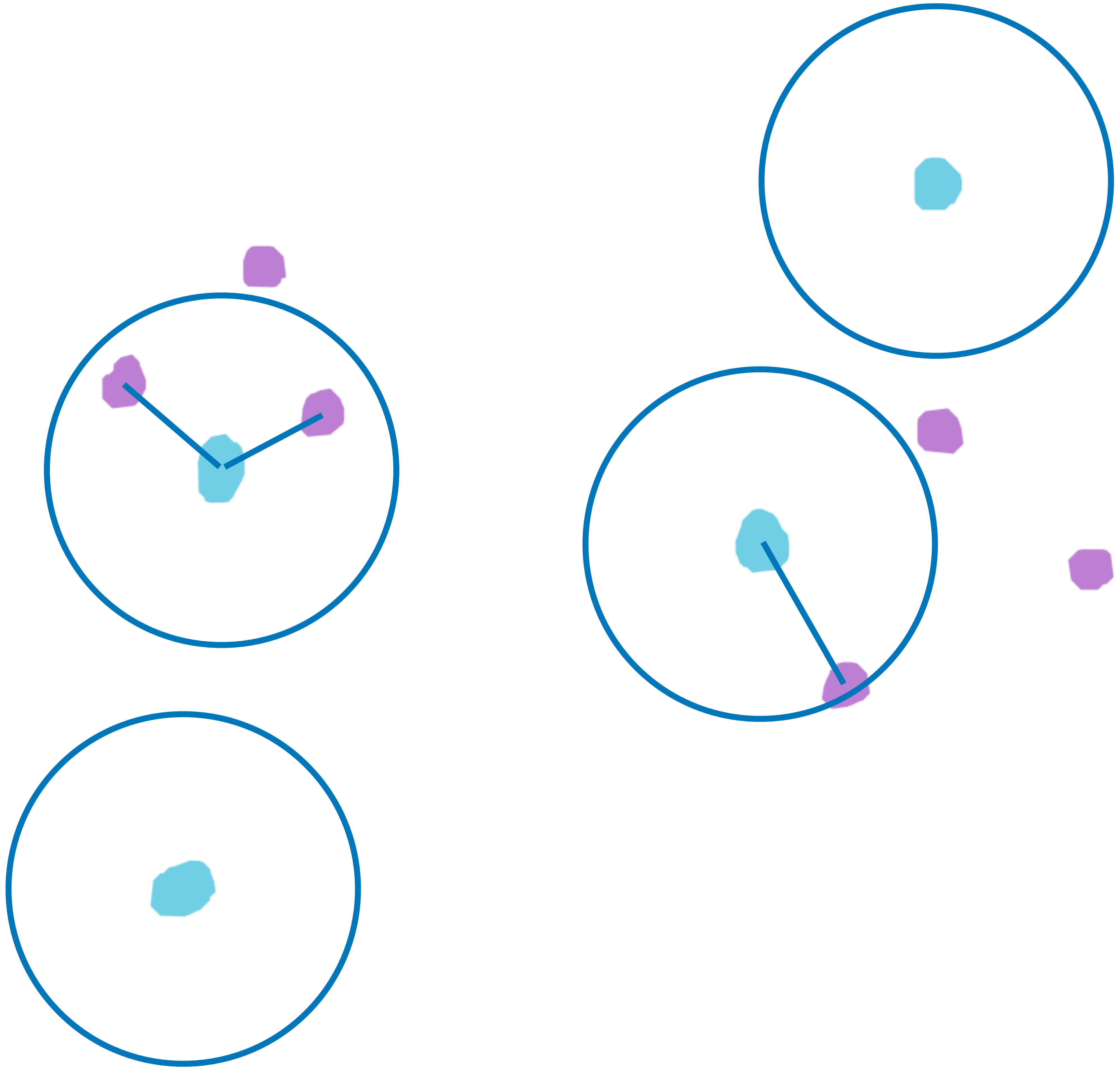
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$

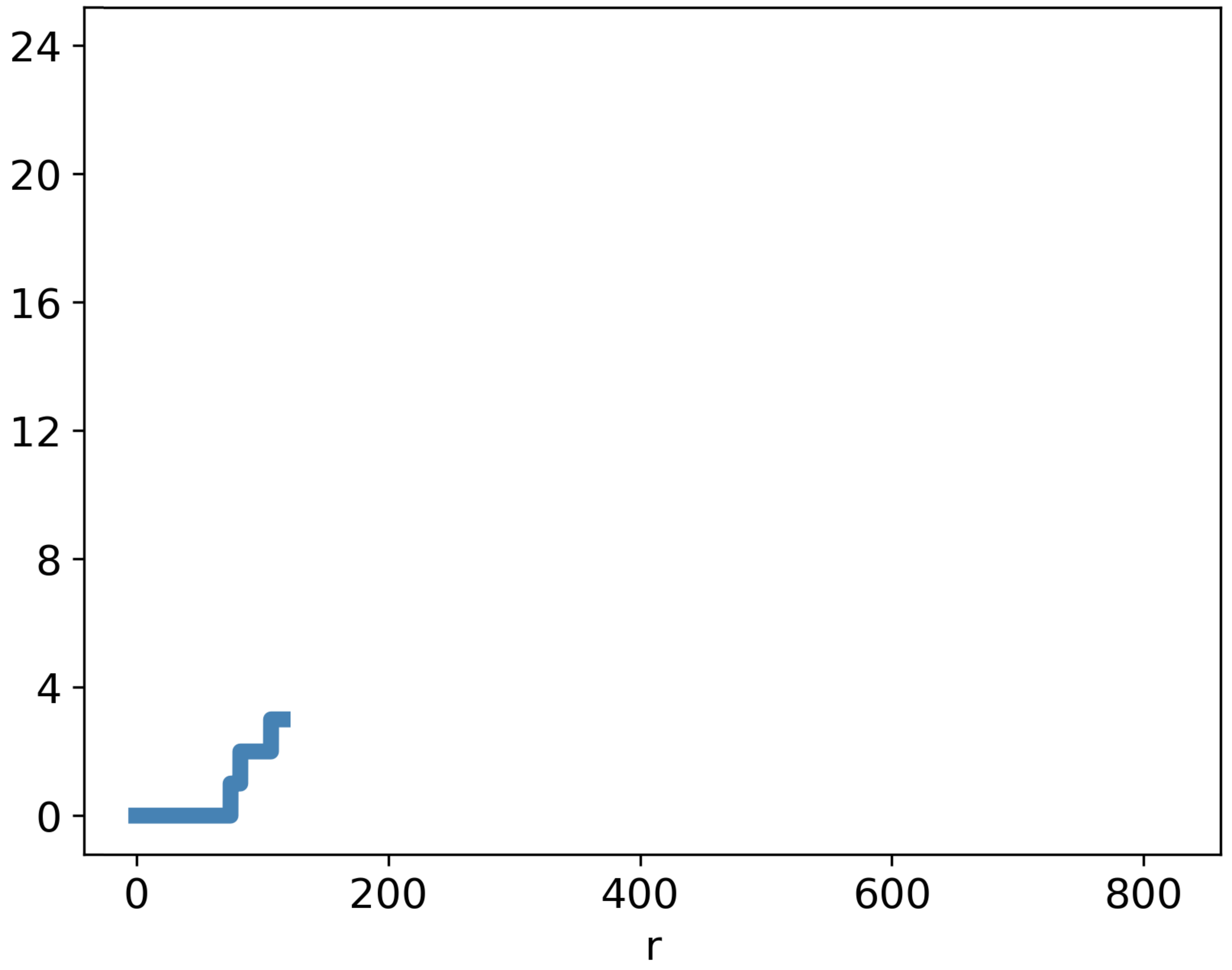




Ripley’s K function

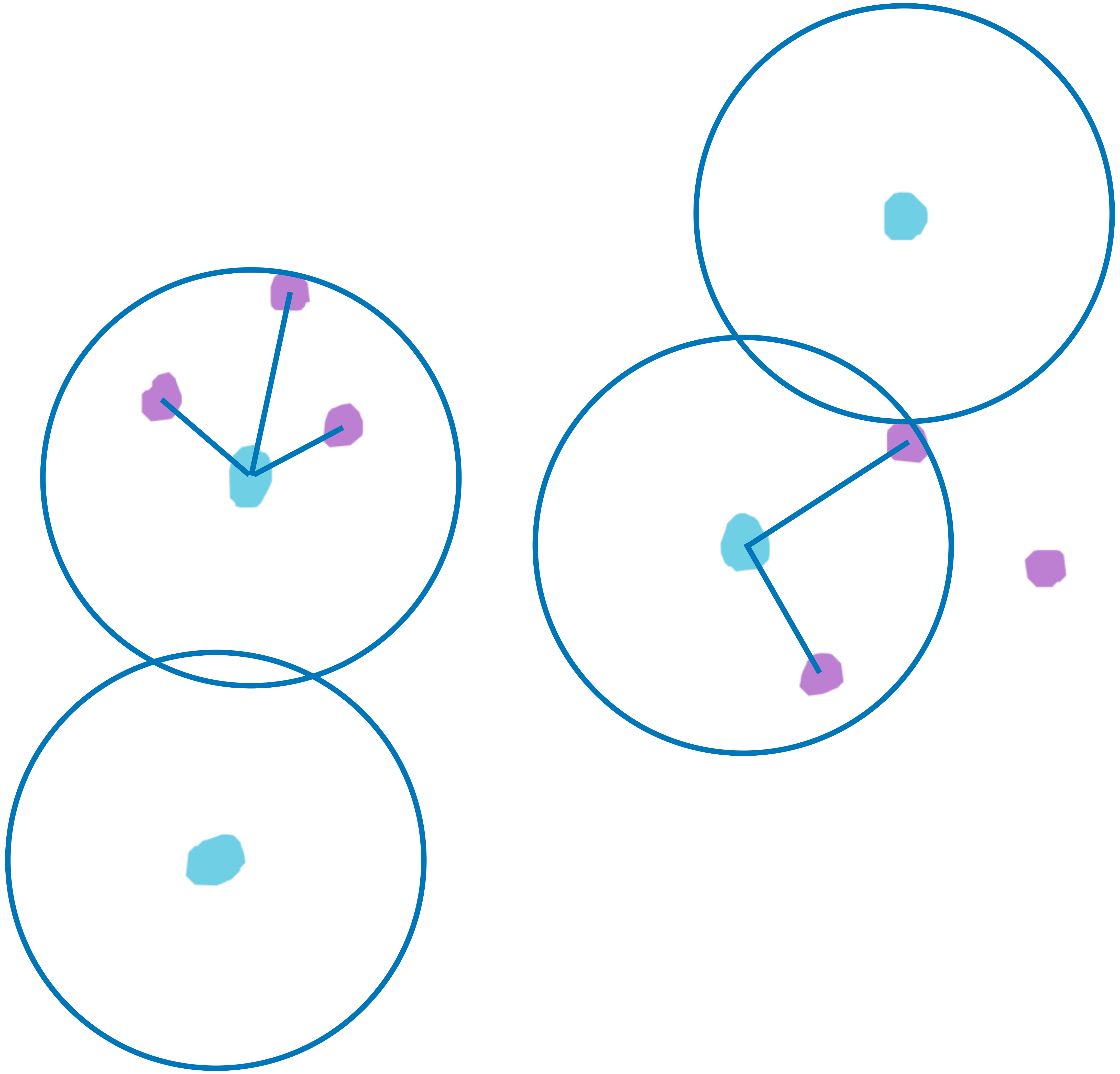
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$

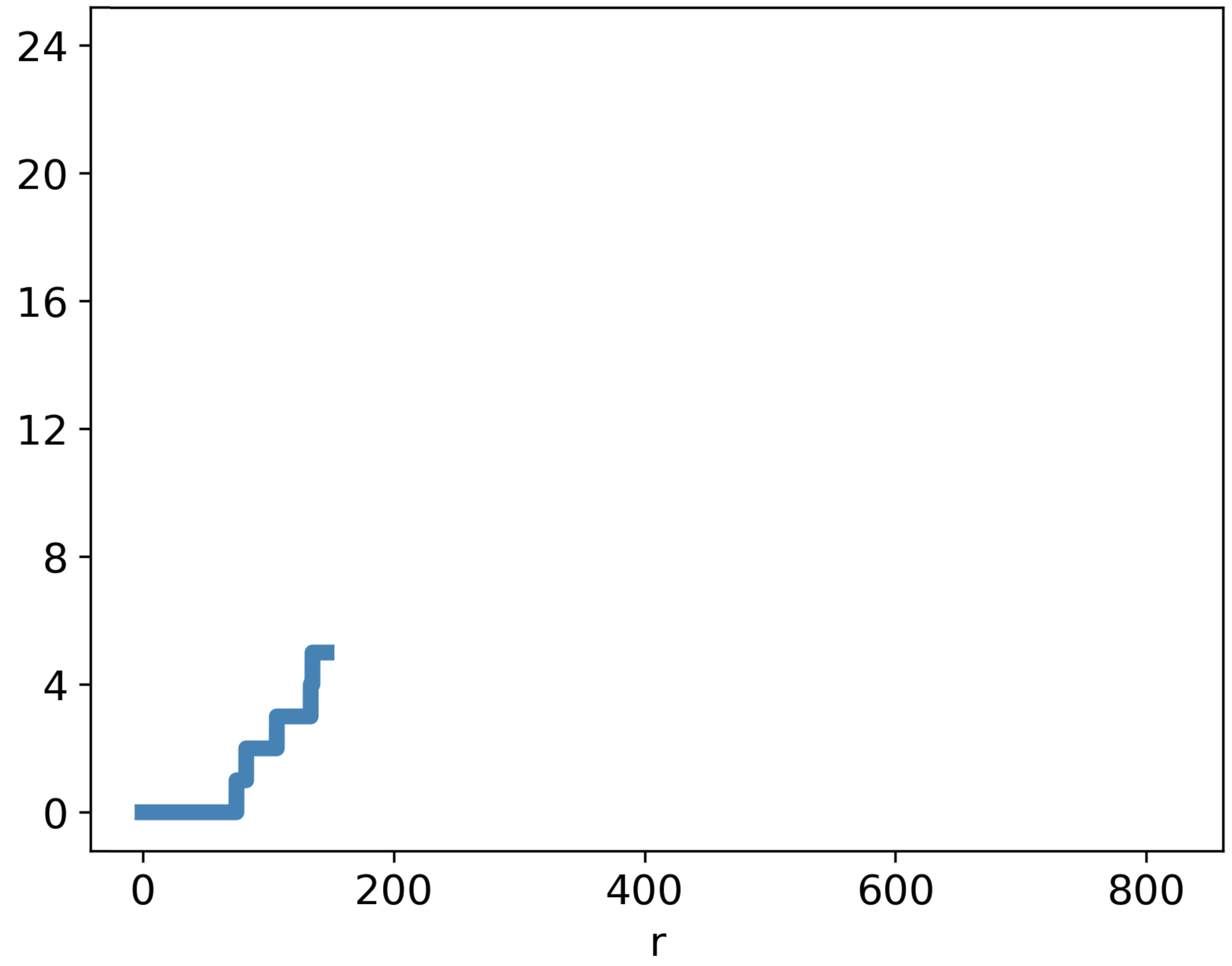




Ripley’s K function

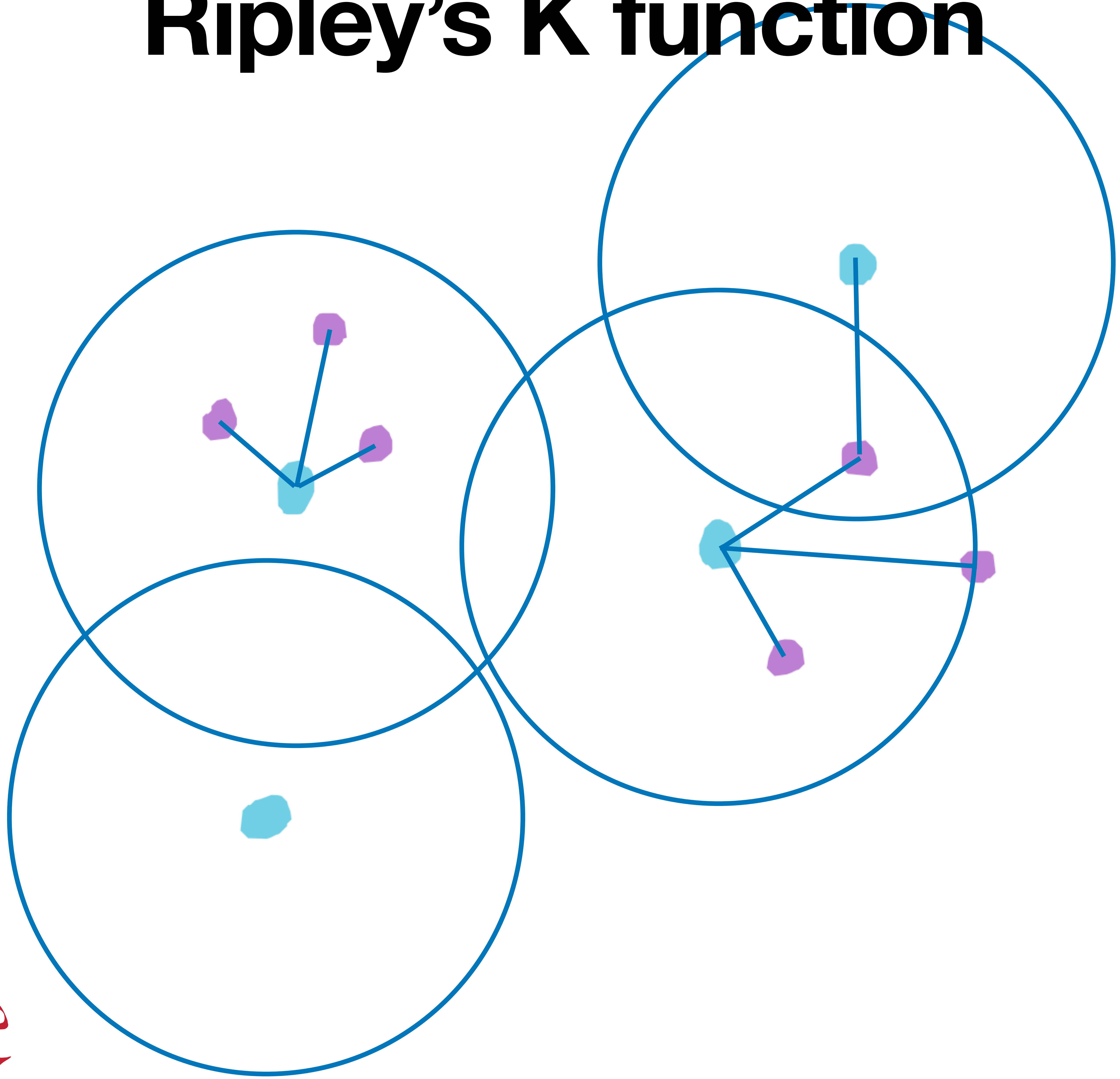
$$S(r) = \frac{|\Omega|}{n_1 n_2}\sum_{i=1}^{n_1}\sum_{j=1}^{n_2}\mathbf{1}(d_{ij} < r)b(i,j,r)$$

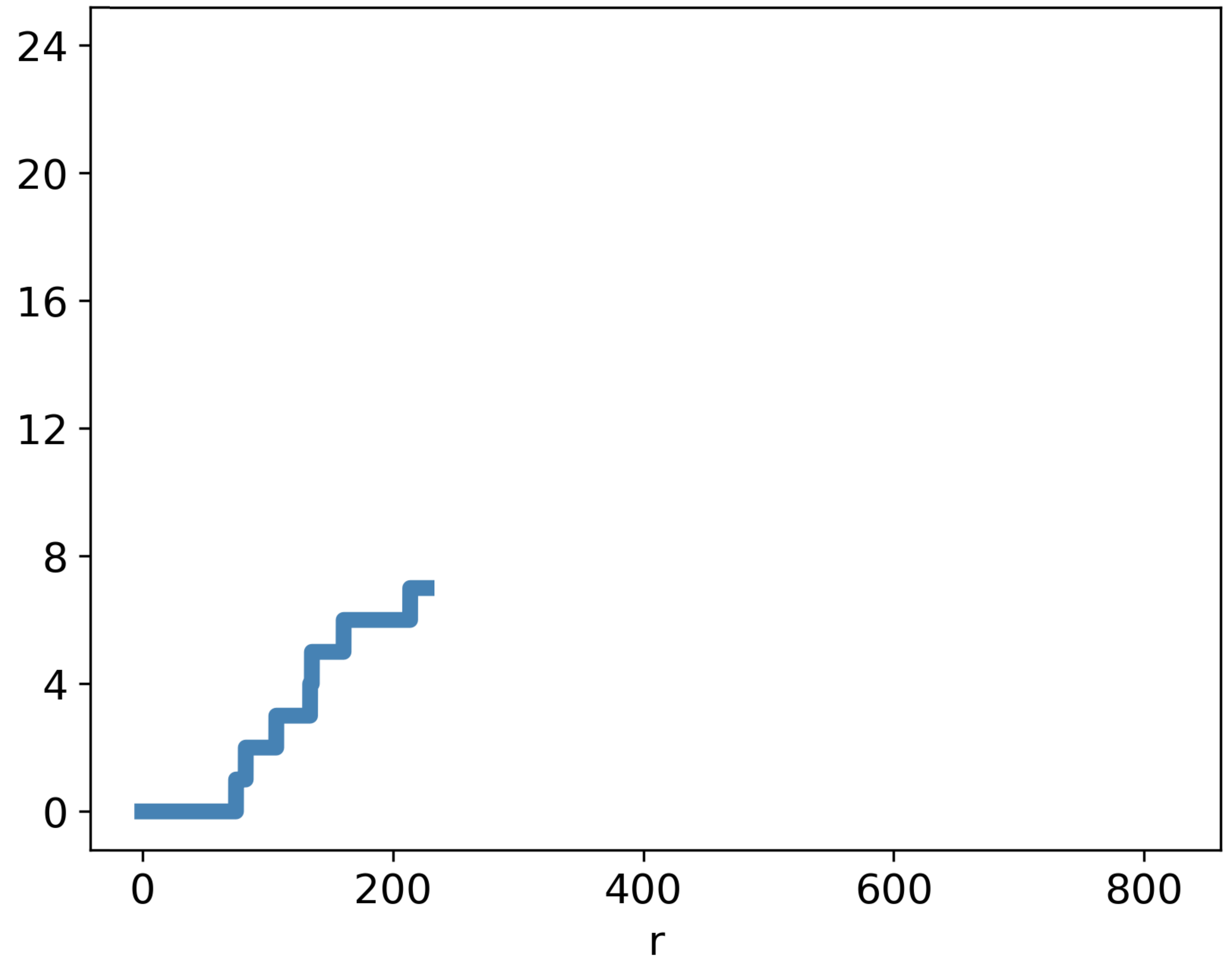




Ripley’s K function

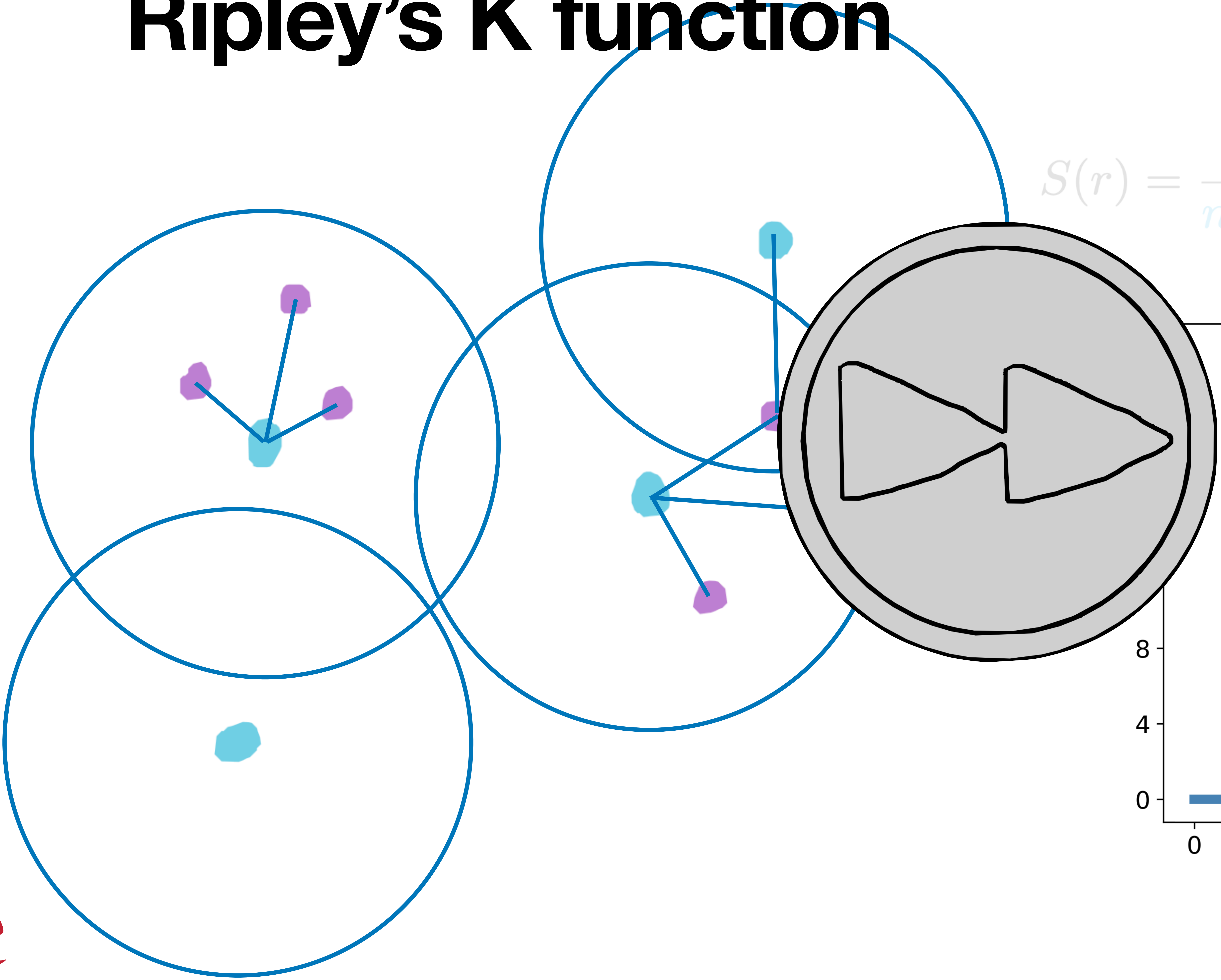
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$

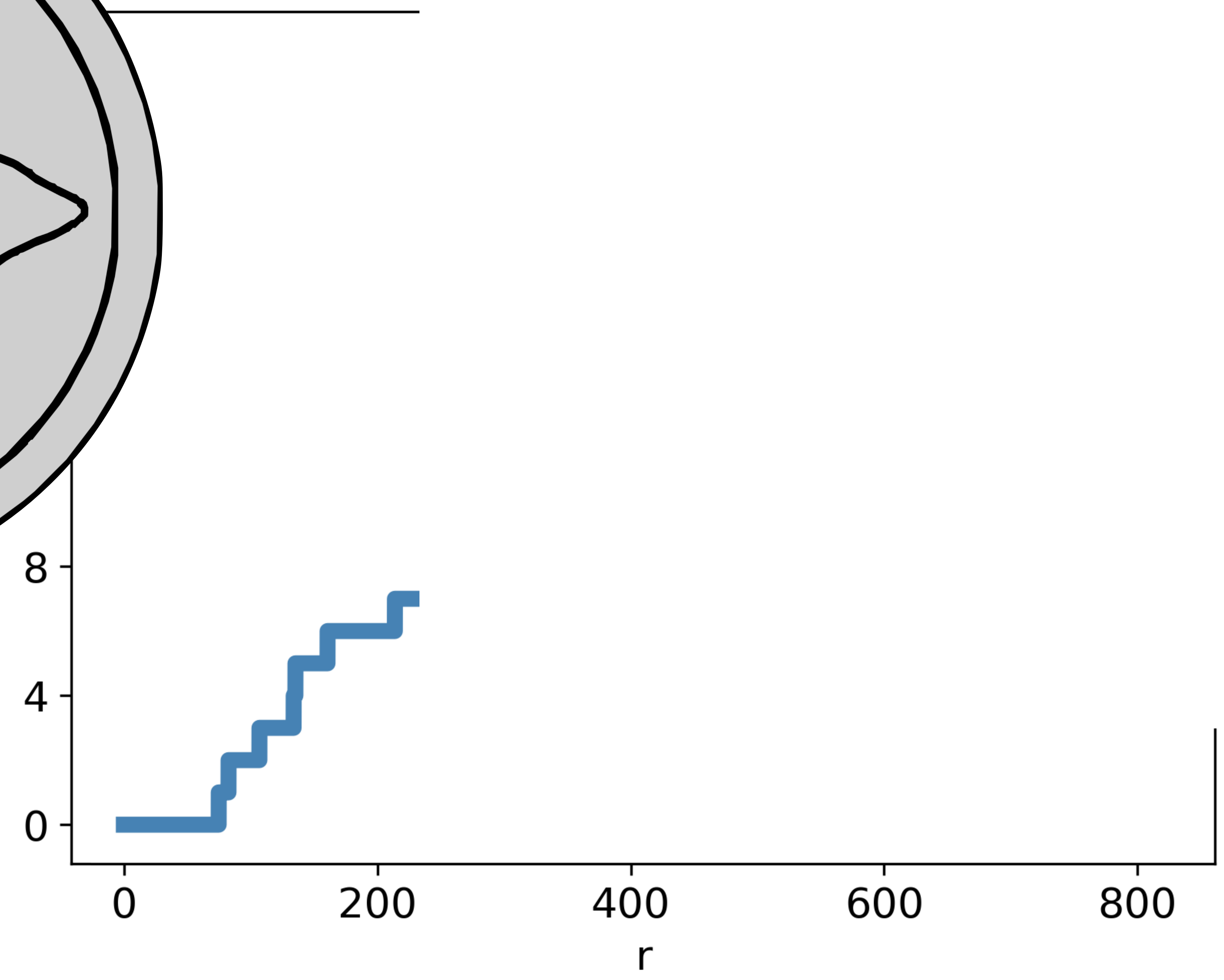




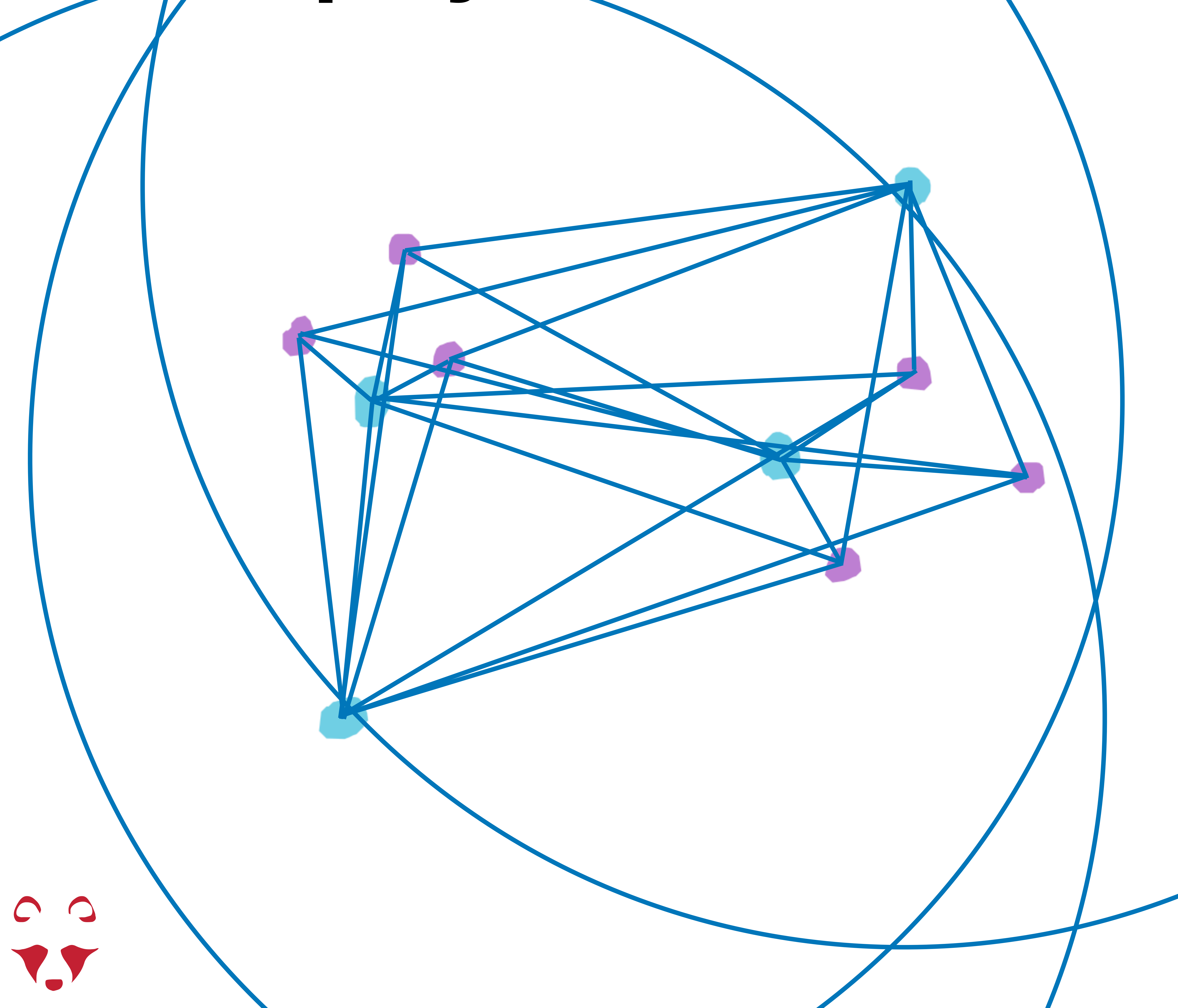
Ripley's K function

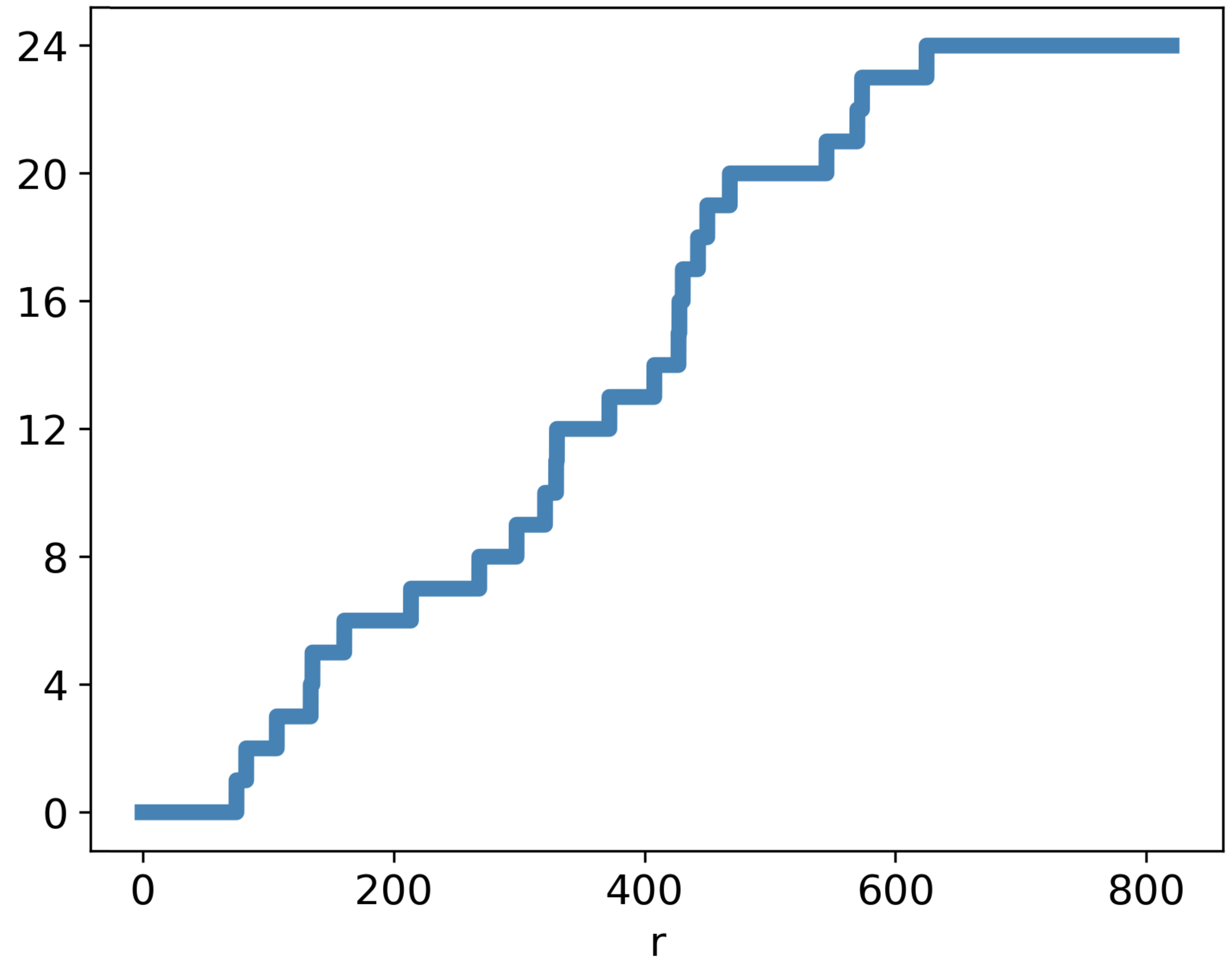
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$





Ripley's K function

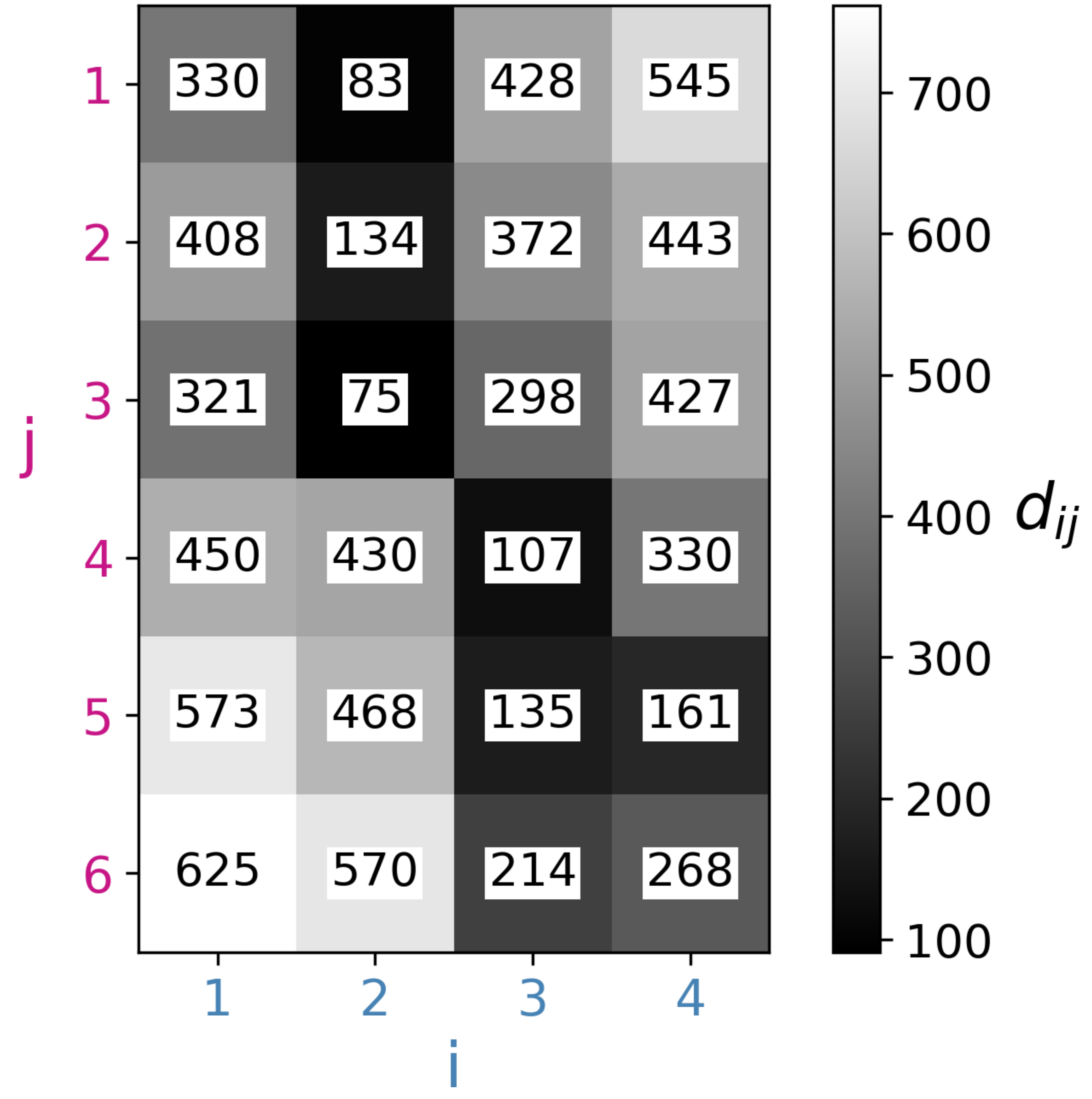


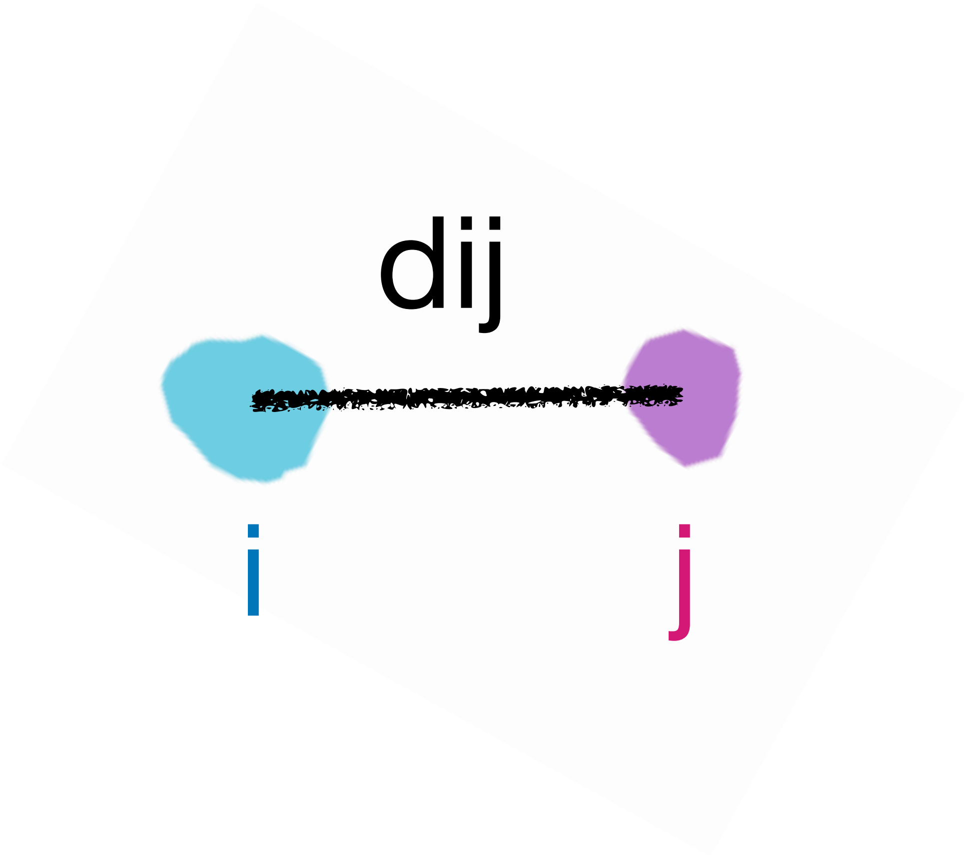


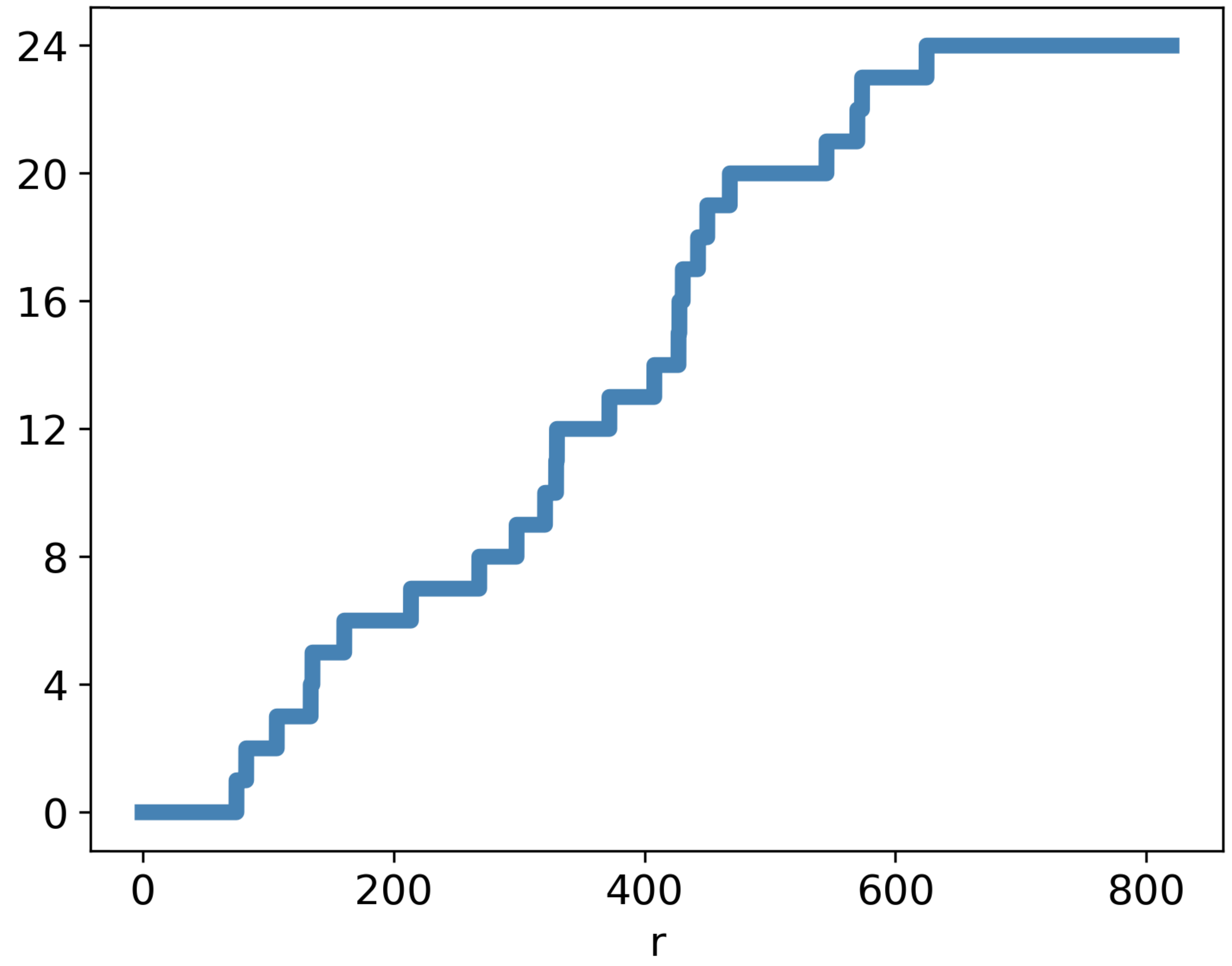
Ripley’s K function

Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r)\, b(i, j, r)$$



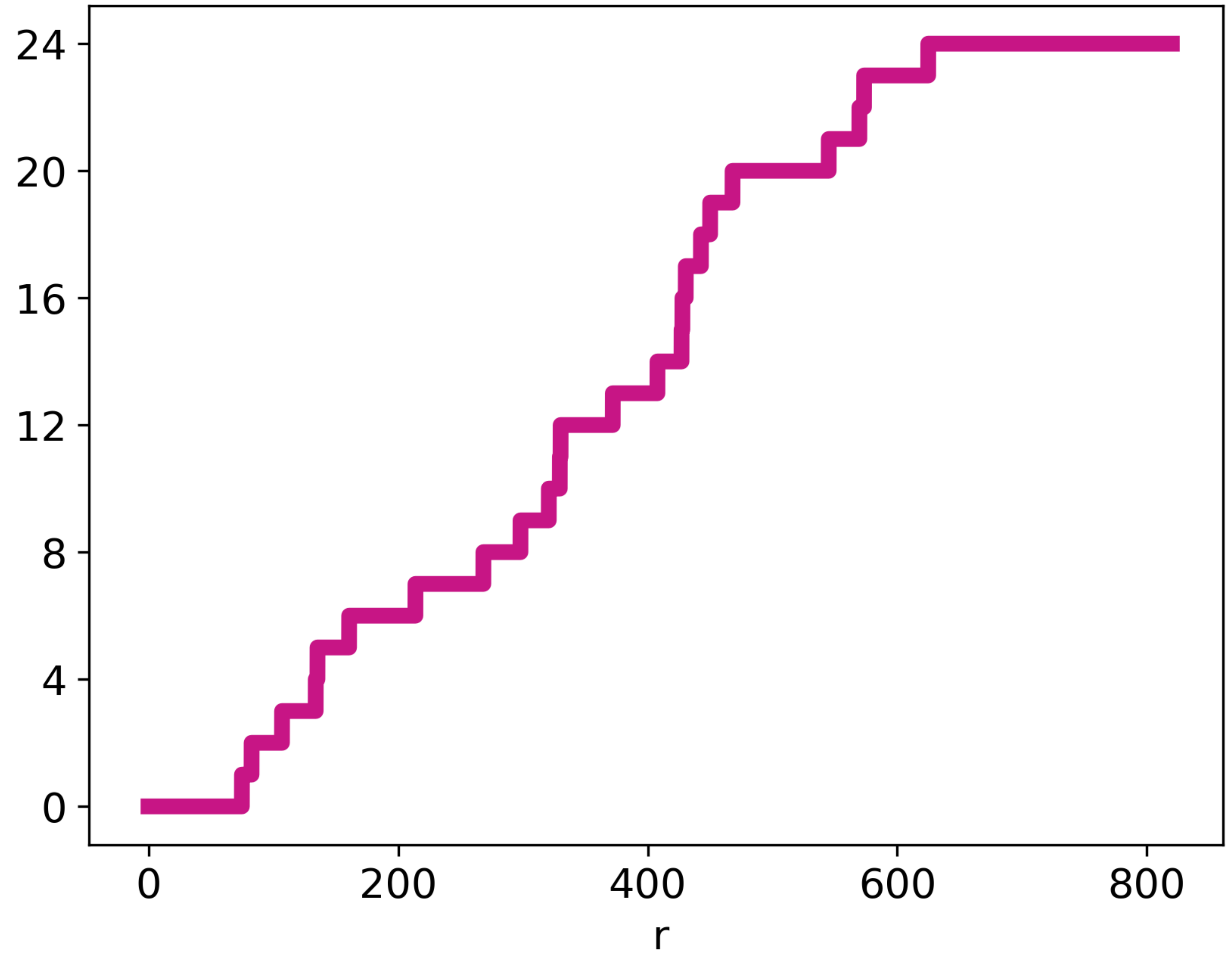




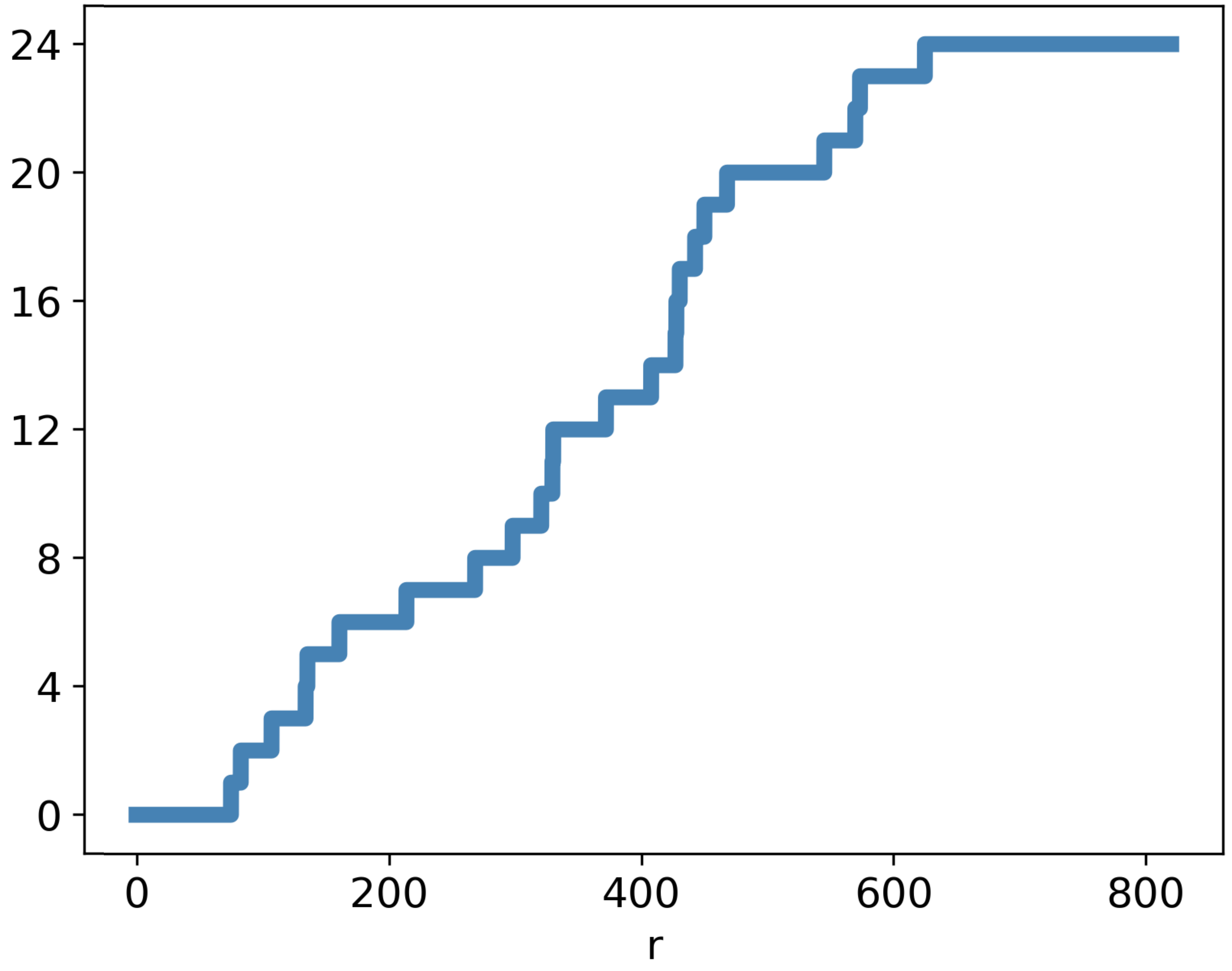
Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r)\, b(i, j, r)$$

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r)\, b(i, j, r)$$

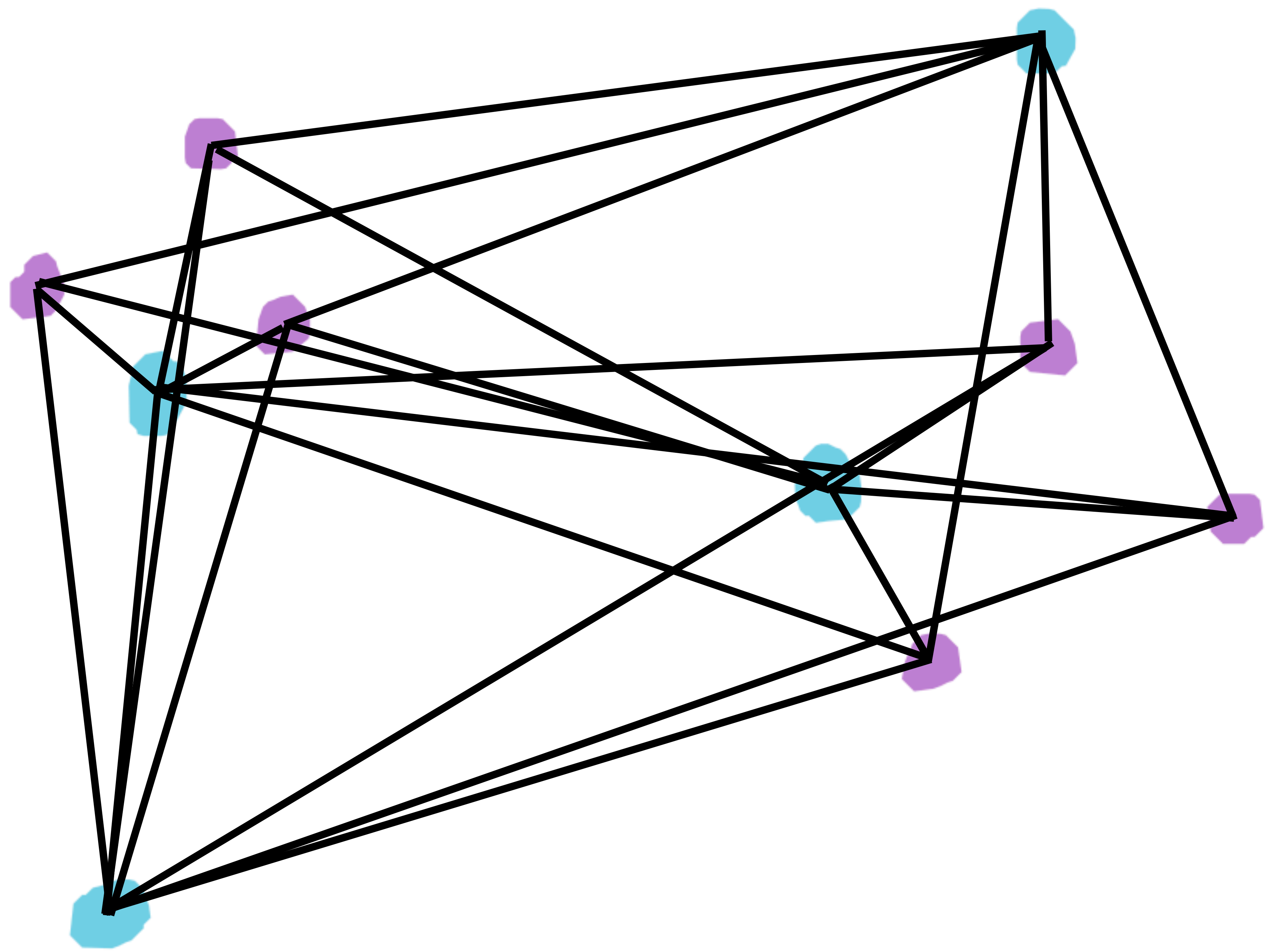


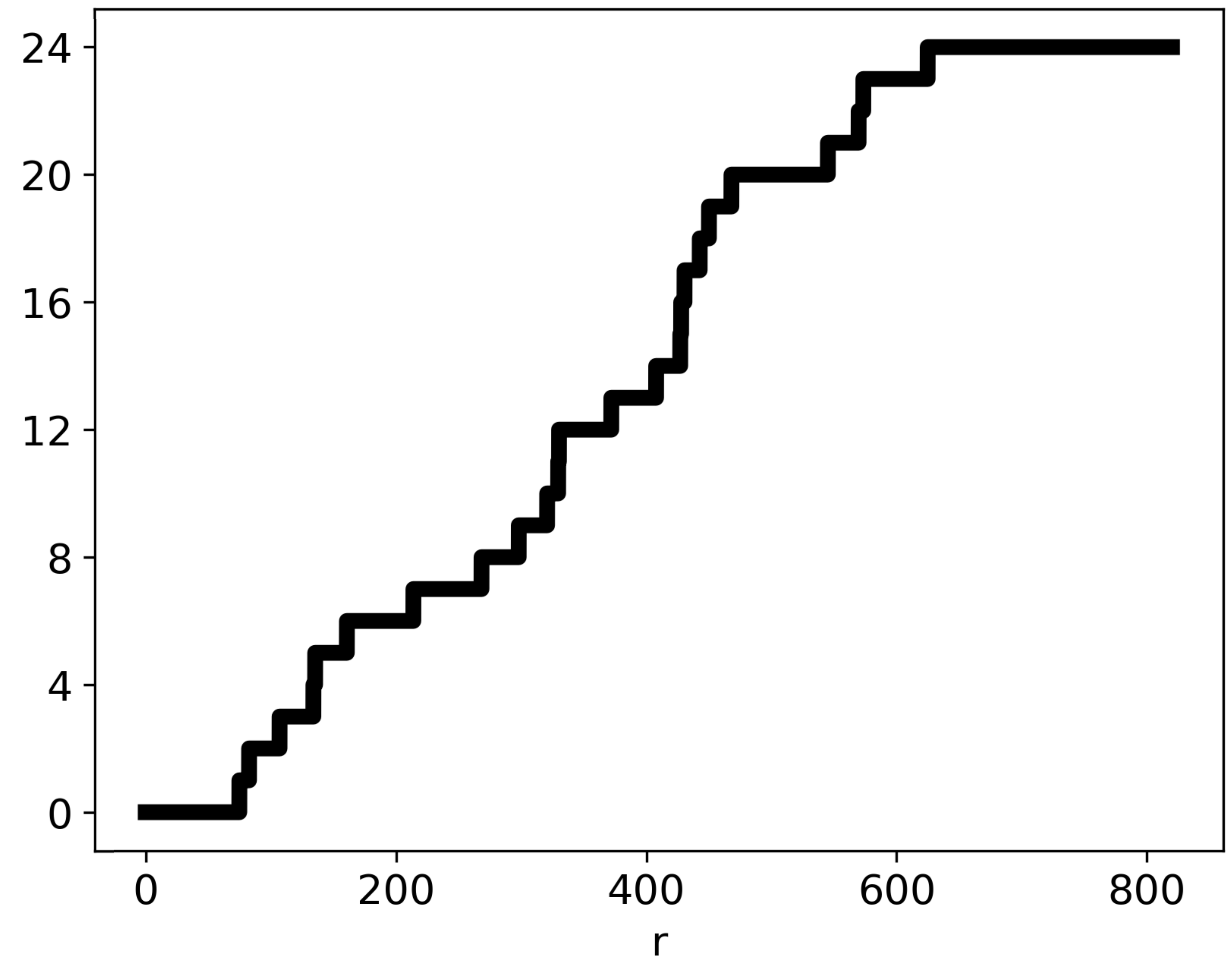
=



Ripley's K function

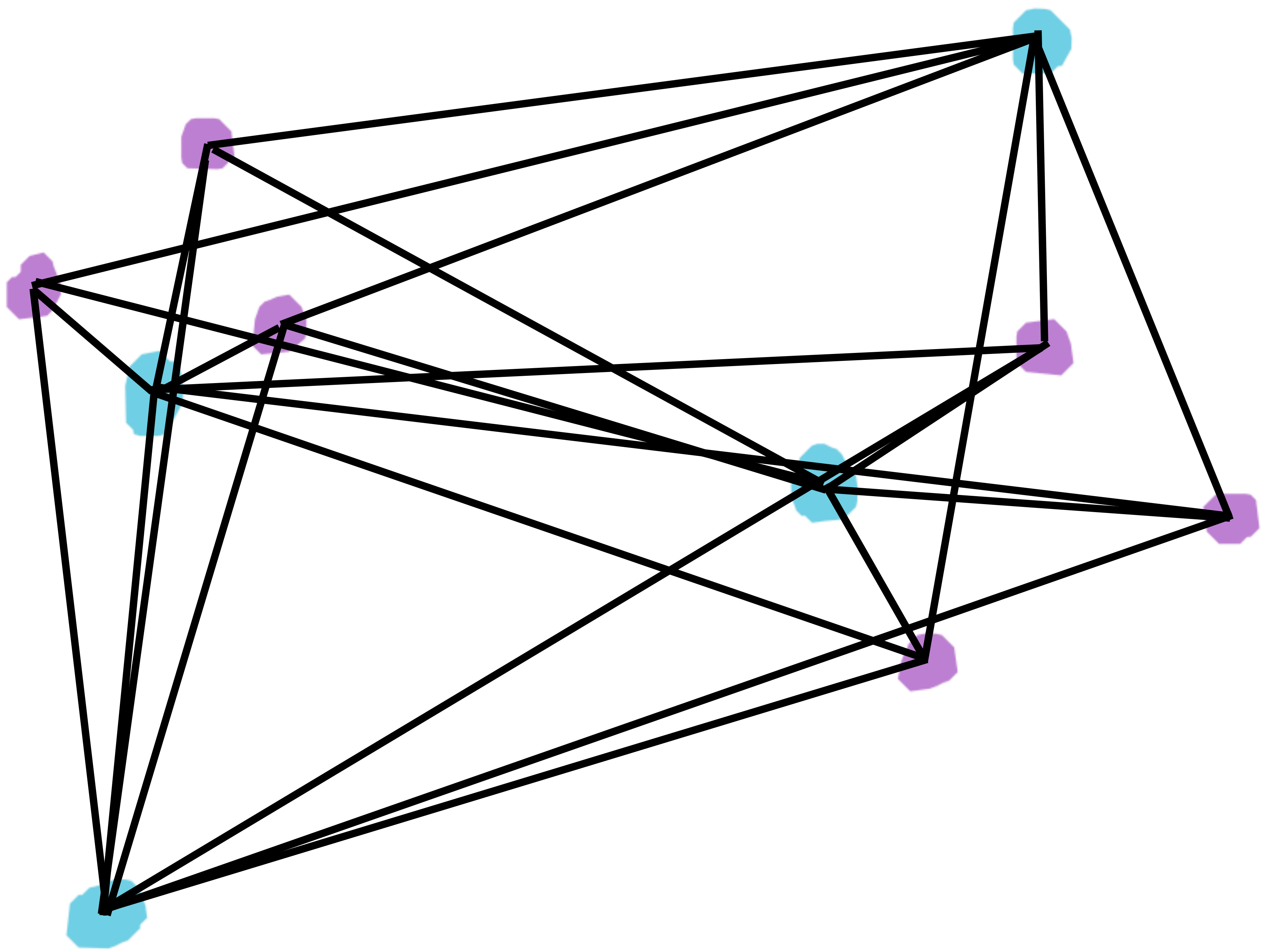
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i,j,r)$$

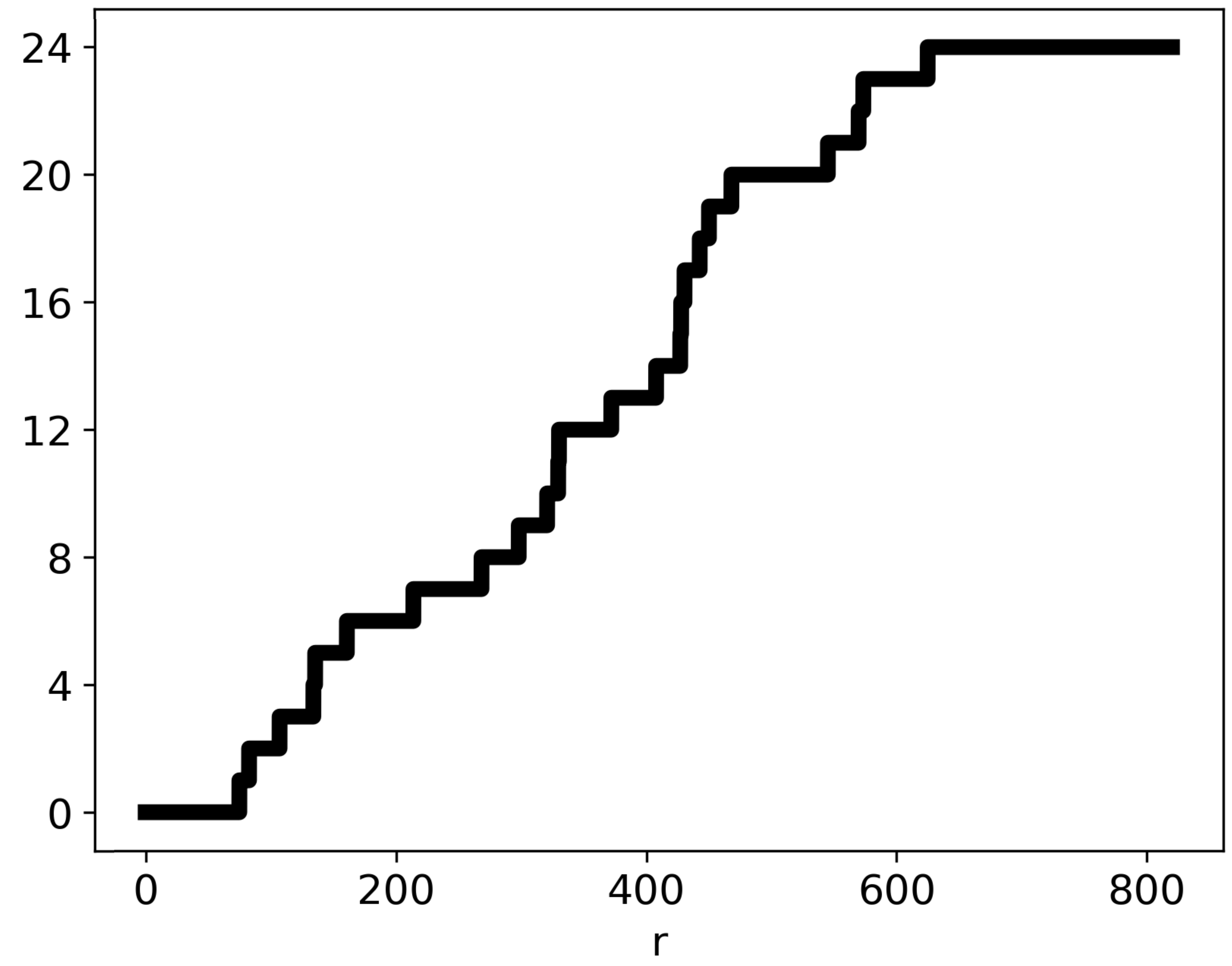




Ripley's K function

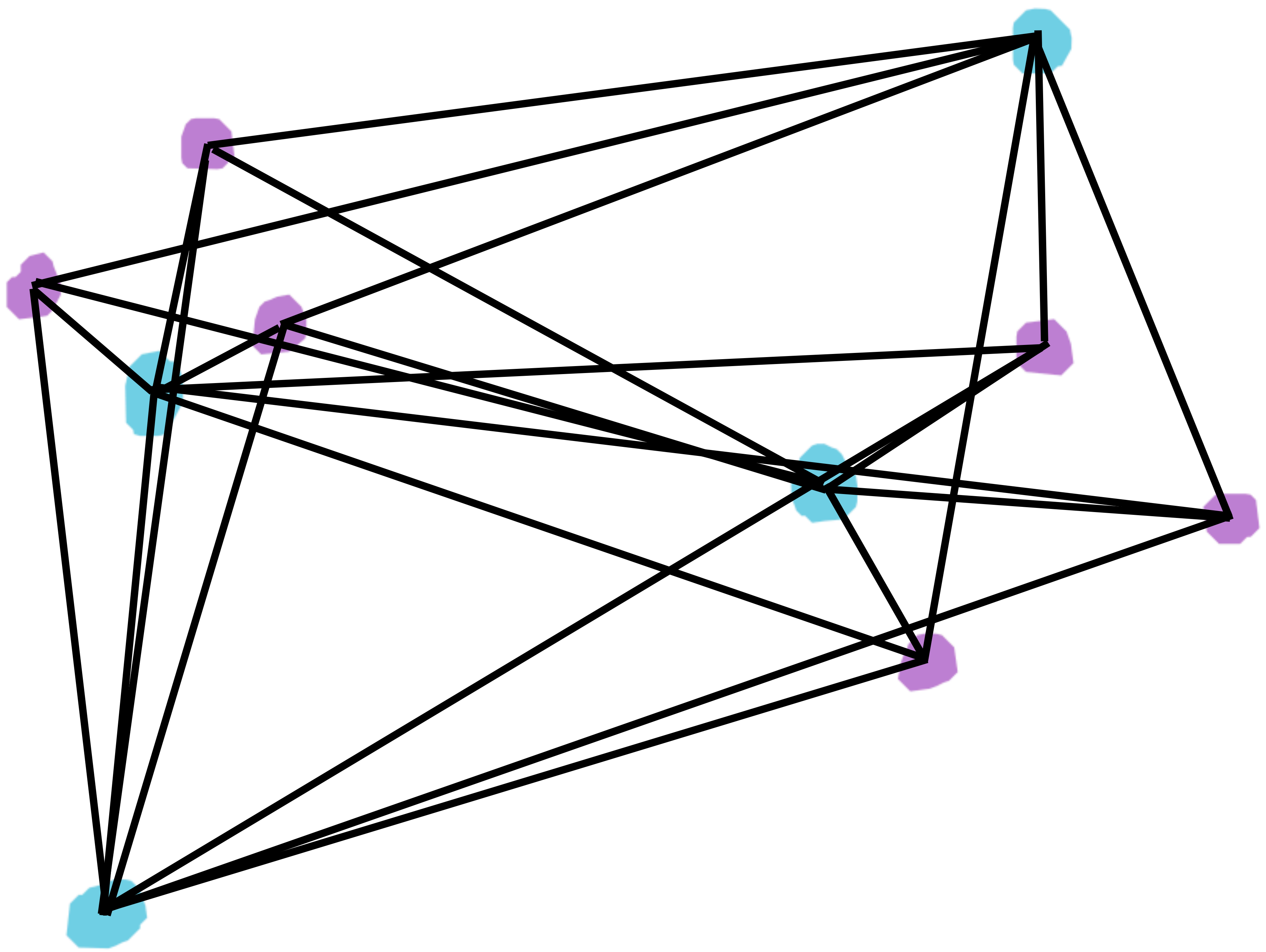
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i,j,r)$$

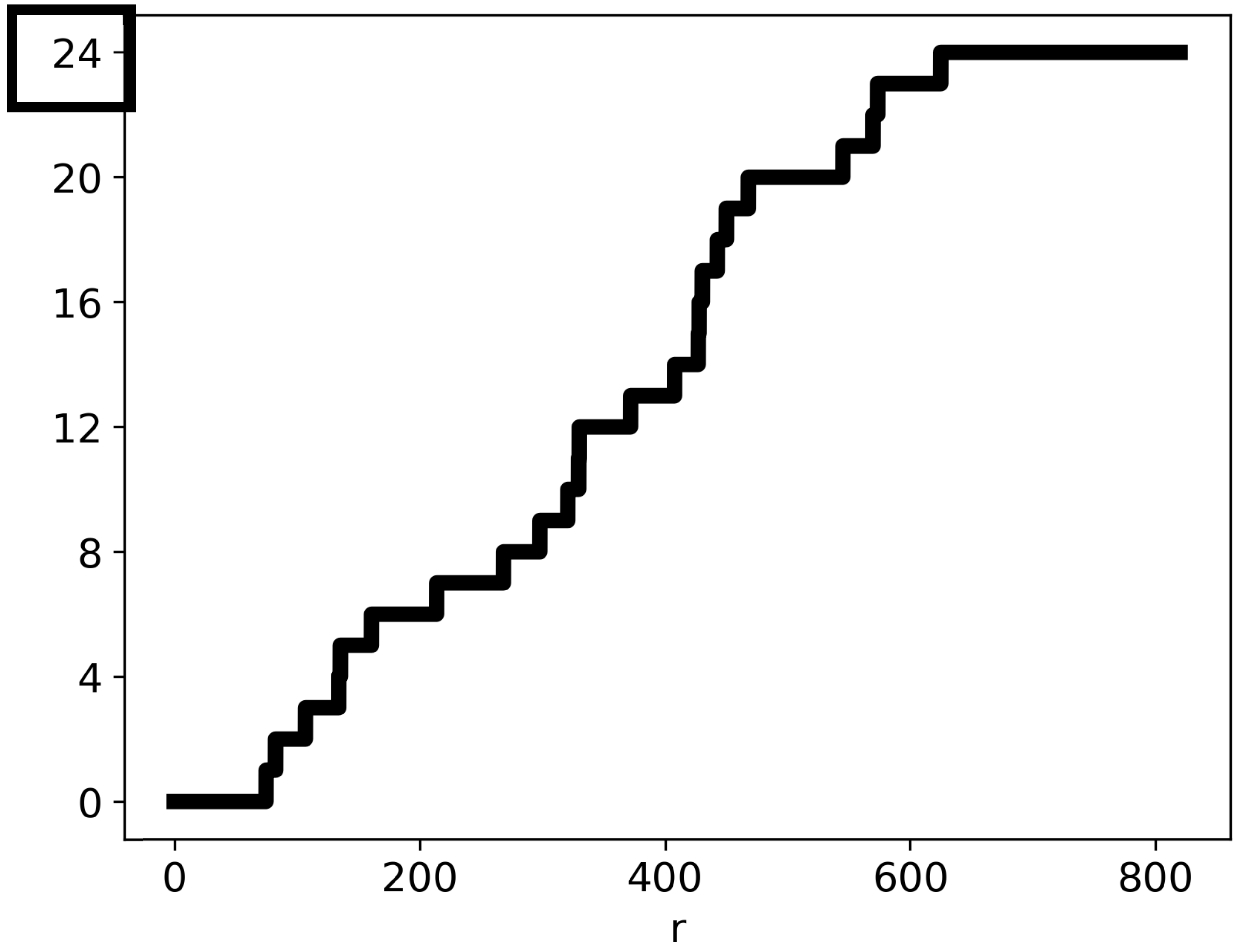




Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i,j,r)$$

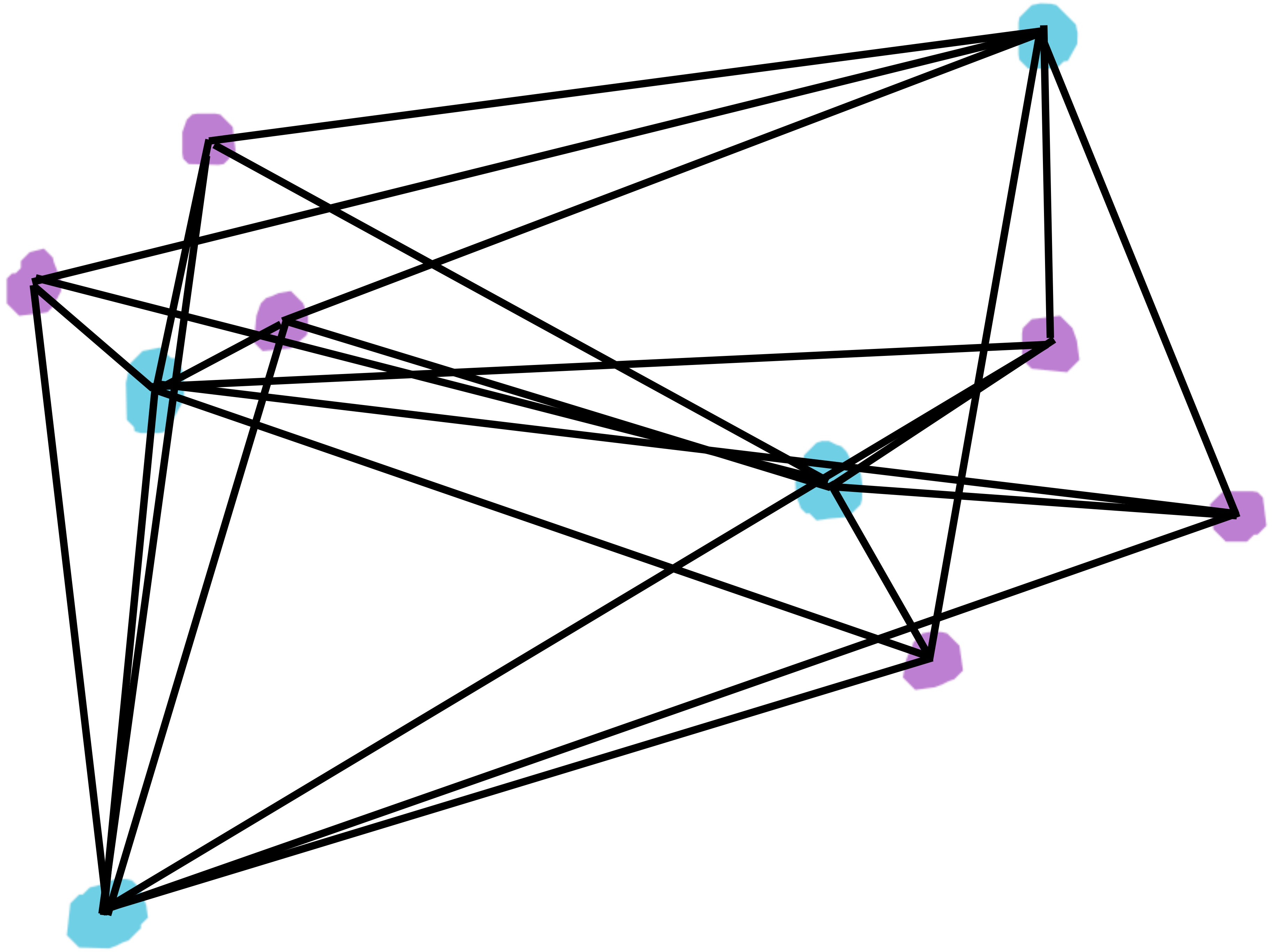


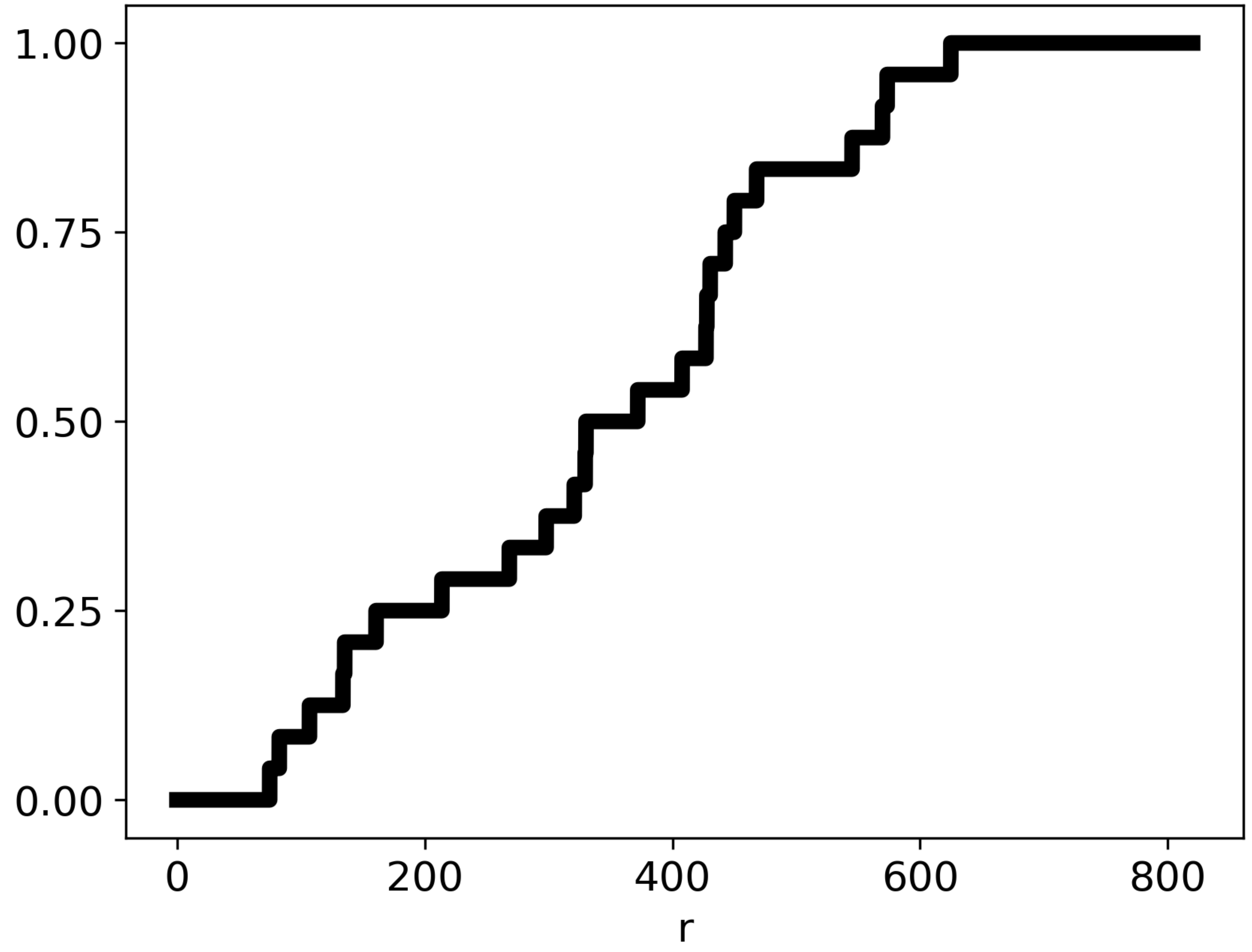


$$n_1 n_2 = \text{n connections} = 24$$

Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$

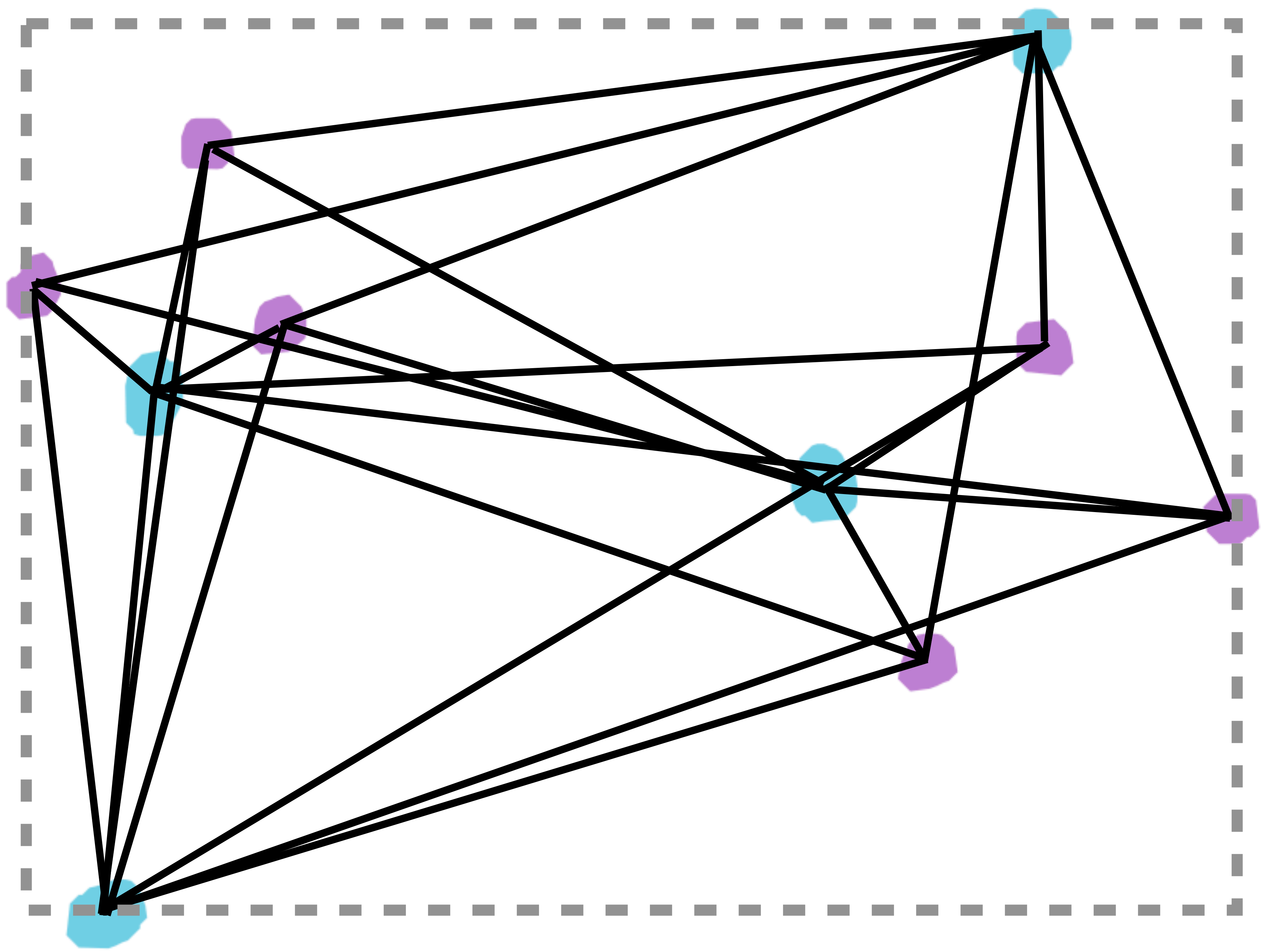




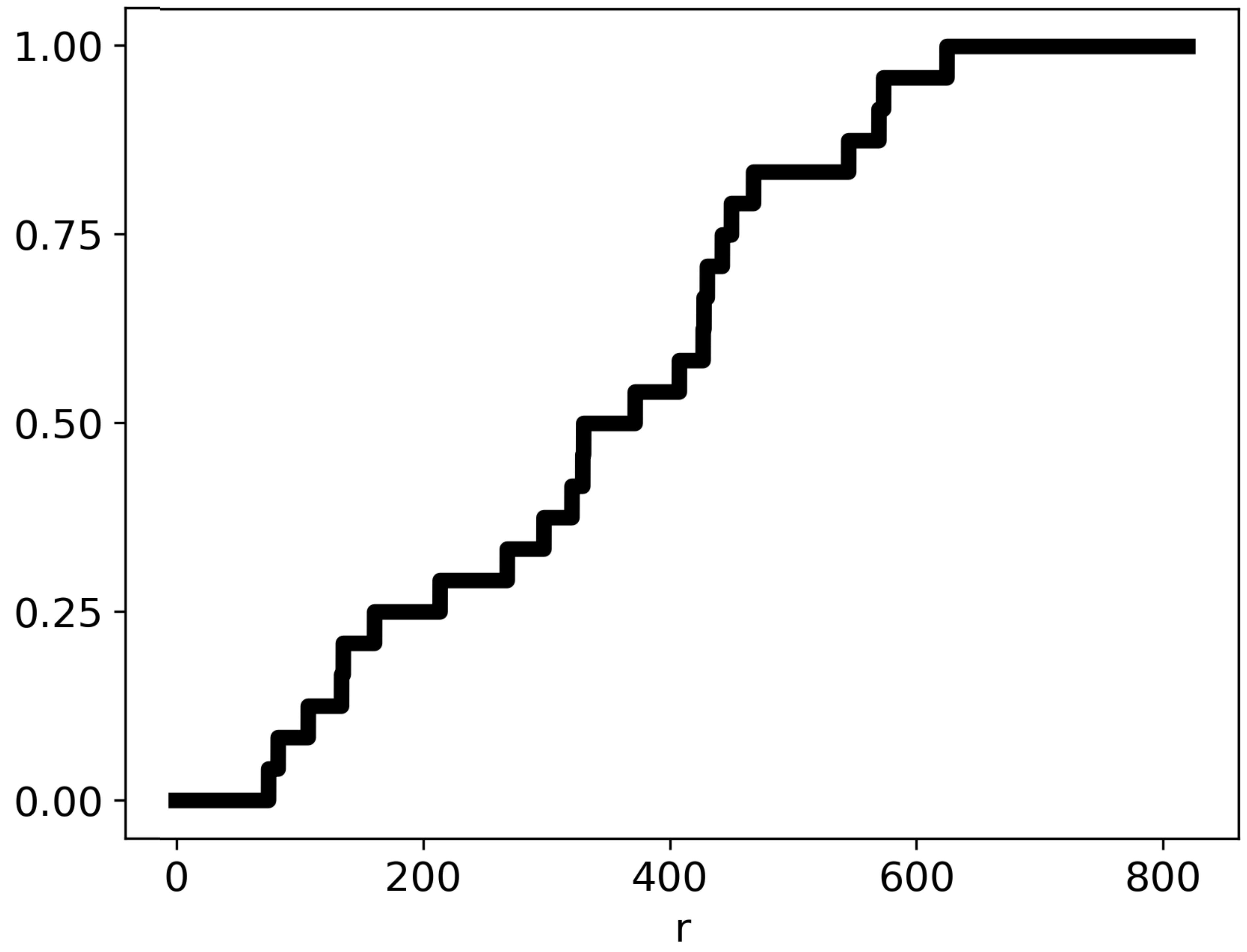
$$n_1 n_2 = \text{n connections} = 24$$

Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i,j,r)$$

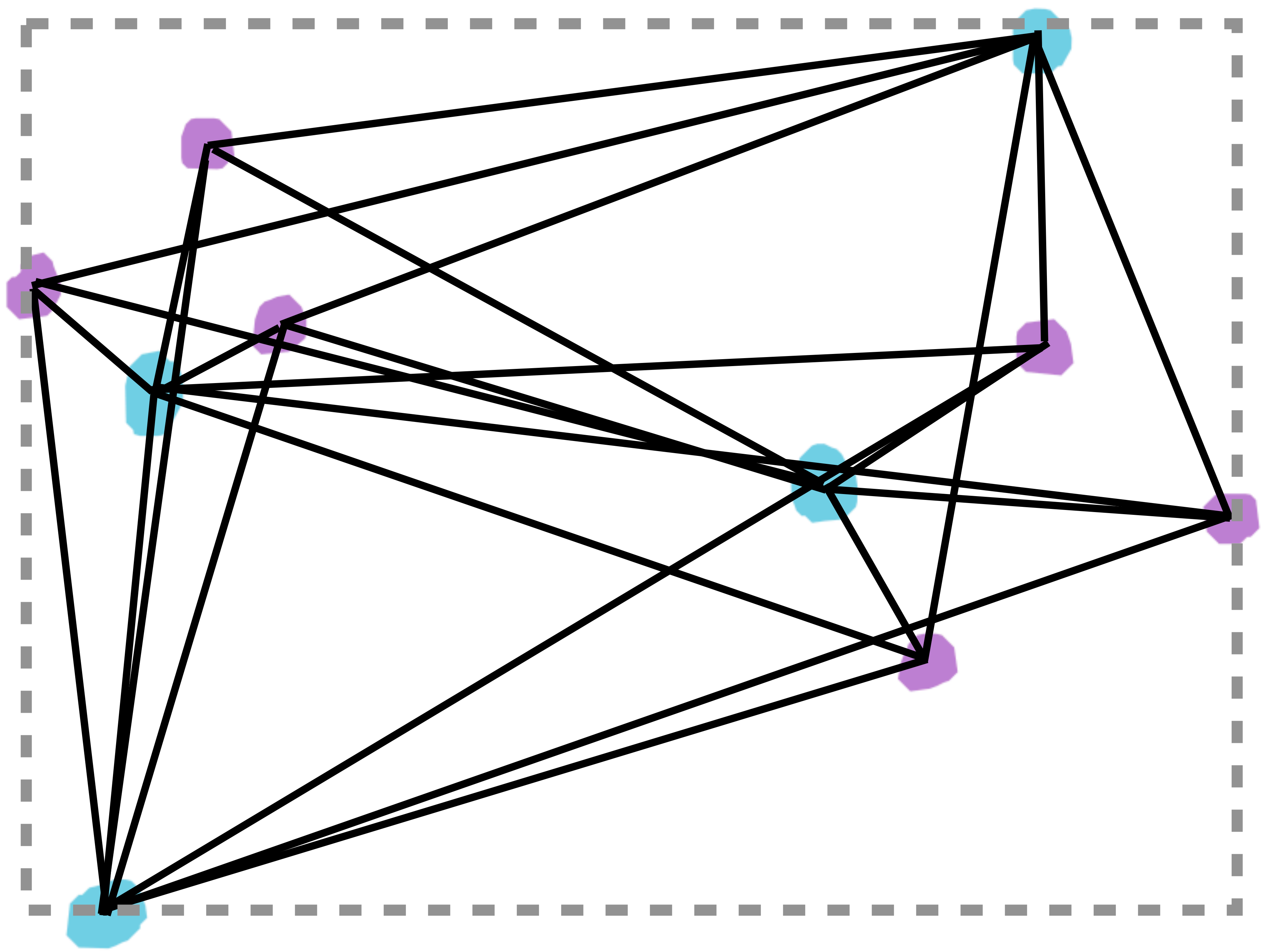


$$|\Omega| = \text{Area of FOV}$$

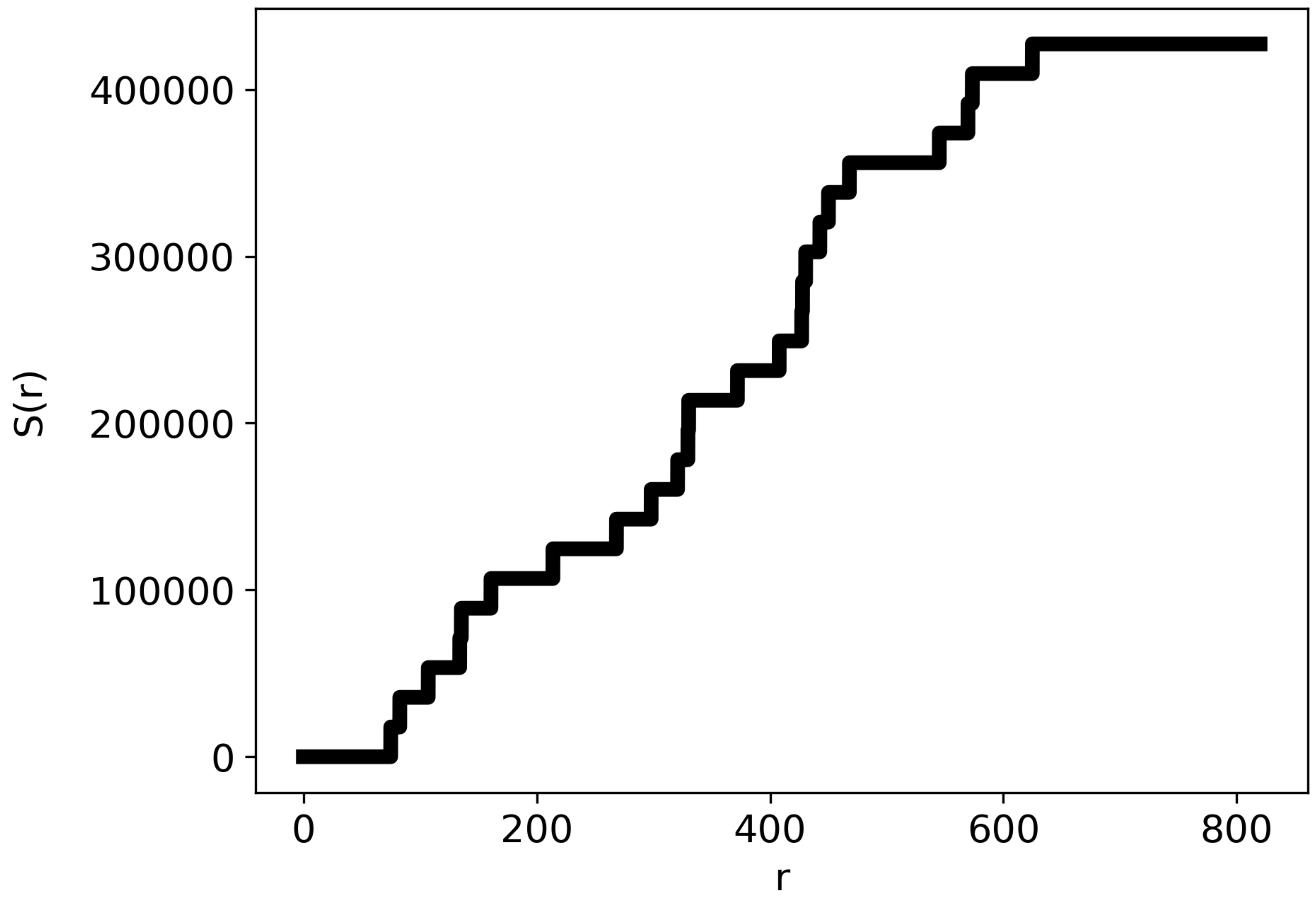


Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i,j,r)$$



$$|\Omega| = \text{Area of FOV}$$

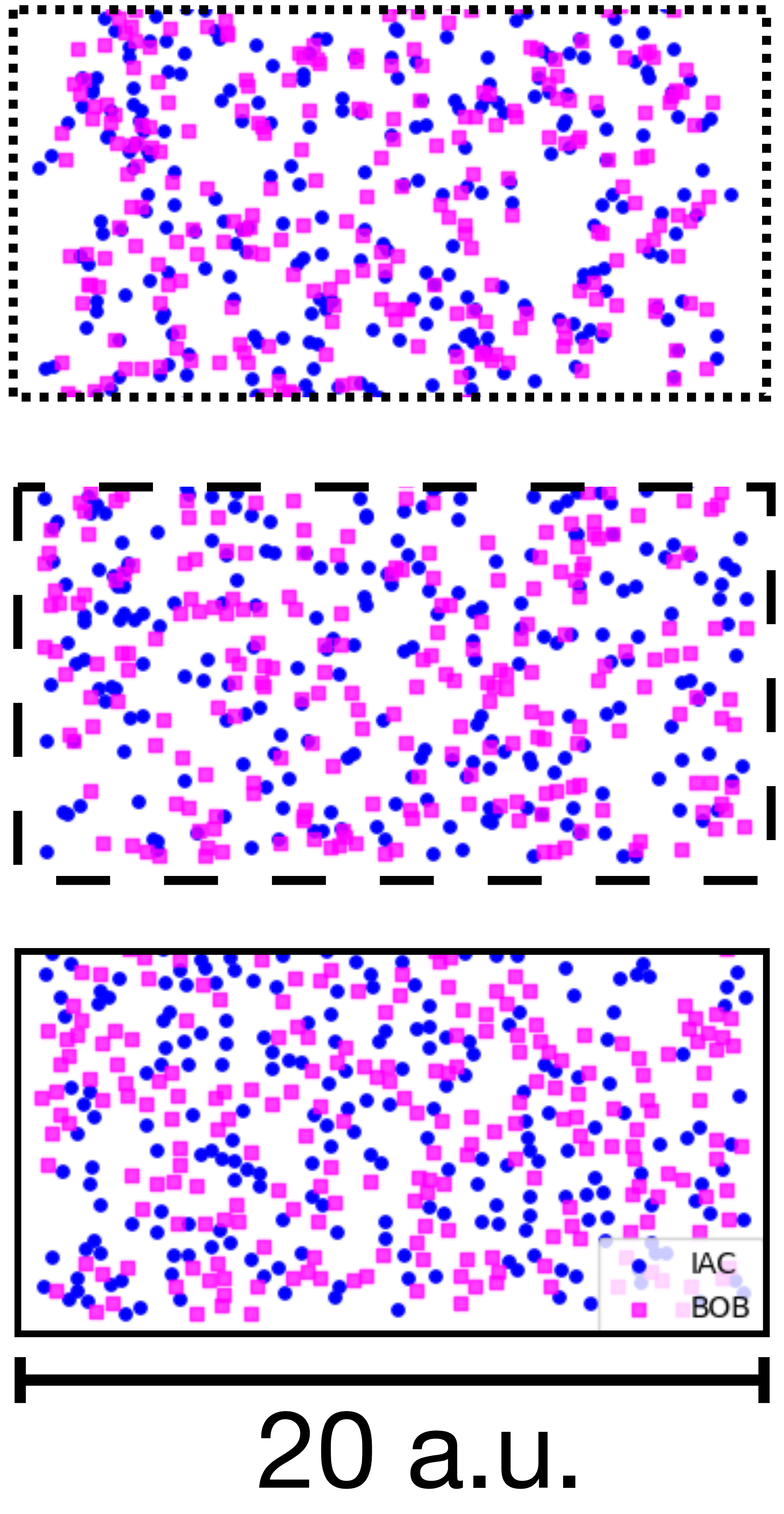


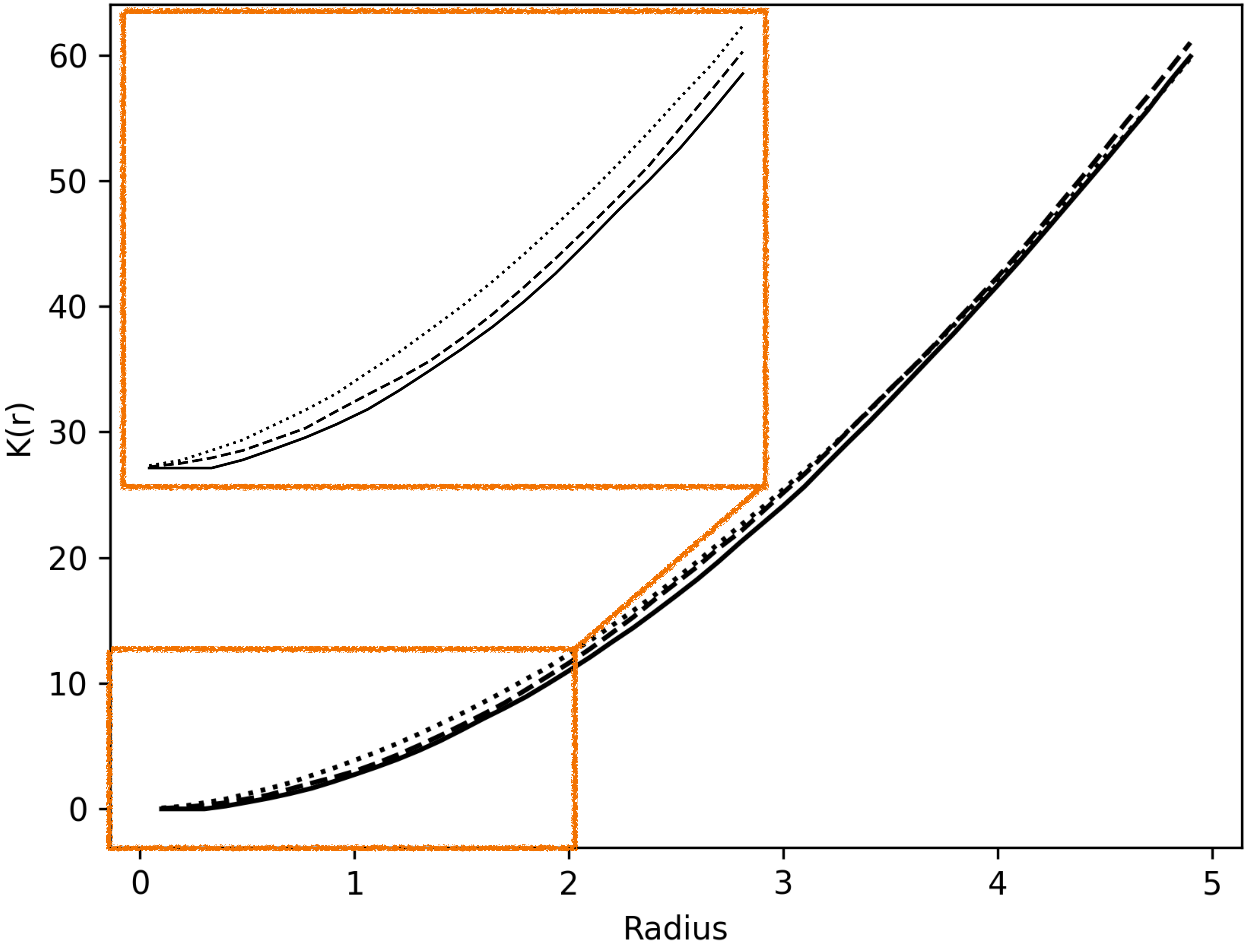
Results: Ripley's K function

Fall — cold

Fall — medium

Fall — warm



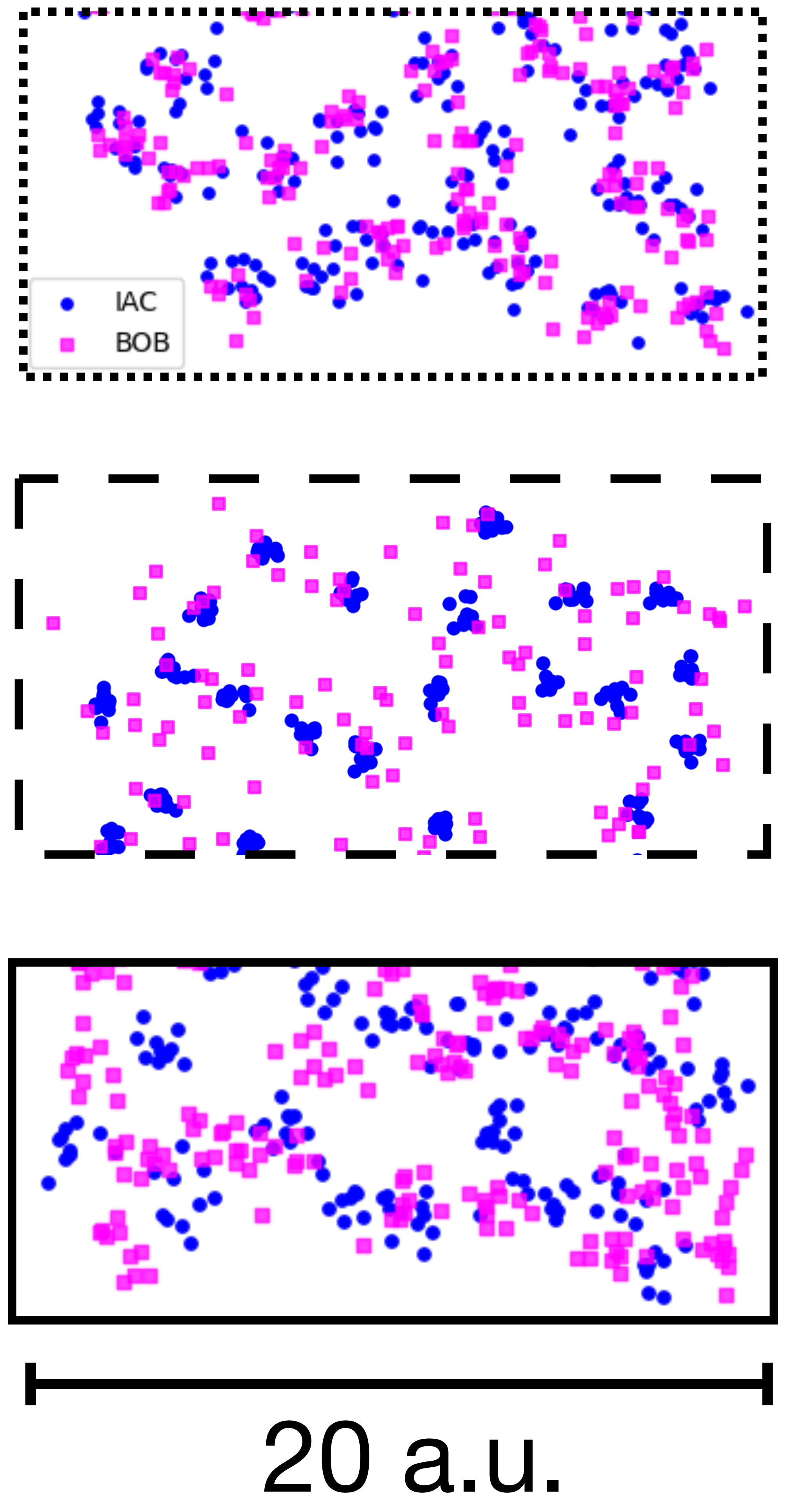


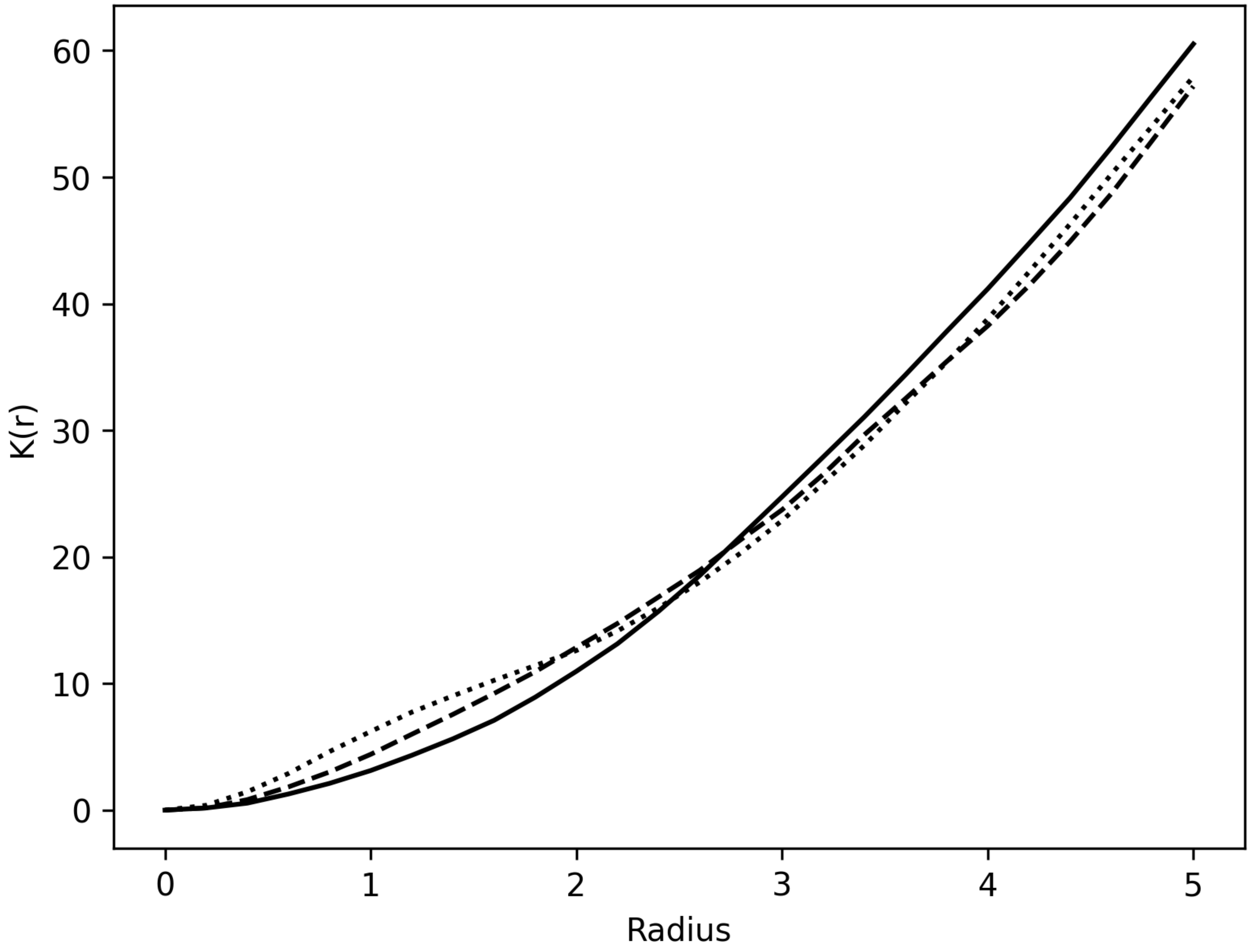
Results: Ripley’s K function

Winter — cold

Winter — medium

Winter — warm



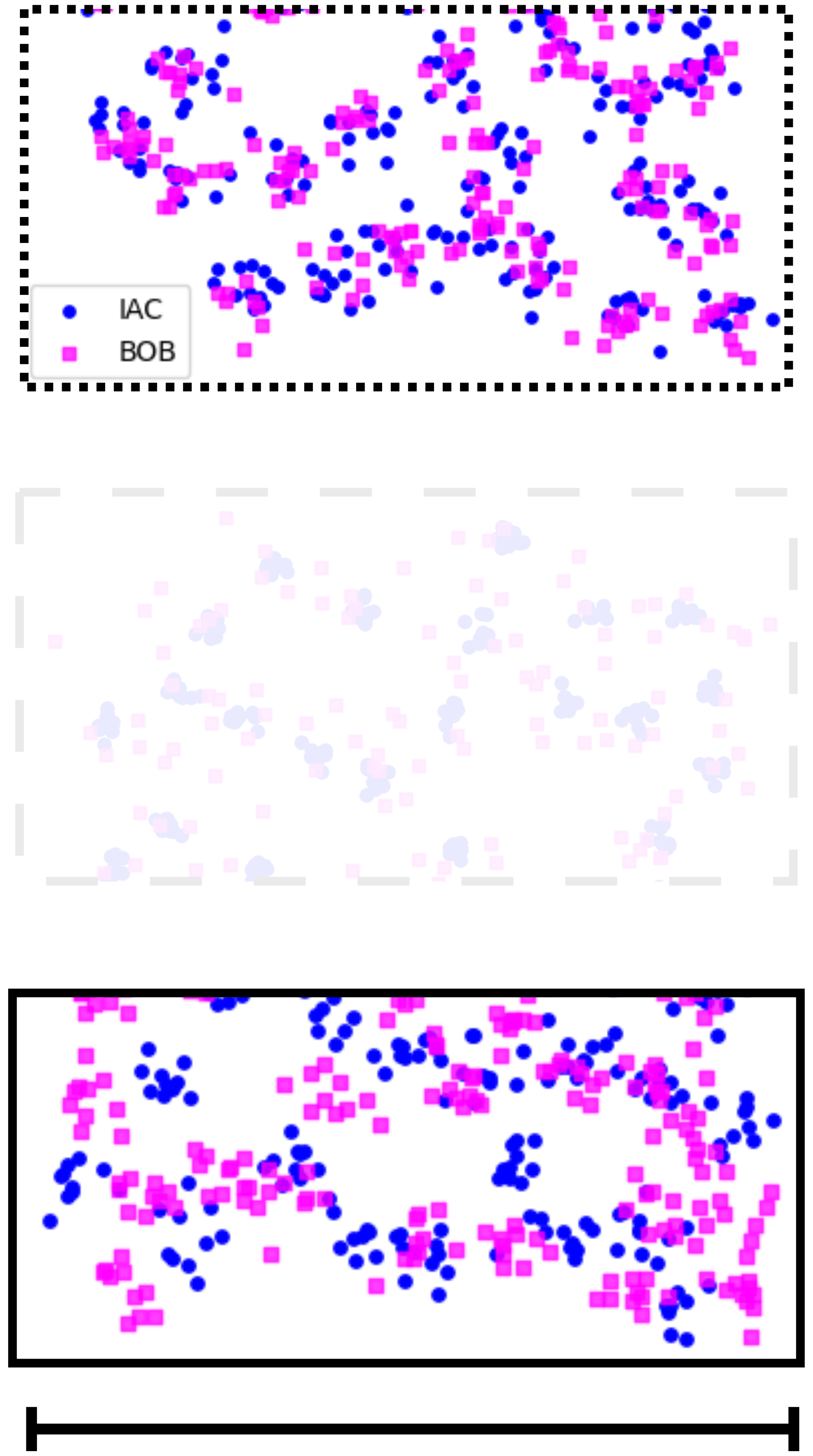


Results: Ripley’s K function

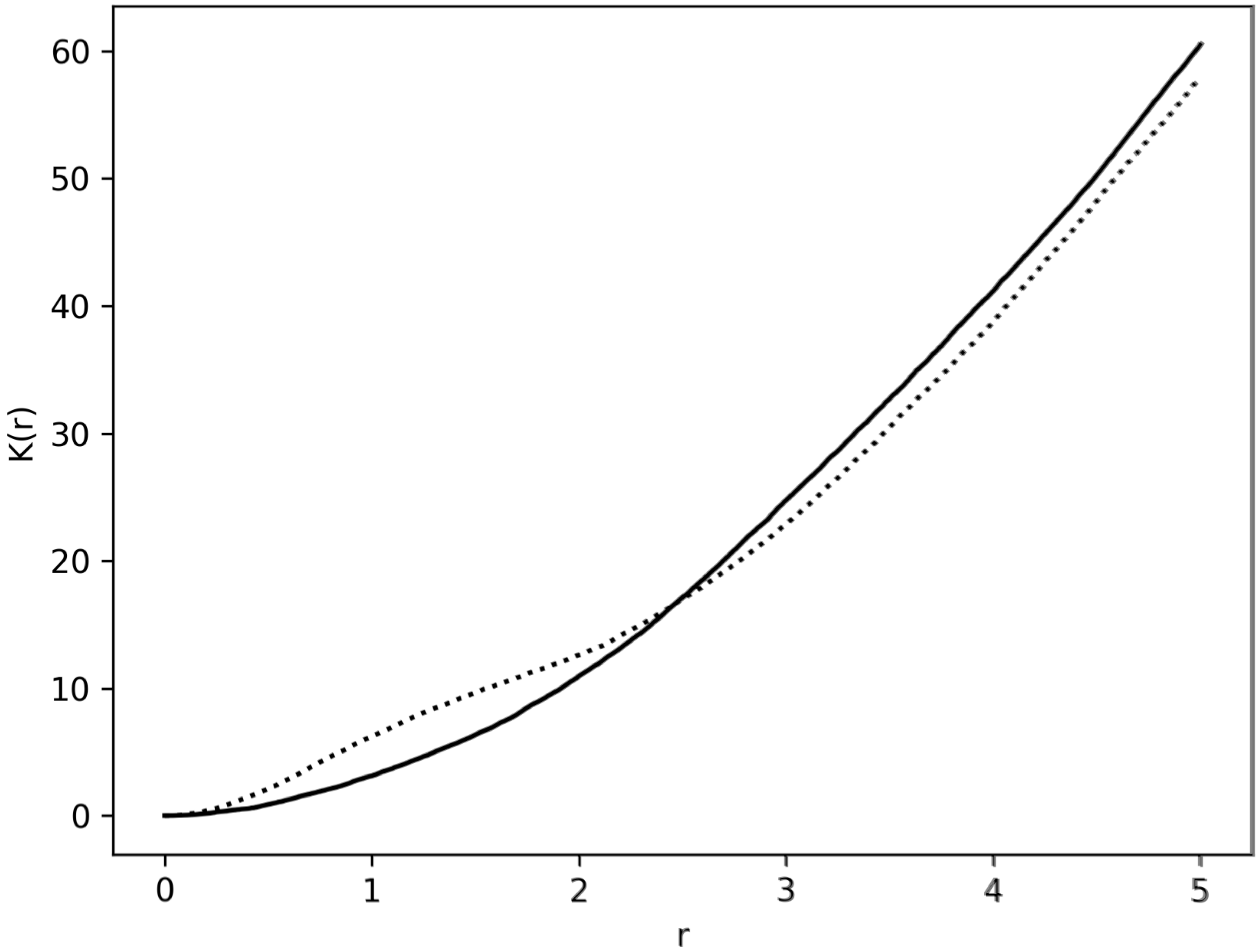
Winter — cold

Winter — medium

Winter — warm



20 a.u.

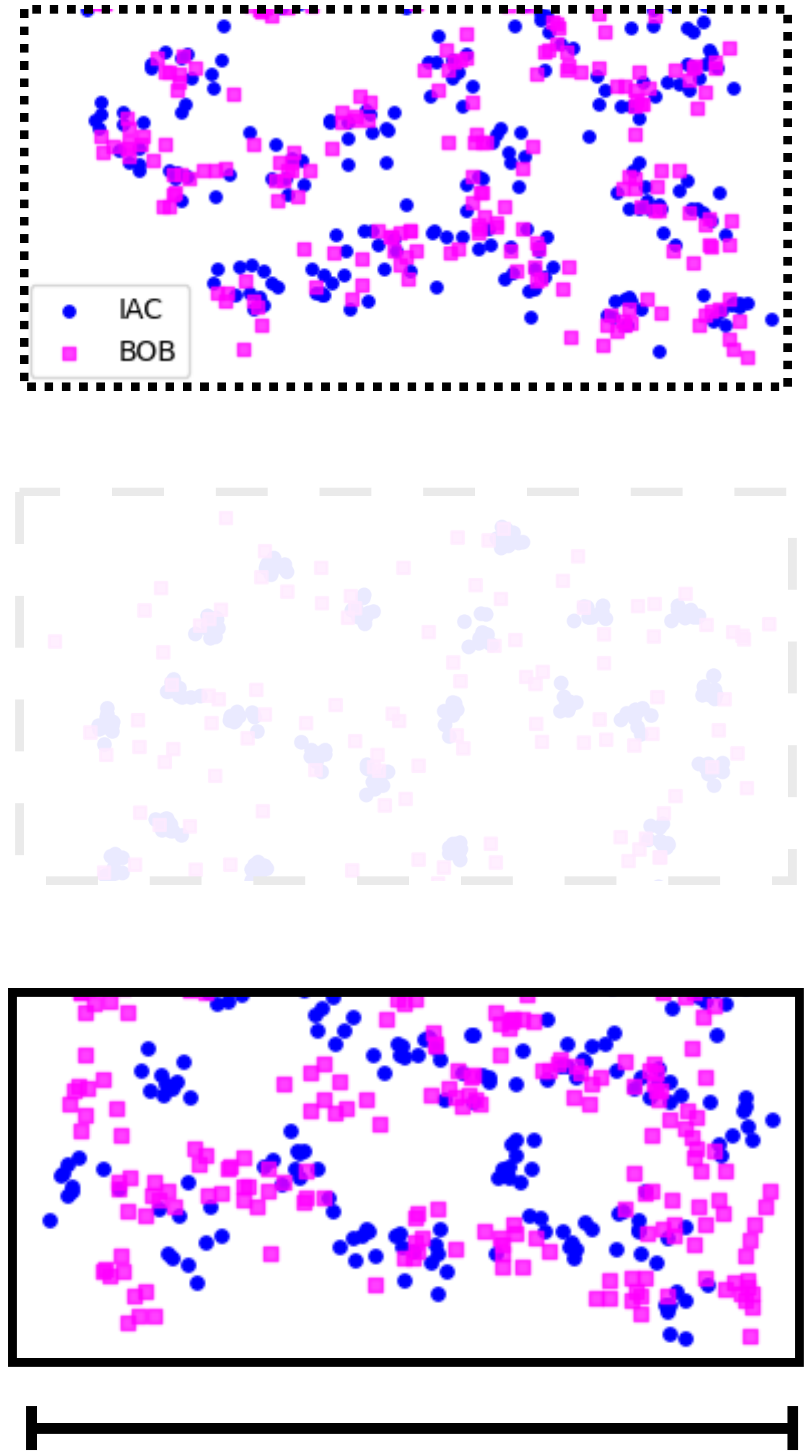


Results: Ripley's K function

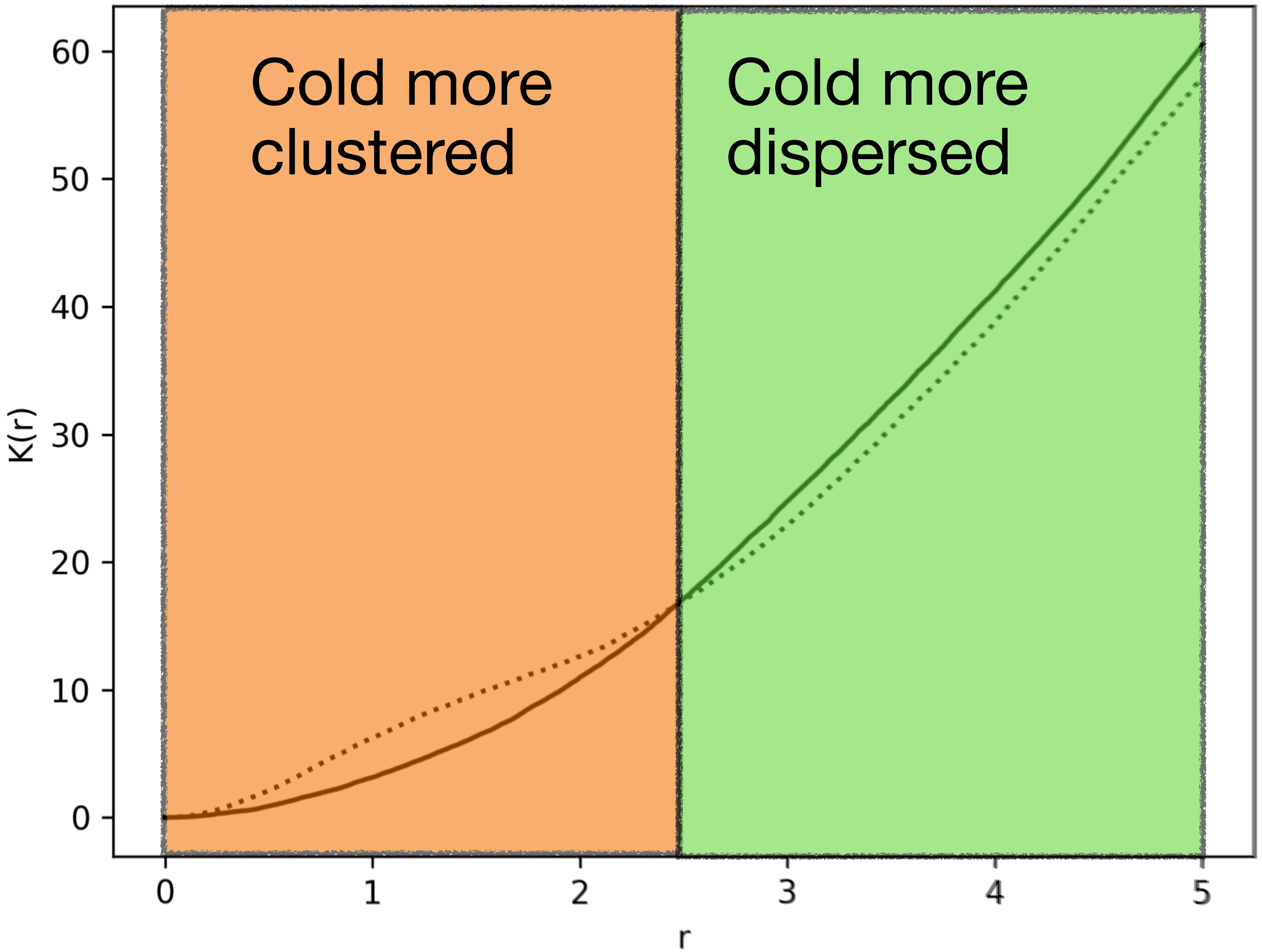
Winter — cold

Winter — medium

Winter — warm



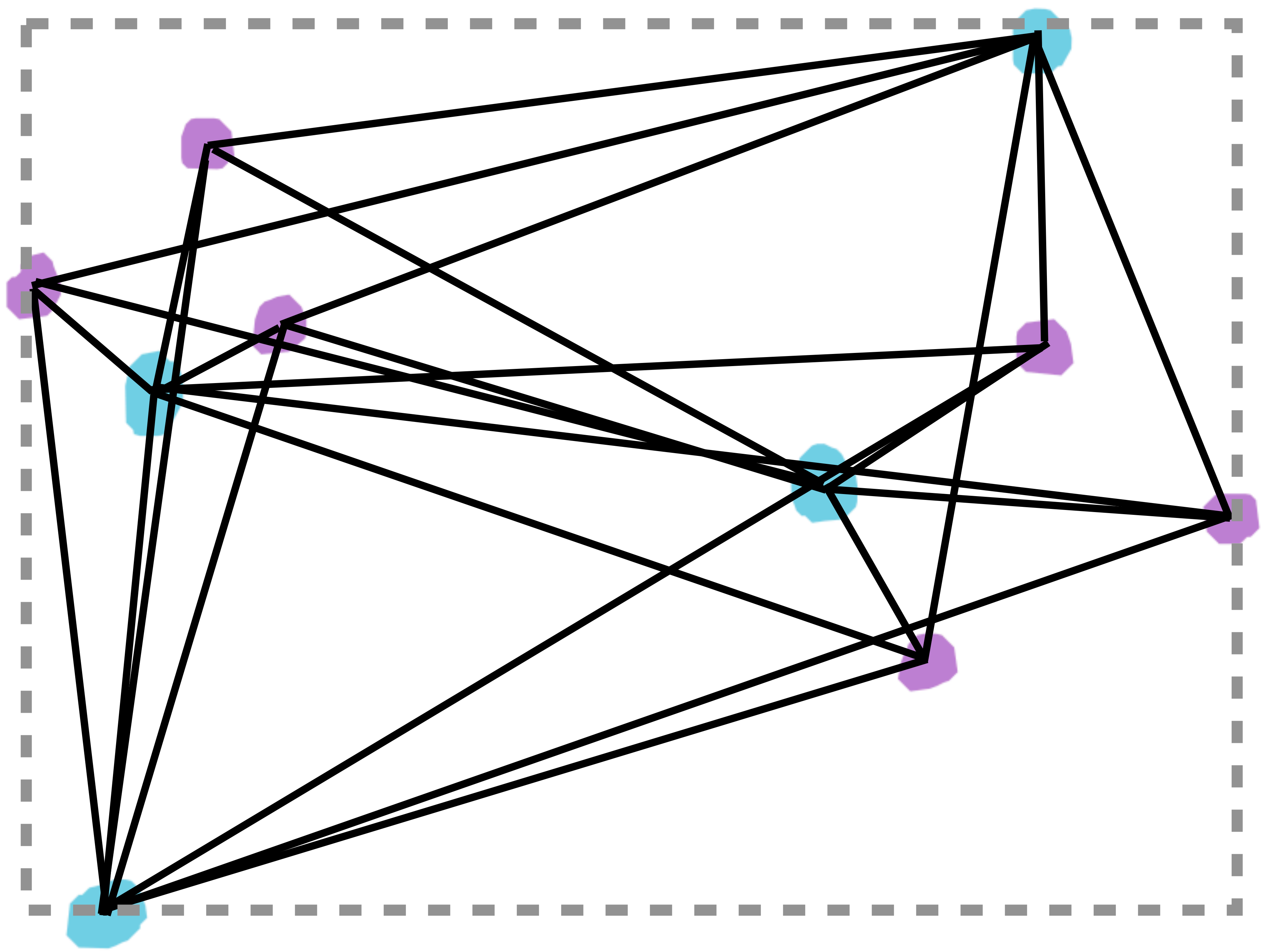
20 a.u.



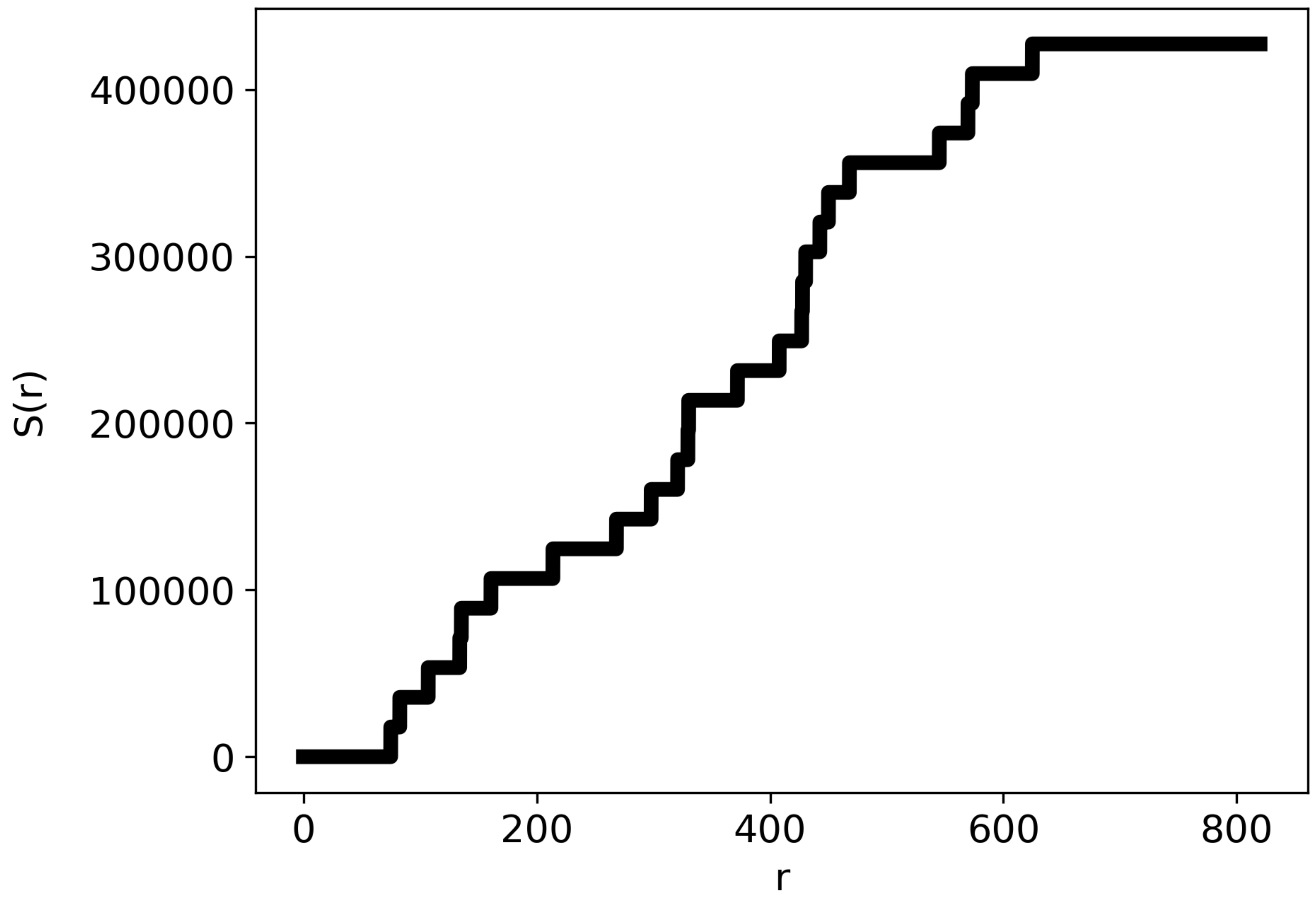
-> 4. Ripley’s K function

Ripley's K function

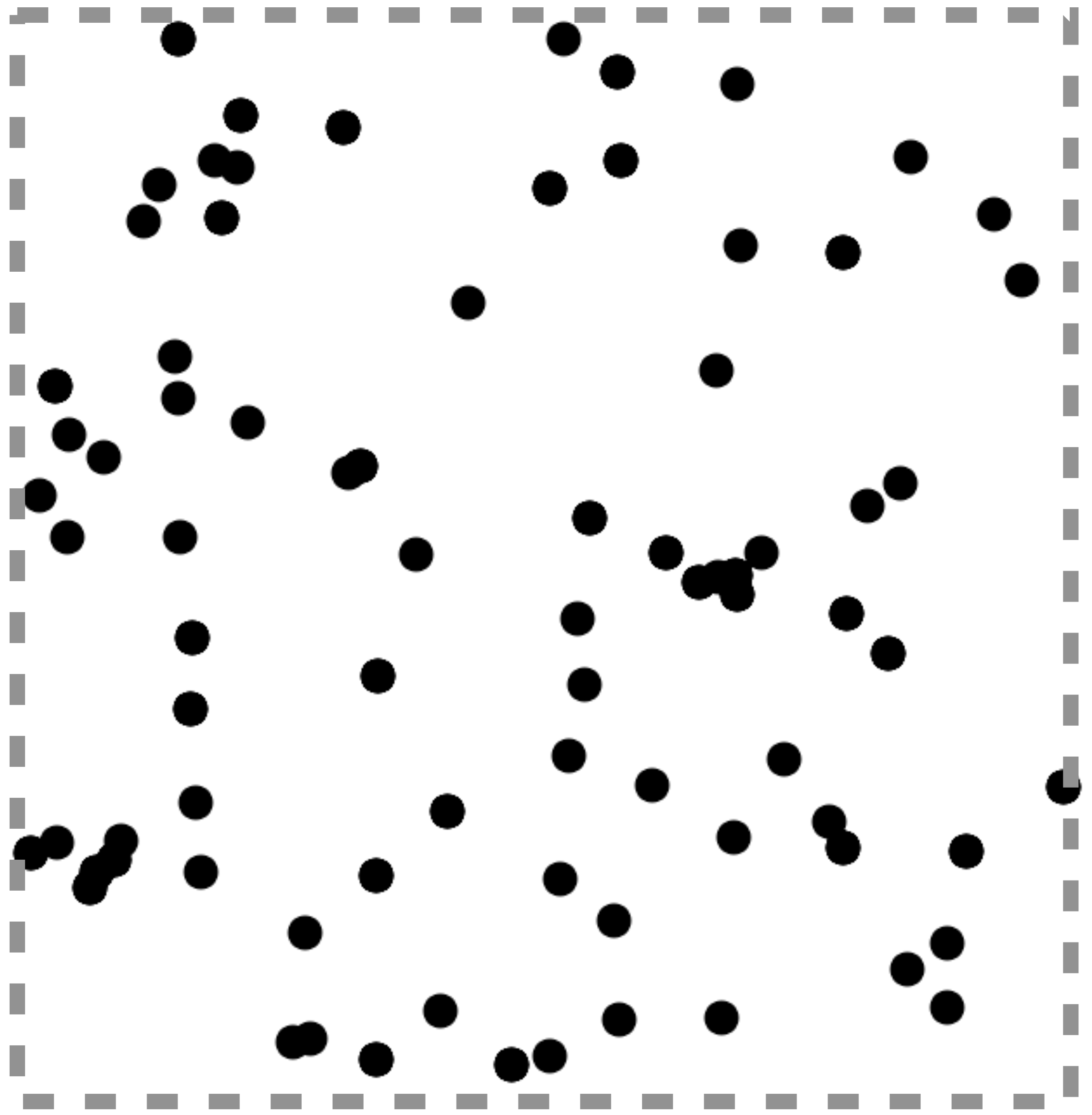
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) b(i, j, r)$$



$|\Omega| = \text{Area of FOV}$



Ripley's K function

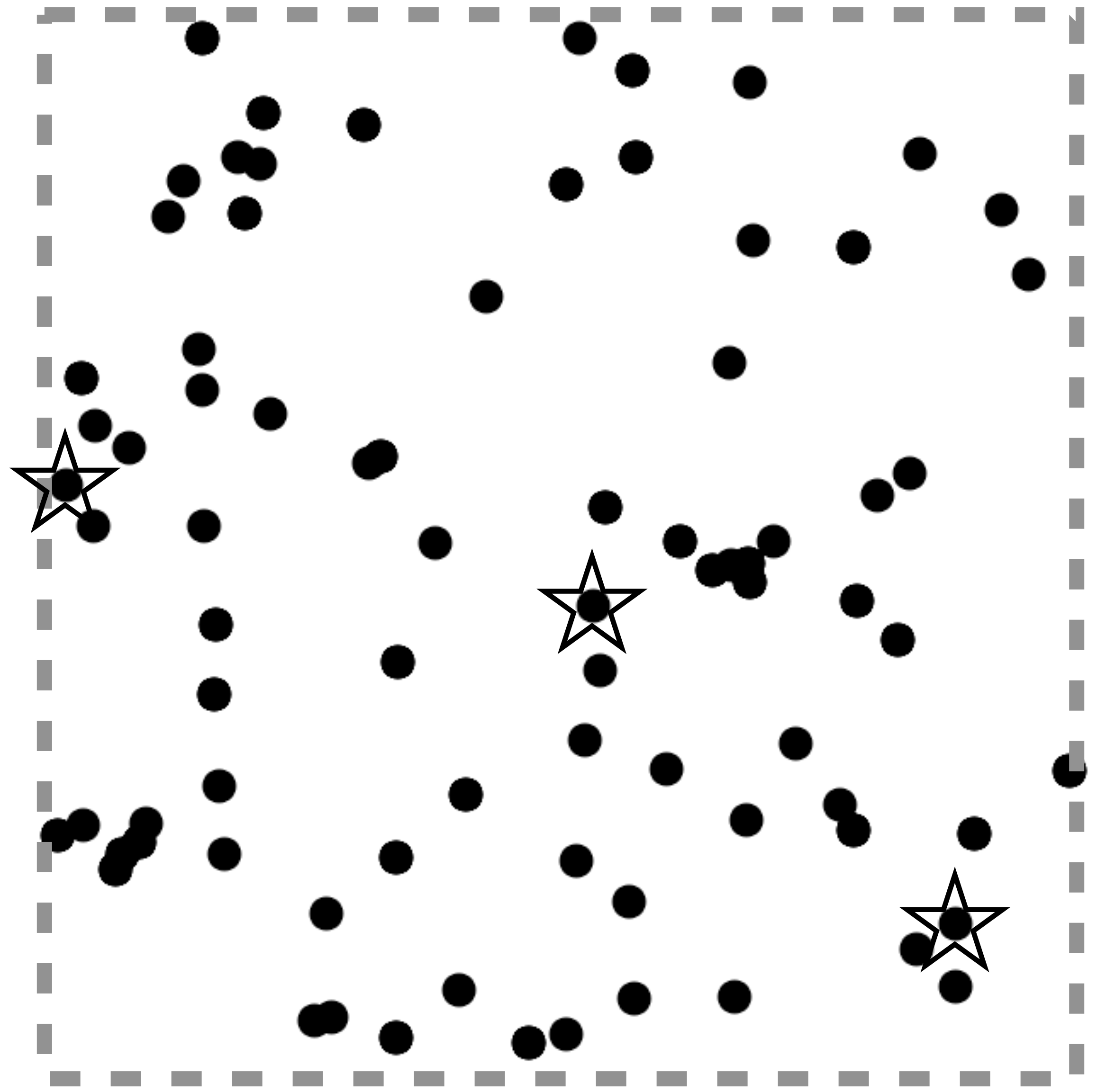


$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$

$$b(i,j,r) = \frac{|c(i,d_{ij})|}{|c(i,d_{ij}) \cap \Omega|}$$

Ripley's K function

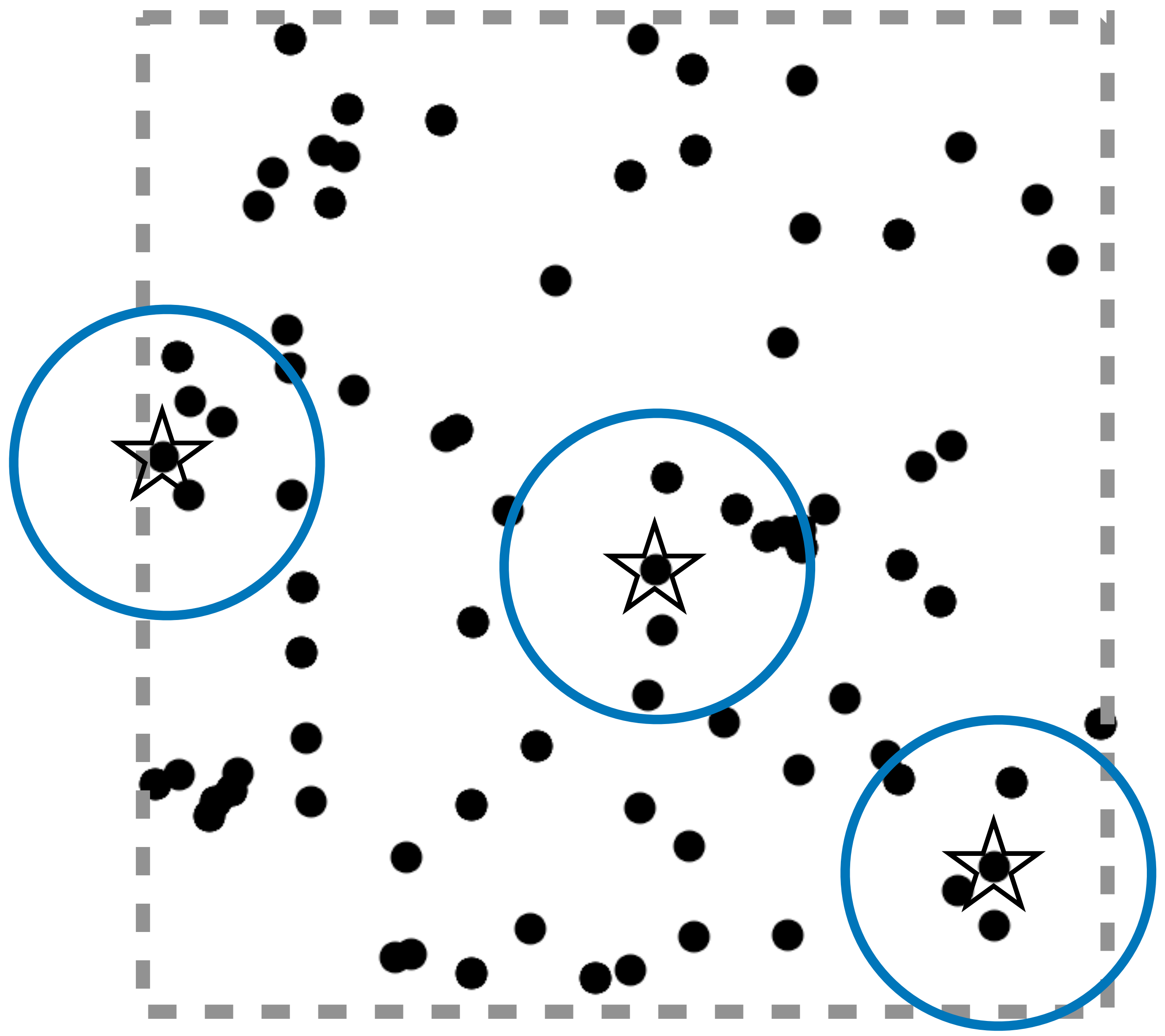
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$



$$b(i,j,r) = \frac{|c(i, d_{ij})|}{|c(i, d_{ij}) \cap \Omega|}$$

Ripley's K function

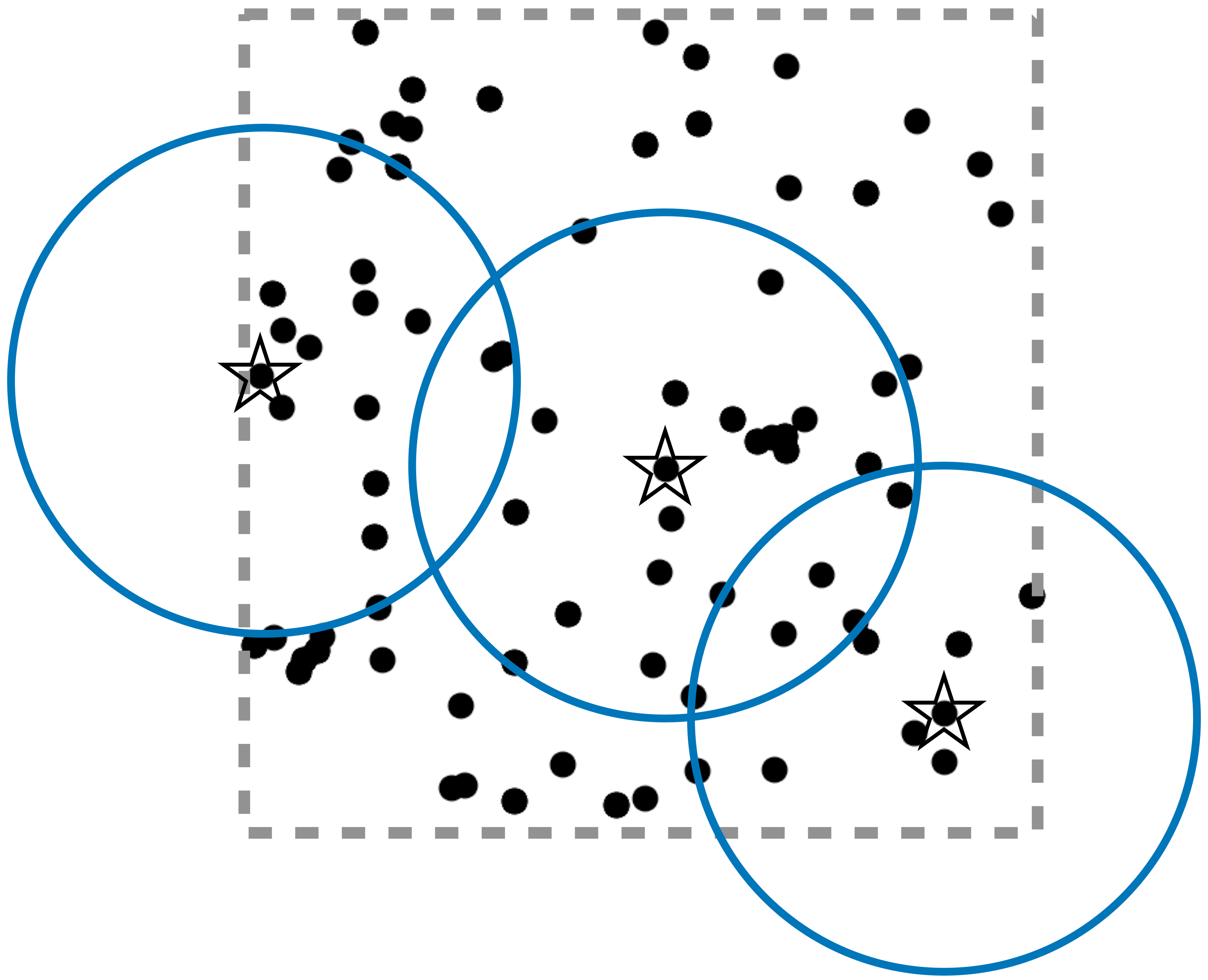
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$



$$b(i,j,r) = \frac{|c(i, d_{ij})|}{|c(i, d_{ij}) \cap \Omega|}$$

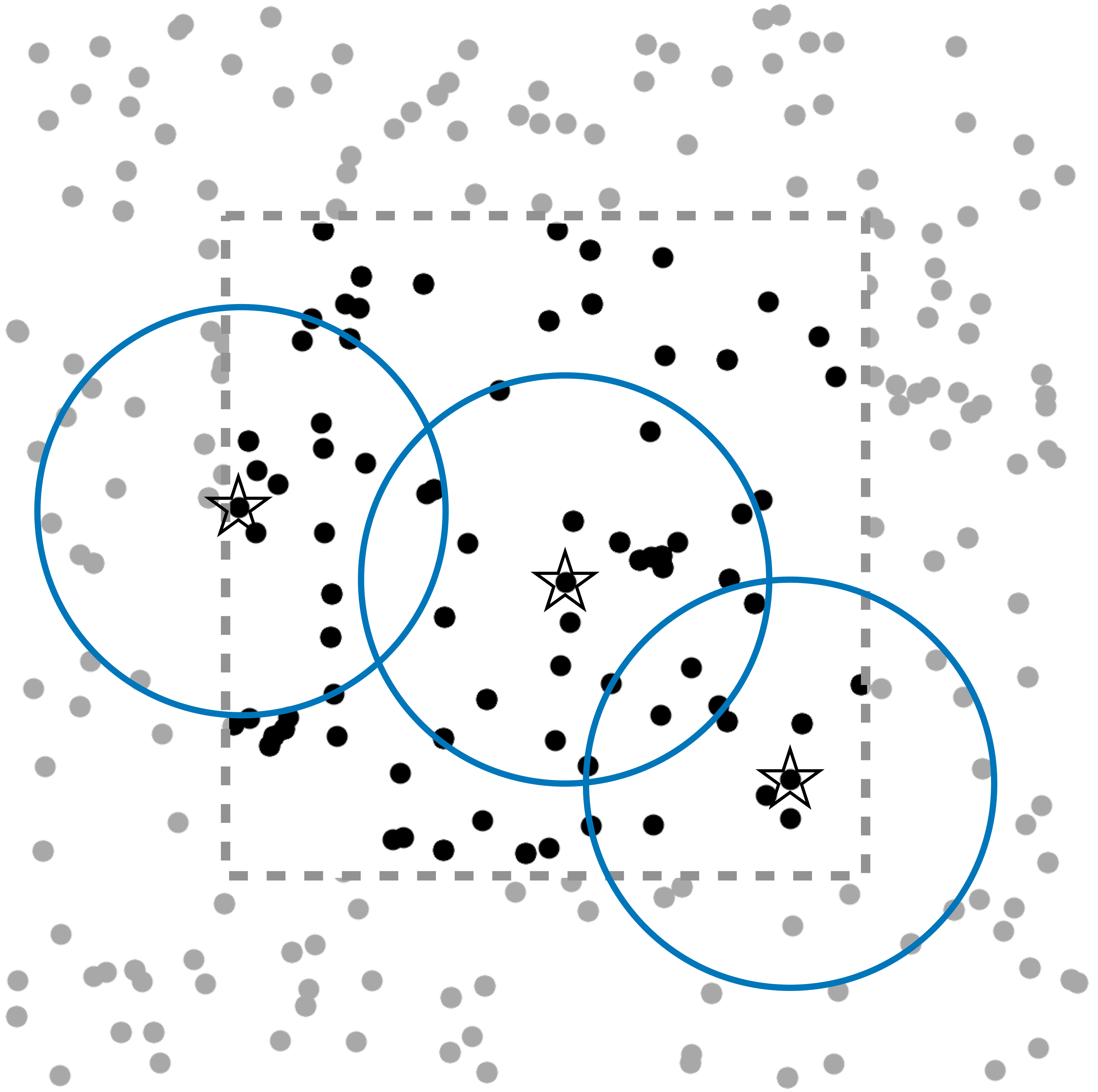
Ripley's K function

$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$



$$b(i,j,r) = \frac{|c(i, d_{ij})|}{|c(i, d_{ij}) \cap \Omega|}$$

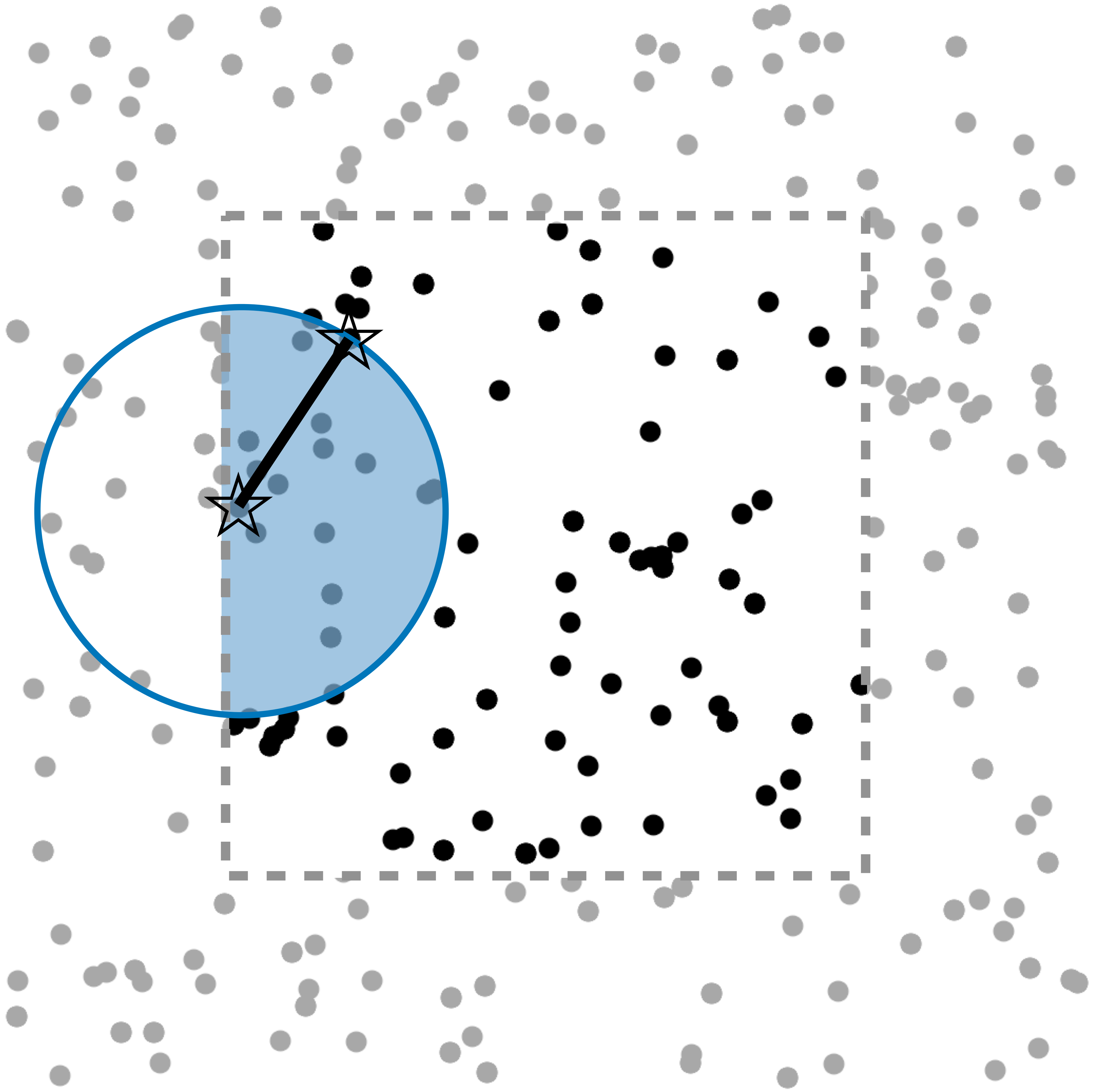
Ripley’s K function



$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$

$$b(i,j,r) = \frac{|c(i,d_{ij})|}{|c(i,d_{ij}) \cap \Omega|}$$

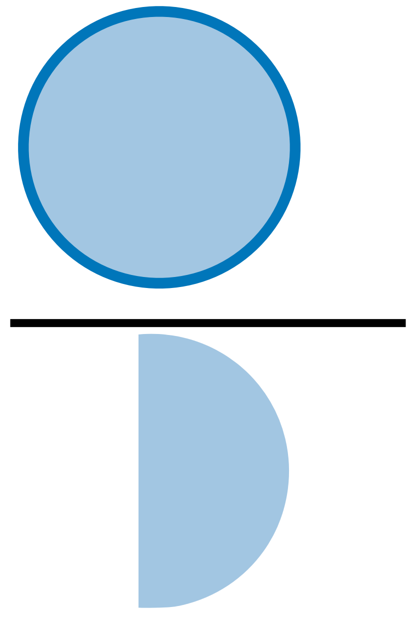
Ripley's K function



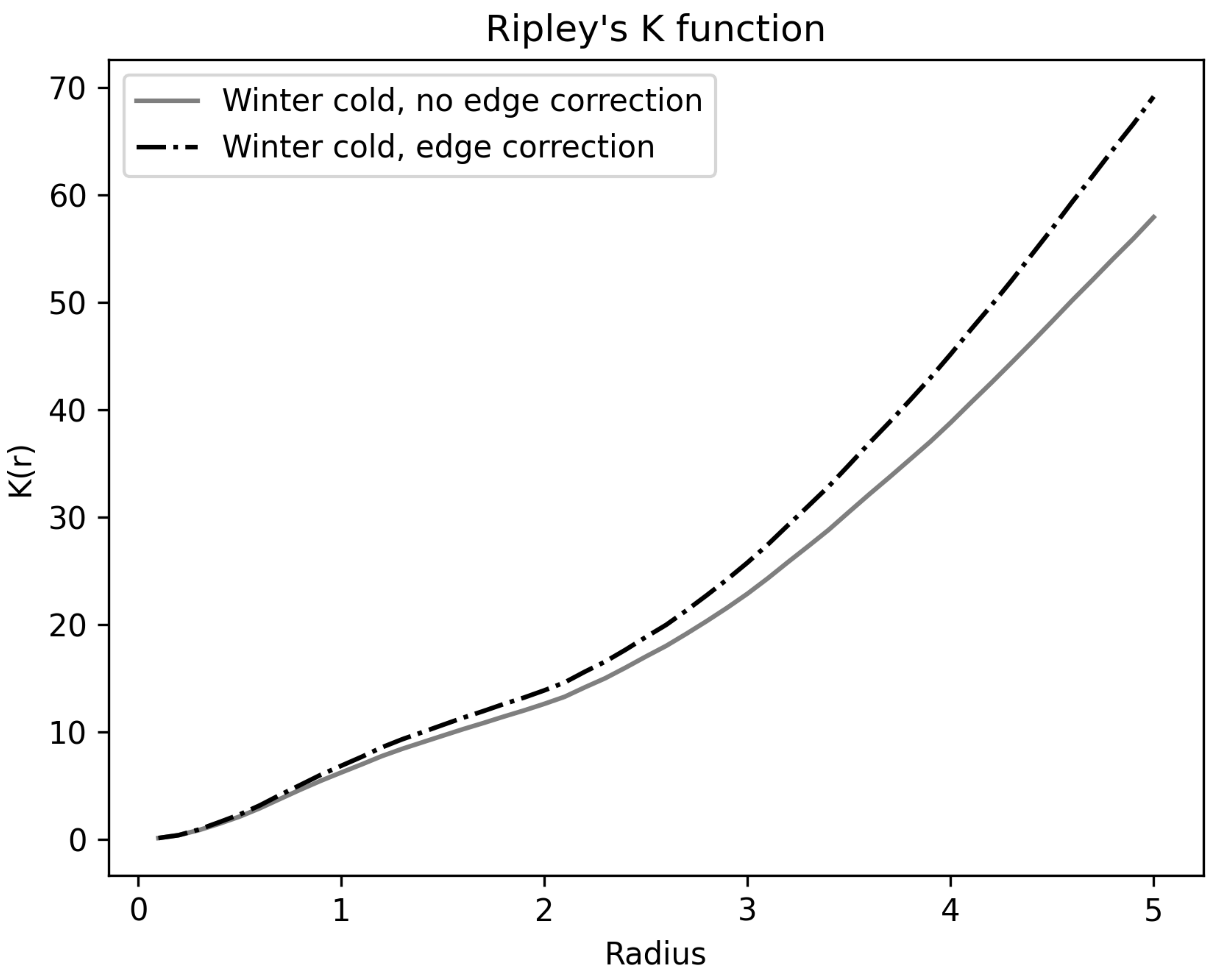
$$S(r) = \frac{|\Omega|}{n_1 n_2} \sum_{i=1}^{n_1} \sum_{j=1}^{n_2} \mathbf{1}(d_{ij} < r) \boxed{b(i,j,r)}$$

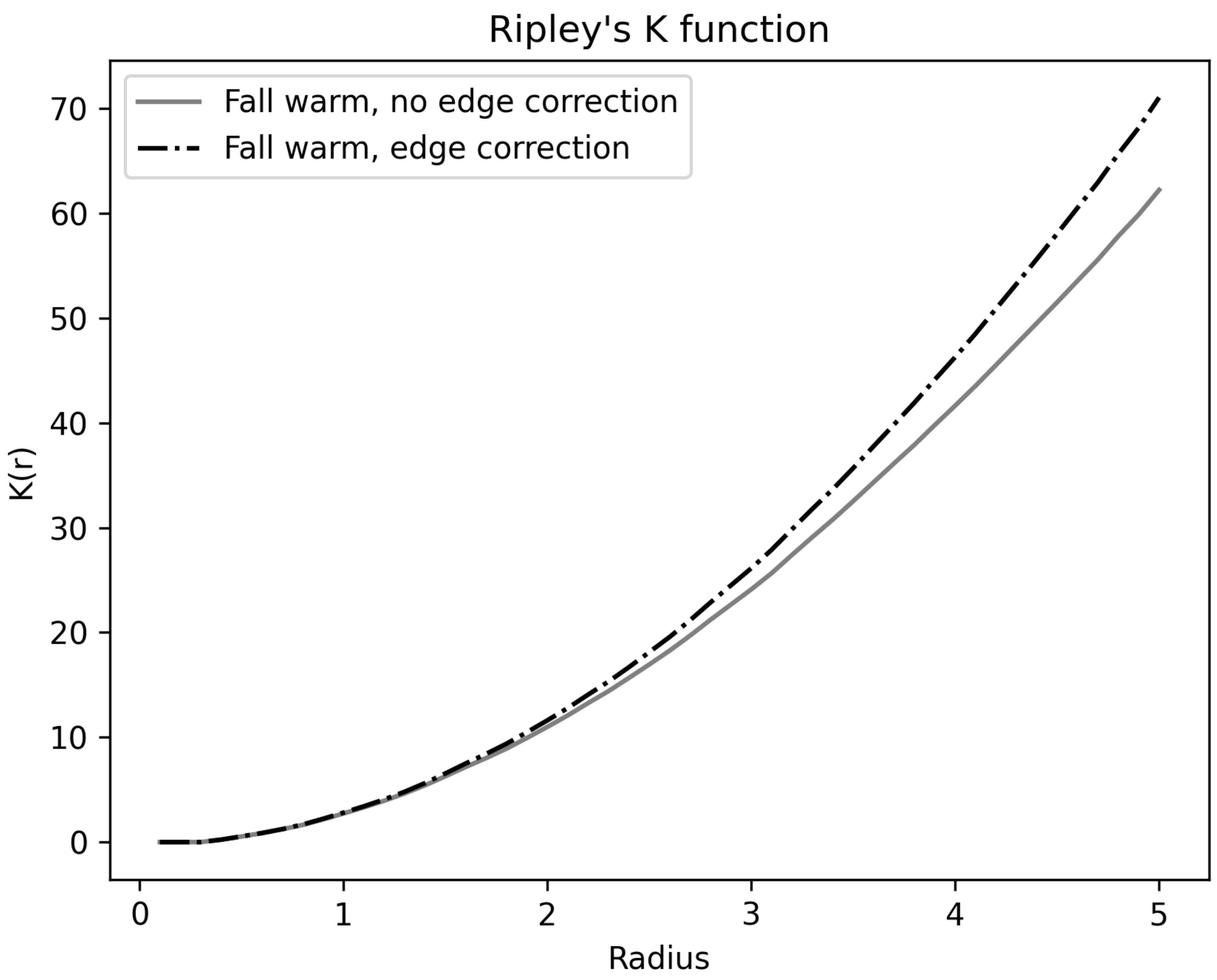
$$b(i,j,r) = \frac{|c(i,d_{ij})|}{|c(i,d_{ij}) \cap \Omega|}$$

=



Ripley’s K function





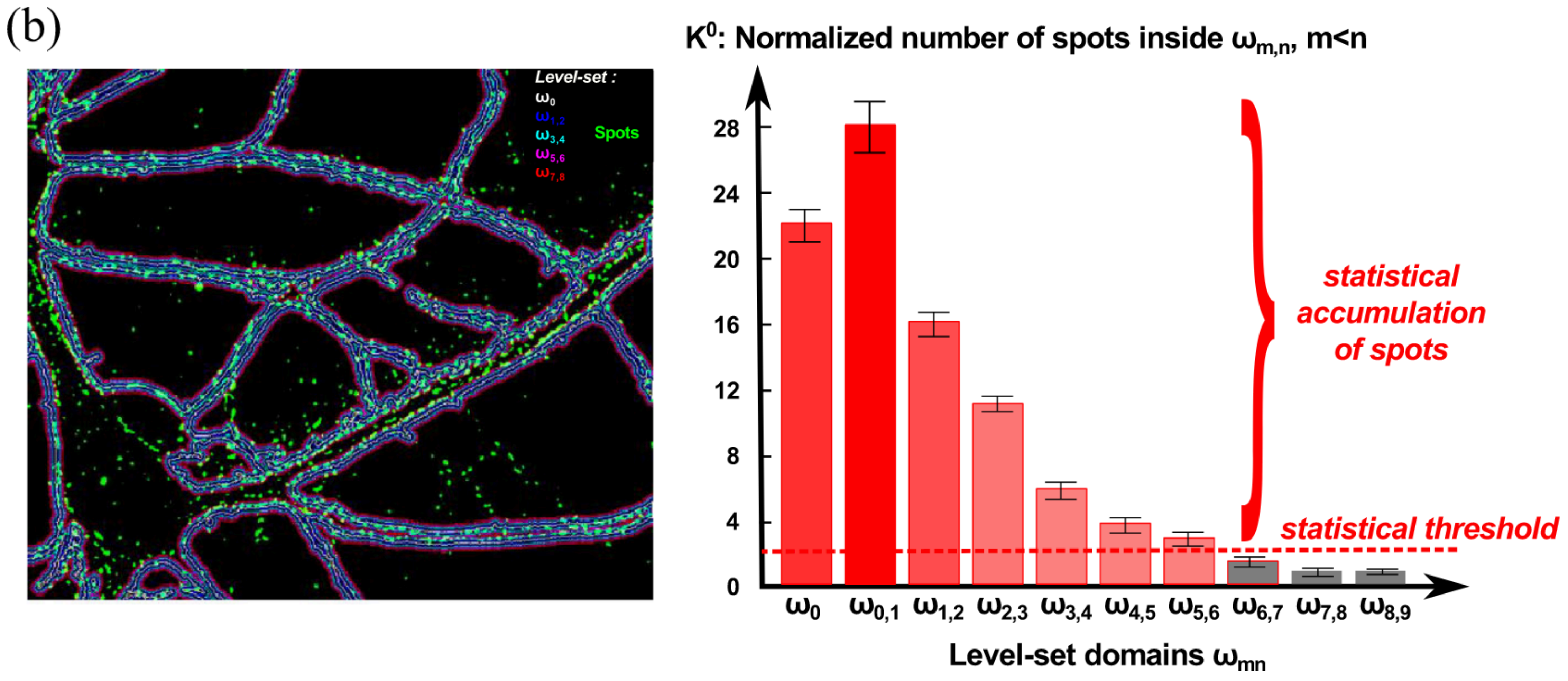
-> Code

-> Code

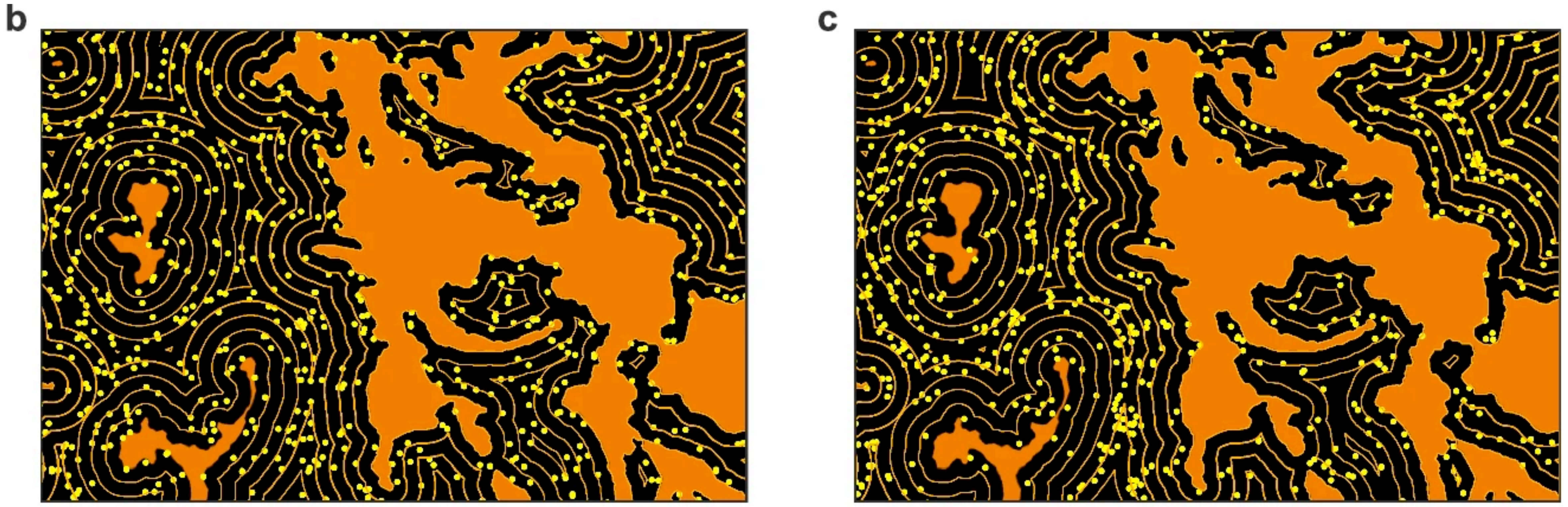
Ripley's K function

- Symmetric: BOB → IAC = IAC → BOB
- Returns: A number for each radius
- Range: Long

Beyond Ripley's K function

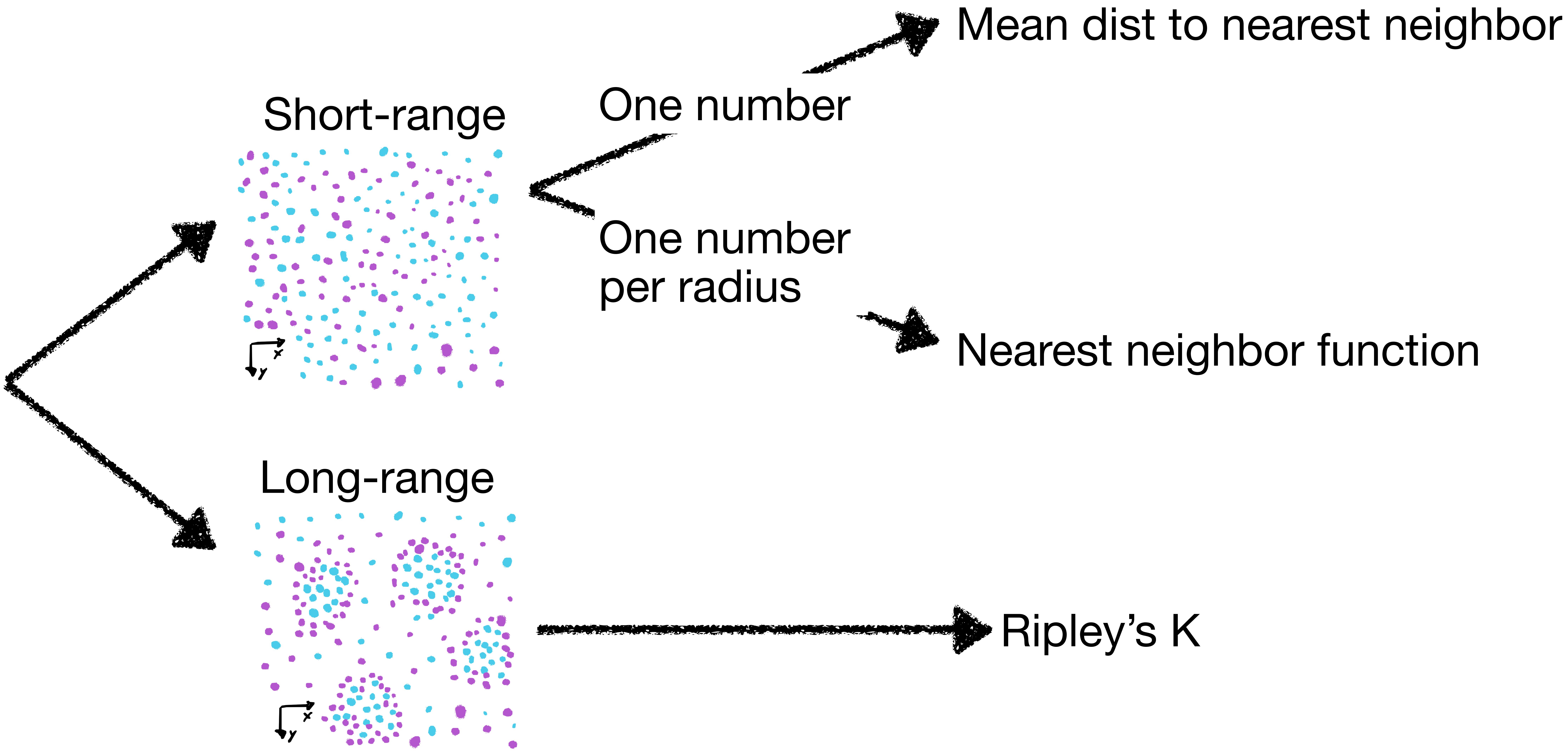


S. Mukherjee, C. Gonzalez-Gomez, L. Danglot, T. Lagache and J. -C. Olivo-Marin, "Generalizing the Statistical Analysis of Objects' Spatial Coupling in Bioimaging," in *IEEE Signal Processing Letters*, vol. 27, pp. 1085-1089, 2020, doi: 10.1109/LSP.2020.3003821.



Benimam, M.M., Meas-Yedid, V., Mukherjee, S. *et al.* Statistical analysis of spatial patterns in tumor microenvironment images. *Nat Commun* **16**, 3090 (2025). https://doi.org/10.1038/s41467-025-57943-y

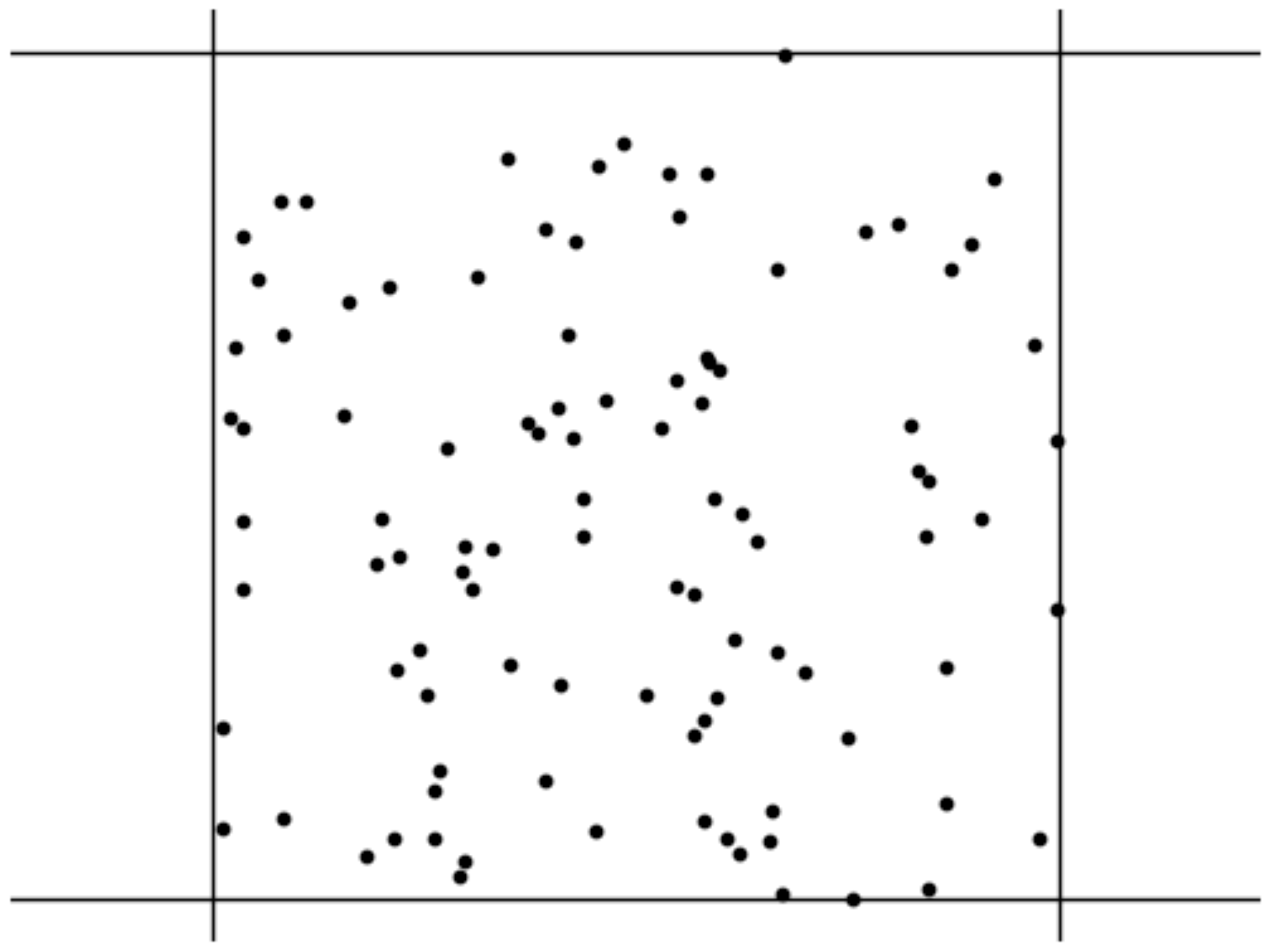
Summary



Short-range
Long-range
One number
One number
per radius
Mean dist to nearest neighbor
Nearest neighbor function
Ripley's K

Validation — the null distribution

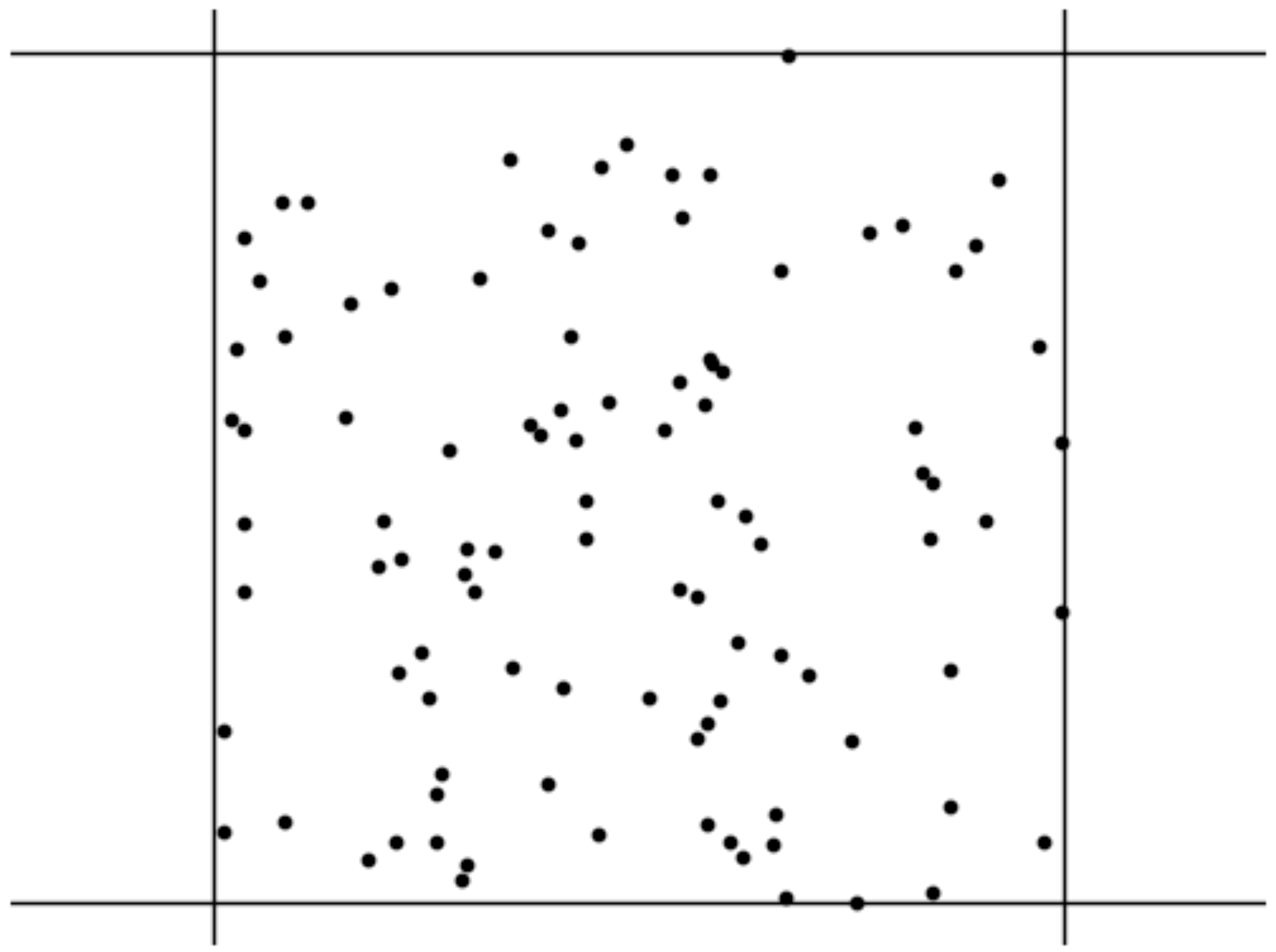
- How are proteins distributed that
  - Don’t interact with each other
  - Or their surrounding



What is the evidence that my points may or may not be distributed like this?

Validation — the null distribution

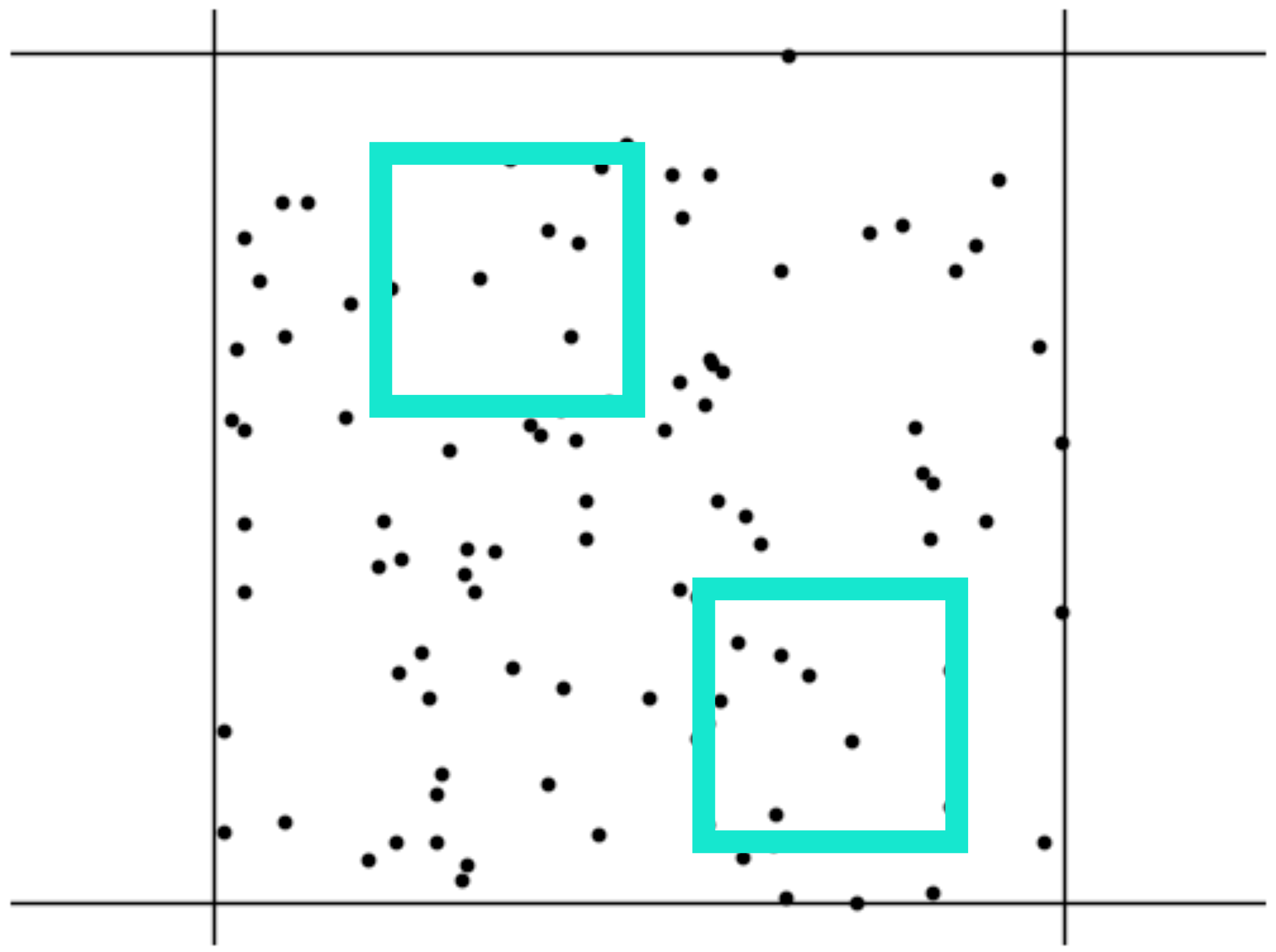
- How are proteins distributed that
  - Don't interact with each other
  - Or their surrounding
- To find out, you blindly throw darts at a board



What is the evidence that my points may or may not be distributed like this?

Validation — the null distribution

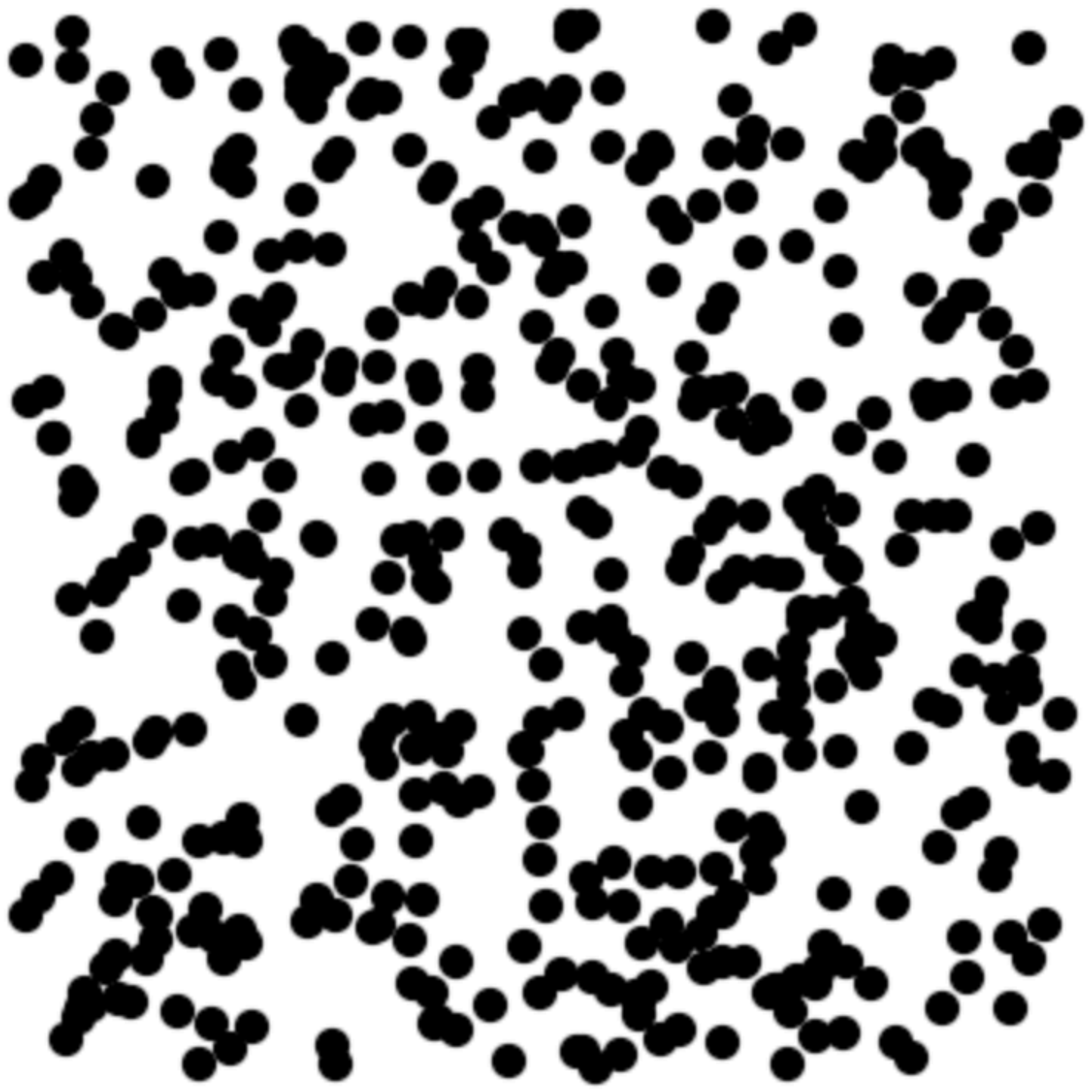
- How are proteins distributed that
  - Don't interact with each other
  - Or their surrounding
- To find out, you blindly throw darts at a board
- The chance of a dart landing is the same, no matter where on the board



What is the evidence that my points may or may not be distributed like this?

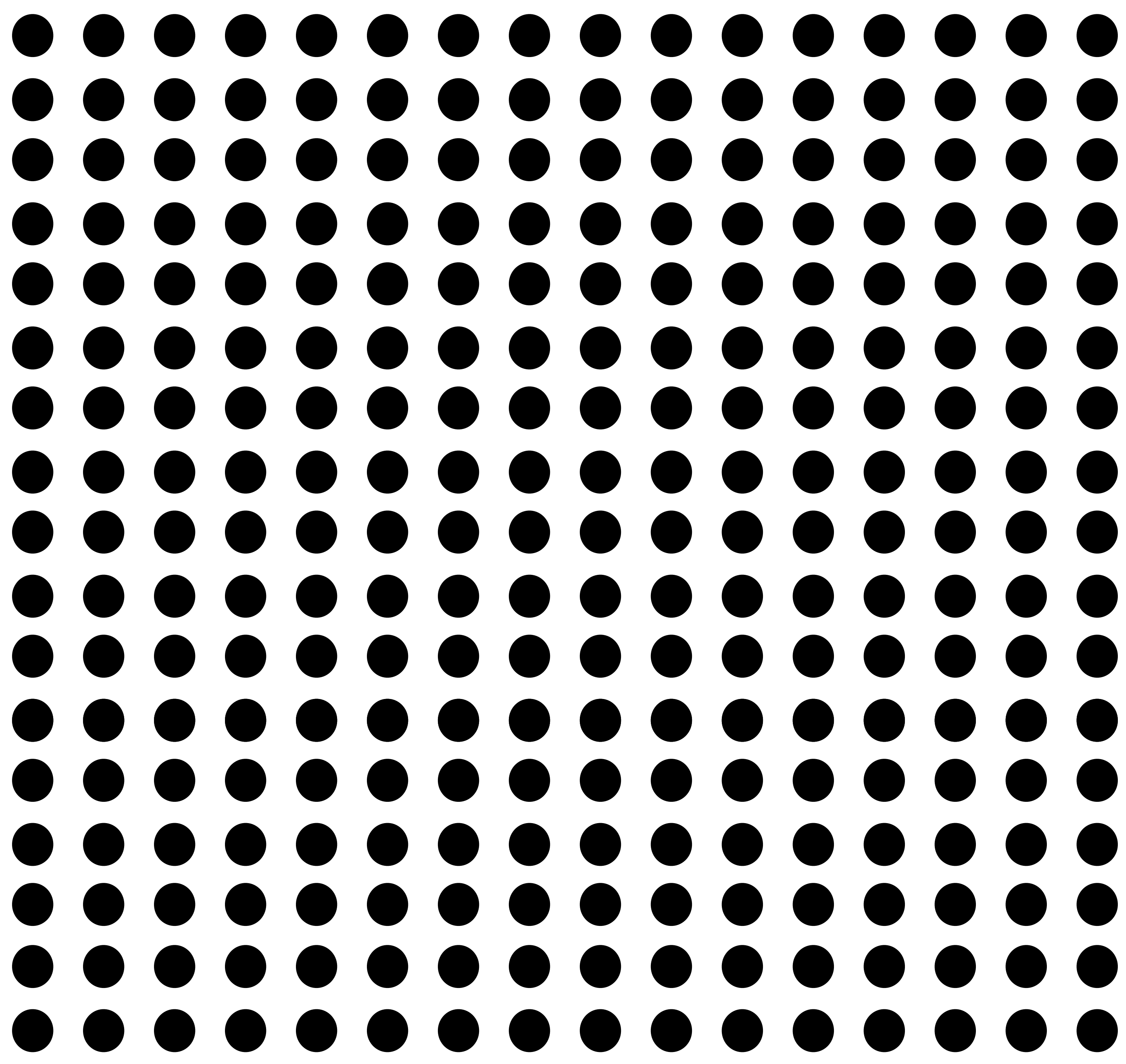
Validation — the null distribution

"Uniformly distributed"



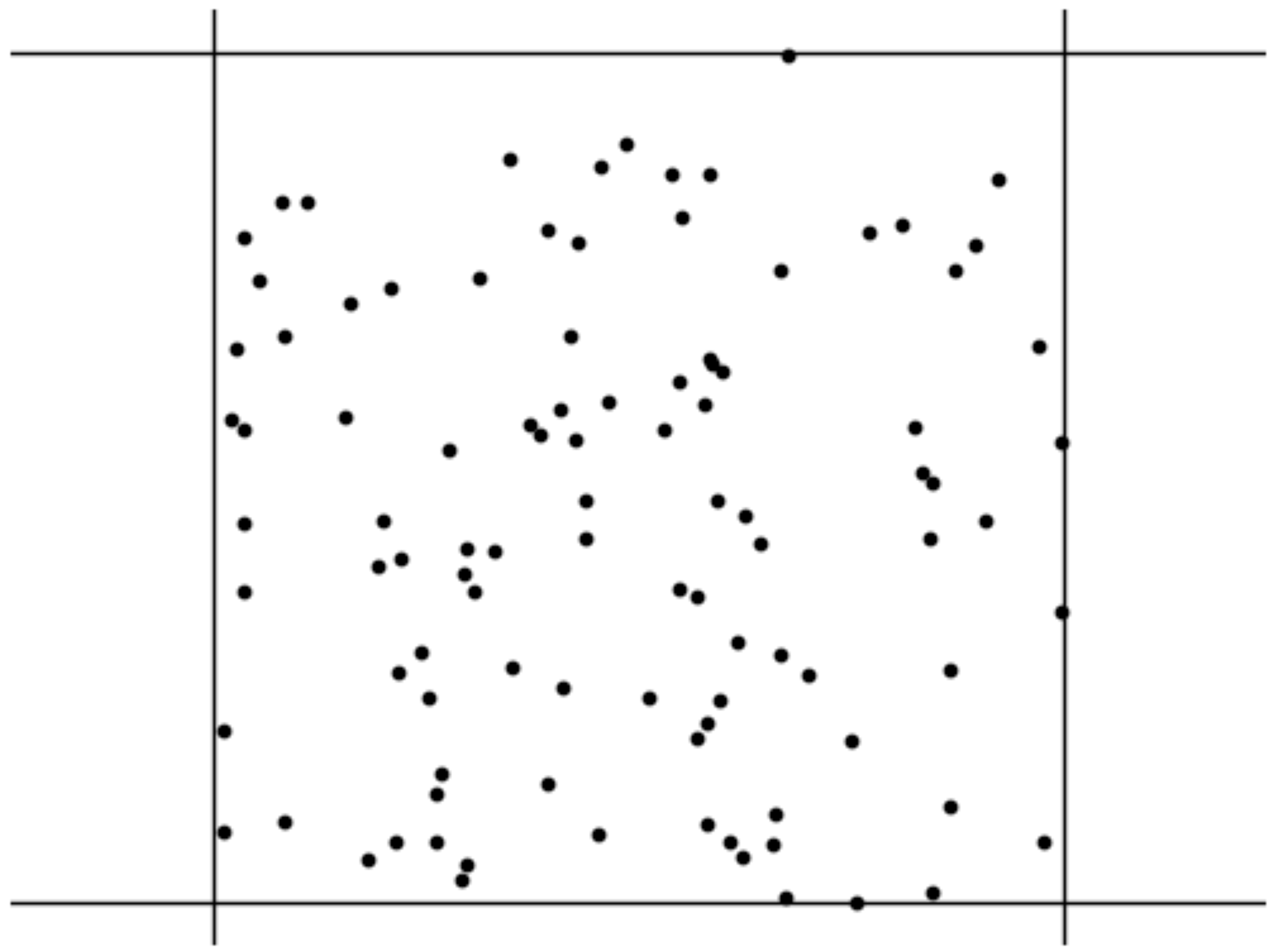
≠

"Uniformly spaced"



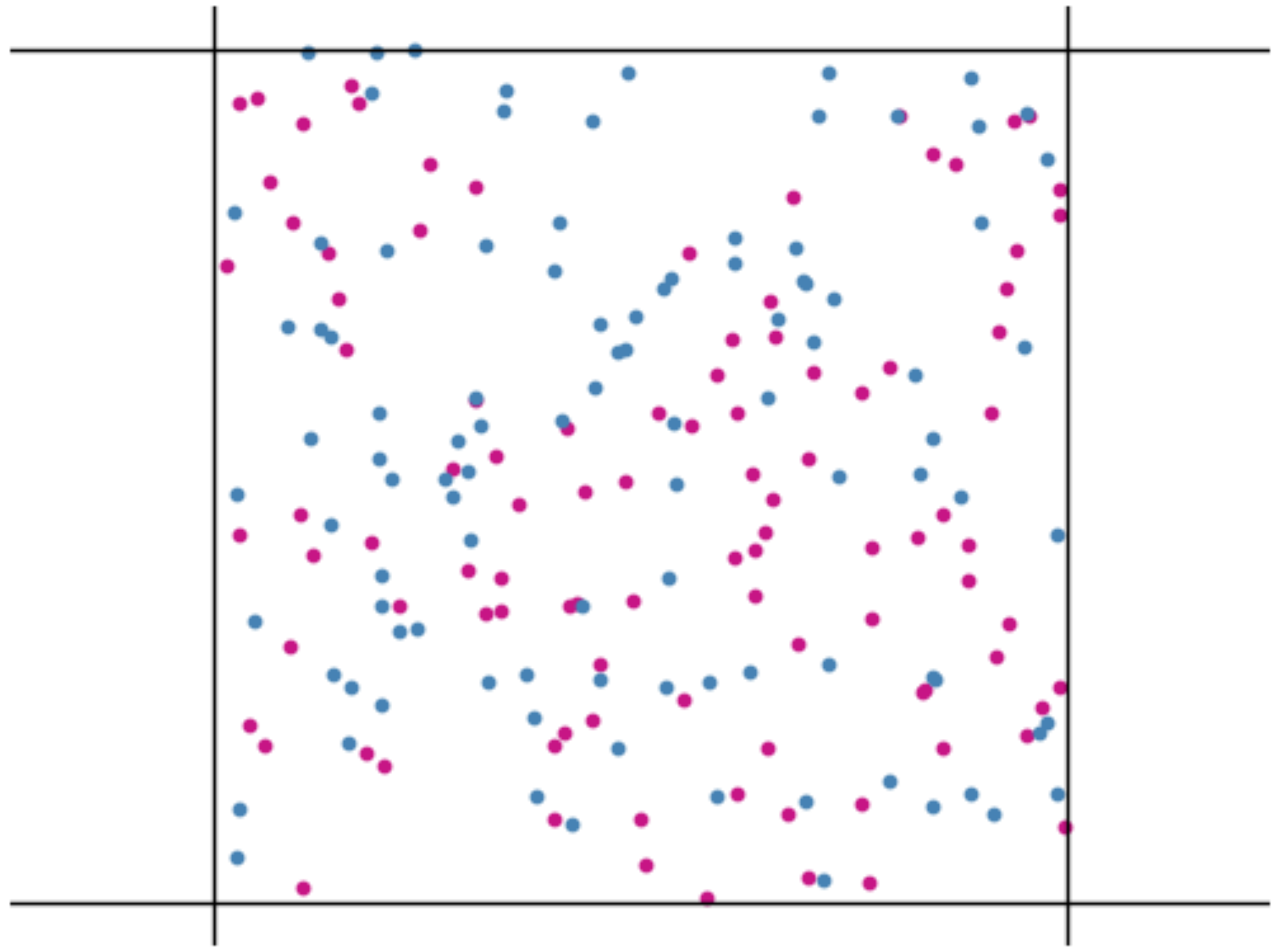
Validation — the null distribution

- How are proteins distributed that
  - Don’t interact with each other
  - Or their surrounding
- To find out, you blindly throw darts at a board
- The chance of a dart landing is the same, no matter where on the board



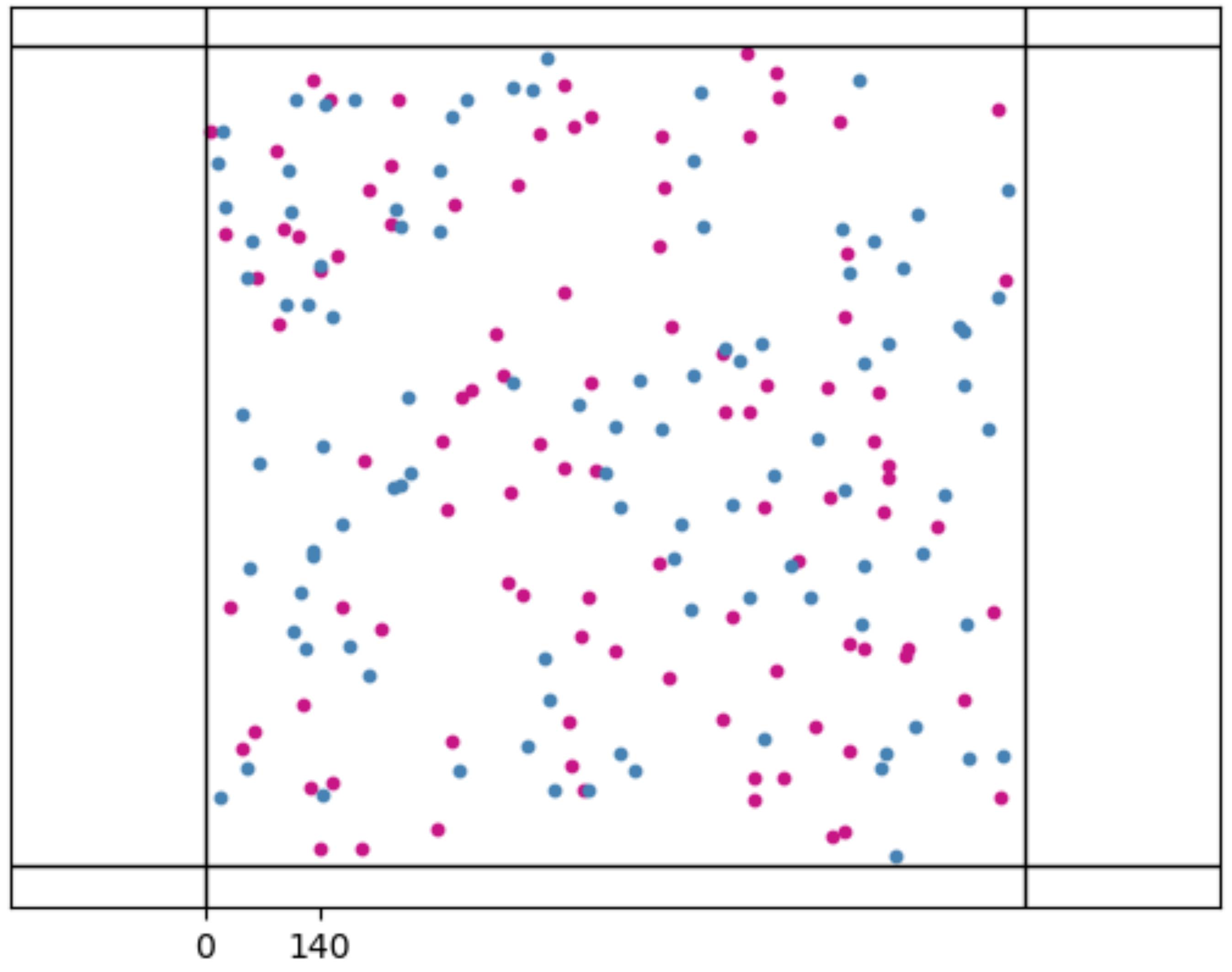
Validation — the null distribution

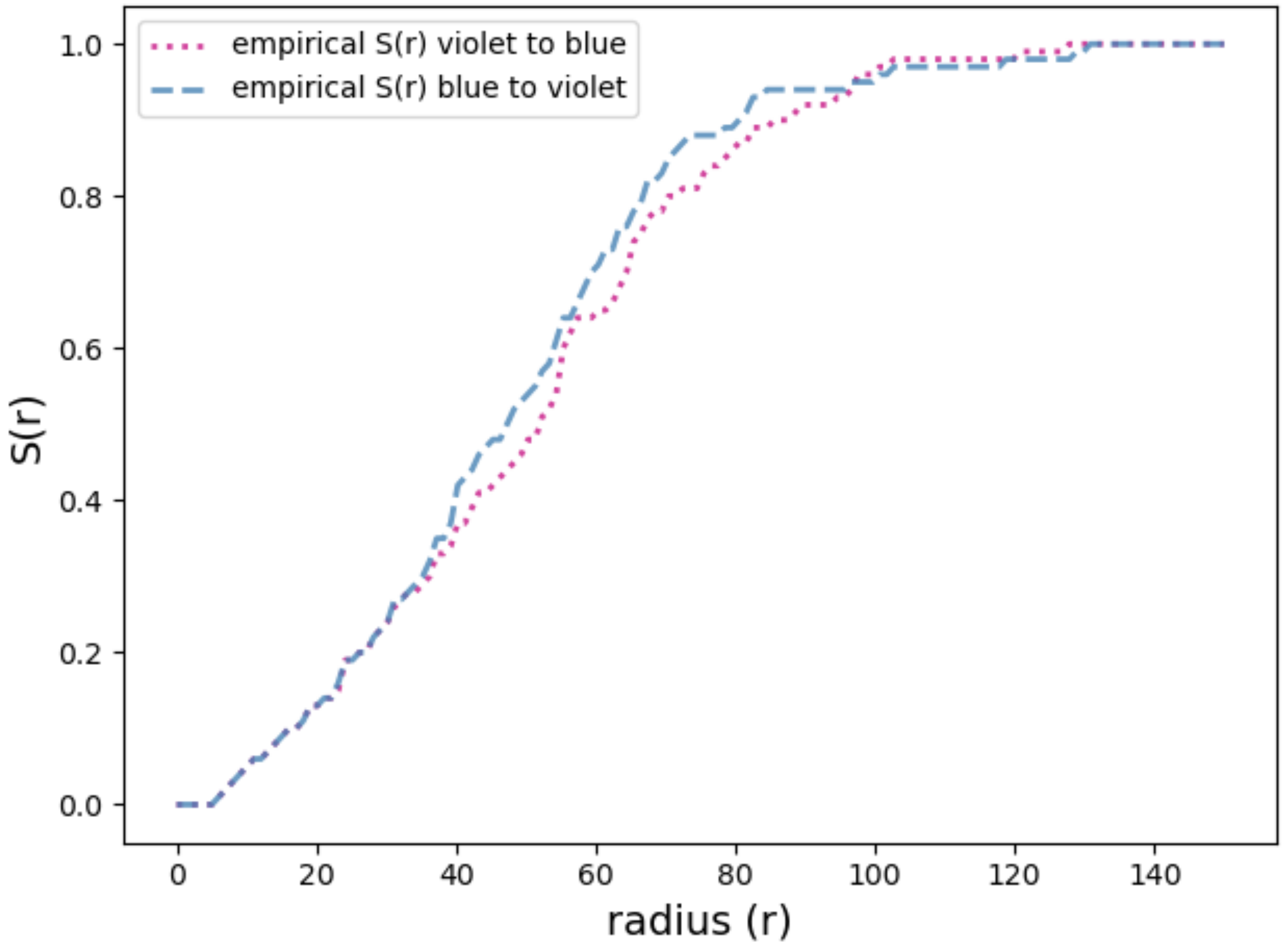
- How are proteins distributed that
  - Don’t interact with each other
  - Or their surrounding
- To find out, you blindly throw darts at a board
- The chance of a dart landing is the same, no matter where on the board
- The darts can have multiple colors



Validation — the null distribution

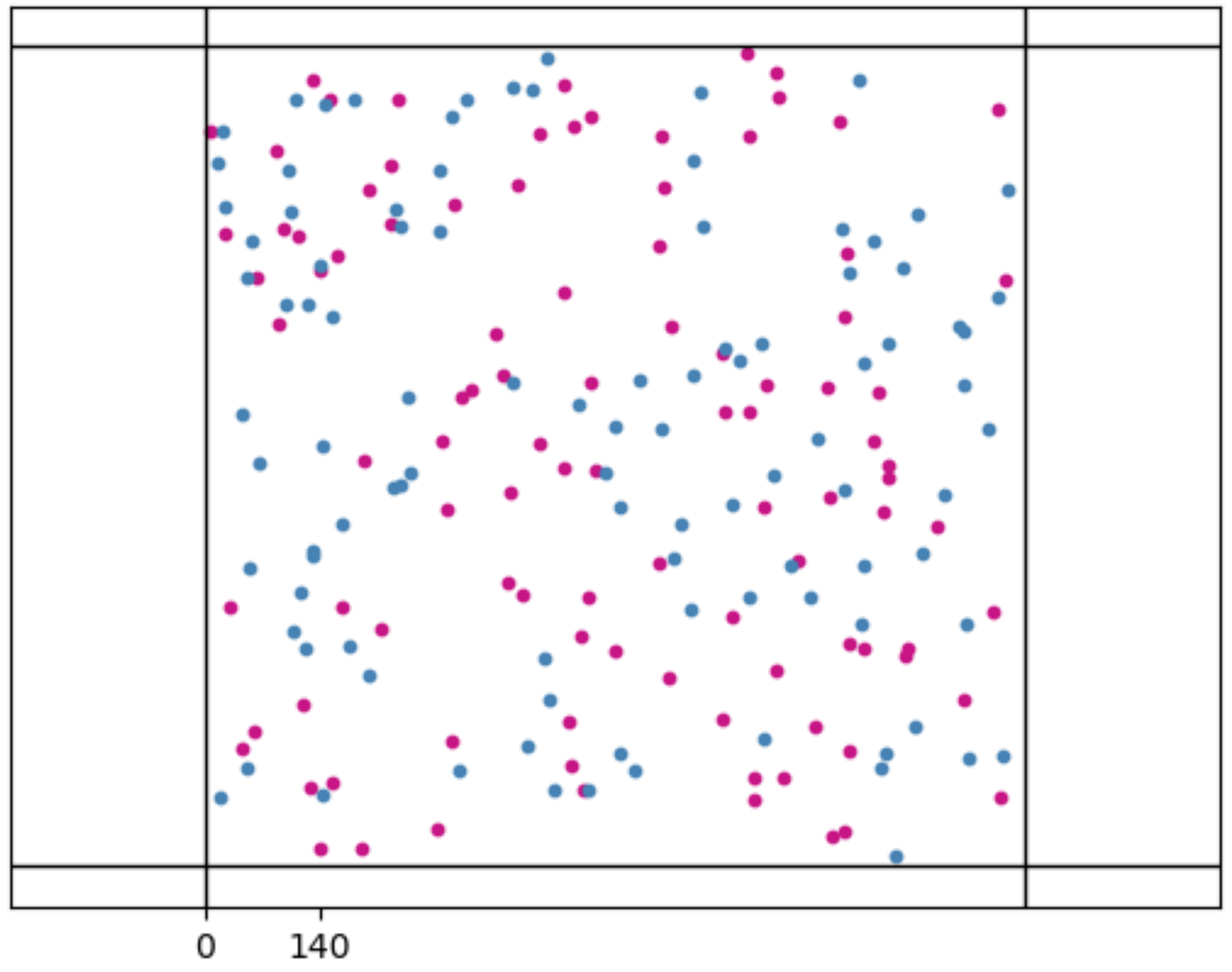
Empirical null distributions

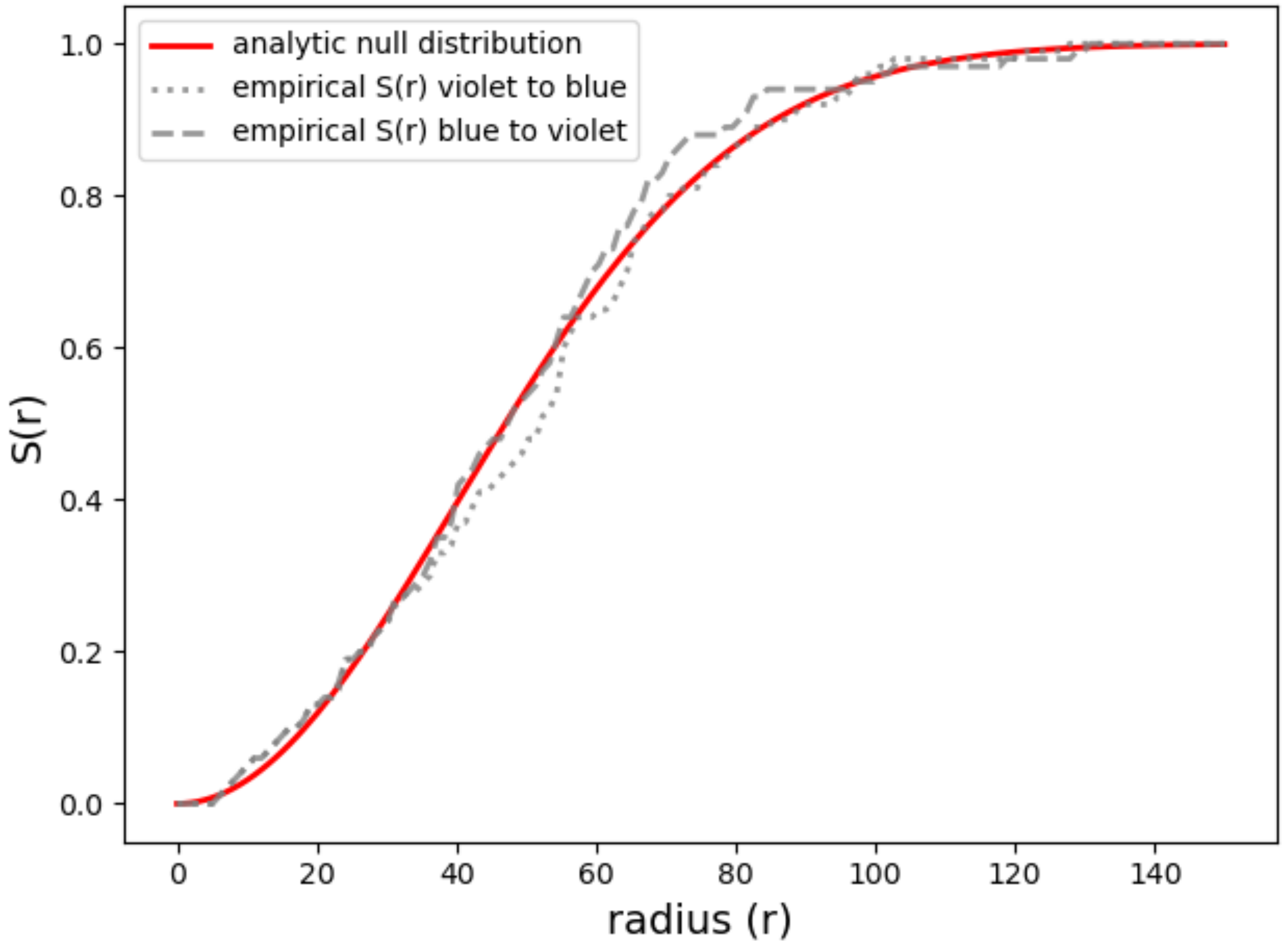




Validation — the null distribution

Analytic null distribution





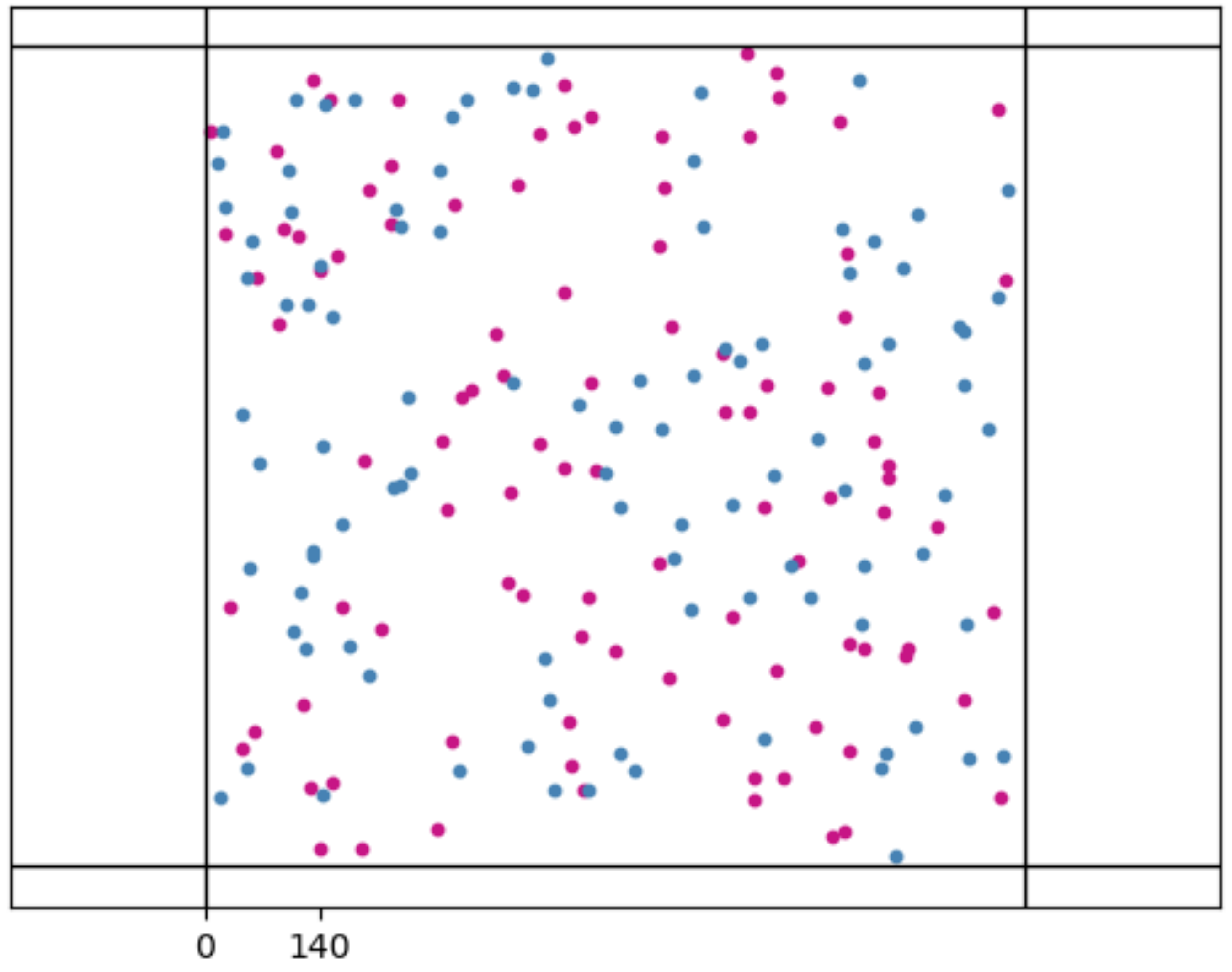
$$S(r) = 1 - e^{-\left(\frac{n_2}{|\Omega|}\right)\pi r^2}$$

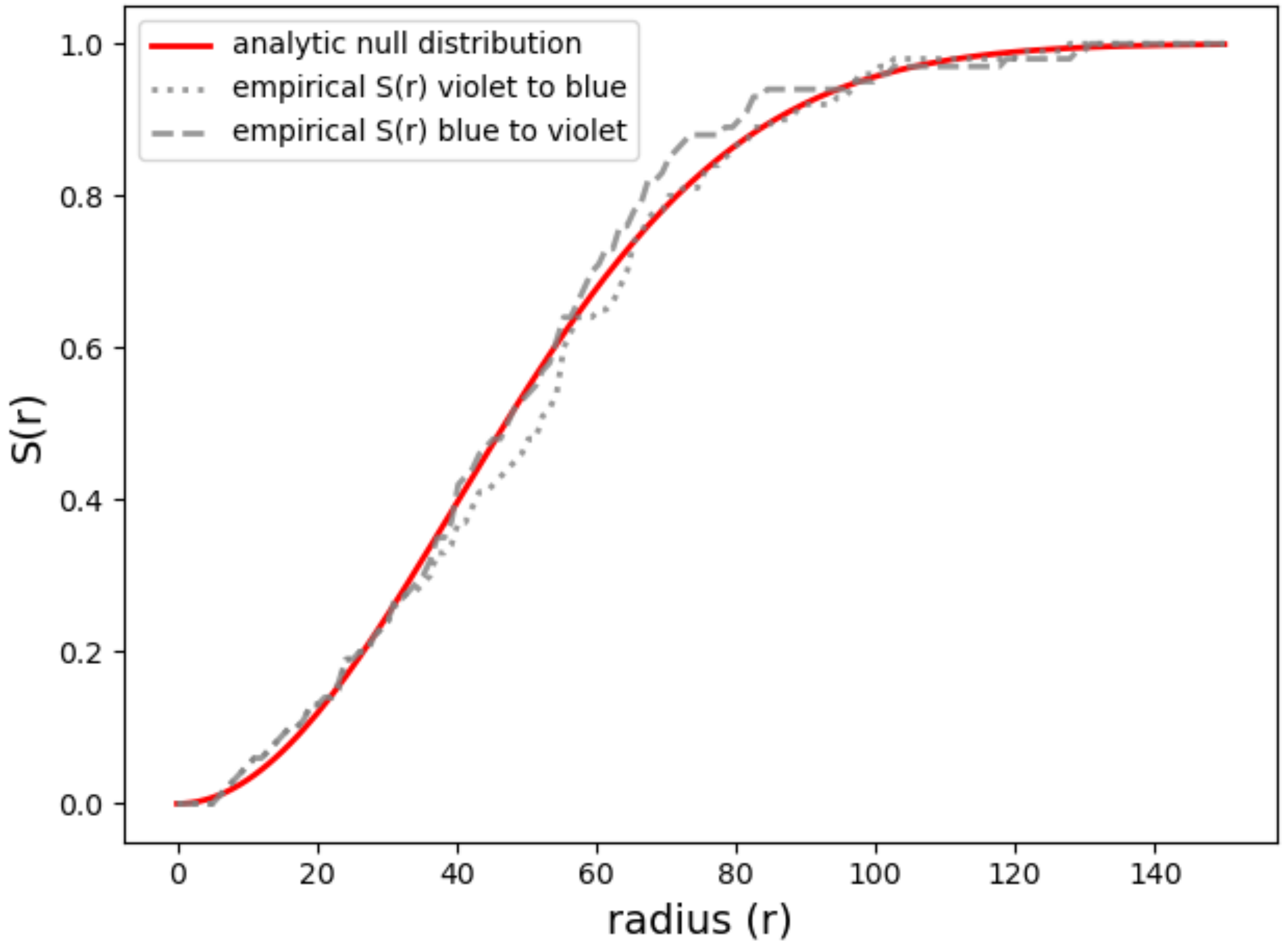
Density of points n_2

You dont need any data to compute the analytic null distribution

Validation — the null distribution

Analytic null distribution



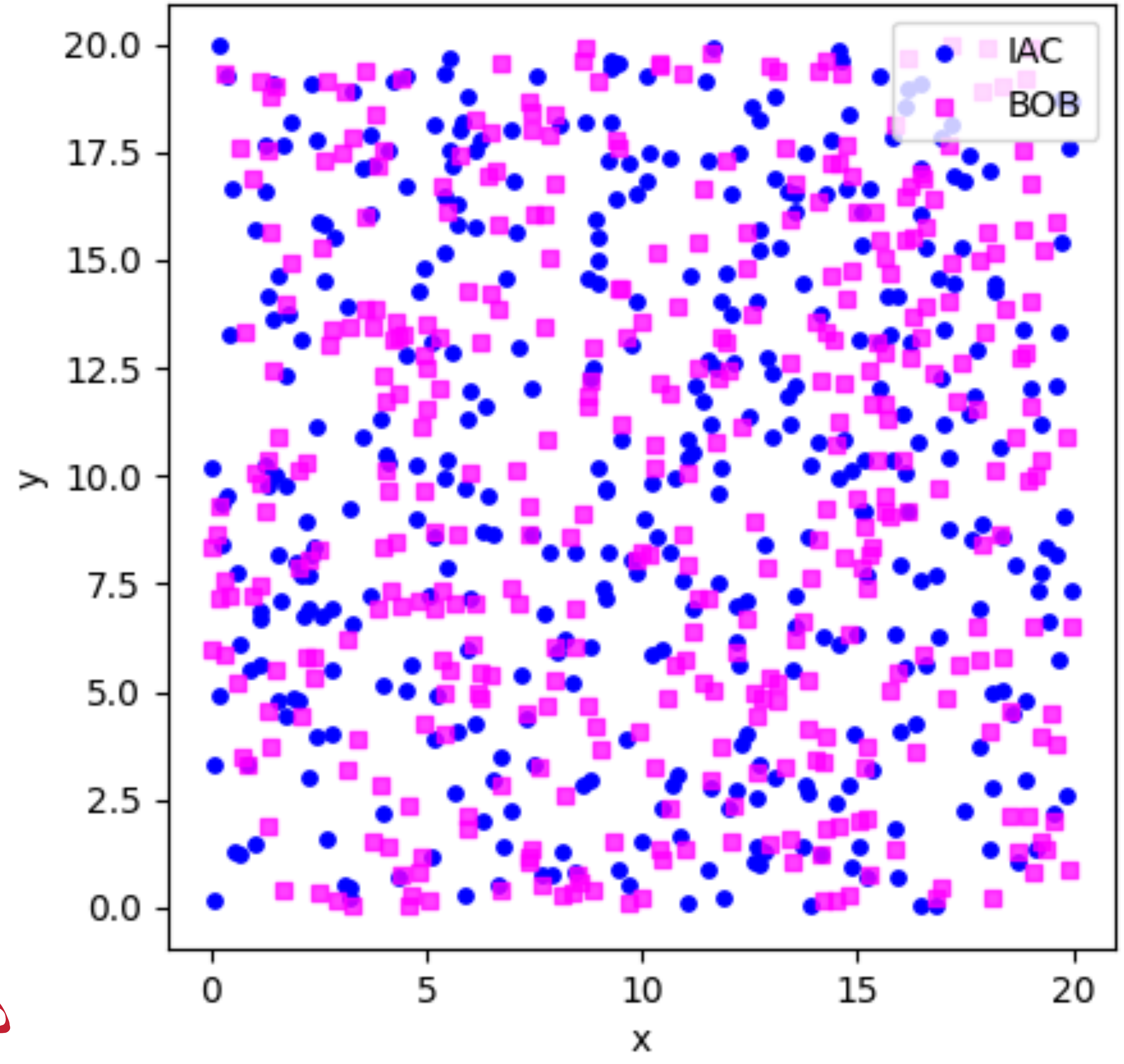


$$S(r) = 1 - e^{-\frac{n_2}{|\Omega|}\pi r^2}$$

Area of a circle with radius r

You dont need any data to compute the analytic null distribution

Validation — the null distribution



BOB = 400

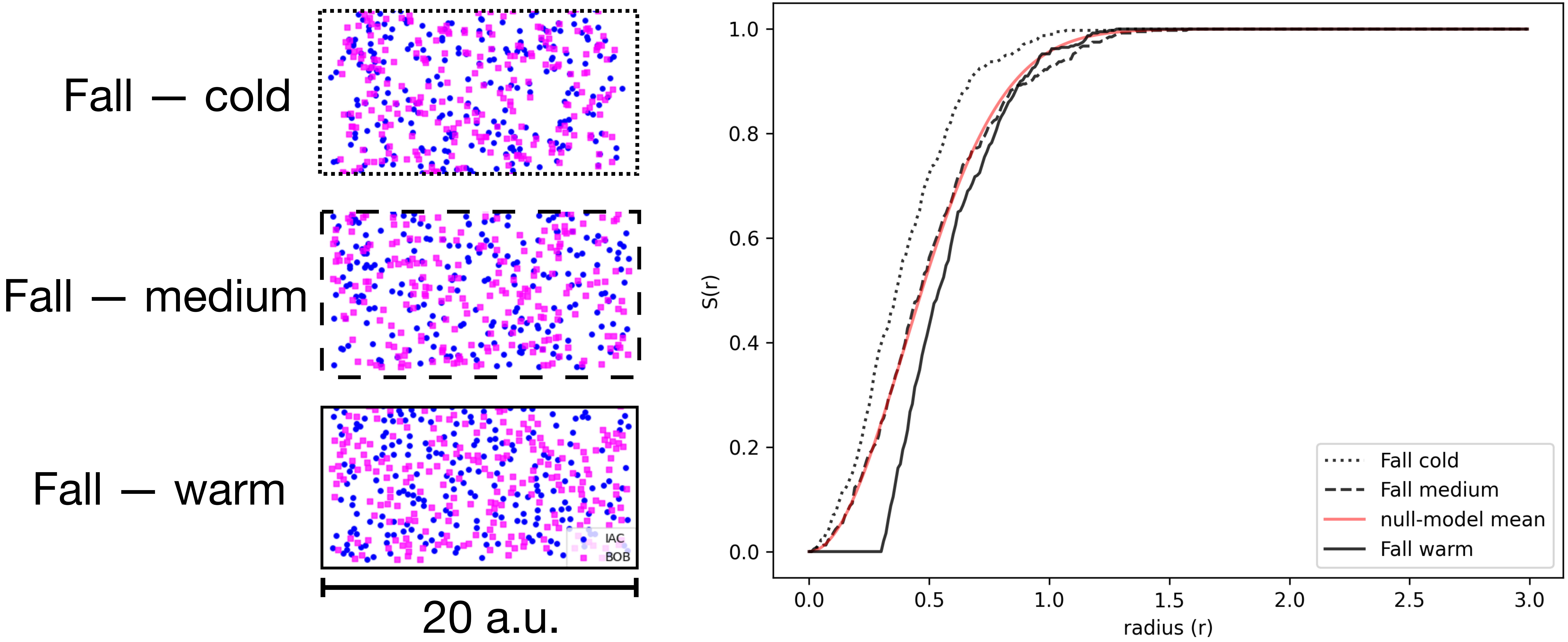
IAC = 400

$$S(r) = 1 - e^{-\frac{n_2}{|\Omega|}\pi r^2}$$

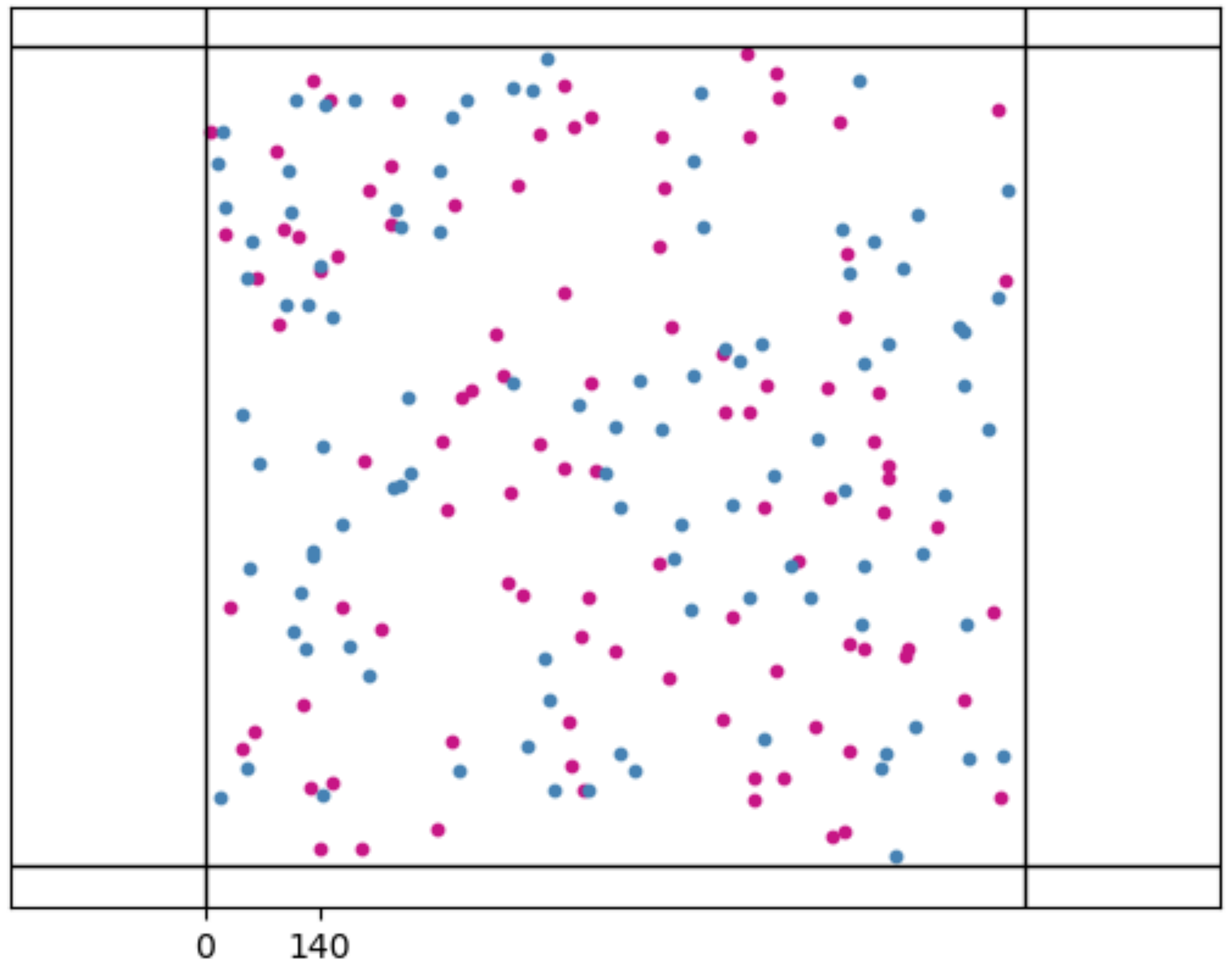
Exercise: Find good values for n2 and |Ω|

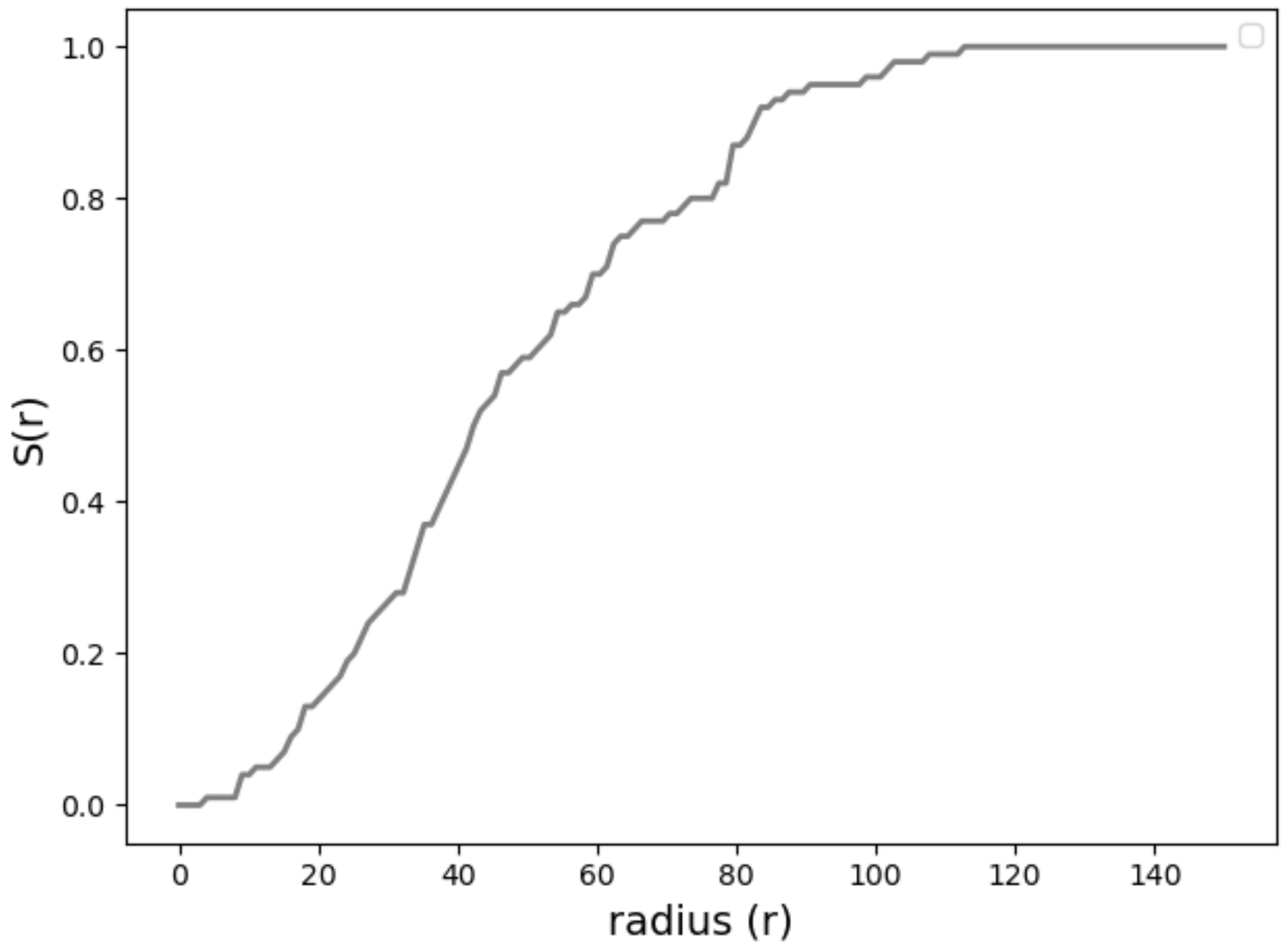
```
n = 1345345  # number of points in the dataset
area = 4056780  # area of the FOV

nulldist = getnulldist(
    n=n, area=area, radii=radii
)  # These are not good values for n and area. Change them!
```

Results — the null distribution



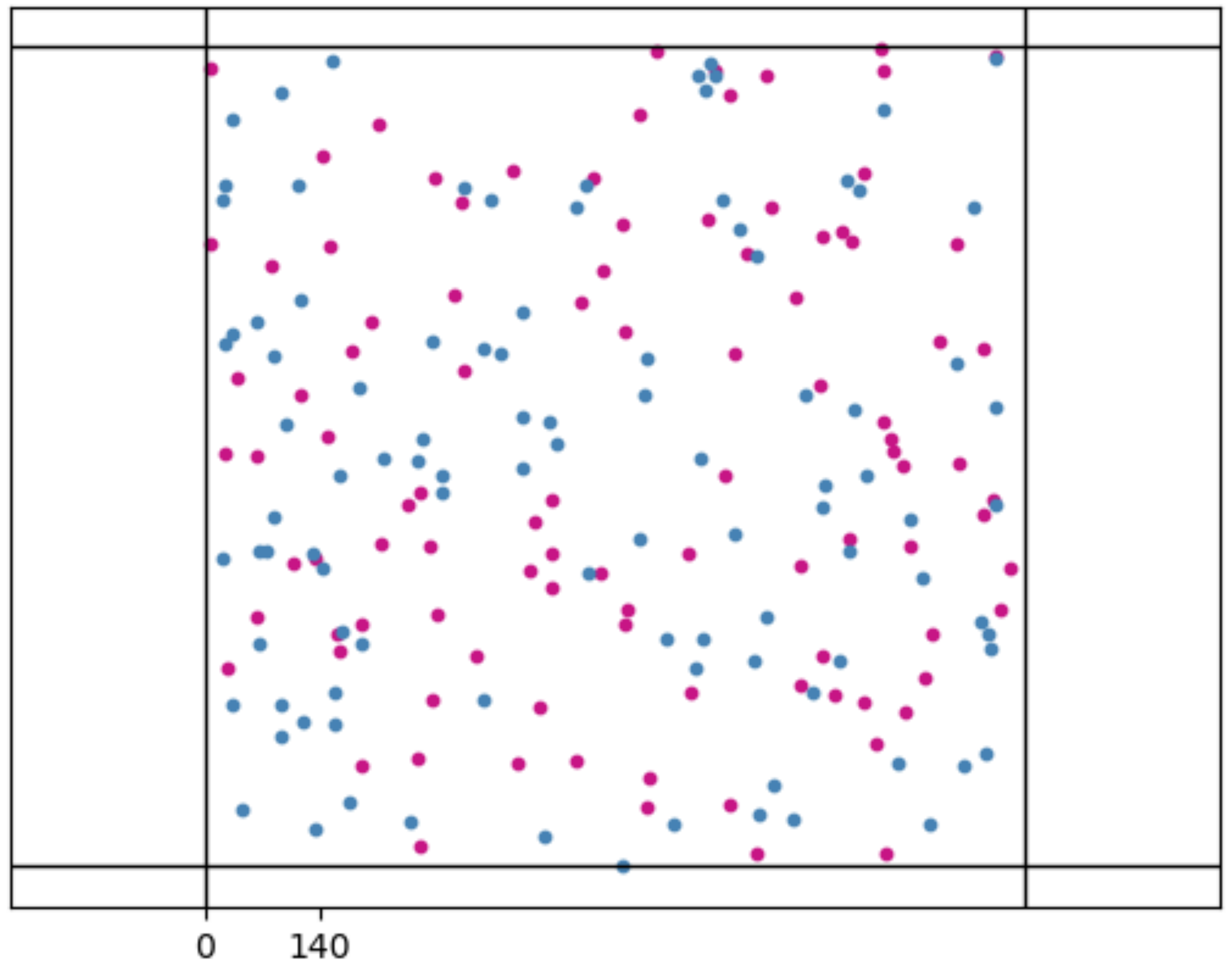
Monte-Carlo-based significance testing

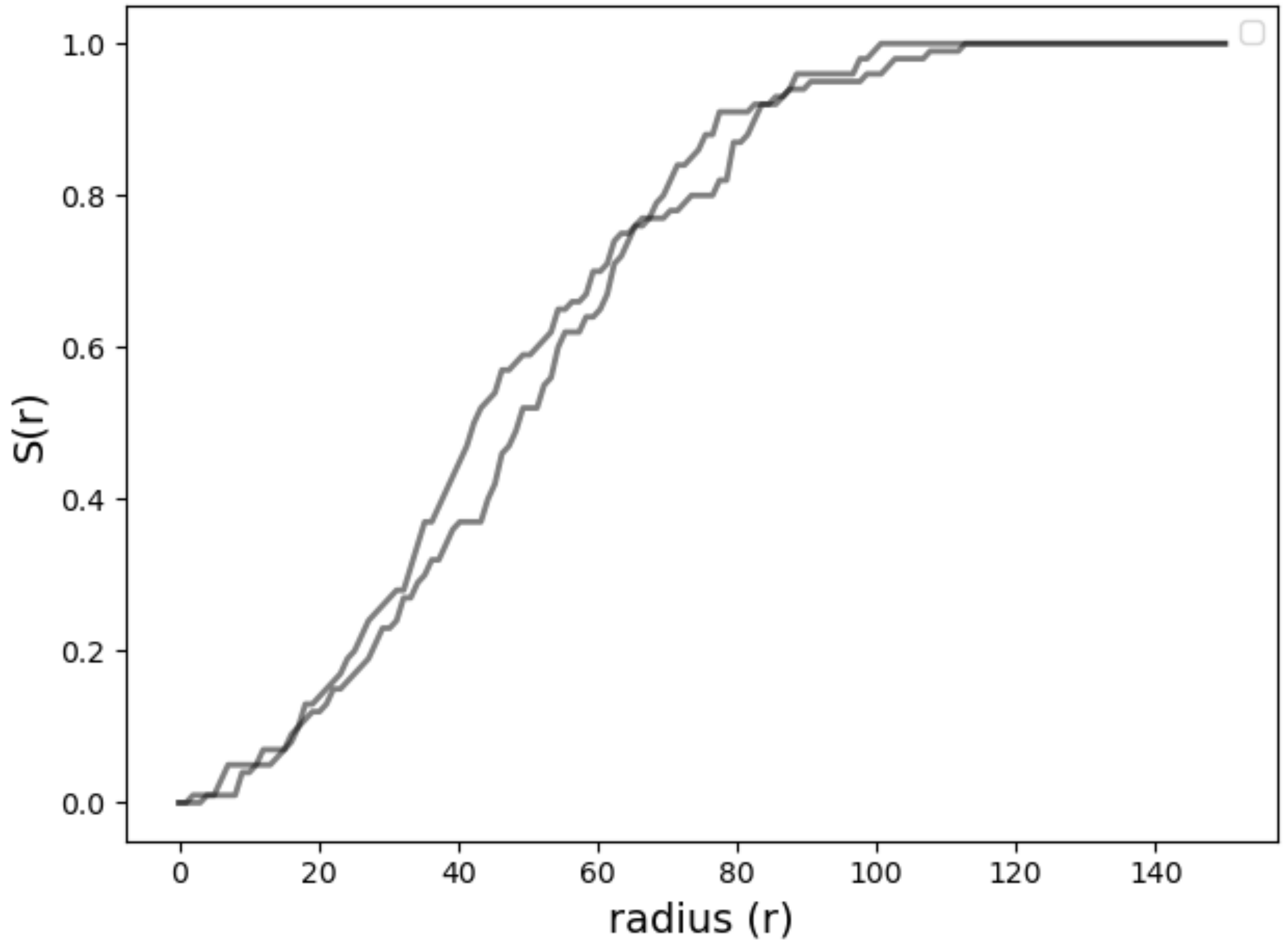




1

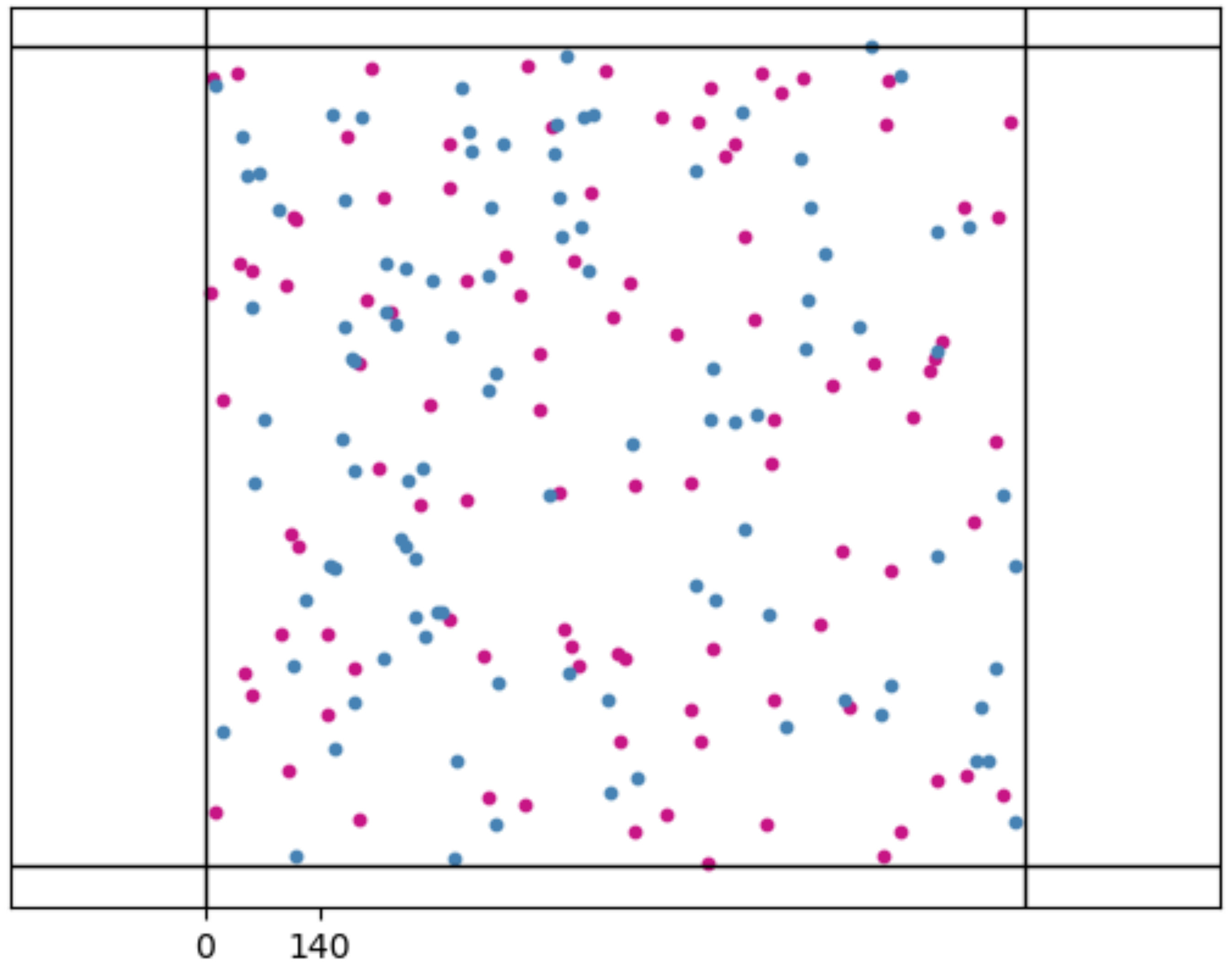
Monte-Carlo-based significance testing

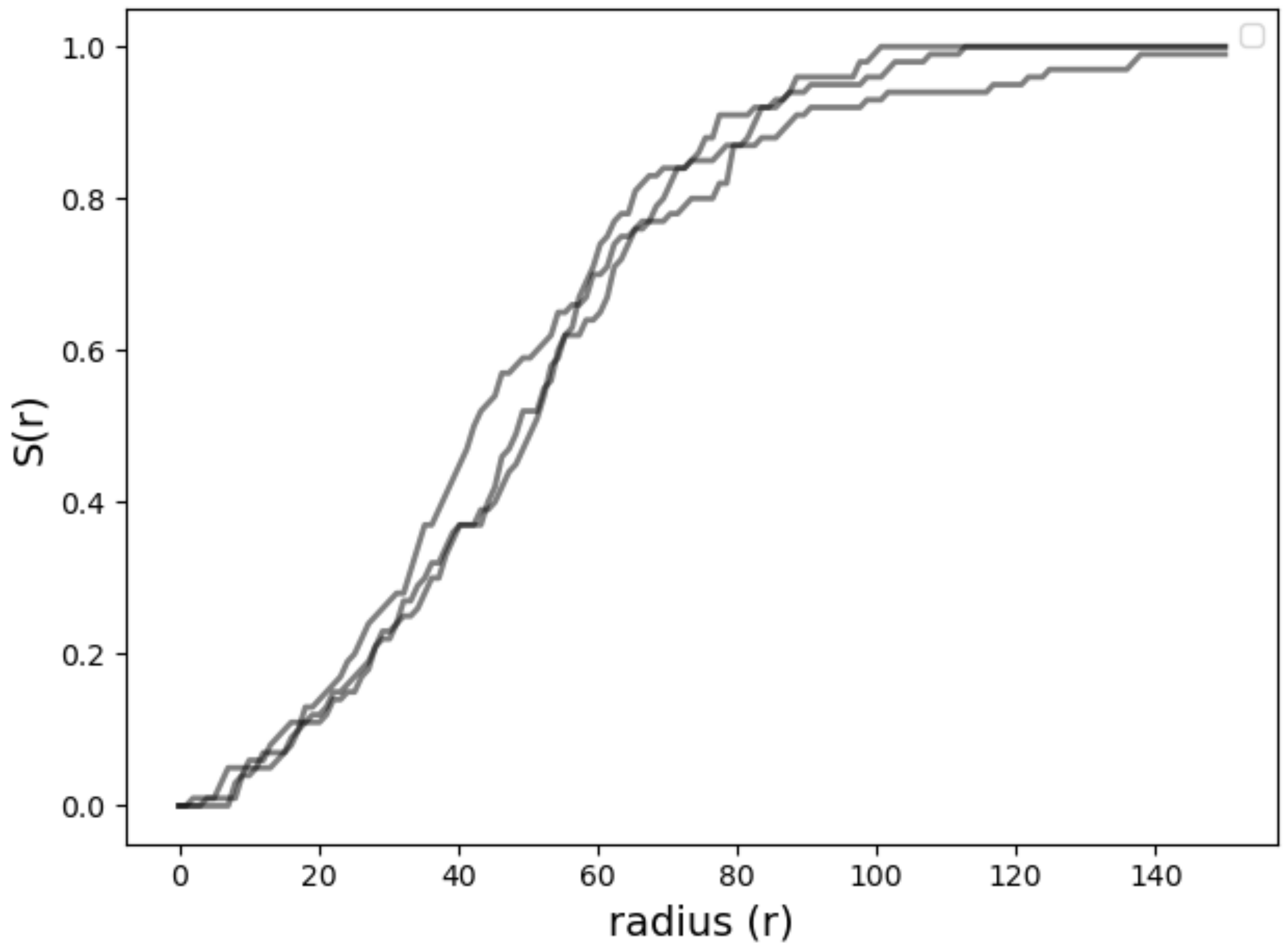




2

Monte-Carlo-based significance testing

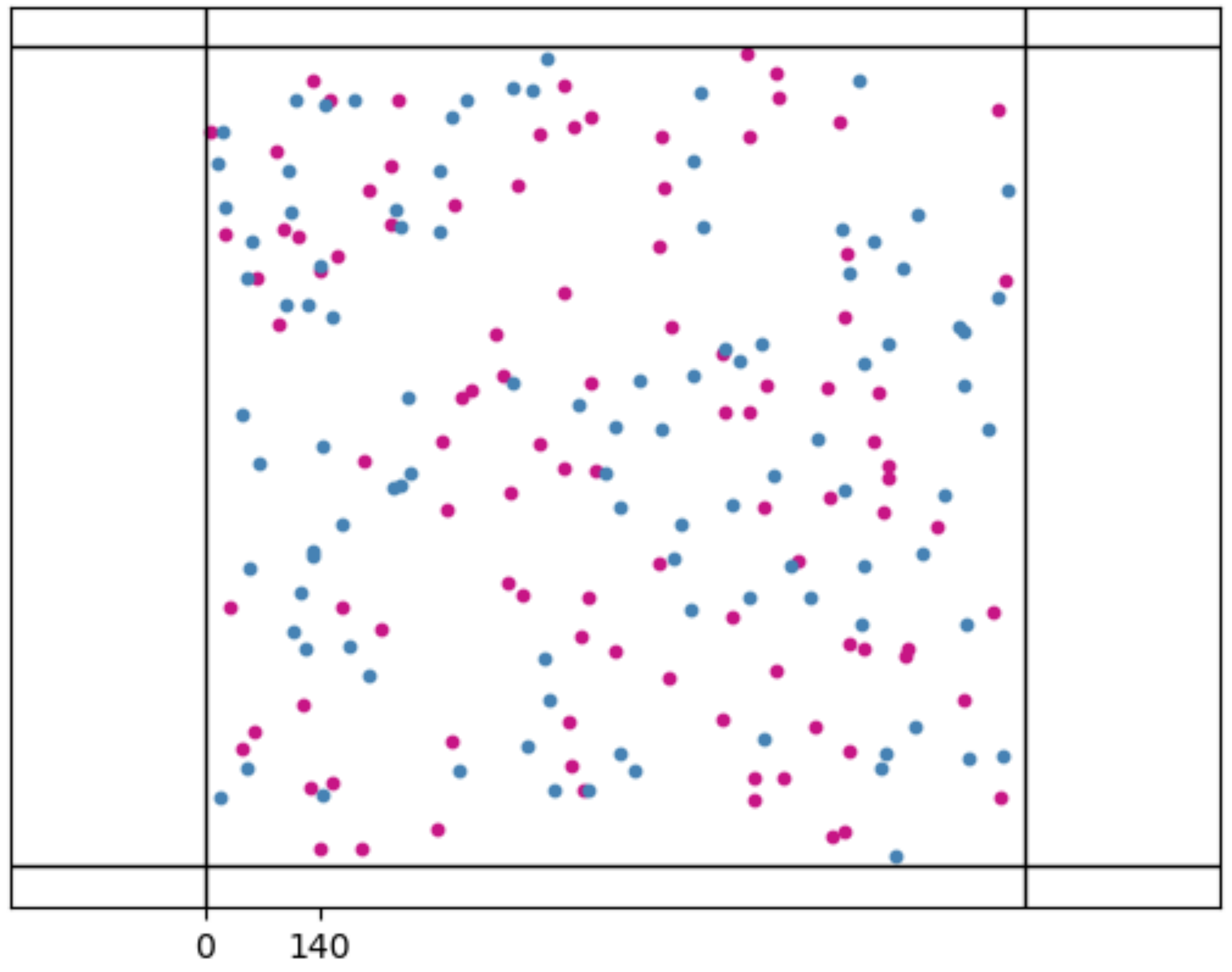


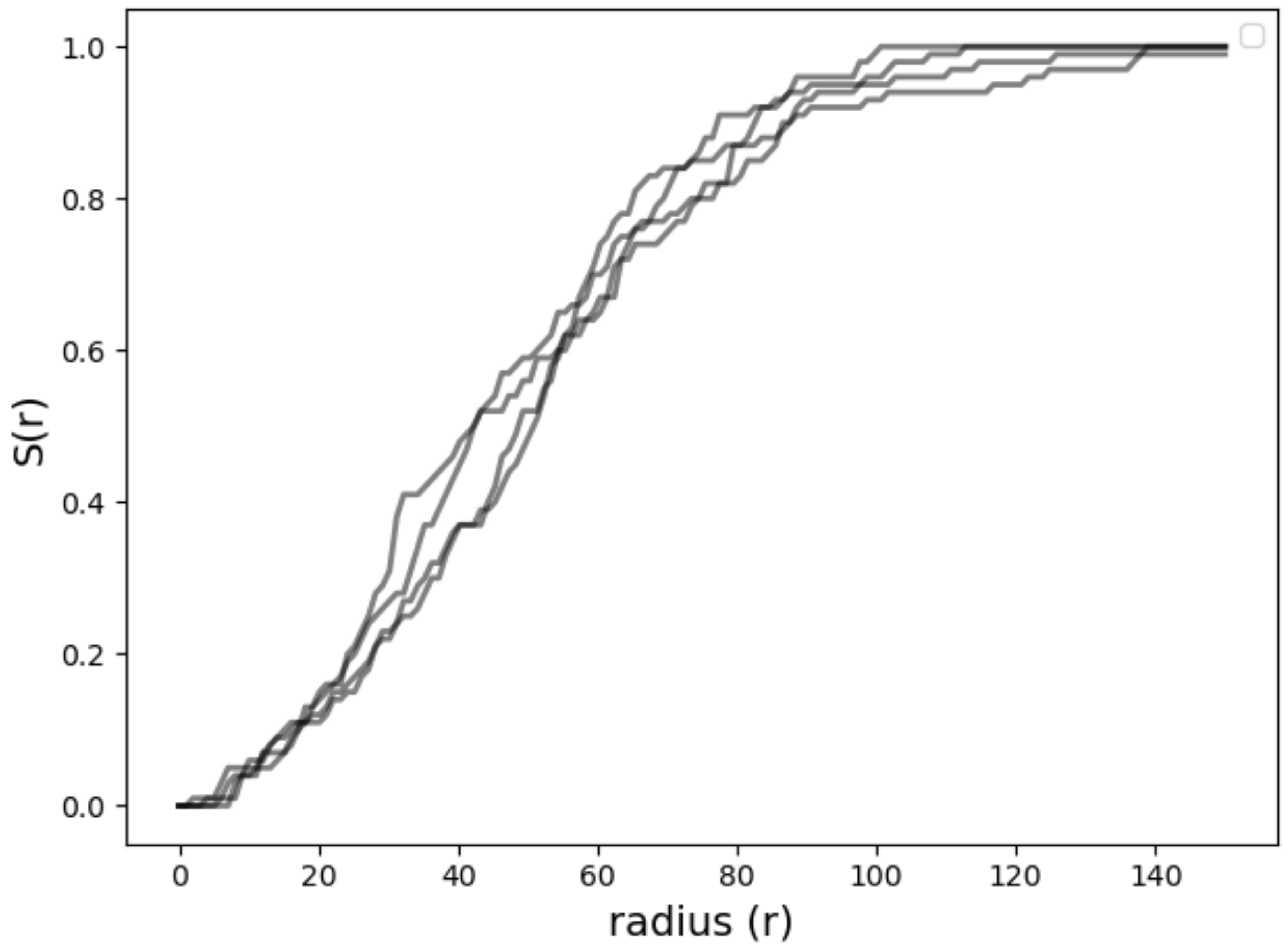


3

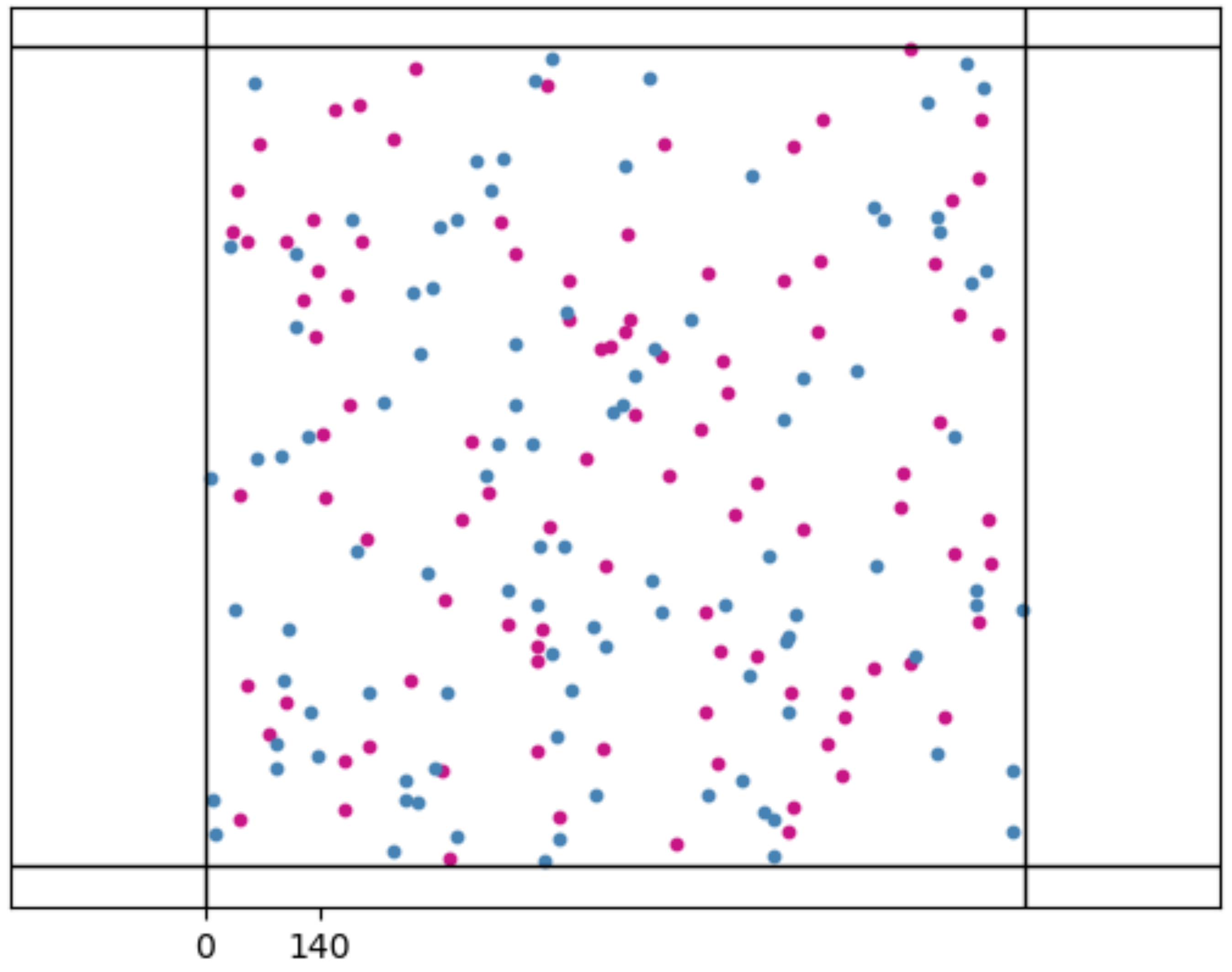
Monte-Carlo-based significance testing

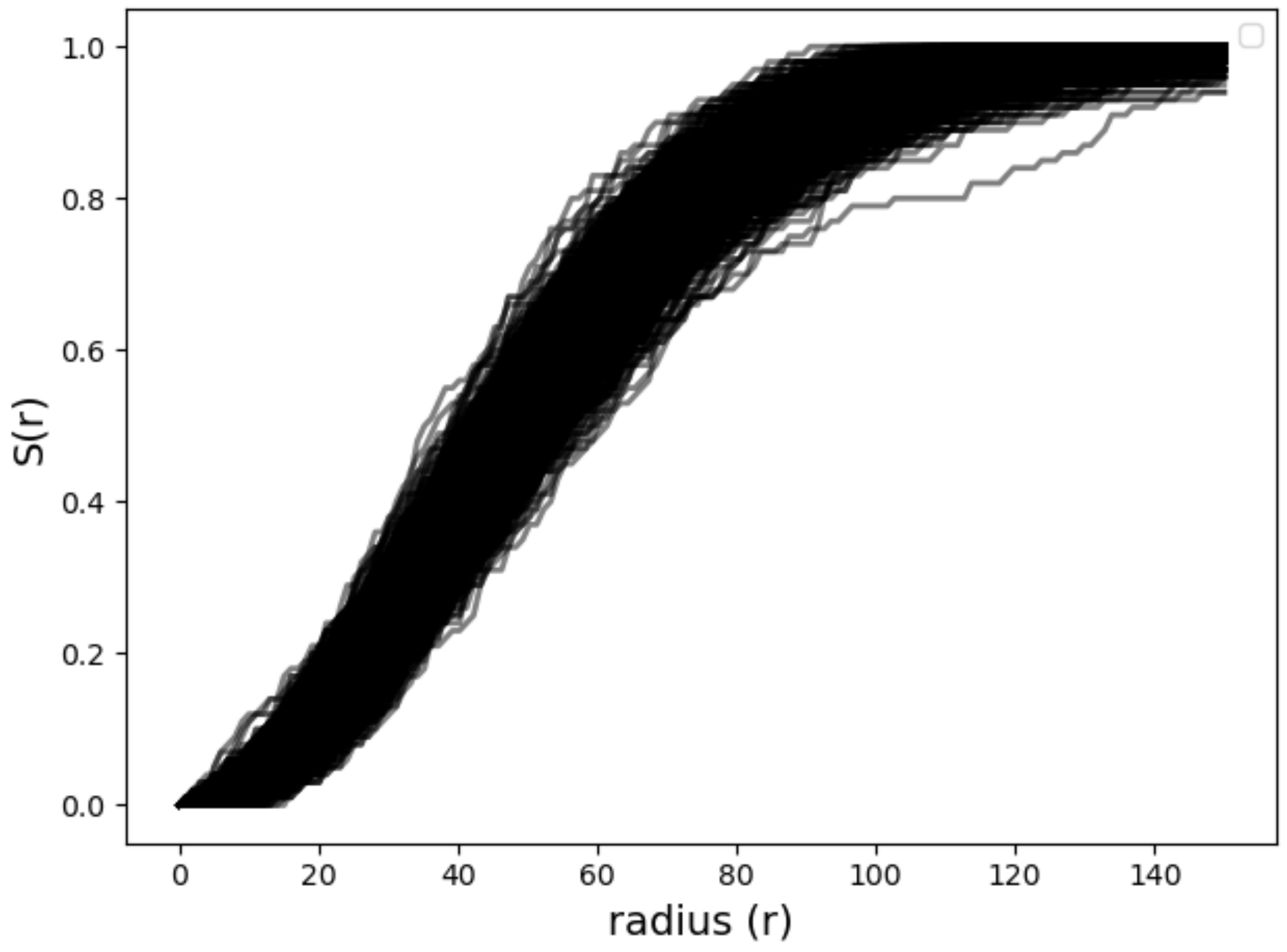
4





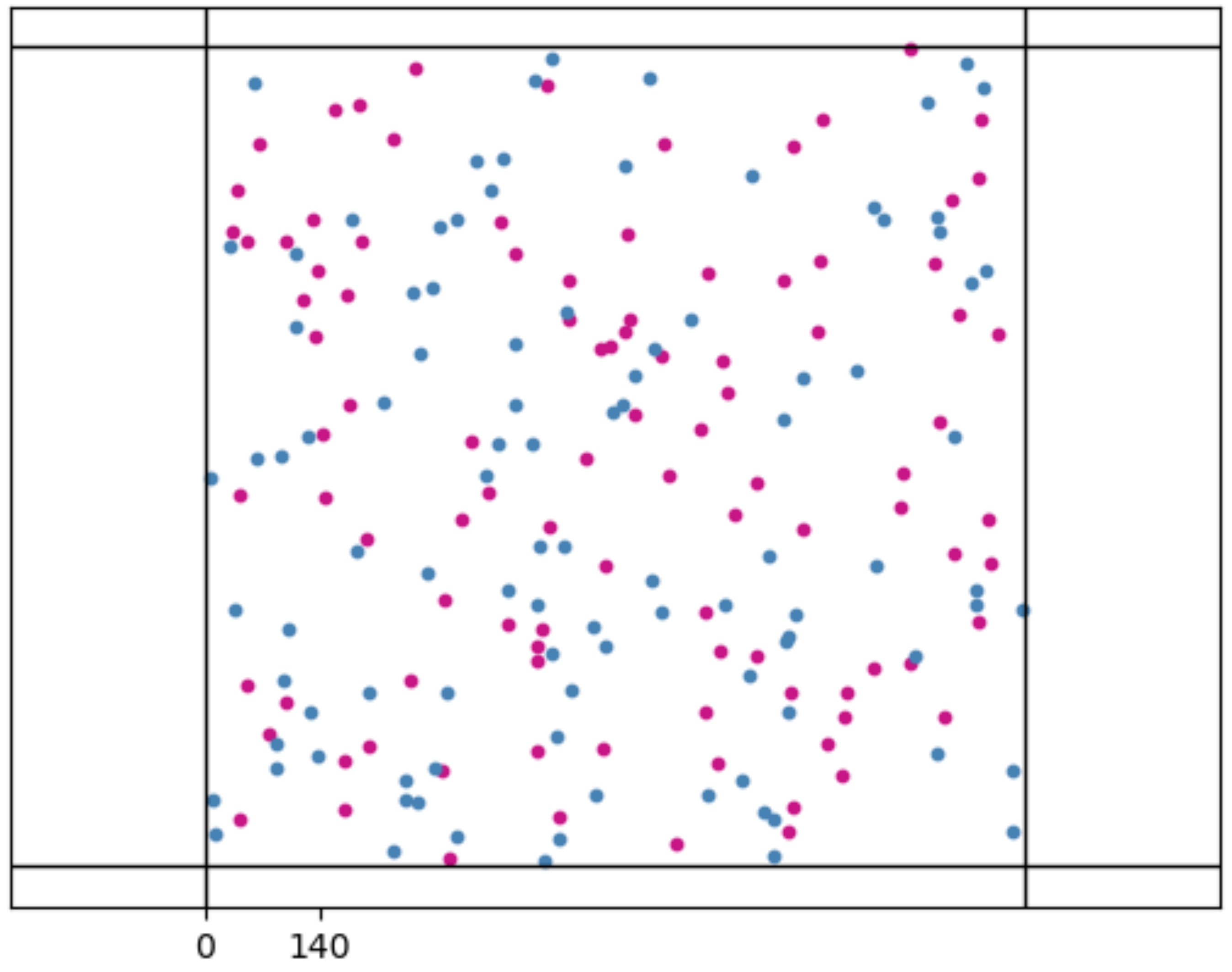
Monte-Carlo-based significance testing

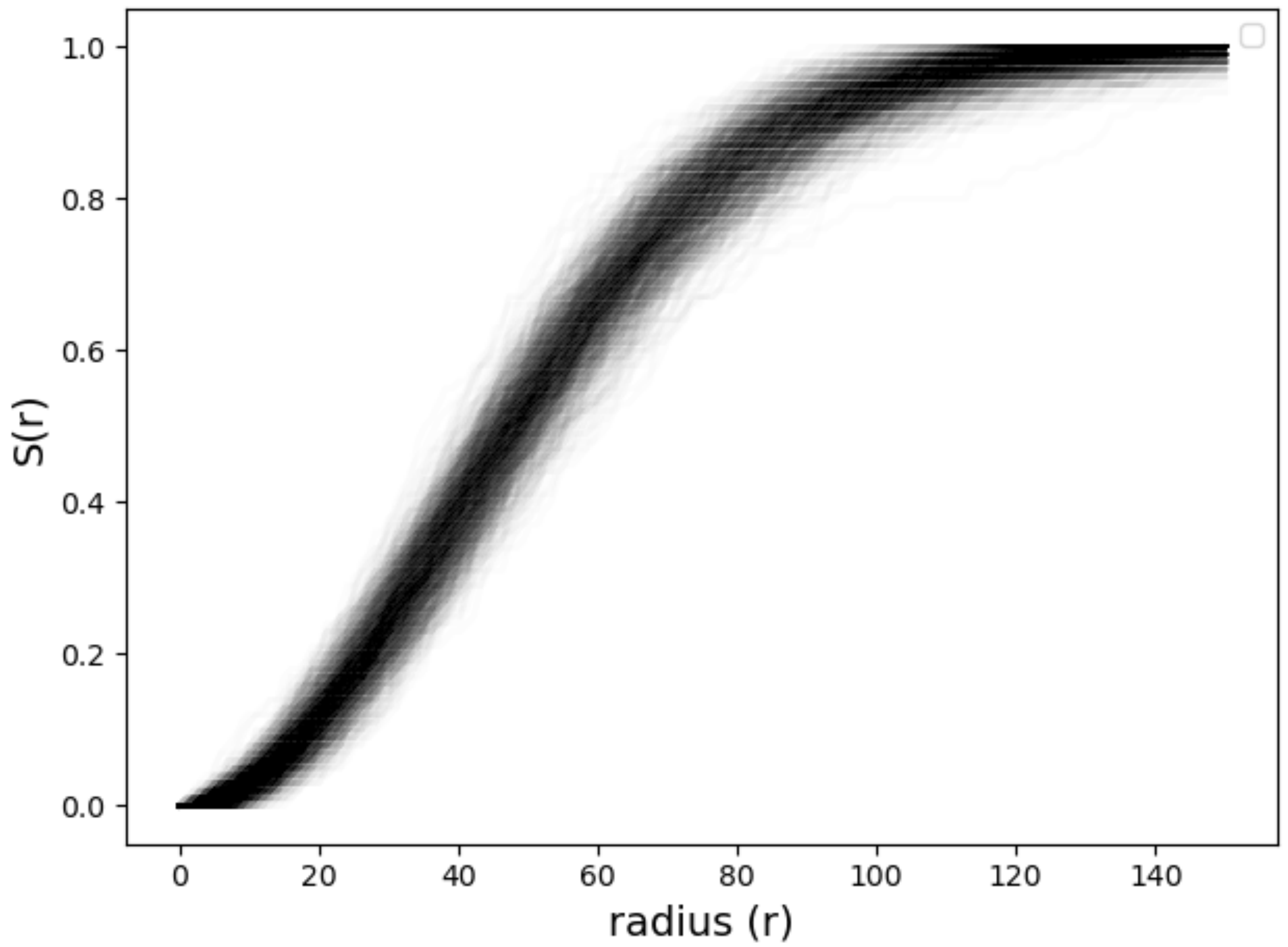




1000

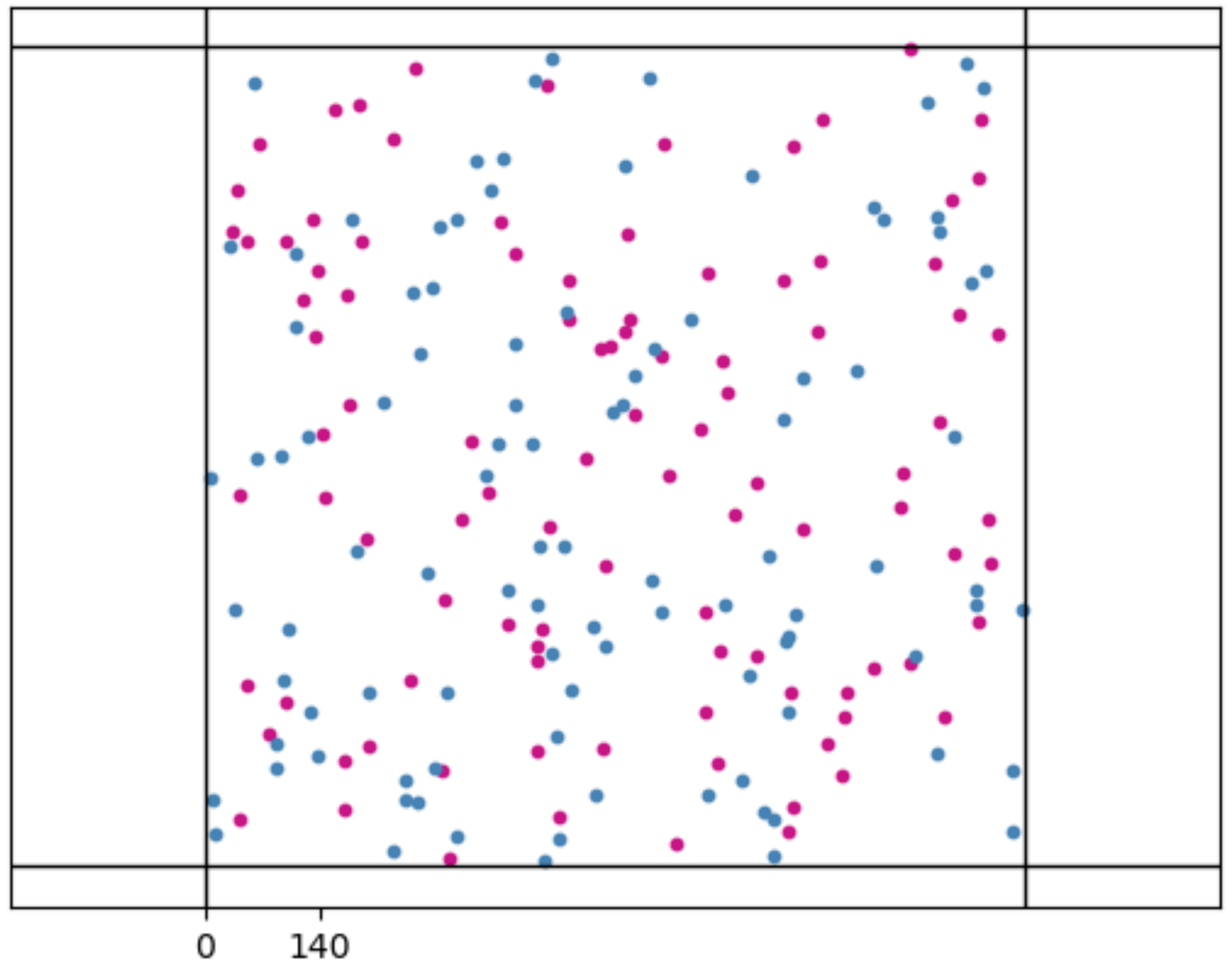
Monte-Carlo-based significance testing

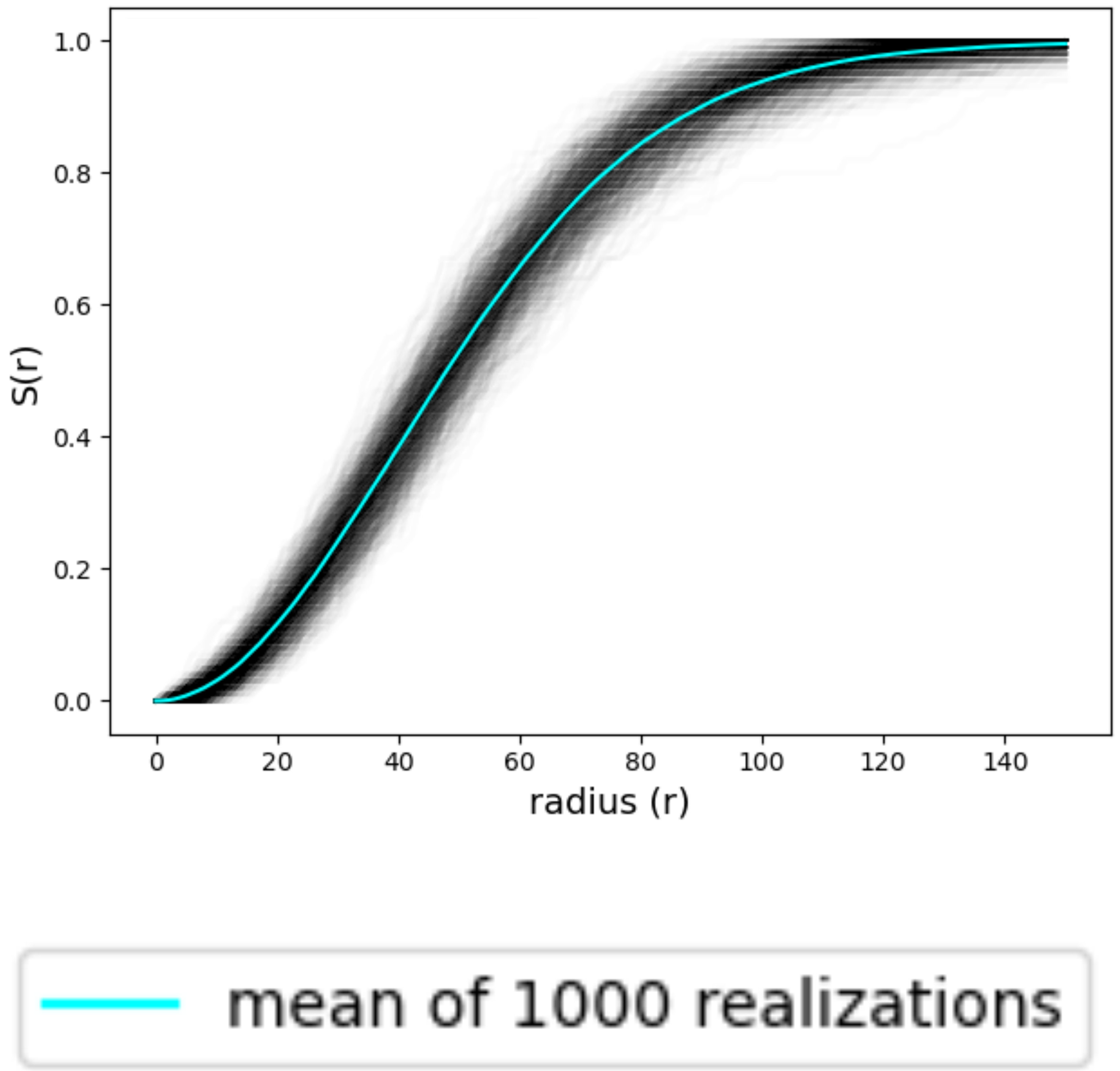




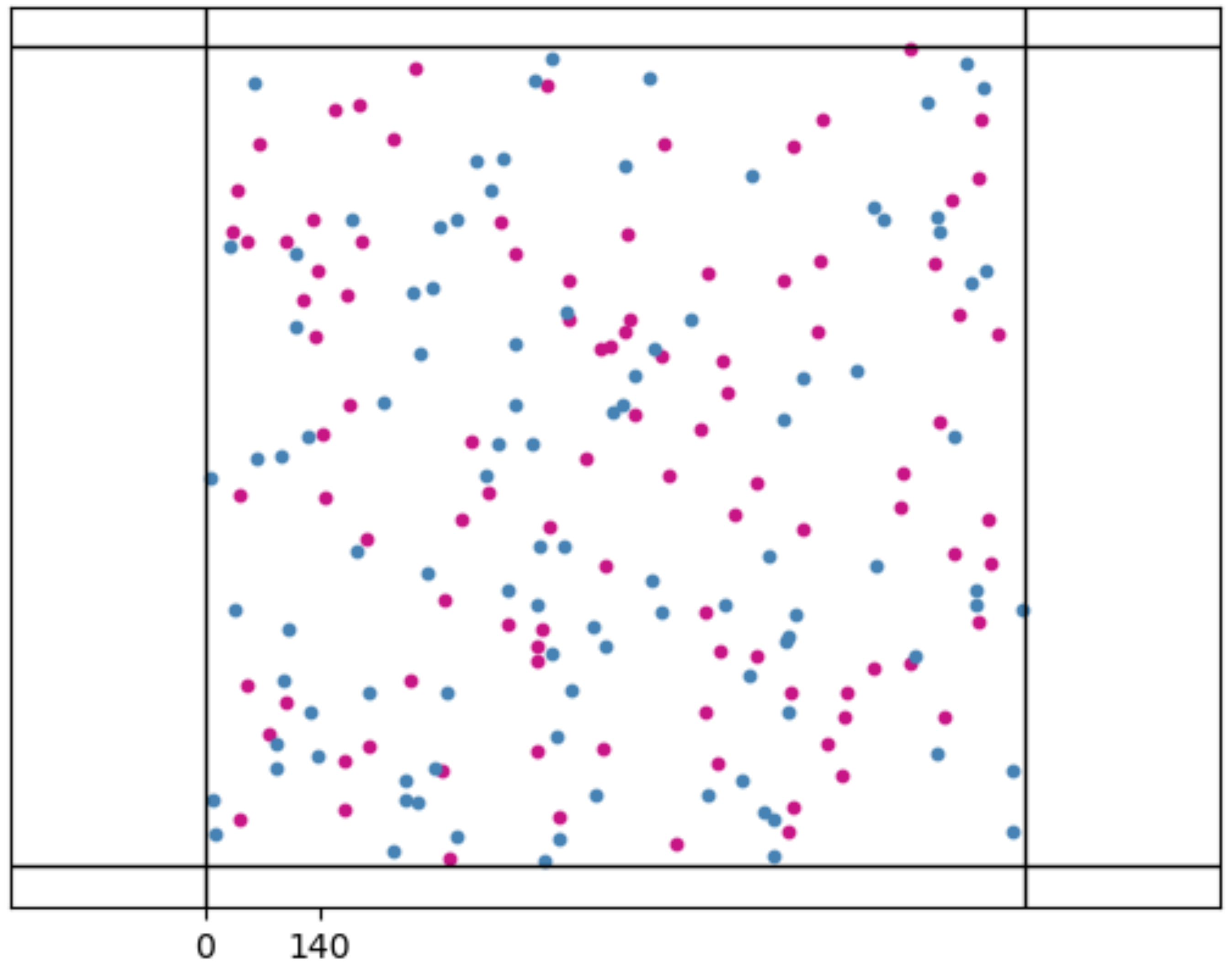
1000

Monte-Carlo-based significance testing

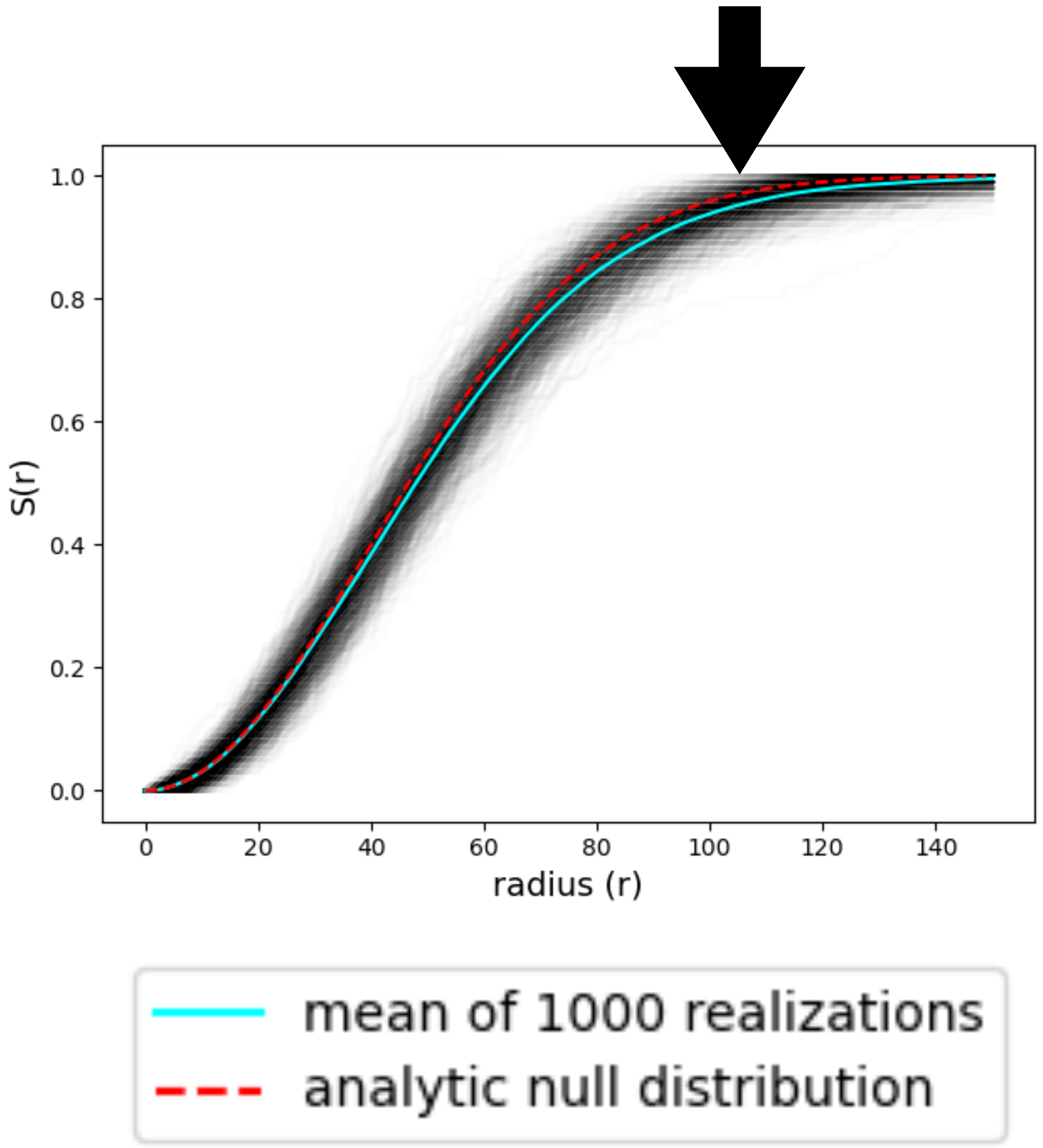




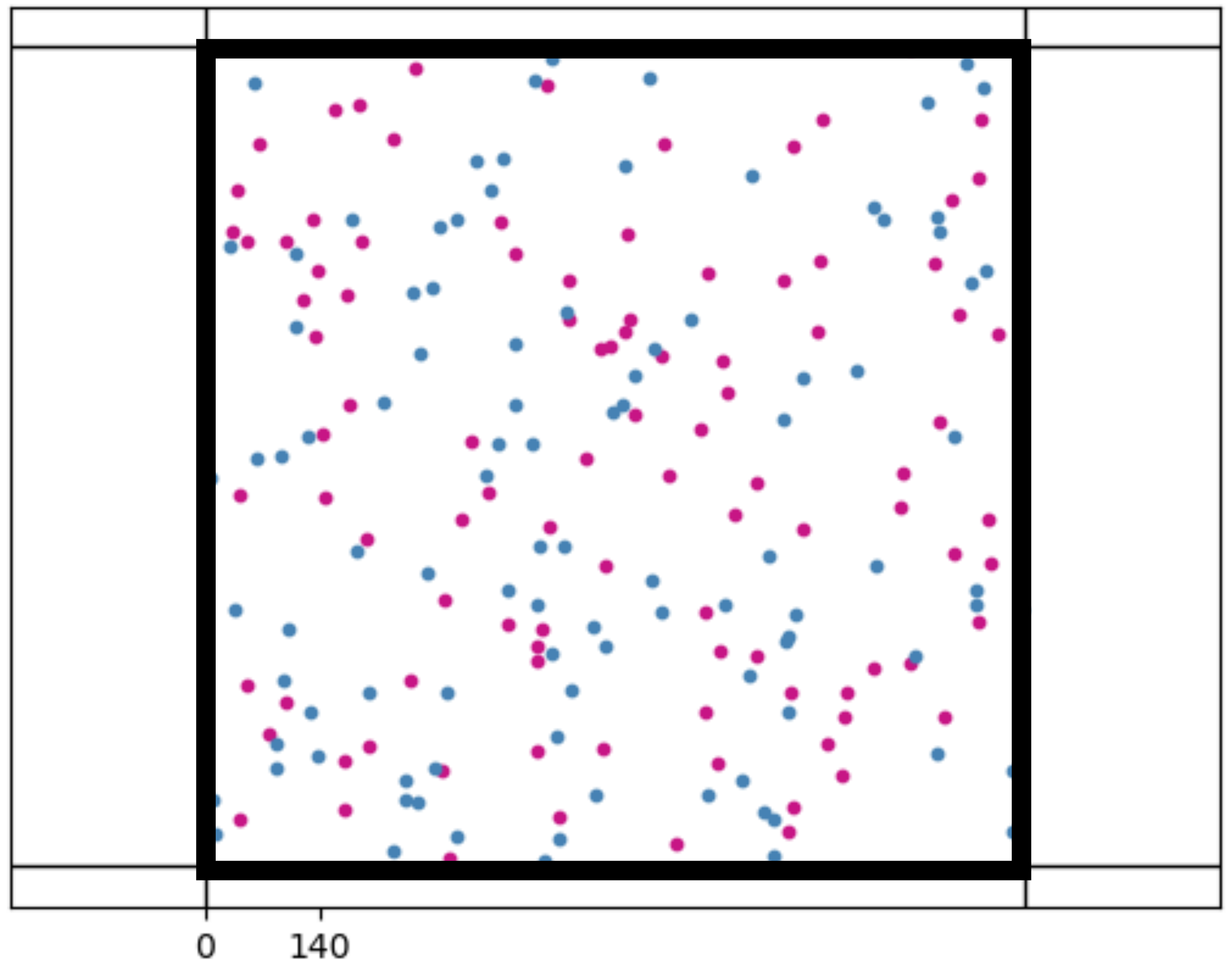
Monte-Carlo-based significance testing



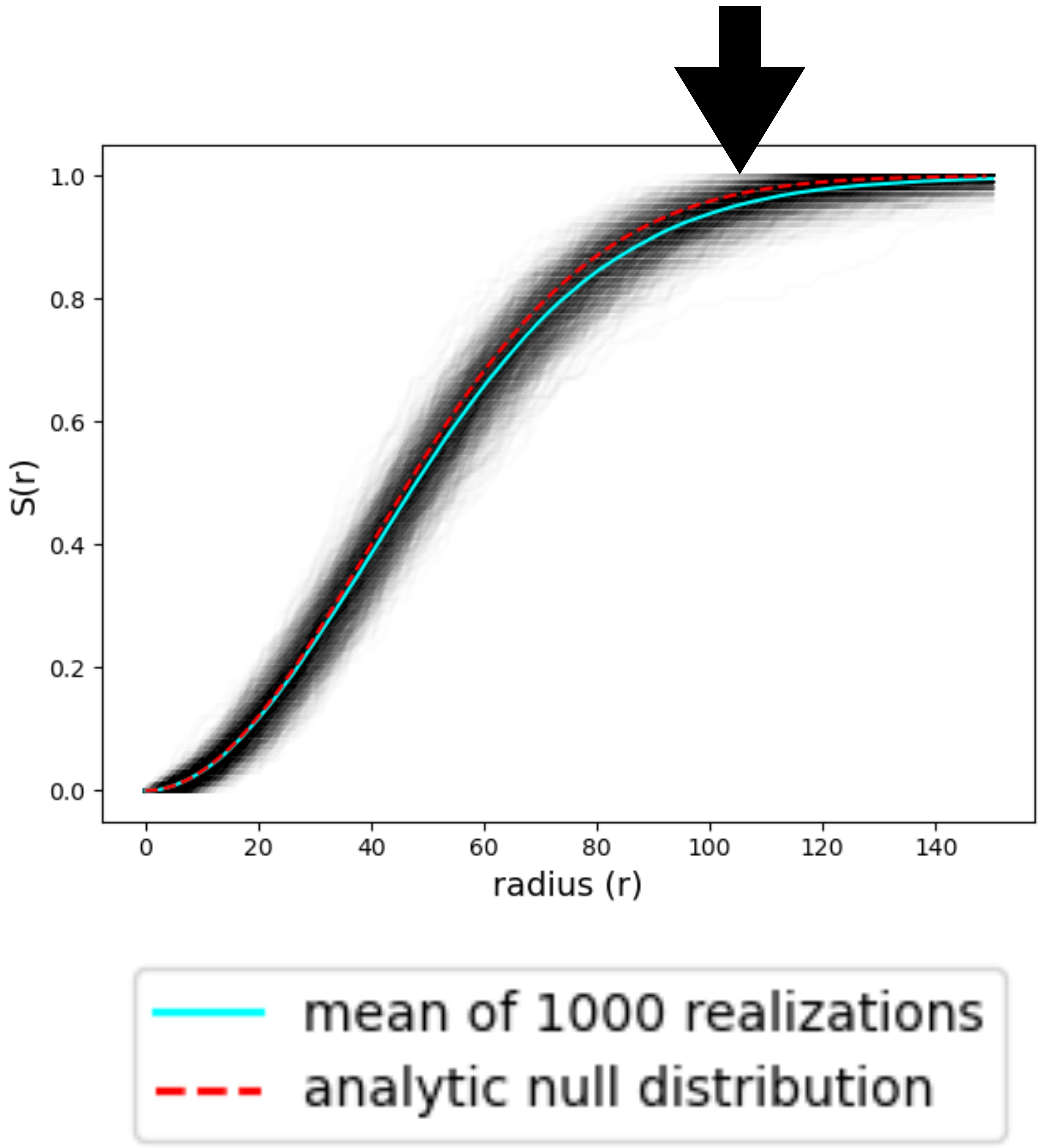
Why?



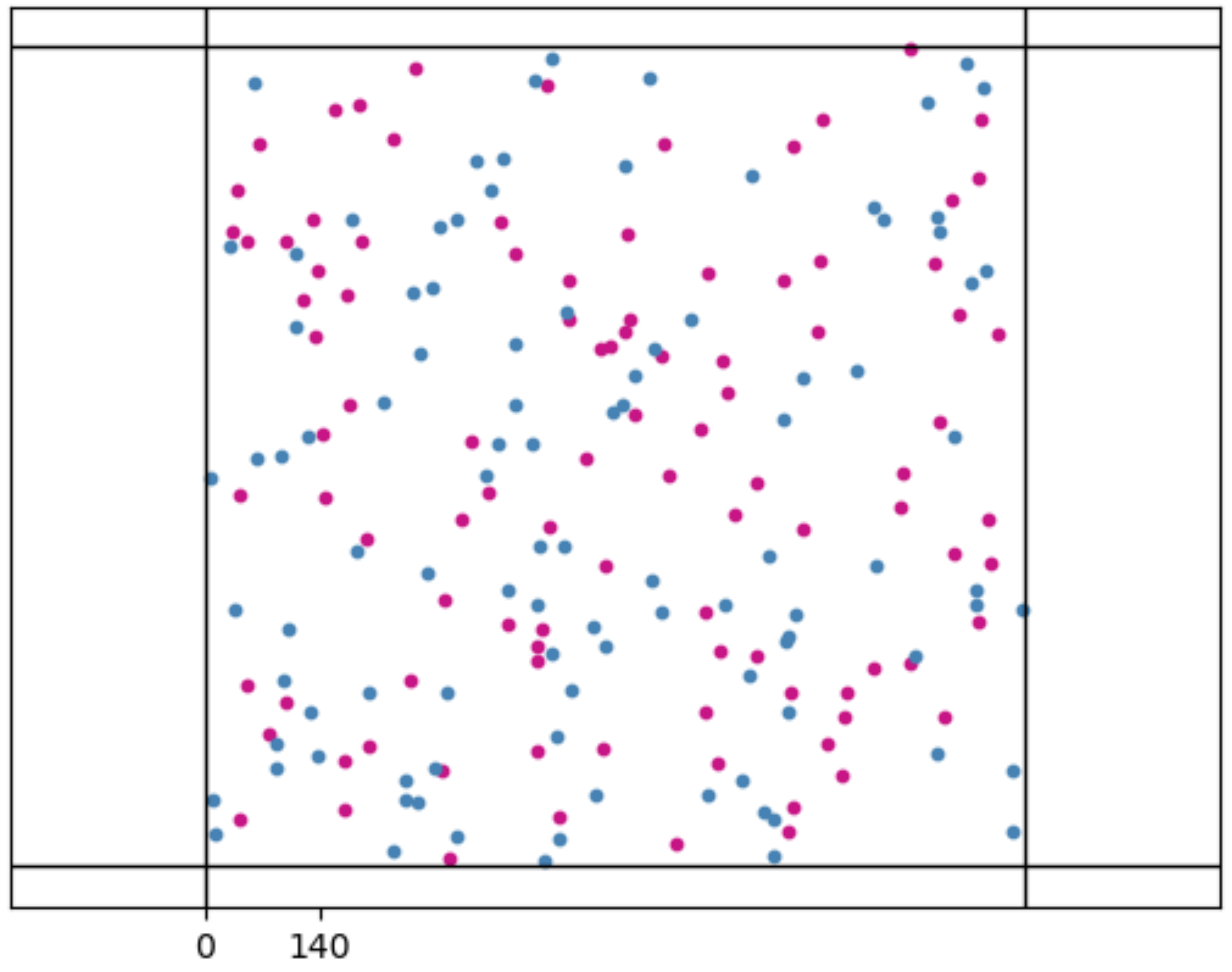
Monte-Carlo-based significance testing

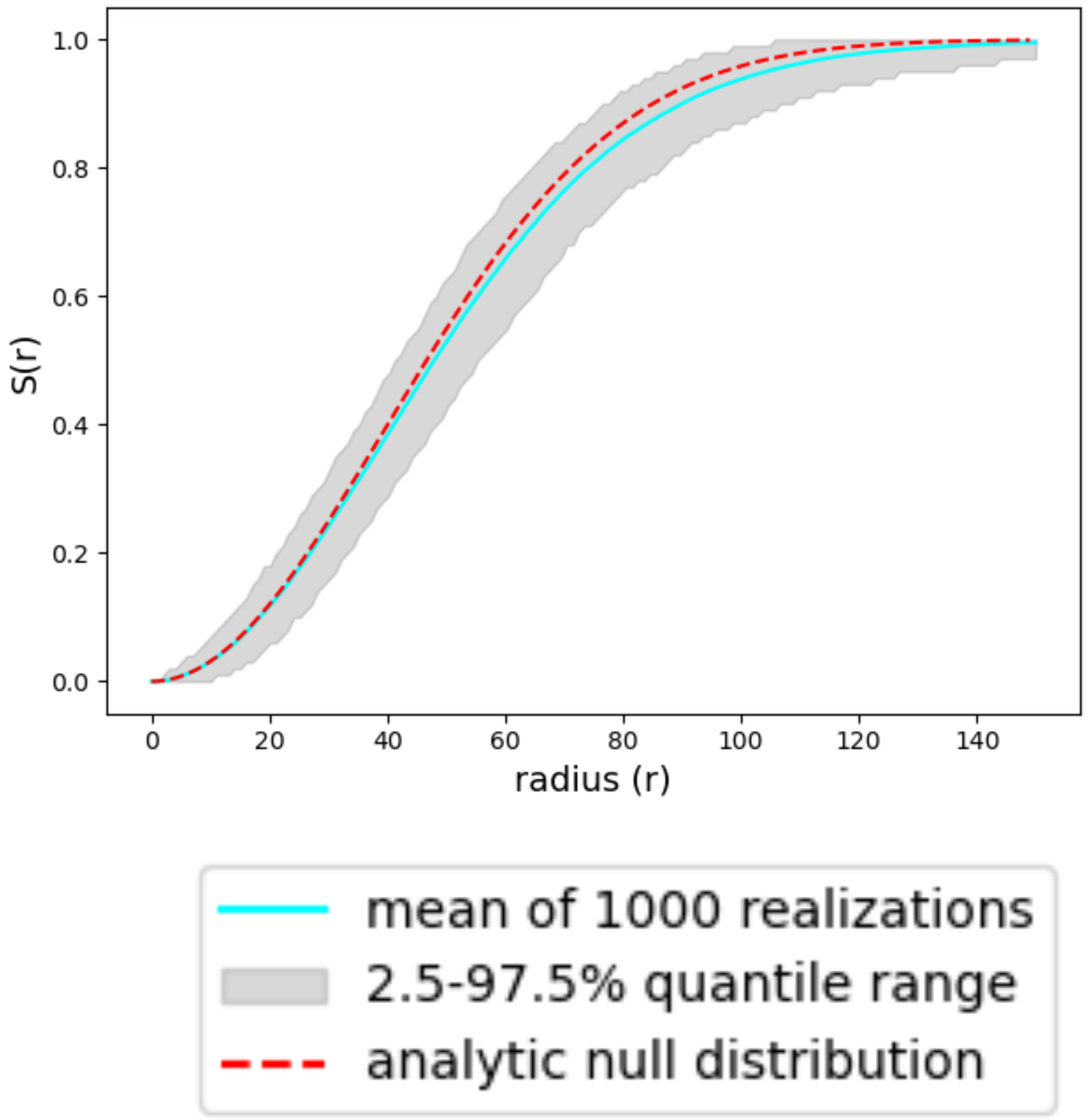


Why?



Monte-Carlo-based significance testing

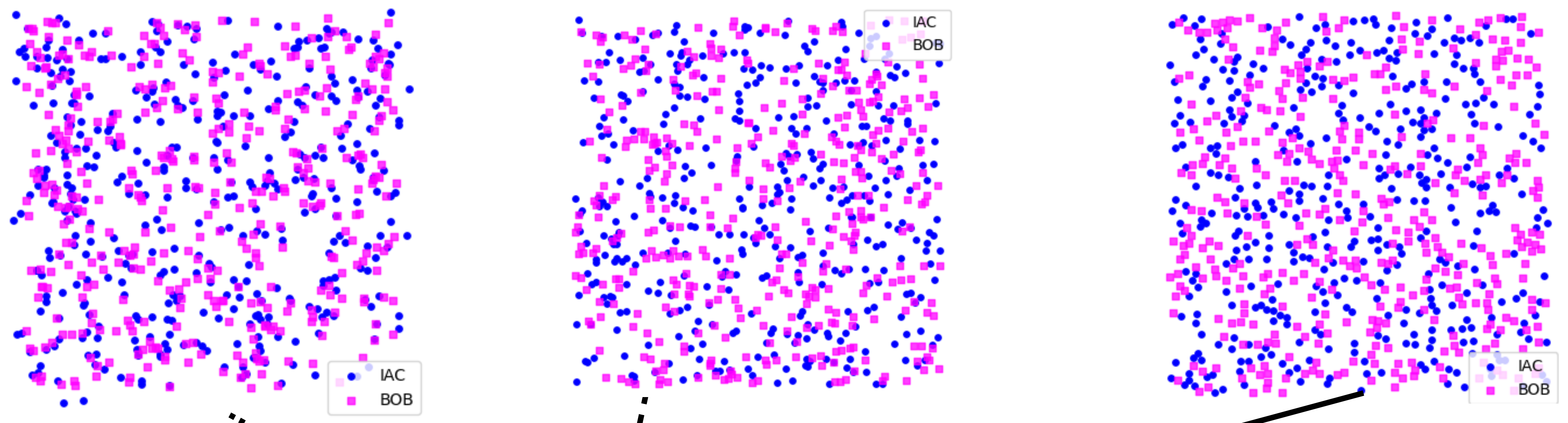


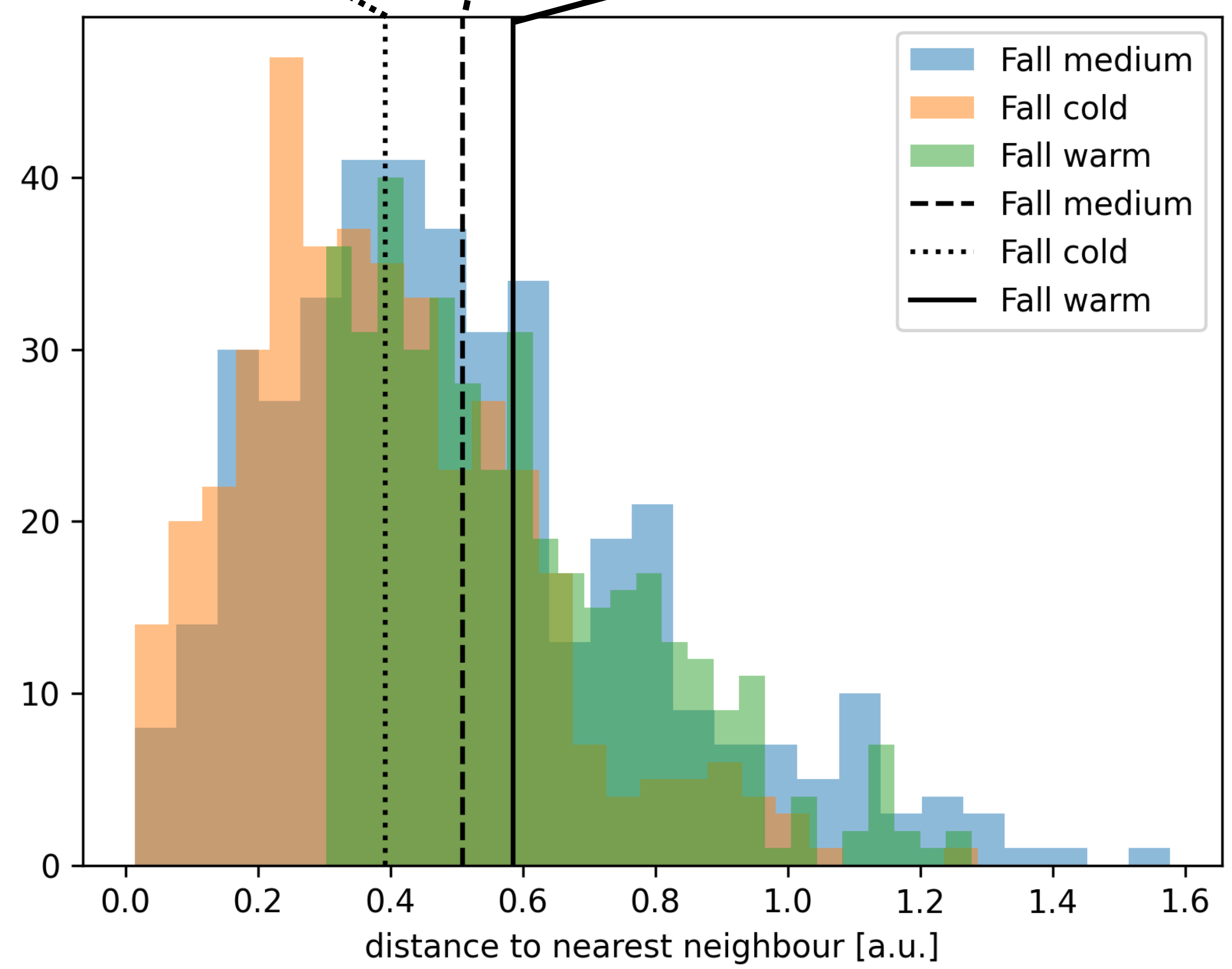


Exercise: throw darts at a board

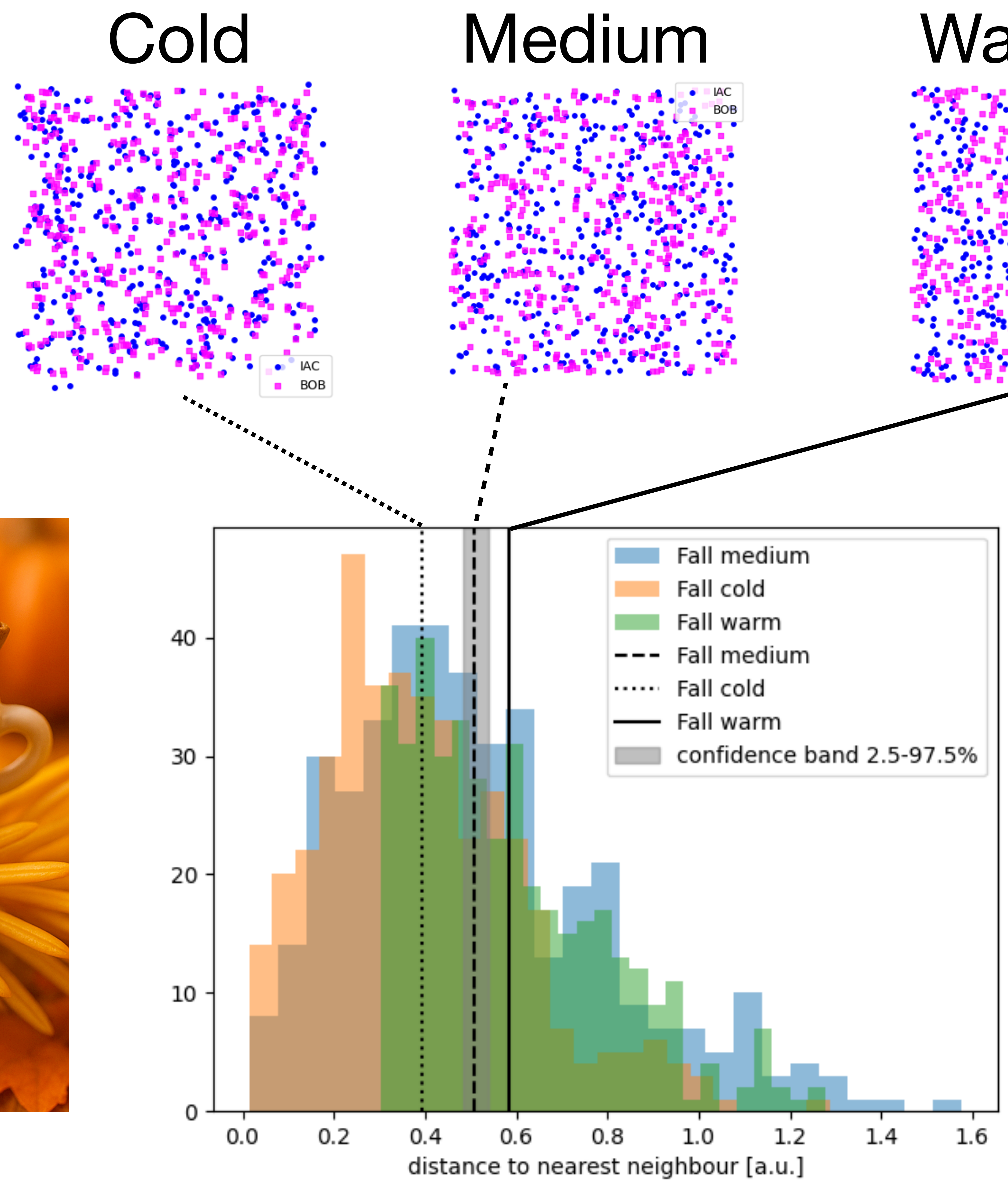
Results: Mean distance IAC -> BOB

Cold Medium Warm





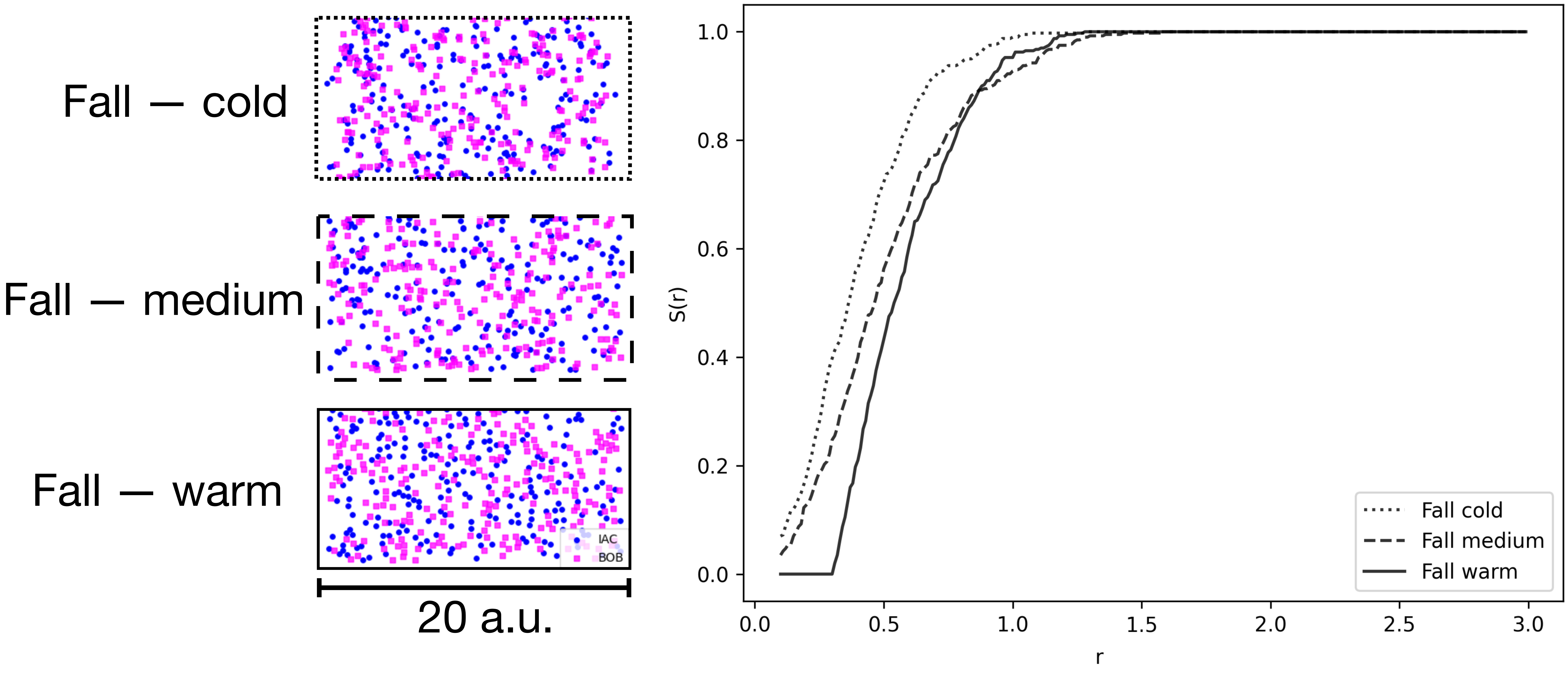
Results: Mean distance IAC -> BOB



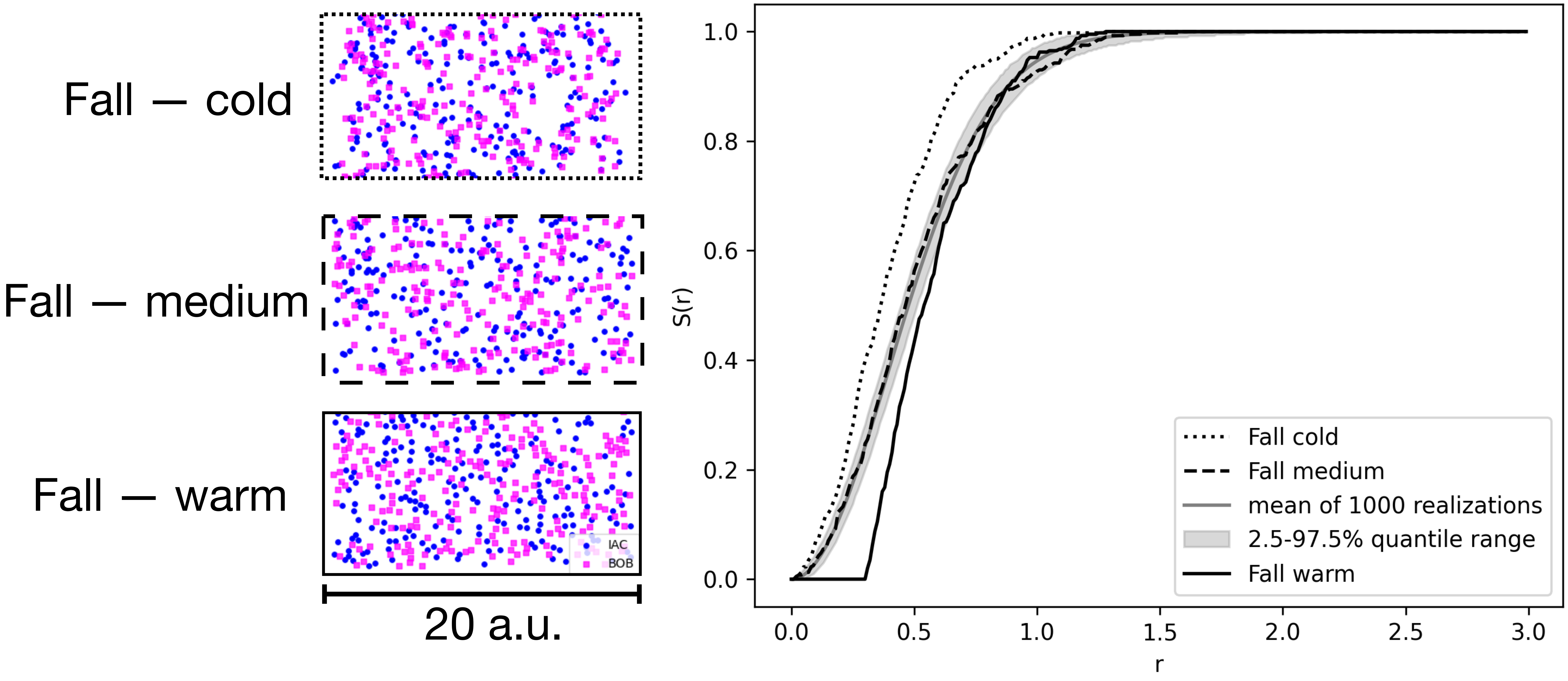
Cold IAC->BOB are closer to each other than random under the 95% significance level

Warm IAC->BOB are further apart from each other than random under the 95% significance level

Results: Nearest neighbor function

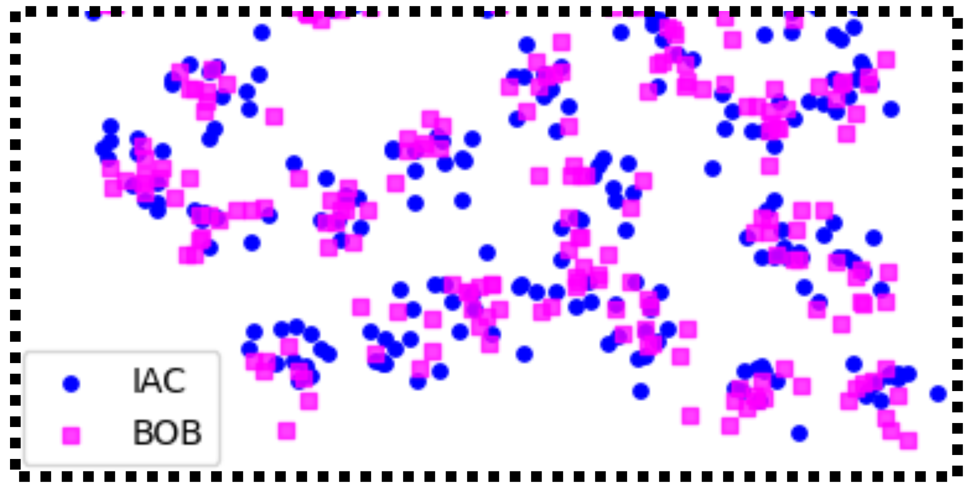


Results: Nearest neighbor function



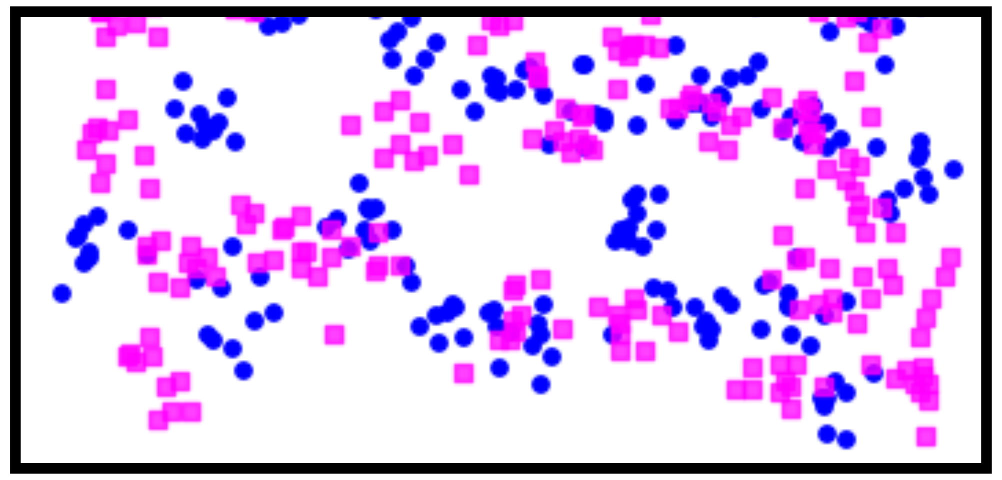
Results: Ripley's K function

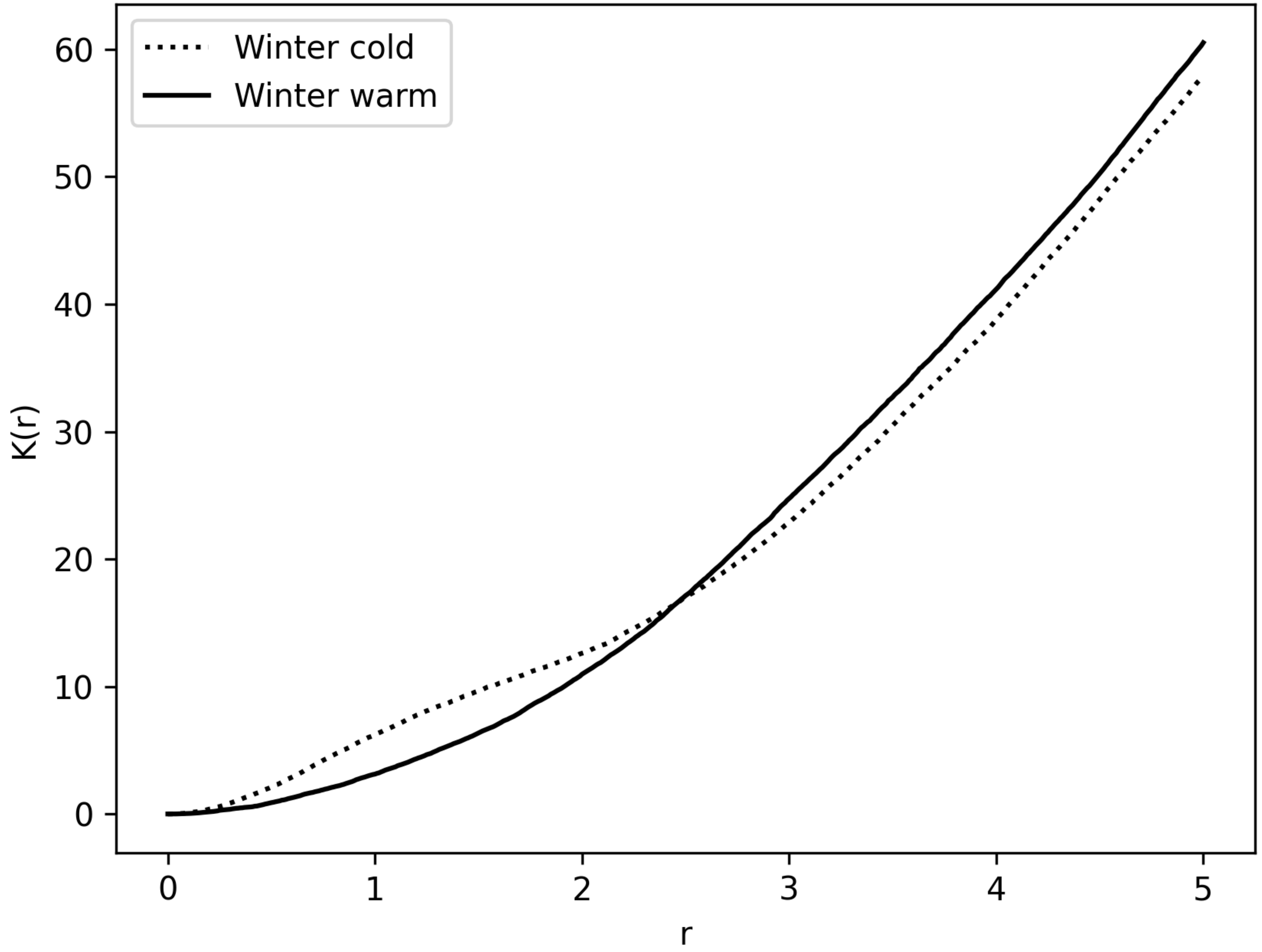
Winter — cold



Winter — medium

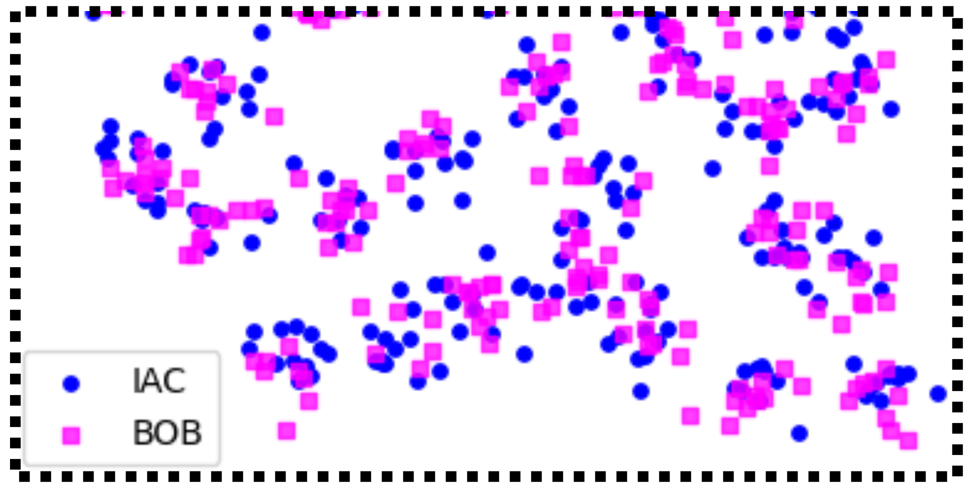
Winter — warm





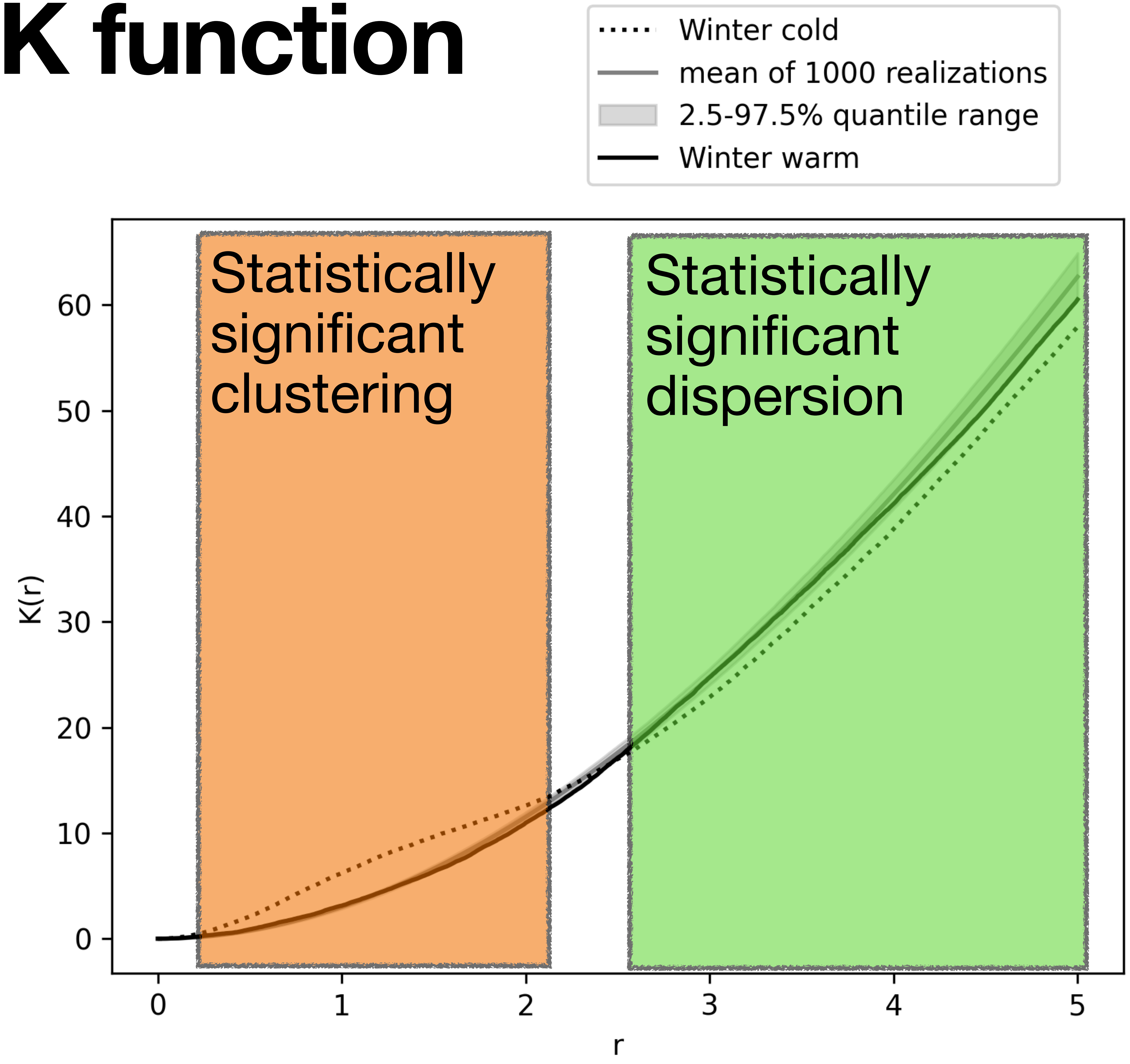
Results: Ripley’s K function

Winter — cold



Winter — medium

Winter — warm



The bigger picture: Monte-Carlo-based significance testing

- Just because your sample isn’t uniformly distributed doesn't mean it is biologically meaningful!
- It is possible to simulate hypotheses beyond uniform distributions.

Python notes

locan

Our implementation of Ripley's K was tested against the Locan library implementation:

https://locan.readthedocs.io/en/latest/tutorials/notebooks/Analysis_Ripley.html#

References

Lagache T, Sauvonnet N, Danglot L, Olivo-Marin JC. Statistical analysis of molecule colocalization in bioimaging. Cytometry A. 2015 Jun;87(6):568-79. doi: 10.1002/cyto.a.22629. Epub 2015 Jan 20. PMID: 25605428.

Ripley, B. D. “The Second-Order Analysis of Stationary Point Processes.” *Journal of Applied Probability*, vol. 13, no. 2, 1976, pp. 255–66. *JSTOR*, https://doi.org/10.2307/3212829. Accessed 13 July 2025.