

Introduction & Test results

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Introduction

- Educational background: Completed a master's degree at the University of Belgrade's Faculty of Mathematics, specializing in mathematics and computer science

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- Current employment: Working at Endava where our client is Adobe
- Career aspiration: Eager to expand knowledge in bioinformatics and actively seeking opportunities to work in a bioinformatics company

Data

- Data used in this task: Mouse embryo brain

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- Data contains 59704 cells and 4 columns with values:

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 - cell name (cell_id)

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 - spatial coordinates (x, y)
 - cell type (sim anno)

	cell_ID	x	y	sim anno
0	CELL.61	-5591.0	-1156.0	Ery
1	CELL.84	-5538.0	-1185.0	Ery
2	CELL.105	-5496.0	-1206.0	Ery
3	CELL.131	-5519.0	-1235.0	Ery
4	CELL.132	-5546.0	-1235.0	Ery
...
59699	CELL.70553	-1463.0	-8763.0	DorsHb RGC
59700	CELL.70555	-1175.0	-8770.0	Mixed GlioB
59701	CELL.70556	-1365.0	-8764.0	Mixed GlioB
59702	CELL.70557	-1425.0	-8776.0	DorsHb RGC
59703	CELL.70558	-1396.0	-8774.0	DorsHb RGC

59704 rows × 4 columns

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	cell_ID	x	y	sim anno
0	CELL.523	-8598.0	-1609.0	Ery
1	CELL.662	-8135.0	-1686.0	Ery
2	CELL.918	-8628.0	-1775.0	Ery
3	CELL.933	-8745.0	-1779.0	Ery
4	CELL.942	-8786.0	-1779.0	Ery
...
31107	CELL.68191	-9542.0	-8167.0	Ery
31108	CELL.68234	-9631.0	-8172.0	Hb Glu NeuB
31109	CELL.68264	-9610.0	-8173.0	Mb RGC
31110	CELL.68268	-9493.0	-8171.0	Ery
31111	CELL.68334	-9395.0	-8176.0	Mb RGC

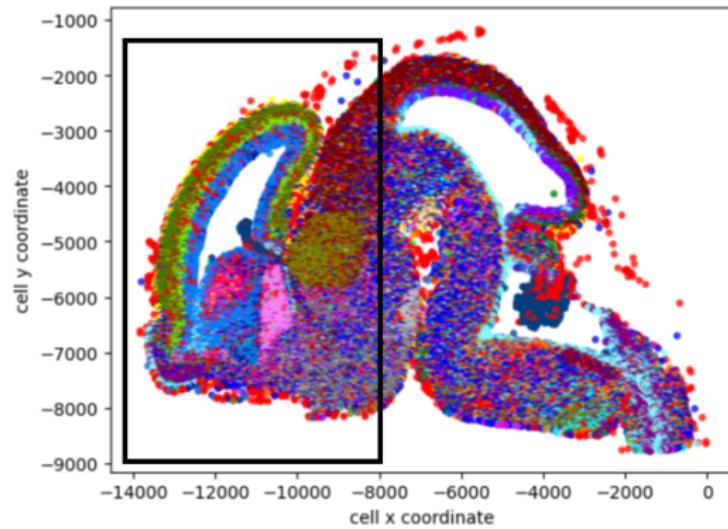
31112 rows x 4 columns

Data

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- The task:
 - Categorize cells into spatial communities by evaluating the percentage of cell types found in their immediate surroundings
 - Calculate the homogeneity score for each community and decide whether the community is homogeneous (community with very similar percentages of cell types in all of its parts) or heterogeneous (community in which percentages of cell types vary significantly across different parts of it)

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- The task:
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 - Calculate the homogeneity score for each community and decide whether the community is homogeneous (community with very similar percentages of cell types in all of its parts) or heterogeneous (community in which percentages of cell types vary significantly across different parts of it)
 - Calculate for each community the mixture (count and percentage) of cell types that are present in it

Calculation of the percentage of cell types in cell's neighborhood

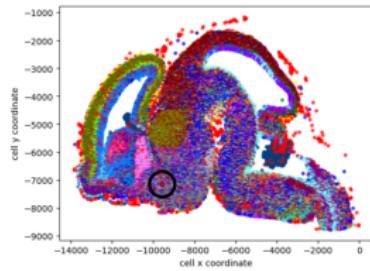
- Input: Cell cell (red dot on the image below) and integer neighborhood_radius (radius of black circle on the image below)

Calculation of the percentage of cell types in cell's neighborhood

- Input: Cell `cell` (red dot on the image below) and integer `neighborhood_radius` (radius of black circle on the image below)
- Output: list `types_percentage`, where `types_percentage[k]` represents the percentage of cells in the neighborhood of the cell (indicated by the black circle in the image below) that belong to the cell type with number `k`. All cell types in the tissue are mapped to integers from 0 to `num_of_types - 1`

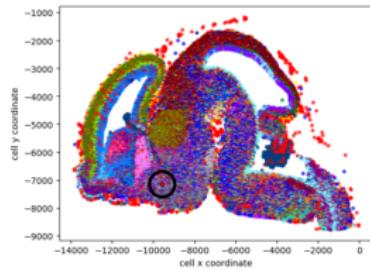
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- After this step, we have a list of the percentage of cell types in each cell's neighborhood, allowing us to compare them based on this information

Comparing cells based on the percentage of cell types in their neighborhood

- Distance functions used: Manhattan, Euclidean and Hamming

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- Distance functions used: Manhattan, Euclidean and Hamming
- For the Hamming distance, there is a parameter called `hamming_param`. For example, if `types_percentage = [0.1, 0.5, 0.4]` and `hamming_param = 0.3`, then the modified percentages will be `[0, 1, 1]`. In this case, only the first element (0.1) is less than 0.3, so it will be set to 0, and the rest will be set to 1. And these modified percentages of cells will be compared when comparing two cells using the Hamming distance.

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- Clustering algorithm: Hierarchical clustering (Agglomerative) and Leiden clustering

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- For Agglomerative clustering there are two optional parameters:
 - `linkage_method` (single, average, ward, centroid) where default value is single
 - `threshold` where default value is None, and in such cases, the optimal threshold will be calculated using the Silhouette method

Clustering cells based on the similarity of cell types present in their neighborhood.

- Clustering algorithm: Hierarchical clustering (Agglomerative) and Leiden clustering
- Only the results for the Agglomerative clustering will be presented, as it is better suited for the current problem
- For Agglomerative clustering there are two optional parameters:
 - `linkage_method` (single, average, ward, centroid) where default value is single
 - `threshold` where default value is None, and in such cases, the optimal threshold will be calculated using the Silhouette method
- Agglomerative clustering will be performed on the distance matrix `percentage_distance_matrix`, where
`percentage_distance_matrix[cell_i.id_num][cell_j.id_num] = dist(cell_i.types_percentage, cell_j.types_percentage)`, and the distance function `dist` can be one of Manhattan, Euclidean, or Hamming distances

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 - neighborhood_radius (mandatory parameter)
 - distance_function (Manhattan which is default, Euclidean and Hamming with parameter)
 - linkage_method (single which is default, average, ward and centroid) and threshold (default is None) for Agglomerative clustering

Result 1

- Results where:

Result 1

- Results where:
 - neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)

Result 1

- Results where:
 - neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)
 - distance_function = Manhattan

Result 1

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 - linkage_method = average

Result 1

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- neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)
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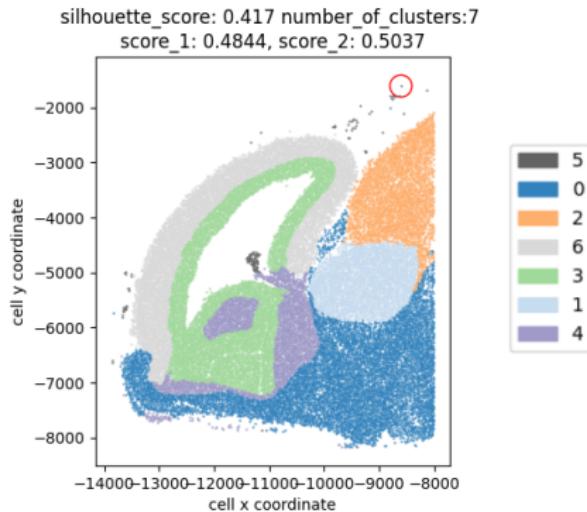


Figure: Clusters

Result 1 - Clusters statistics

- The mean of distribution of percentage distances between all cells is 1.48, as shown in the image below

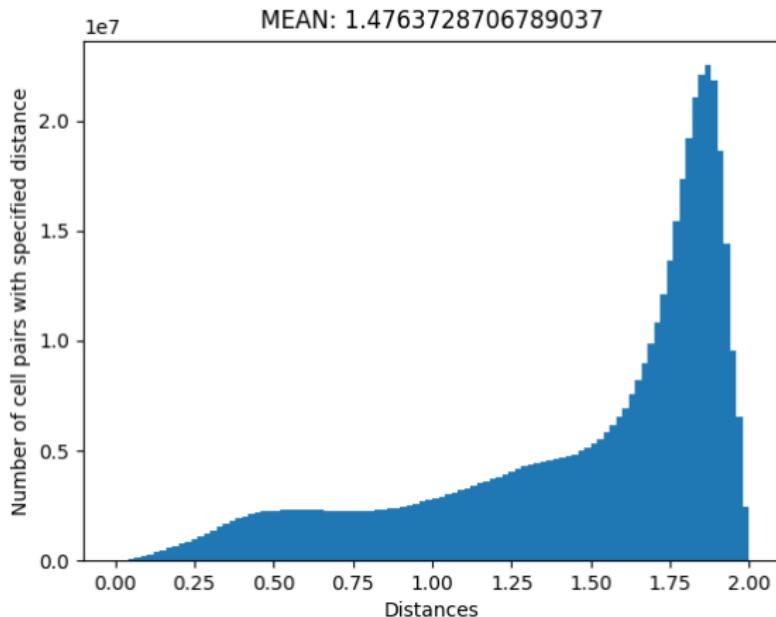


Figure: Distribution of percentage distances between all cells

Result 1 - Homogeneous or Heterogeneous clusters

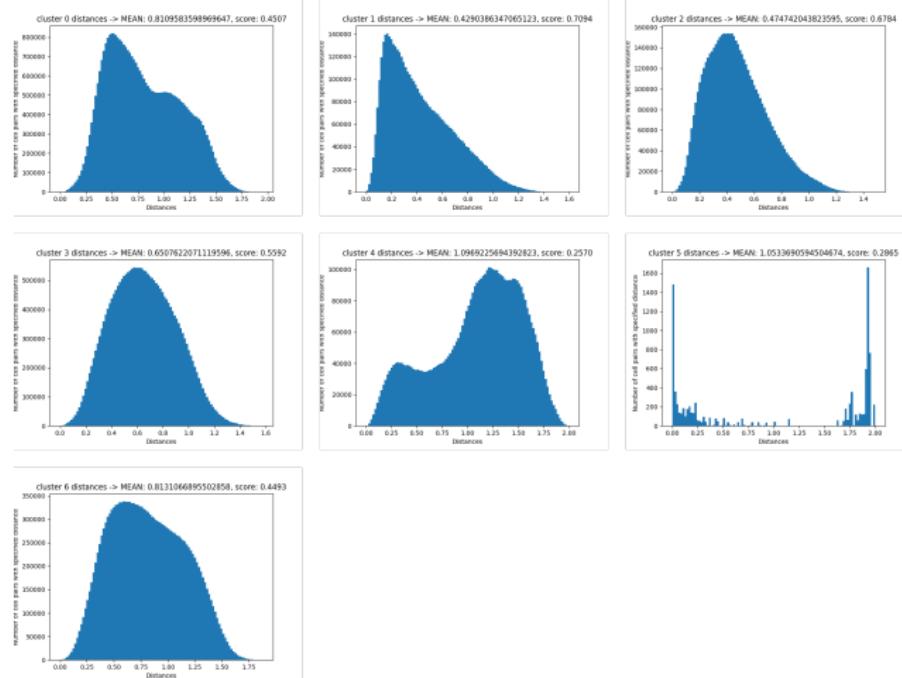


Figure: Distribution of percentage distances between cells for each cluster

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- This was done to determine whether a cluster is homogeneous or heterogeneous. If a cluster is homogeneous, there should be a significant number of percentage distances between cells that are close to zero as this indicates that neighborhood of those cells are similar

Result 1 - Homogeneous or Heterogeneous clusters

- In the image above, distributions of percentage distances between cells for each cluster are displayed
- This was done to determine whether a cluster is homogeneous or heterogeneous. If a cluster is homogeneous, there should be a significant number of percentage distances between cells that are close to zero as this indicates that neighborhood of those cells are similar
- If there is a significant number of homogeneous clusters, it indicates the effectiveness of the clustering, as our goal is to group cells with very similar surroundings.

Cluster homogeneity score

- Cluster c homogeneity score

$$HS(c) = \begin{cases} 1 - m(c)/M & , \text{ if } m(c) < M \\ 0 & , \text{ otherwise} \end{cases}$$

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 - if $HS(c) > 0.5$, then cluster c is homogeneous
 - otherwise, cluster c is heterogeneous

Total homogeneity score

- Total homogeneity score of clustering C

$$THS(C) = \frac{\sum_{c \in C} HS(c) \cdot n(c)}{N}$$

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- Total homogeneity score takes values from 0 to 1 meaning: value close to 1 indicates that the clustering is of high quality, while a value close to 0 suggests that the clustering is of poor quality.

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- From the image above, we can observe that we have 7 clusters from which 1, 2, and 3 are homogeneous, and cluster 0 and 6 are close to homogeneous

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- Furthermore, clusters 4 and 5 have the lowest homogeneity score and are heterogeneous clusters

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- From the image above, we can observe that we have 7 clusters from which 1, 2, and 3 are homogeneous, and cluster 0 and 6 are close to homogeneous
- Furthermore, clusters 4 and 5 have the lowest homogeneity score and are heterogeneous clusters
- Total homogeneity score of this clustering is 0.5 and silhouette score is 0.42

Result 1 (statistics) - Number of different cell types in each cluster

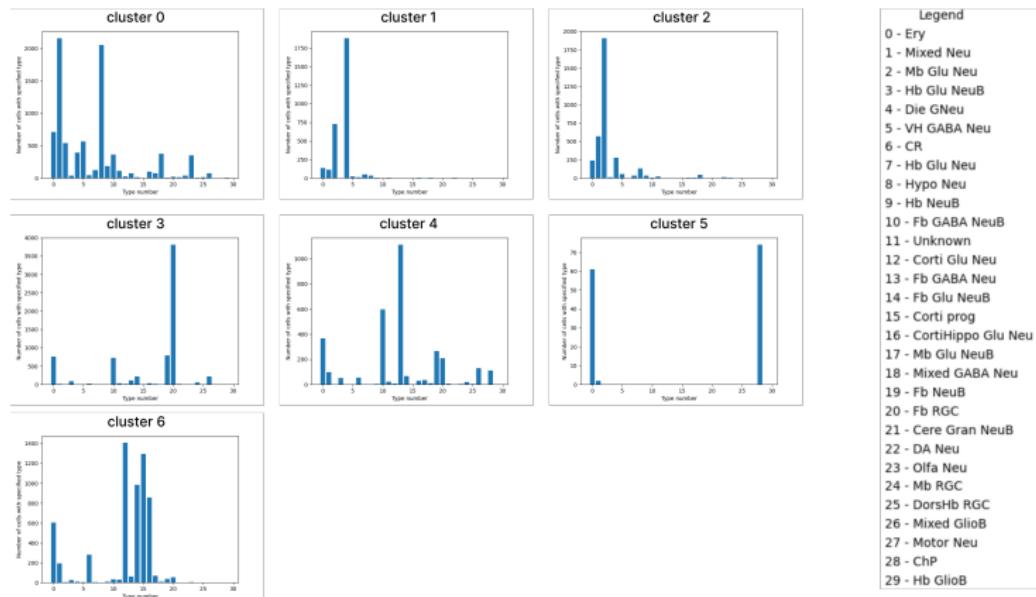


Figure: Number of different cell types in each cluster

Result 1 (statistics) - Percentage of different cell types in each cluster

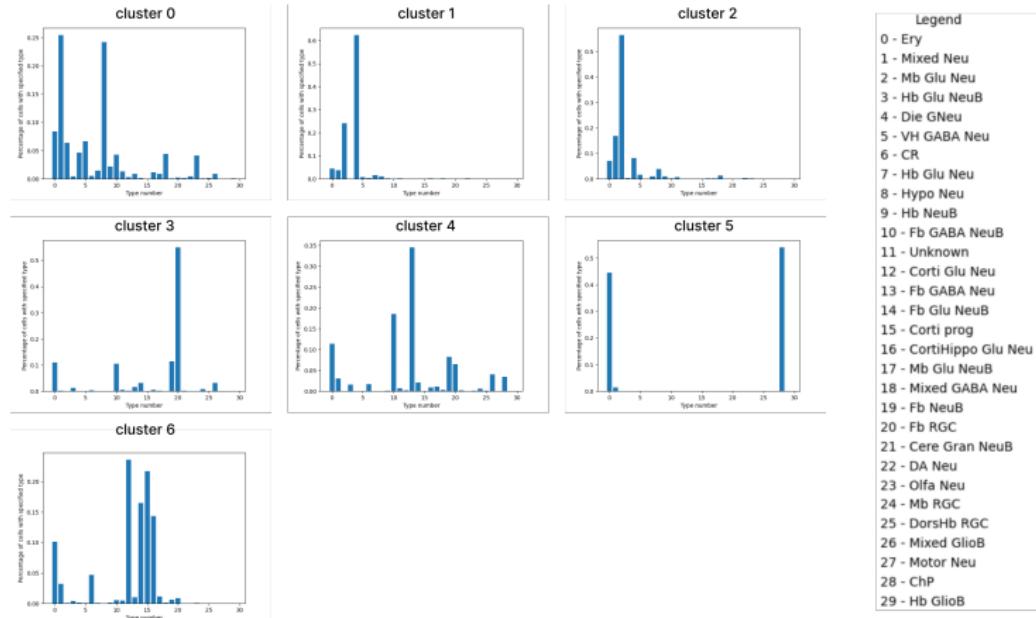


Figure: Percentage of different cell types in each cluster

Result 1 - Decreasing the threshold

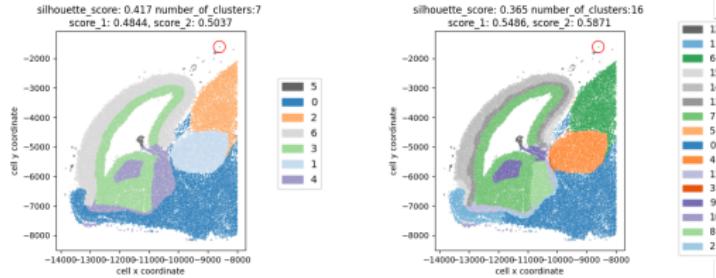


Figure: Clusters with threshold 64 and 55, respectively

Result 1 - Decreasing the threshold

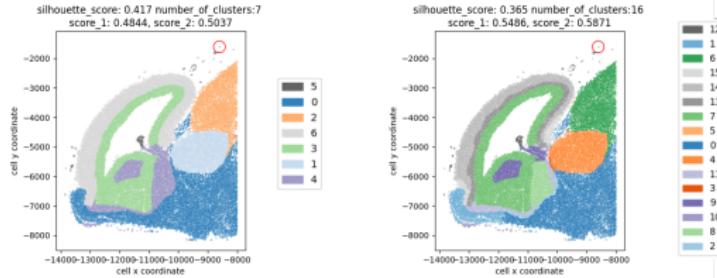


Figure: Clusters with threshold 64 and 55, respectively

- Decreasing the threshold (from 68 to 55) did result in a higher number of clusters with slight improvement in homogeneity score

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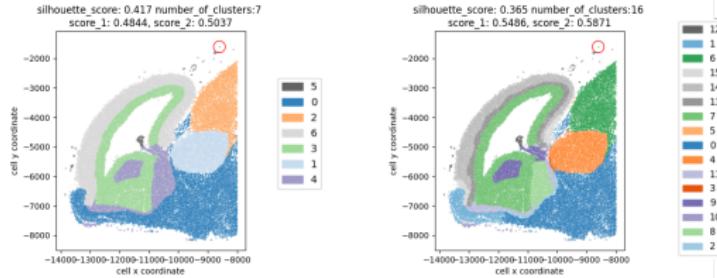


Figure: Clusters with threshold 64 and 55, respectively

- Decreasing the threshold (from 68 to 55) did result in a higher number of clusters with slight improvement in homogeneity score
- The gray cluster (cluster 6) from the previous clustering has now been divided into clusters 13 and 14. This division has resulted in higher homogeneity scores for clusters 13 (0.62) and 14 (0.6) than what was observed for the single cluster 6 (0.45) in the previous clustering.

Result 1 - Decreasing the threshold

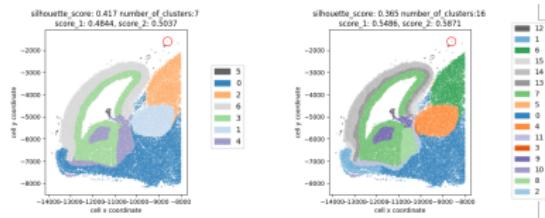


Figure: Clusters with threshold 68 and 55, respectively

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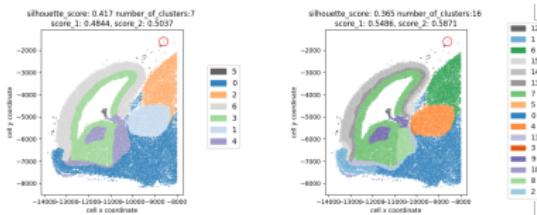


Figure: Clusters with threshold 68 and 55, respectively

- The purple cluster (cluster 4) from the previous clustering has now been divided into clusters 8 , 9 and 11 . This division has resulted in higher homogeneity scores for clusters 8 (0.64), 9 (0.45) and 11 (0.46) than what was observed for the single cluster 4 (0.26) in the previous clustering.

Result 1 - Decreasing the threshold

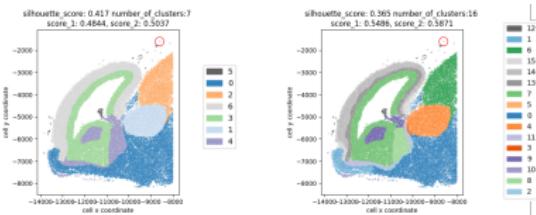


Figure: Clusters with threshold 68 and 55, respectively

- The purple cluster (cluster 4) from the previous clustering has now been divided into clusters 8 , 9 and 11 . This division has resulted in higher homogeneity scores for clusters 8 (0.64), 9 (0.45) and 11 (0.46) than what was observed for the single cluster 4 (0.26) in the previous clustering.
- The blue cluster (cluster 0) from the previous clustering has now been divided into clusters 0, 1 and 2. This division has resulted in higher homogeneity scores for clusters 0 (0.56), and 2 (0.54) and lower for cluster 1 (0.39) than what was observed for the single cluster 0 (0.45) in the previous clustering.

Result 1 - Decreasing the threshold

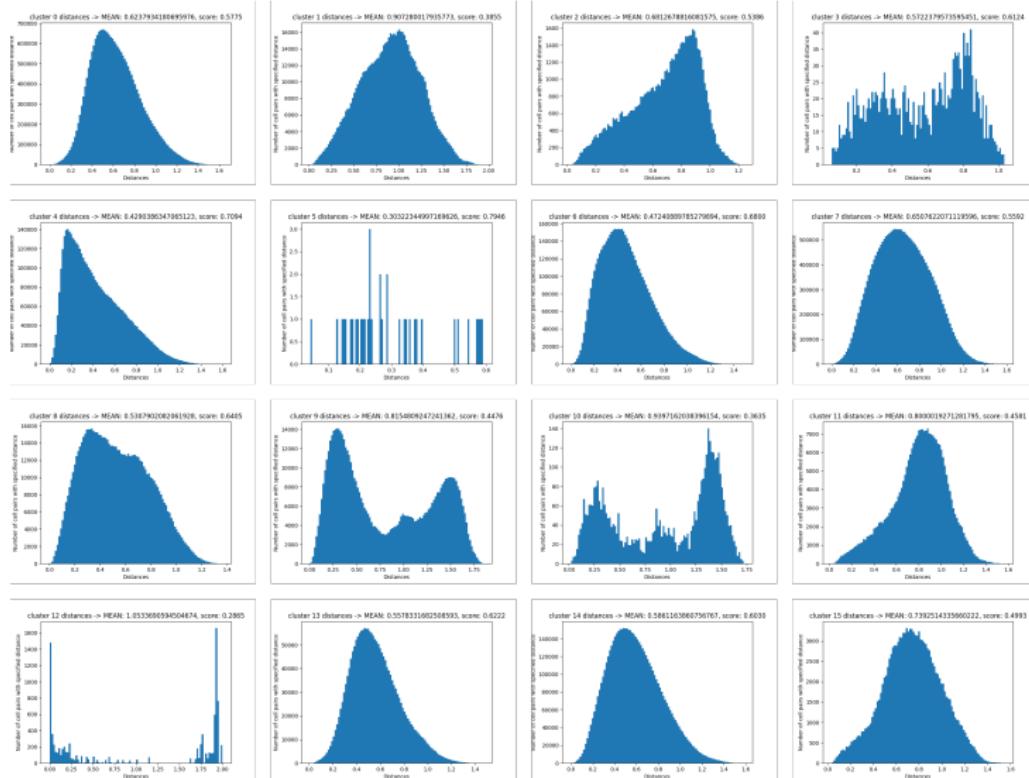


Figure: Distribution of percentage distances between cells for each cluster

Result 1 - Decreasing the threshold

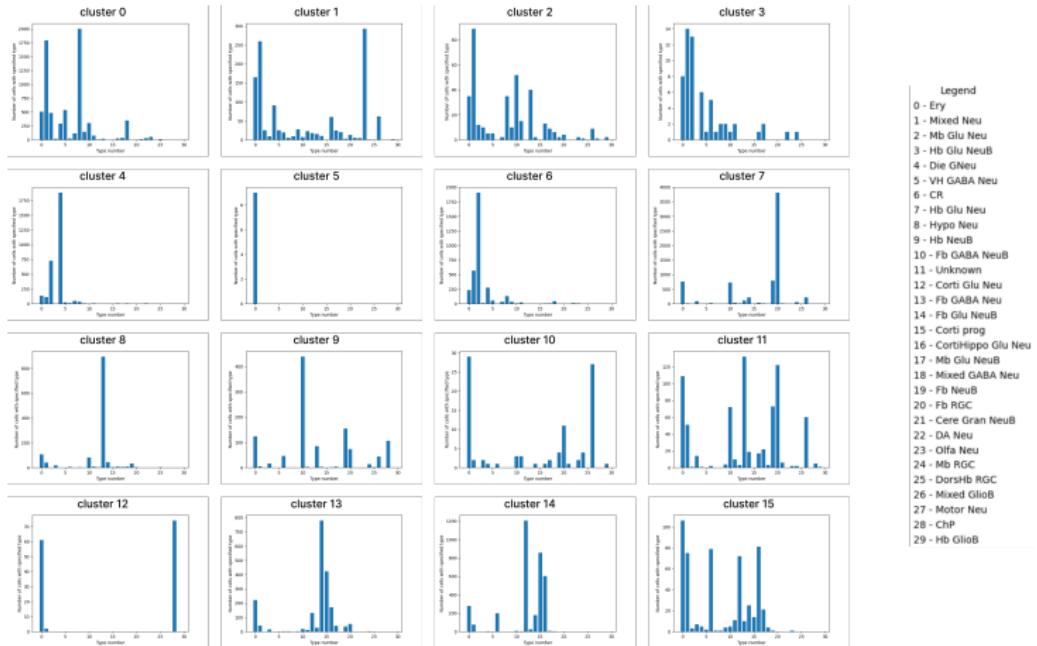


Figure: Number of different cell types in each cluster

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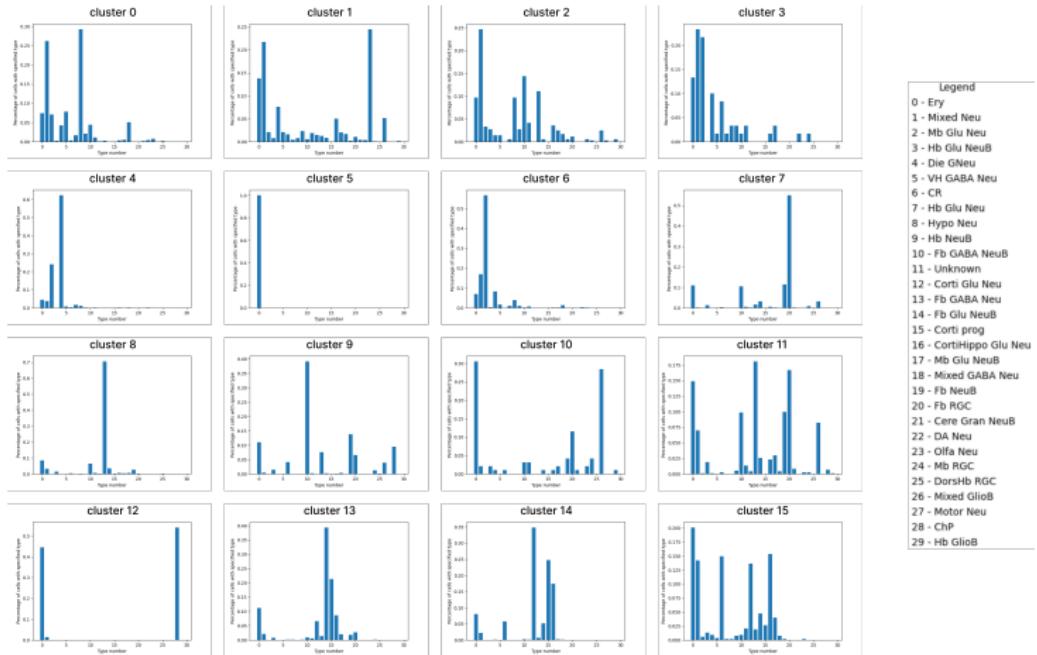


Figure: Percentage of different cell types in each cluster

Result 1 - Decreasing the neighborhood radius

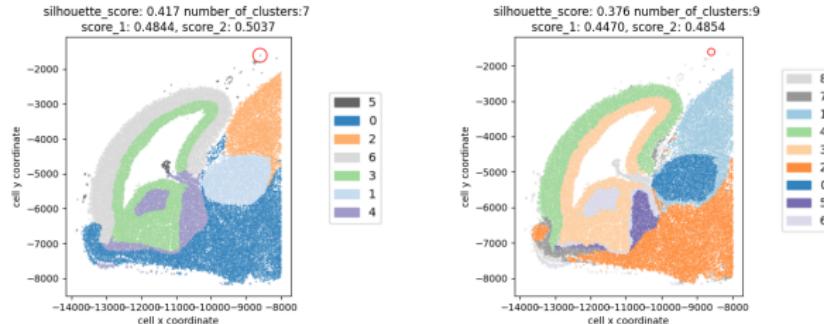


Figure: Clusters with neighborhood_radius 200 and 100, respectively

Result 1 - Decreasing the neighborhood radius

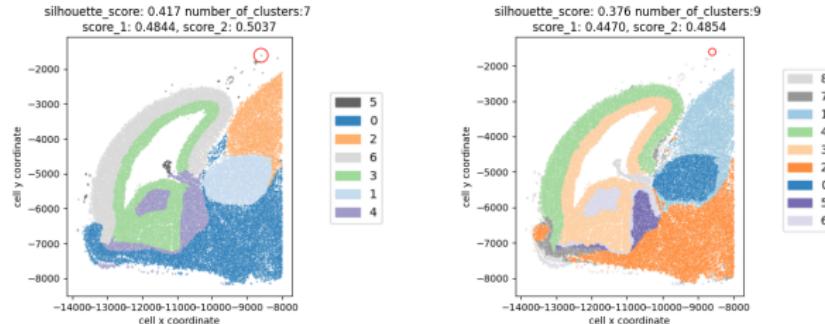


Figure: Clusters with neighborhood_radius 200 and 100, respectively

- Decreasing the neighborhood radius (from 200 to 100) did result in higher number of clusters, with a slightly lower silhouette and total homogeneity score.

Result 1 - Decreasing the neighborhood radius

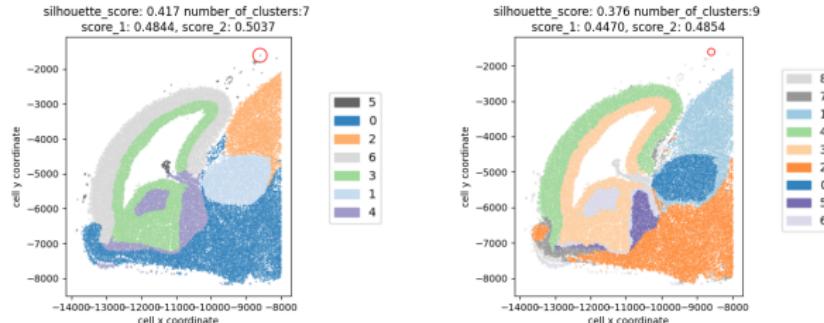


Figure: Clusters with neighborhood_radius 200 and 100, respectively

- Decreasing the neighborhood radius (from 200 to 100) did result in higher number of clusters, with a slightly lower silhouette and total homogeneity score.
- The purple cluster (cluster 4) from the previous clustering has now been divided into clusters 5 and 6. This division has resulted in higher homogeneity scores for clusters 5 (0.59) and 6 (0.36) than what was observed for the single cluster 4 (0.26) in the previous clustering.

Result 1 - Decreasing the neighborhood radius

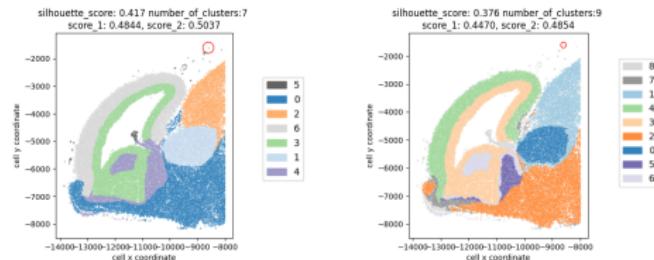


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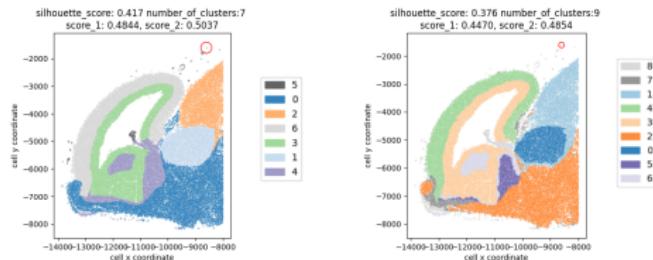


Figure: Clusters with neighborhood_radius 200 and 100, respectively

- The blue cluster (cluster 0) from the previous clustering has now been divided into clusters 2 and 7. This division has resulted in higher homogeneity scores for cluster 2 (0.47) and lower for cluster 7 (0.3) than what was observed for the single cluster 0 (0.45) in the previous clustering.

Result 1 - Decreasing the neighborhood radius

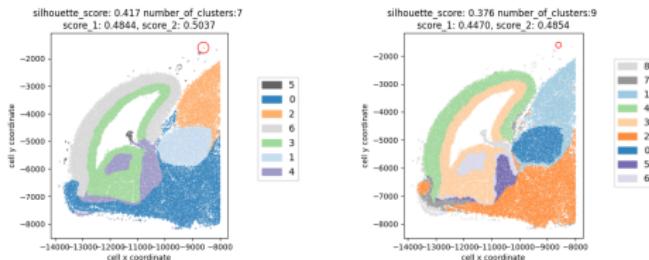


Figure: Clusters with neighborhood_radius 200 and 100, respectively

- The blue cluster (cluster 0) from the previous clustering has now been divided into clusters 2 and 7. This division has resulted in higher homogeneity scores for cluster 2 (0.47) and lower for cluster 7 (0.3) than what was observed for the single cluster 0 (0.45) in the previous clustering.
- The green cluster (cluster 3) from the previous clustering (0.56) has slightly higher homogeneity scores than cluster 3 in new clustering (0.51), same as clusters 6 from the previous clustering (0.45) and cluster 4 from the new clustering (0.37).

Result 1 - Decreasing the neighborhood radius

- The mean of distribution of percentage distances between all cells is 1.58, as shown in the image below

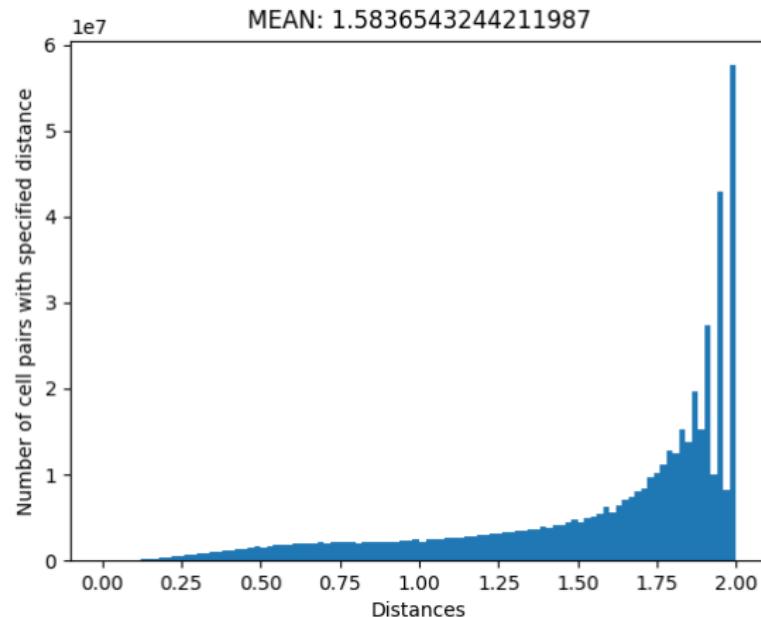


Figure: Distribution of percentage distances between all cells

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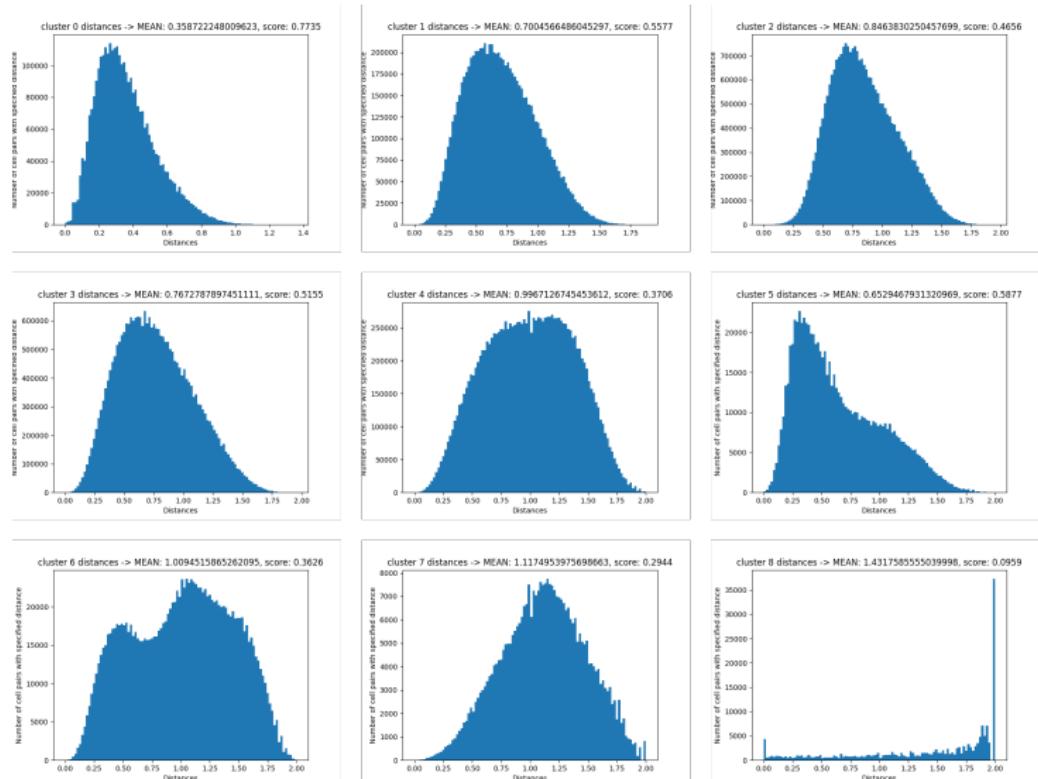


Figure: Distribution of percentage distances between cells for each cluster

Result 2

- Results where:

Result 2

- Results where:
 - neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)

Result 2

- Results where:
 - neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)
 - distance_function = Euclidean

Result 2

- Results where:
 - neighborhood_radius = 200 (the radius that corresponds to the red circle in the image below)
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- distance_function = Euclidean
- linkage_method = centroid

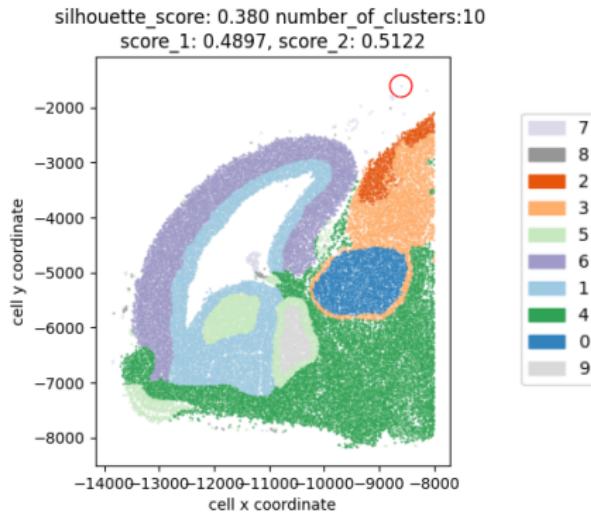


Figure: Clusters

Result 2 - Statistics of clusters

- The mean of distribution of percentage distances between all cells is 0.63, as shown in the image below

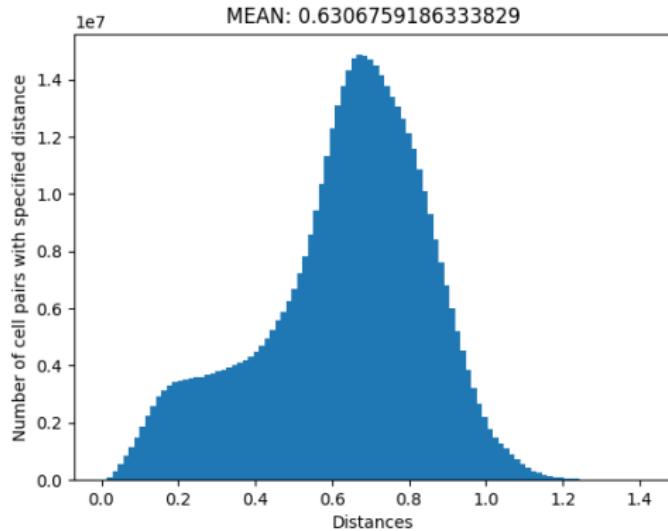


Figure: Distribution of percentage distances between all cells

Result 2 - Homogeneous or Heterogeneous clusters

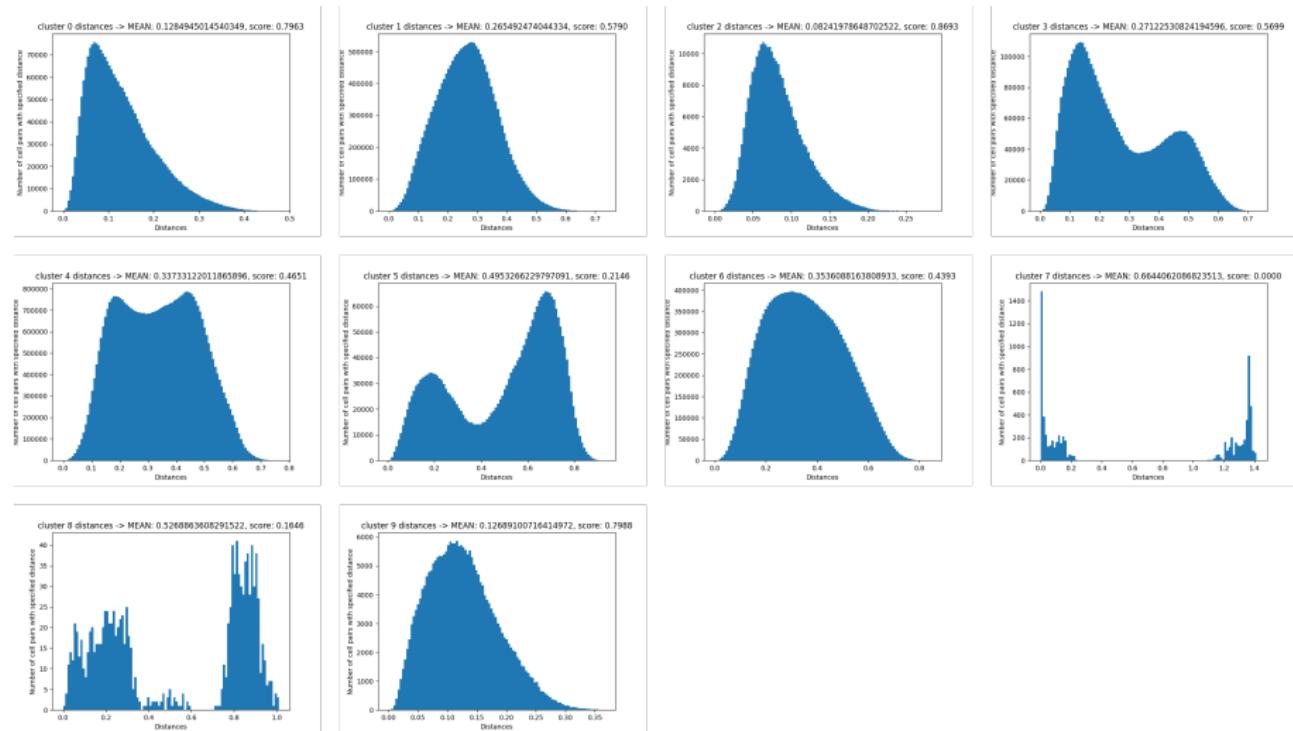


Figure: Distribution of percentage distances between cells for each cluster

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- Distributions for the other clusters are more promising. Cluster 0, 1, 2, 3 and 9 are homogeneous and clusters 4 and 6 are close to homogeneous
- Total homogeneity score of this clustering is 0.51 and silhouette score is 0.38

Result 2 - Number of different cell types in each cluster

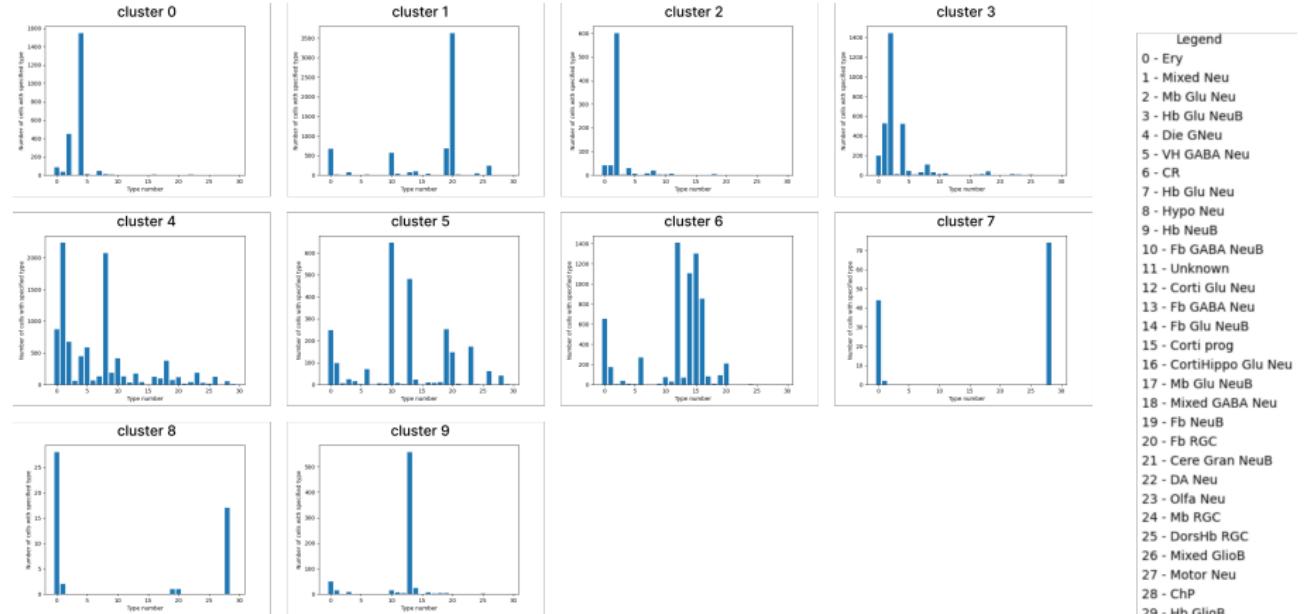


Figure: Number of different cell types in each cluster

Result 2 - Percentage of different cell types in each cluster

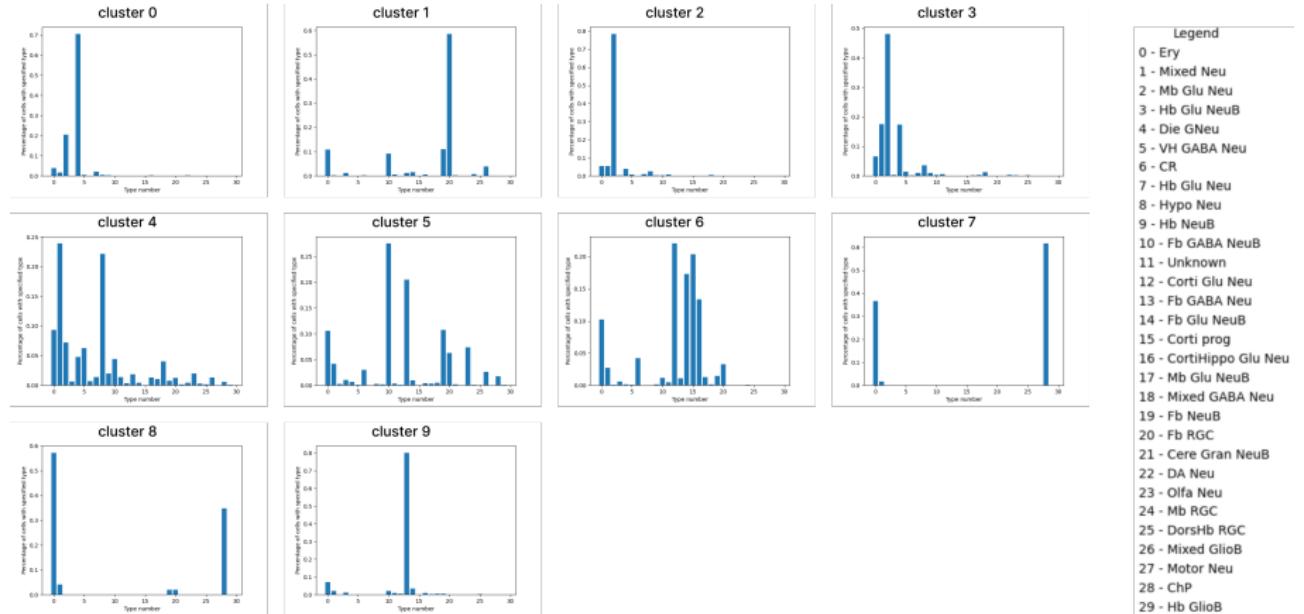


Figure: Percentage of different cell types in each cluster

Result 3

- Results where:

Result 3

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 - neighborhood_radius = 150 (the radius that corresponds to the red circle in the image below)

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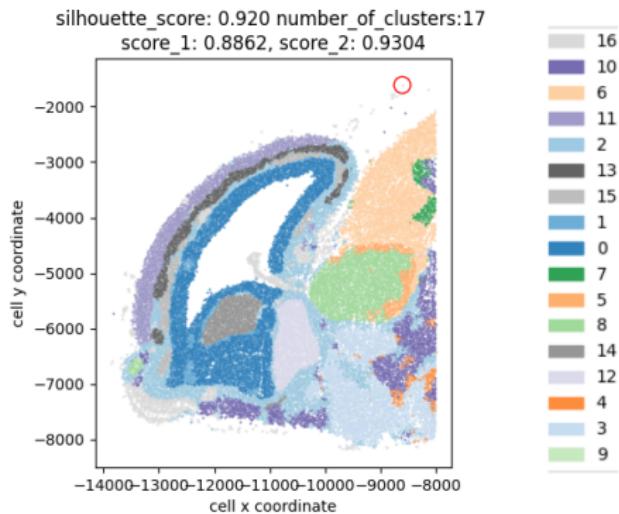


Figure: Clusters

Result 3 - Statistics of clusters

- The mean of distribution of percentage distances between all cells is 0.89, as shown in the image below

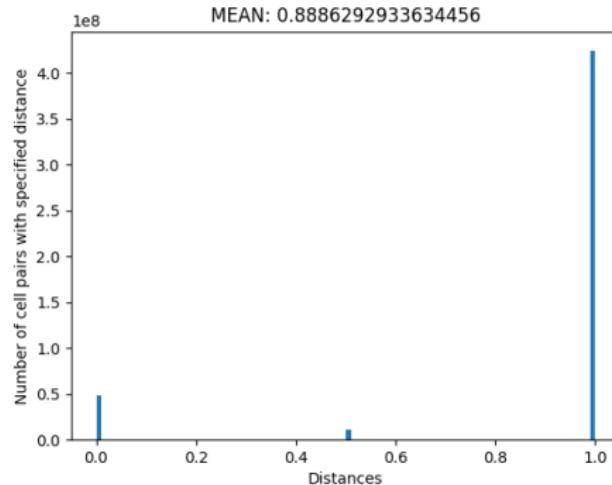


Figure: Distribution of percentage distances between all cells

Result 3 - Homogeneous or Heterogeneous clusters

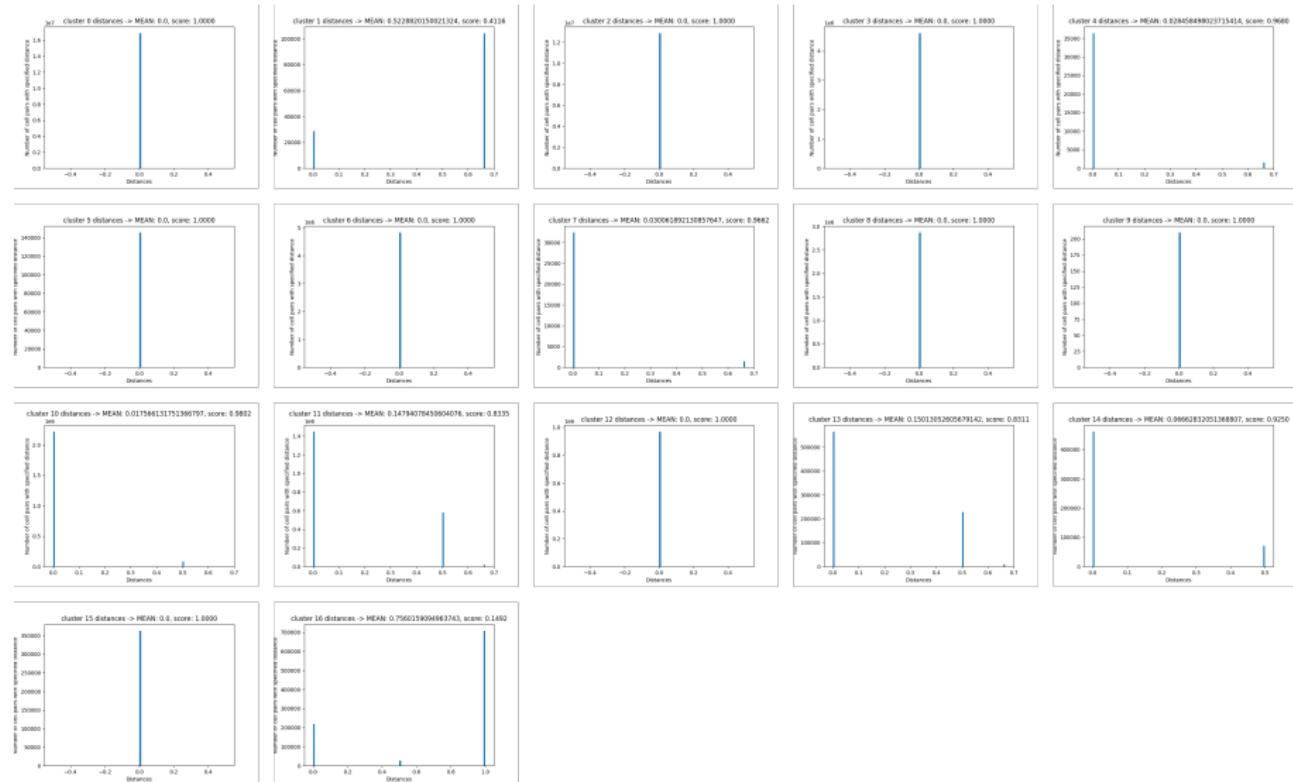


Figure: Distribution of percentage distances between cells for each cluster

Result 3 - Homogeneous or Heterogeneous clusters

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- From the image above, all clusters, except clusters 1 and 16, are homogeneous with high homogeneity scores
- Total homogeneity score of this clustering is 0.93 and silhouette score is 0.92

Result 3 - Number of different cell types in each cluster

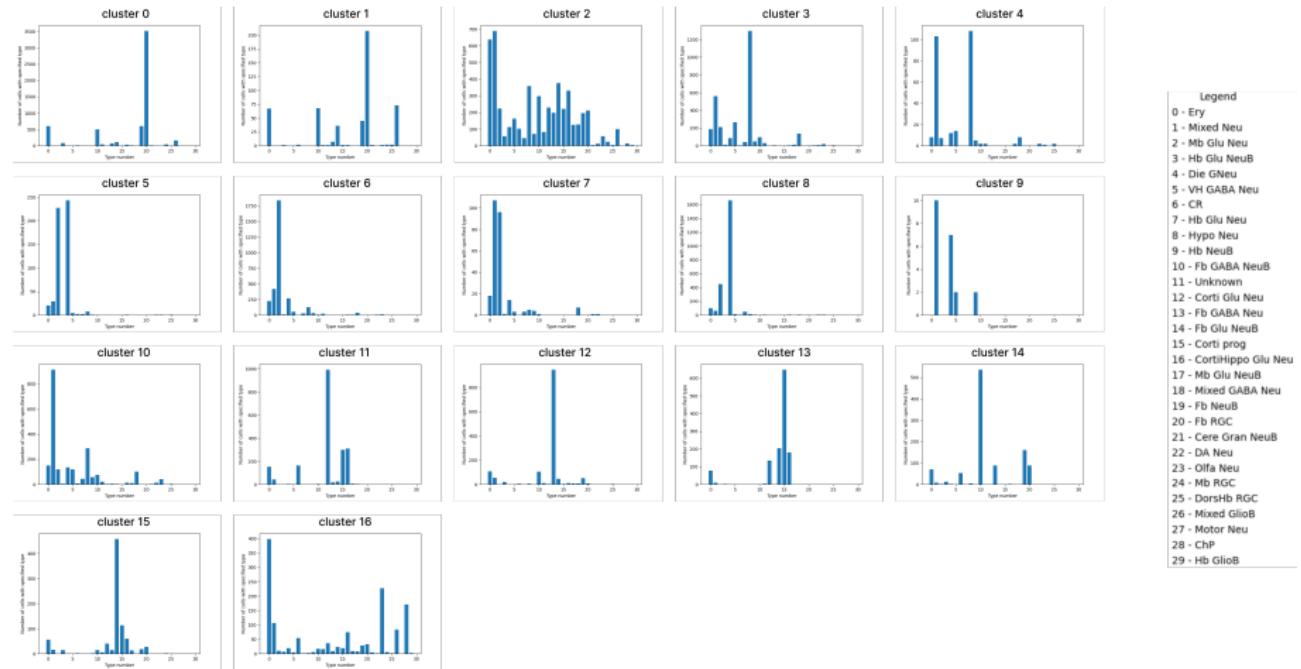


Figure: Number of different cell types in each cluster

Result 3 - Percentage of different cell types in each cluster

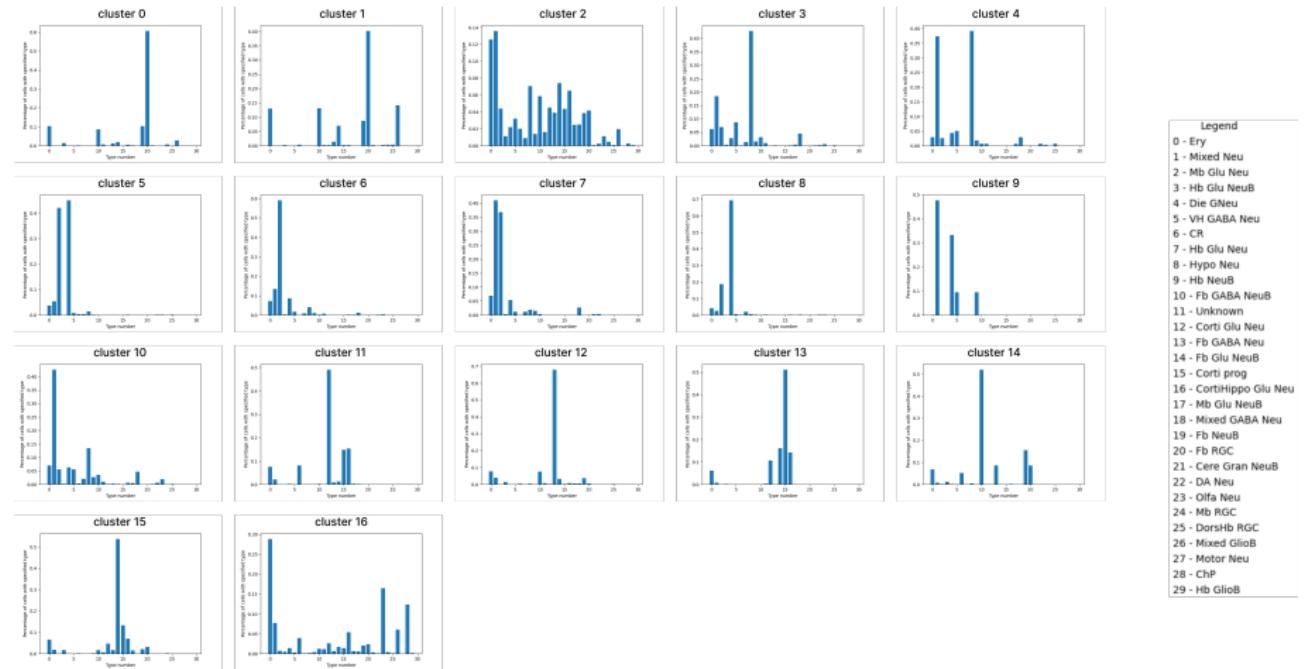


Figure: Percentage of different cell types in each cluster

Result 3 - Increasing the threshold

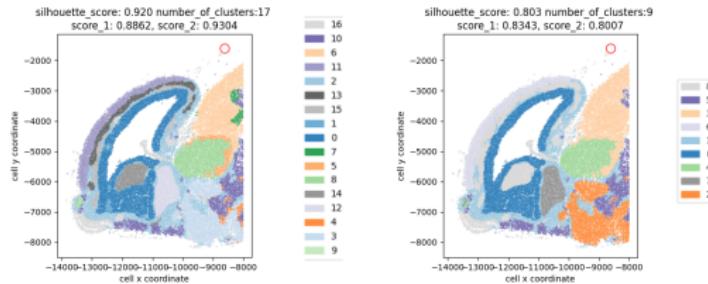


Figure: Clusters with threshold 30 and 45, respectively

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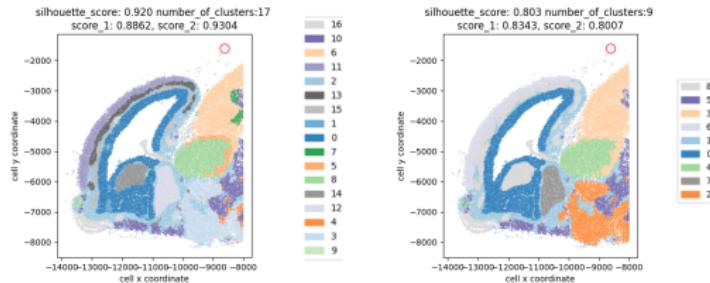


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- Increasing the threshold (from 30 to 45) did result in a lower number of clusters with slightly lower silhouette and total homogeneity score

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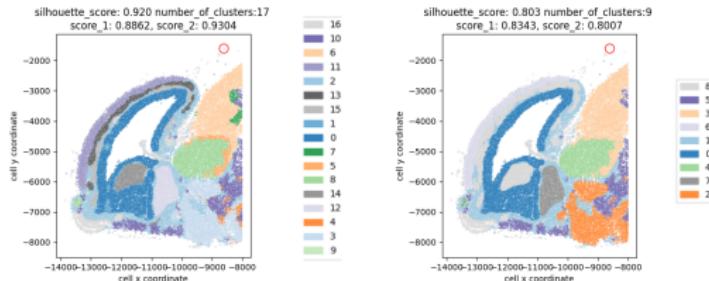


Figure: Clusters with threshold 30 and 45, respectively

- Increasing the threshold (from 30 to 45) did result in a lower number of clusters with slightly lower silhouette and total homogeneity score
- Clusters 0 and 1 from the previous clustering has now been merged into single cluster 0. This has resulted in higher homogeneity score for cluster 0 (0.91) than what was observed for cluster1 (0.45) and slightly lower score than 0 (1) in the previous clustering

Result 3 - Increasing the threshold

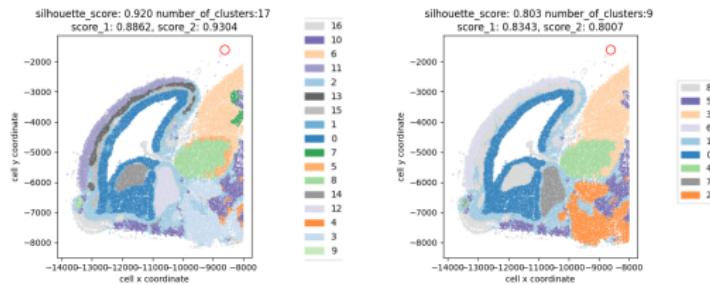


Figure: Clusters with threshold 30 and 45, respectively

Result 3 - Increasing the threshold

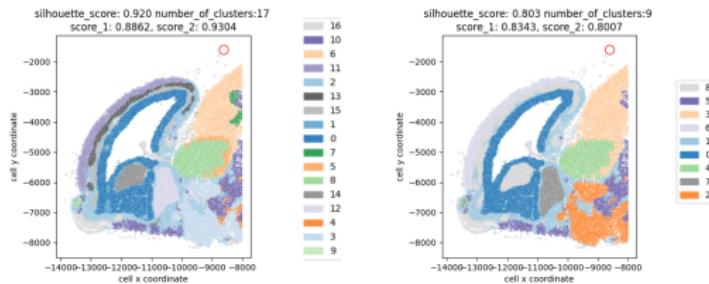


Figure: Clusters with threshold 30 and 45, respectively

- Clusters 13 and 14 and 15 from the previous clustering have now been merged into cluster 8. This has resulted in lower homogeneity score for cluster 8 (0.07) than what was observed for the clusters 13 (0.83), 14 (0.92) and 15 (1) in the previous clustering.

Result 3 - Increasing the threshold

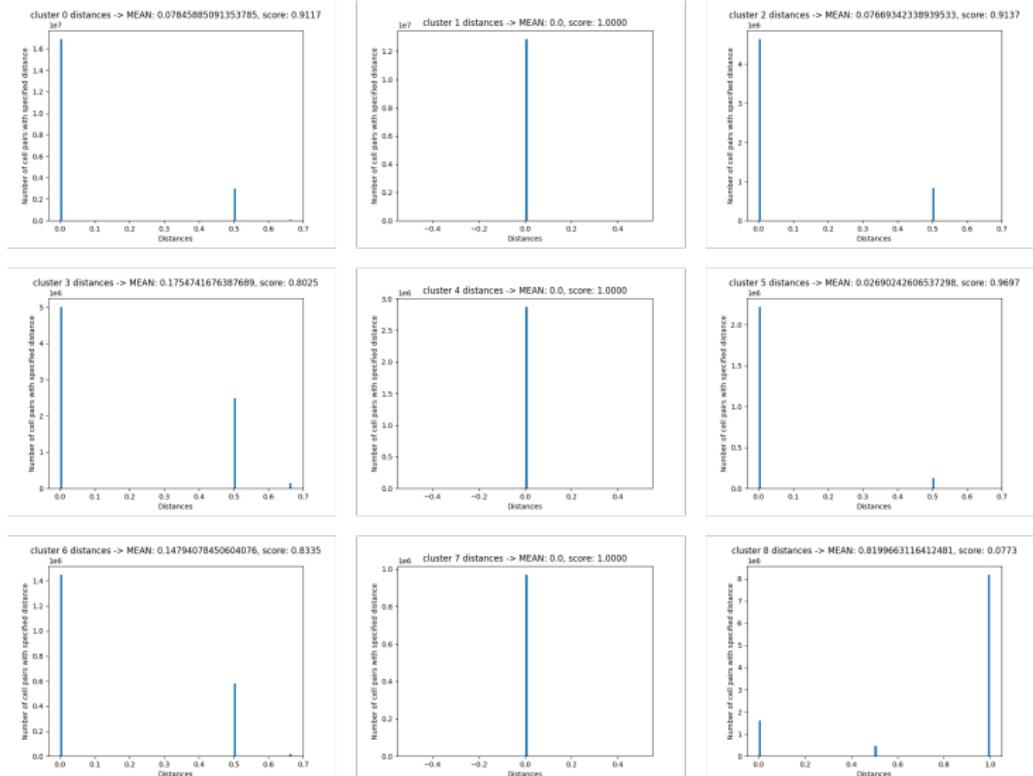


Figure: Distribution of percentage distances between cells for each cluster

Result 3 - Increasing the threshold

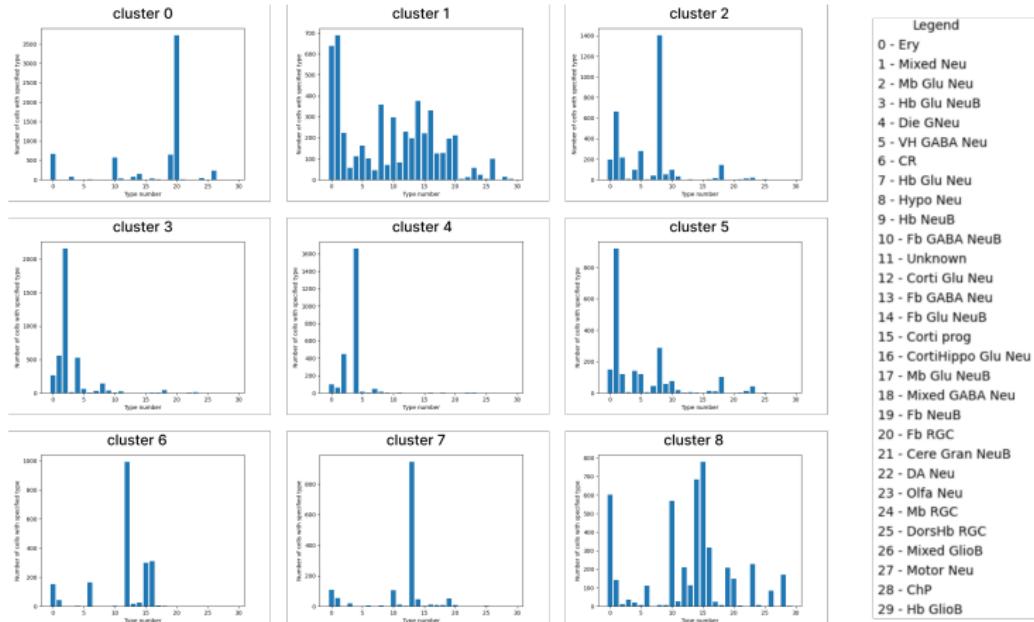


Figure: Number of different cell types in each cluster

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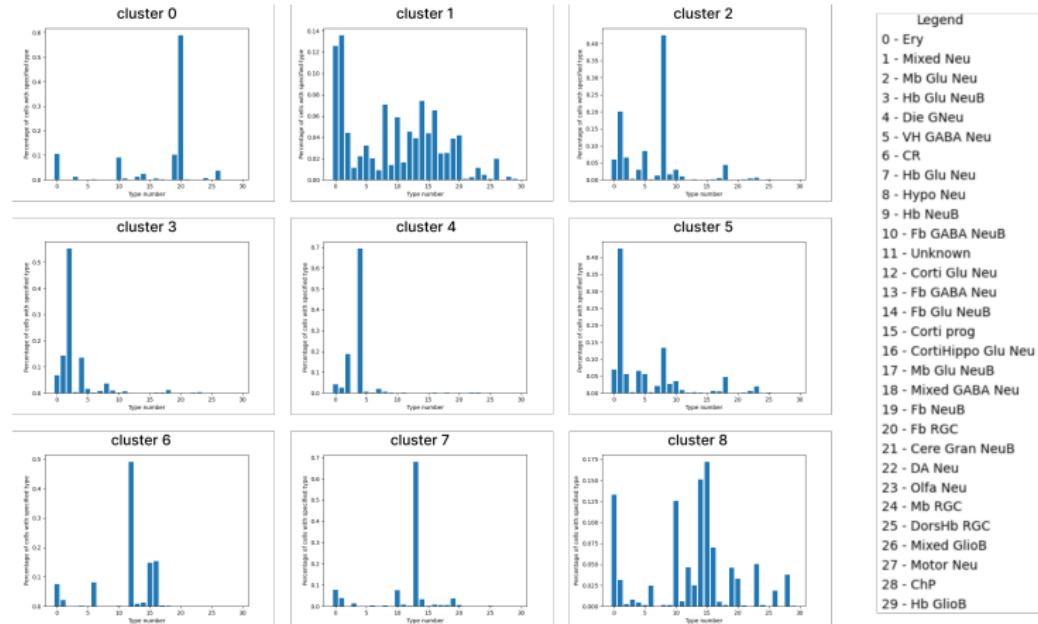


Figure: Percentage of different cell types in each cluster

Conclusion

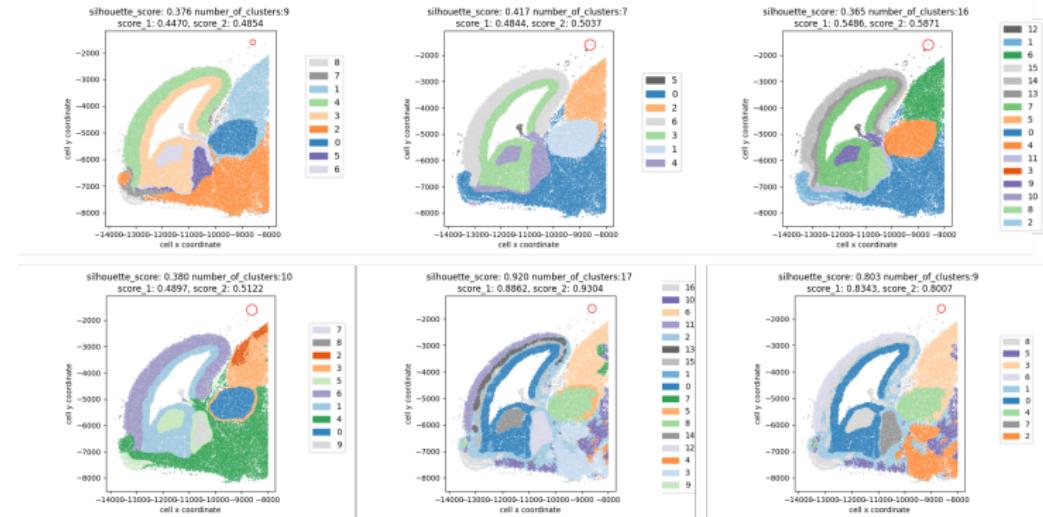


Figure: Clusters for different clusterings - Manhattan (100 200 - different threshold), Euclidean (200) Hamming with parameter 0.3 (150 - different thresholds)

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 - 5, 8, 9, 12 and 7
 - 3, 3, 7, 1, 0 and 0
 - 1, 2, 6, 3, 6 and 3

Conclusion

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 - 0, 1, 4, 0, 8 and 4
 - 5, 8, 9, 12 and 7
 - 3, 3, 7, 1, 0 and 0
 - 1, 2, 6, 3, 6 and 3
- Furthermore, there are clusters that appear as a single cohesive group in one clustering but get divided into smaller, more homogeneous sub-clusters in others

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Investigated correlation between spatial coordinates and gene expressions of the cells while also analyzing their impact on cell type