CodingLab7

June 7, 2025

Neural Data Science

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Summer term 2025

Student names: Aakarsh Nair, Andreas Kotzur, Ahmed Eldably

LLM Disclaimer: Google Gemini , Google Gemini Diffiusion - Planning, Coding, and Verification.

1 Coding Lab 7 : Transcriptomics

[1]:
```
import os
import subprocess

if os.environ.get("COLAB_JUPYTER_IP"):
    if os.path.exists("/content/Neural_Data_Science_Summer_2025/"):
        os.chdir("/content/Neural_Data_Science_Summer_2025/")
    else:
        # Use subprocess.run to execute the git clone command
        subprocess.run(["git", "clone", "https://github.com/aeldably/
 ↪Neural_Data_Science_Summer_2025/"])
    os.chdir("Neural_Data_Science_Summer_2025/notebooks")
    print("This code runs only in Colab")
else:
    print("This code runs outside of Colab")
```

```
This code runs only in Colab
```

[2]:
```
!pip3 install tsnecuda==3.0.1+cu122 -f https://tsnecuda.isx.ai/tsnecuda_stable.
 ↪html
!pip install openTSNE jupyter_black watermark igraph leidenalg
```

```
Looking in links: https://tsnecuda.isx.ai/tsnecuda_stable.html
Collecting tsnecuda==3.0.1+cu122
  Downloading https://s3.us-
west-1.wasabisys.com/tsnecuda/tsnecuda-3.0.1%2Bcu122-py3-none-any.whl (385.6 MB)
```

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Requirement already satisfied: numpy>=1.14.1 in
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Installing collected packages: tsnecuda
Successfully installed tsnecuda-3.0.1+cu122
Collecting openTSNE
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manylinux_2_17_x86_64.manylinux2014_x86_64.whl.metadata (7.8 kB)
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Collecting watermark
  Downloading watermark-2.5.0-py2.py3-none-any.whl.metadata (1.4 kB)
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Collecting texttable>=1.6.2 (from igraph)
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Requirement already satisfied: click>=8.0.0 in /usr/local/lib/python3.11/dist-
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Collecting mypy-extensions>=0.4.3 (from
black>=21->black[jupyter]>=21->jupyter_black)
  Downloading mypy_extensions-1.1.0-py3-none-any.whl.metadata (1.1 kB)
Requirement already satisfied: packaging>=22.0 in
/usr/local/lib/python3.11/dist-packages (from
black>=21->black[jupyter]>=21->jupyter_black) (24.2)
Collecting pathspec>=0.9.0 (from black>=21->black[jupyter]>=21->jupyter_black)
  Downloading pathspec-0.12.1-py3-none-any.whl.metadata (21 kB)
Requirement already satisfied: platformdirs>=2 in
/usr/local/lib/python3.11/dist-packages (from
black>=21->black[jupyter]>=21->jupyter_black) (4.3.8)
Collecting tokenize-rt>=3.2.0 (from black[jupyter]>=21->jupyter_black)
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Collecting jedi>=0.16 (from ipython>=6.0->watermark)
  Downloading jedi-0.19.2-py2.py3-none-any.whl.metadata (22 kB)
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/usr/local/lib/python3.11/dist-packages (from ipython>=6.0->watermark) (3.0.51)
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pexpect>4.3->ipython>=6.0->watermark) (0.7.0)
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toolkit!=3.0.0,!=3.0.1,<3.1.0,>=2.0.0->ipython>=6.0->watermark) (0.2.13)
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Downloading pathspec-0.12.1-py3-none-any.whl (31 kB)
Downloading tokenize_rt-6.2.0-py2.py3-none-any.whl (6.0 kB)
Installing collected packages: texttable, tokenize-rt, pathspec, mypy-
extensions, jedi, igraph, leidenalg, black, watermark, openTSNE, jupyter_black
Successfully installed black-25.1.0 igraph-0.11.8 jedi-0.19.2
jupyter_black-0.4.0 leidenalg-0.10.2 mypy-extensions-1.1.0 openTSNE-1.0.2
pathspec-0.12.1 texttable-1.7.0 tokenize-rt-6.2.0 watermark-2.5.0
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[14]:

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Collecting jupyter_black
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Collecting watermark
  Downloading watermark-2.5.0-py2.py3-none-any.whl.metadata (1.4 kB)
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(3.8 kB)
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Requirement already satisfied: matplotlib-inline in
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/usr/local/lib/python3.11/dist-packages (from scikit-learn>=0.20->openTSNE)
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Requirement already satisfied: parso<0.9.0,>=0.8.4 in
/usr/local/lib/python3.11/dist-packages (from
jedi>=0.16->ipython>=6.0->watermark) (0.8.4)
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Installing collected packages: texttable, tokenize-rt, pathspec, mypy-
extensions, jedi, igraph, leidenalg, black, watermark, openTSNE, jupyter_black
Successfully installed black-25.1.0 igraph-0.11.8 jedi-0.19.2
jupyter_black-0.4.0 leidenalg-0.10.2 mypy-extensions-1.1.0 openTSNE-1.0.2
pathspec-0.12.1 texttable-1.7.0 tokenize-rt-6.2.0 watermark-2.5.0
```

[3]:
```
import numpy as np
import pylab as plt
import pandas as pd
import matplotlib.pyplot as plt

# We recommend using openTSNE for experiments with t-SNE
# https://github.com/pavlin-policar/openTSNE
from openTSNE import TSNE

#%matplotlib inlinep

%load_ext jupyter_black

%load_ext watermark
%watermark --time --date --timezone --updated --python --iversions --watermark␣
 ↪-p sklearn
```

```
Last updated: 2025-06-07 16:48:40UTC

Python implementation: CPython
Python version       : 3.11.13
IPython version      : 7.34.0

sklearn: 1.6.1

pandas    : 2.2.2
openTSNE  : 1.0.2
numpy     : 2.0.2
matplotlib: 3.10.0

Watermark: 2.5.0
```

[4]:
```
plt.style.use("../matplotlib_style.txt")
```

2 Introduction

In this notebook you are going to work with transcriptomics data, in particular single-cell RNA sequencing (scRNA-seq) data from the paper by Harris et al. (2018). They recorded the transcriptomes of 3,663 inhibitory cells in the hippocampal area CA1. Their analysis divided these cells into 49 fine-scale clusters coresponding to different cell subtypes. They asigned names to these cluster in a hierarchical fashion according to strongly expressed gene in each clusters. The figure below shows the details of their classification.

You will first analyze some of the most relevant statistics of UMI gene counts distributions, and afterwards follow the standard pipeline in the field to produce a visualization of the data.

2.1 Load data

Download the data from ILIAS, move it to the `data/` directory and unzip it there. The read counts can be found in `counts`, with rows corresponding to cells and columns to genes. The cluster assignments for every individual cell can be found in `clusters`, along with the colors used in the publication in `clusterColors`.

[5]:
```
# LOAD HARRIS ET AL DATA

# Load gene counts
data = pd.read_csv("../data/nds_cl_7/harris-data/expression.tsv", sep="\t")
genes = data.values[:, 0]
cells = data.columns[1:-1]
counts = data.values[:, 1:-1].transpose().astype("int")
data = []

# Kick out all genes with all counts = 0
genes = genes[counts.sum(axis=0) > 0]
counts = counts[:, counts.sum(axis=0) > 0]
print(counts.shape)

# Load clustering results
data = pd.read_csv("../data/nds_cl_7/harris-data/analysis_results.tsv",␣
 ↪sep="\t")
clusterNames, clusters = np.unique(data.values[0, 1:-1], return_inverse=True)

# Load cluster colors
data = pd.read_csv("../data/nds_cl_7/harris-data/colormap.txt", sep="\s+",␣
 ↪header=None)
clusterColors = data.values

# Note: the color order needs to be reversed to match the publication
clusterColors = clusterColors[::-1]

# Taken from Figure 1 - we need cluster order to get correct color order
clusterOrder = [
    "Sst.No",
    "Sst.Npy.C",
    "Sst.Npy.Z",
    "Sst.Npy.S",
    "Sst.Npy.M",
    "Sst.Pnoc.Calb1.I",
    "Sst.Pnoc.Calb1.P",
    "Sst.Pnoc.P",
    "Sst.Erbb4.R",
    "Sst.Erbb4.C",
    "Sst.Erbb4.T",
    "Pvalb.Tac1.N",
    "Pvalb.Tac1.Ss",
    "Pvalb.Tac1.Sy",
    "Pvalb.Tac1.A",
    "Pvalb.C1ql1.P",
    "Pvalb.C1ql1.C",
    "Pvalb.C1ql1.N",
    "Cacna2d1.Lhx6.R",
    "Cacna2d1.Lhx6.V",
    "Cacna2d1.Ndnf.N",
    "Cacna2d1.Ndnf.R",
    "Cacna2d1.Ndnf.C",
    "Calb2.Cry",
    "Sst.Cry",
    "Ntng1.S",
    "Ntng1.R",
    "Ntng1.C",
    "Cck.Sema",
    "Cck.Lmo1.N",
    "Cck.Calca",
    "Cck.Lmo1.Vip.F",
    "Cck.Lmo1.Vip.C",
    "Cck.Lmo1.Vip.T",
    "Cck.Ly",
    "Cck.Cxcl14.Calb1.Tn",
    "Cck.Cxcl14.Calb1.I",
    "Cck.Cxcl14.S",
    "Cck.Cxcl14.Calb1.K",
    "Cck.Cxcl14.Calb1.Ta",
    "Cck.Cxcl14.V",
    "Vip.Crh.P",
    "Vip.Crh.C1",
    "Calb2.Vip.G",
    "Calb2.Vip.I",
    "Calb2.Vip.Nos1",
    "Calb2.Cntnap5a.R",
    "Calb2.Cntnap5a.V",
    "Calb2.Cntnap5a.I",
]

reorder = np.zeros(clusterNames.size) * np.nan
for i, c in enumerate(clusterNames):
    for j, k in enumerate(clusterOrder):
        if c[: len(k)] == k:
            reorder[i] = j
            break
clusterColors = clusterColors[reorder.astype(int)]
```

```
(3663, 17965)
```

3 Task 1: Data inspection

Before we use t-SNE or any other advanced visualization methods on the data, we first want to have a closer look on the data and plot some statistics. For most of the analysis we will compare the data to a Poisson distribution.

3.0.1 1.1. Relationship between expression mean and fraction of zeros

Compute actual and predicted gene expression. The higher the average expression of a gene, the smaller fraction of cells will show a 0 count. Plot the data and explain what you see in the plot.

(3 pts)

[6]:
```
# ------------------------------------------------------
# Compute actual and predicted gene expression (1 pt)
# ------------------------------------------------------

# Compute the average expression for each gene
mean_expression = np.mean(counts, axis=0)

# Compute the fraction of zeros for each gene
fraction_zeros = np.mean(counts == 0, axis=0)
```

[7]:
```
# Compute the Poisson prediction
# (what is the expected fraction of zeros in a Poisson distribution with a␣
 ↪given mean?)
poisson_prediction = np.exp(-mean_expression)
```

[8]:
```
# --------------------------------------------------
# plot the data and the Poisson prediction (1 pt)
# --------------------------------------------------

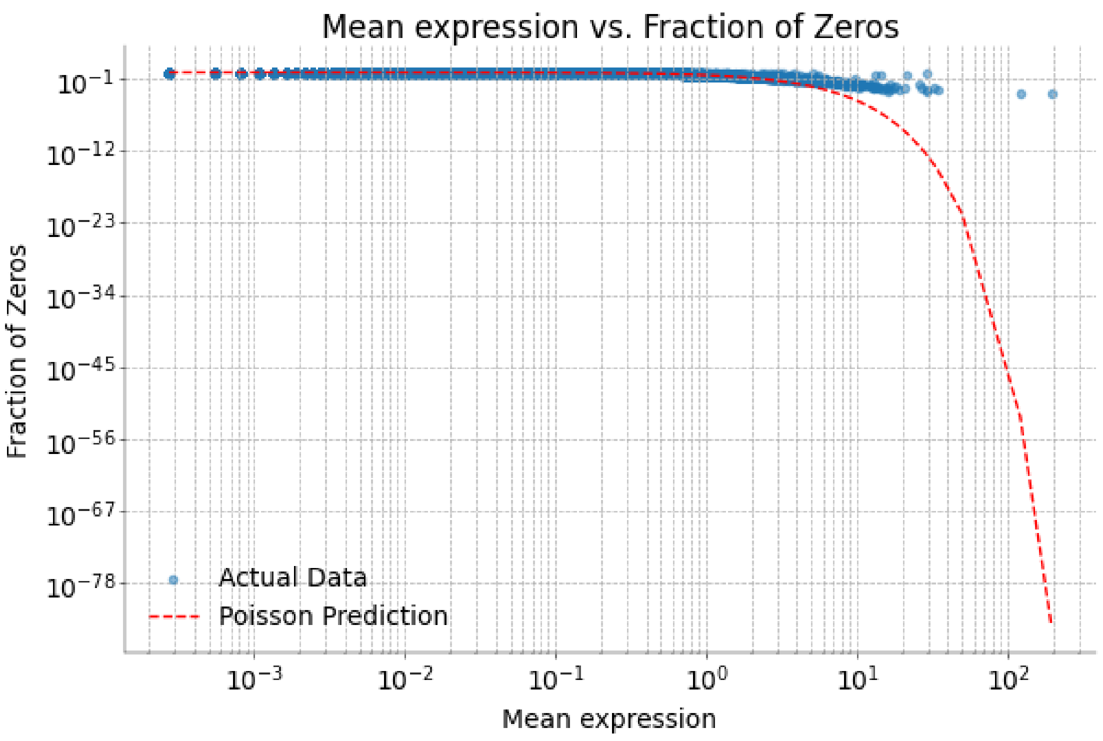
fig, ax = plt.subplots(figsize=(6, 4))
# add plot

# 1. Plot the actual data as a scatter plot
ax.scatter(mean_expression, fraction_zeros, alpha=0.5, label="Actual Data")

# 2. Plot the Poisson prediction as a line plot
#    (Sorting the values makes the line clean)
sorted_indices = np.argsort(mean_expression)
ax.plot(
    mean_expression[sorted_indices],
    poisson_prediction[sorted_indices],
    color="red",
    linestyle="--",
    label="Poisson Prediction",
)
```

```
# 3. Set scales and labels
ax.set_xscale("log")
ax.set_yscale("log")
ax.set_xlabel("Mean expression")
ax.set_ylabel("Fraction of Zeros")
ax.set_title("Mean expression vs. Fraction of Zeros")
ax.grid(True, which="both", linestyle="--", linewidth=0.5)
ax.legend()

plt.savefig("../images/lab7-mean_vs_fraction_zeros.png", dpi=300,␣
 ↪bbox_inches="tight")
plt.show()
```



Explanation (1 pt) …

The plot shows the relationship between the **average expression** of each gene and the **fraction of cells where that gene was not detected** (a zero count). As expected, there is a clear inverse relationship: genes with **higher mean expression are detected in a larger fraction** of cells, and thus have a lower fraction of zeros.

The red dashed line shows the theoretical relationship predicted by a Poisson distribution. The actual data points consistently lie **above** this line, indicating that for any given mean expression level, the fraction of zero counts is **higher** than the Poisson model would predict. This phenomenon is known as **overdispersion** or **zero-inflation** and is a classic characteristic of single-cell RNA sequencing data. It suggests that the simple Poisson model is insufficient to capture the full variability of the data

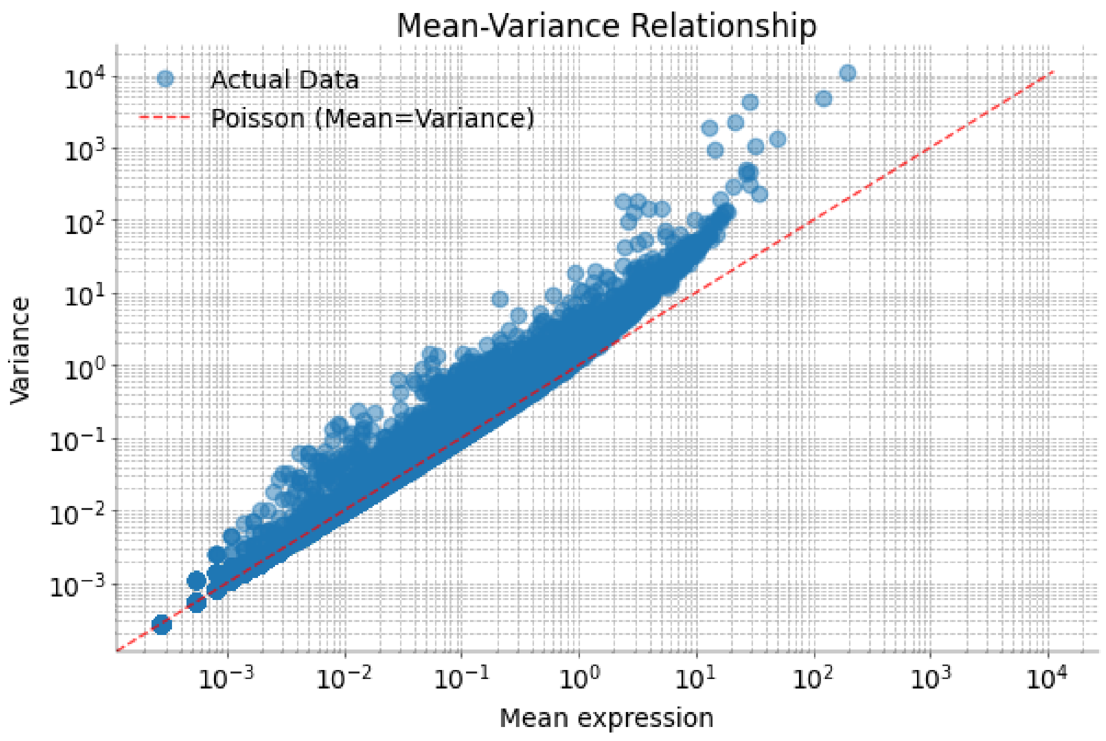
3.0.2 1.2. Mean-variance relationship

If the expression follows Poisson distribution, then the mean should be equal to the variance. Plot the mean-variance relationship and interpret the plot.

(2.5 pts)

[9]:
```python
# -------------------------------------------------------------------
# Compute the variance of the expression counts of each gene (0.5 pt)
# -------------------------------------------------------------------
variance = np.var(counts, axis=0)
```

[10]:
```python
# -------------------------------------------------------------
# Plot the mean-variance relationship on a log-log plot (1 pt)
# Plot the Poisson prediction as a line
# -------------------------------------------------------------

fig, ax = plt.subplots(figsize=(6, 4))
plt.plot(mean_expression, variance, "o", alpha=0.5, label="Actual Data")
lims = [
    np.min([plt.xlim(), plt.ylim()]),  # find the min of the axes
    np.max([plt.xlim(), plt.ylim()]),  # find the max of the axes
]
# Plot the y=x line
plt.plot(lims, lims, "r--", alpha=0.75, label="Poisson (Mean=Variance)")

plt.xscale("log")
plt.yscale("log")
plt.xlabel("Mean expression")
plt.ylabel("Variance")
plt.title("Mean-Variance Relationship")
plt.grid(True, which="both", linestyle="--", linewidth=0.5)
plt.legend()
plt.savefig("../images/lab7-mean_vs_variance.png", dpi=300, bbox_inches="tight")
plt.show()
```



Explanation (1 pt) …

This plot displays the relationship between the **mean** and variance of each gene's expression on a log-log scale. The red dashed line represents the theoretical expectation for a Poisson distribution, where the **variance is equal to the mean**.

The plot clearly shows that nearly all genes lie above this line, indicating that their **variance is greater than their mean**. This is a classic sign of **overdispersion**, which is a common feature of single-cell transcriptomics data. The simple Poisson model is therefore not a sufficient description of the data's variability.

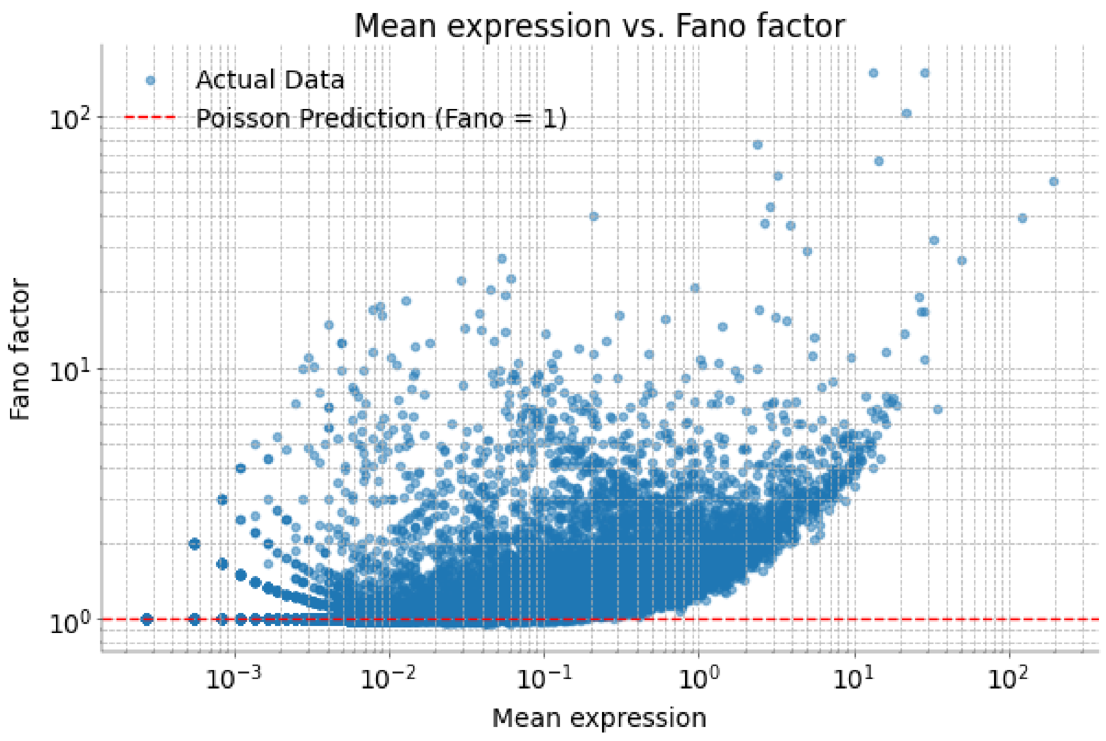
3.0.3 1.3. Relationship between the mean and the Fano factor

Compute the Fano factor for each gene and make a scatter plot of expression mean vs. Fano factor in log-log coordinates, and interpret what you see in the plot. If the expression follows the Poisson distribution, then the Fano factor (variance/mean) should be equal to 1 for all genes.

(2.5 pts)

[11]:
```
# --------------------------------------------
# Compute the Fano factor for each gene (0.5 pt)
# --------------------------------------------

fano = np.var(counts, axis=0) / np.mean(counts, axis=0)
```

[12]:
```
# -------------------------------
# plot fano-factor vs mean (1 pt)
# incl. fano factor
# -------------------------------
# Plot a Poisson prediction as line
# Use the same style of plot as above.

fig, ax = plt.subplots(figsize=(6, 4))

plt.scatter(mean_expression, fano, alpha=0.5, label="Actual Data")
plt.xscale("log")
plt.yscale("log")
plt.xlabel("Mean expression")
plt.ylabel("Fano factor")
plt.title("Mean expression vs. Fano factor")
plt.grid(True, which="both", linestyle="--", linewidth=0.5)
plt.axhline(y=1, color="red", linestyle="--", label="Poisson Prediction (Fano =␣
 ↪1)")
plt.legend()
plt.savefig("../images/lab7-mean_vs_fano.png", dpi=300, bbox_inches="tight")
plt.show()
```



Explanation (1 pt) ...

This plot visualizes the **Fano factor (variance/mean)** against the **mean expression** for each gene. The red line at a Fano factor of 1 indicates the expected value if the data followed a Poisson distribution, where variance equals the mean.

The plot confirms the findings from the previous two sections:

For genes with **very low expression**, the Fano factor is **close to 1**, behaving similarly to a Poisson process. For the majority of genes, however, the Fano factor is significantly greater than 1, indicating that their **variance is much larger than their mean**. This trend of increasing Fano factor with increasing mean expression is a clear sign of the **data's overdispersion** and reinforces that a simple Poisson model is not adequate.

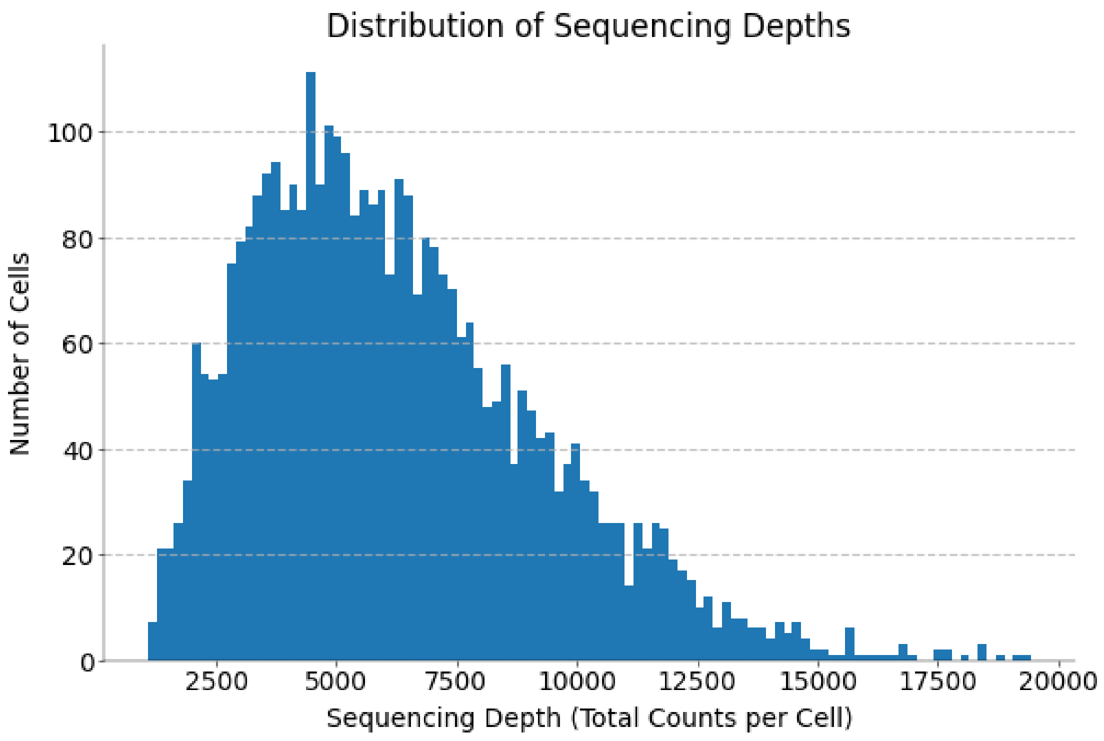
3.0.4 1.4. Histogram of sequencing depths

Different cells have different sequencing depths (sum of counts across all genes) because the efficiency can change from droplet to droplet due to some random expreimental factors. Make a histogram of sequencing depths.

(1.5 pts)

[13]:
```
# -------------------------------
# Compute sequencing depth (0.5 pt)
# -------------------------------
sequencing_depths = np.sum(counts, axis=1)
```

[14]:
```
# ------------------------------------------
# Plot histogram of sequencing depths (1 pt)
# ------------------------------------------
fig, ax = plt.subplots(figsize=(6, 4))

# Create the histogram
ax.hist(sequencing_depths, bins=100)

# Add labels and a title for clarity
ax.set_xlabel("Sequencing Depth (Total Counts per Cell)")
ax.set_ylabel("Number of Cells")
ax.set_title("Distribution of Sequencing Depths")
ax.grid(axis="y", linestyle="--", alpha=0.7)

plt.savefig(
    "../images/lab7-sequencing_depth_histogram.png", dpi=300,␣
  ↪bbox_inches="tight"
)
plt.show()
```



3.0.5 1.5. Fano factors after normalization

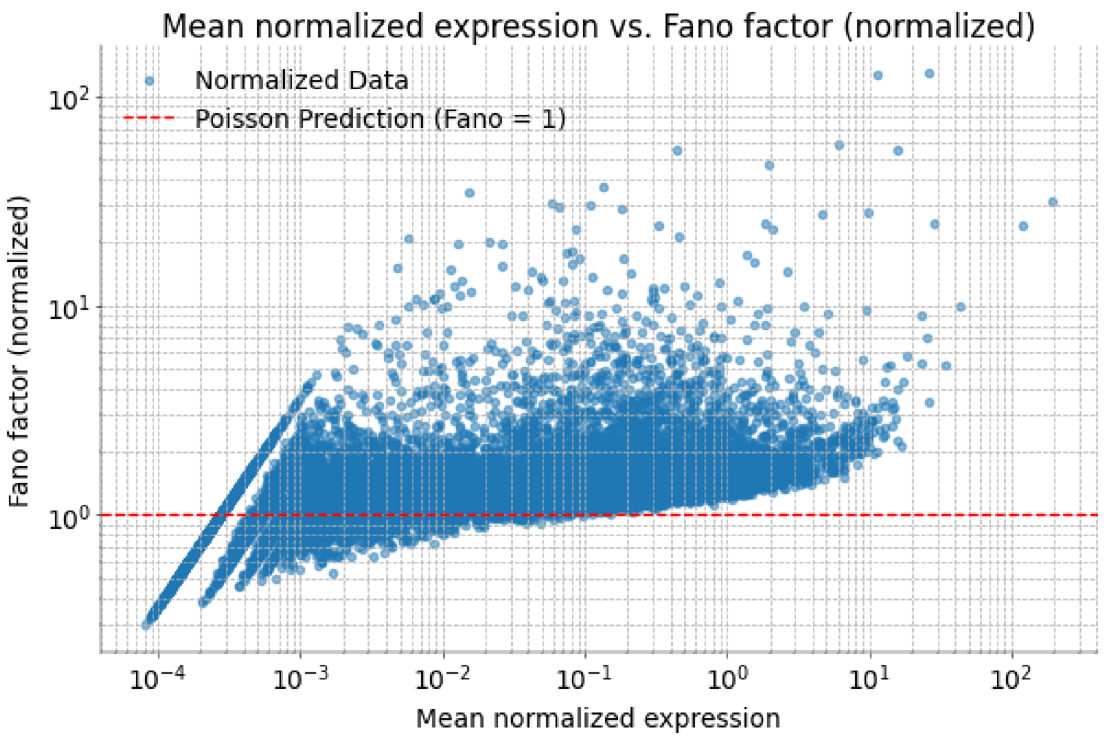
Normalize counts by the sequencing depth of each cell and multiply by the median sequencing depth. Then make the same expression vs Fano factor plot as above. After normalization by sequencing depth, Fano factor should be closer to 1 (i.e. variance even more closely following the mean). This can be used for feature selection.

(2.5 pts)

[15]:
```
# --------------------------------------------------
# compute normalized counts and fano factor (1 pt)
# --------------------------------------------------
median_depth = np.median(sequencing_depths)

normalized_counts = counts / sequencing_depths[:, np.newaxis] * median_depth
mean_normalized = np.mean(normalized_counts, axis=0)
variance_normalized = np.var(normalized_counts, axis=0)

fano_normalized = variance_normalized / mean_normalized
```

[16]:
```
# -----------------------------------------------------------
# plot normalized counts and find the top 10 genes (1 pt)
# hint: keep appropriate axis scaling in mind
# -----------------------------------------------------------
```

```
fig, ax = plt.subplots(figsize=(6, 4))
# add plot
plt.scatter(mean_normalized, fano_normalized, alpha=0.5, label="Normalized␣
    ↪Data")
plt.xscale("log")
plt.yscale("log")
plt.xlabel("Mean normalized expression")
plt.ylabel("Fano factor (normalized)")
plt.title("Mean normalized expression vs. Fano factor (normalized)")
plt.grid(True, which="both", linestyle="--", linewidth=0.5)
plt.axhline(y=1, color="red", linestyle="--", label="Poisson Prediction (Fano =␣
    ↪1)")
plt.legend()
plt.savefig("../images/lab7-mean_vs_fano_normalized.png", dpi=300,␣
    ↪bbox_inches="tight")
plt.show()
```



[17]:
```
# -----------------------------------------------------------------------
# Find top-10 genes with the highest normalized Fano factor (0.5 pts)
# Print them sorted by the Fano factor starting from the highest
# Gene names are stored in the `genes` array
# ---------------------------------------------------------------------
sorted_indices = np.argsort(fano_normalized)
top_10_indices = sorted_indices[-10:]

top_10_genes = genes[top_10_indices]

print("Top 10 genes with the highest normalized Fano factor:")
print(top_10_genes[::-1])  # [::-1] reverses the list to show highest first
```

```
Top 10 genes with the highest normalized Fano factor:
['Sst' 'Npy' 'Vip' 'Cck' 'Cpne2' 'Pcp4' 'Ptpn23' 'Pdzd9' 'Malat1' 'Armc2']
```

4 Task 2: Low dimensional visualization

In this task we will construct a two dimensional visualization of the data. First we will normalize the data with some variance stabilizing transformation and study the effect that different approaches have on the data. Second, we will reduce the dimensionality of the data to a more feasible number of dimensions (e.g. $d = 50$) using PCA. And last, we will project the PCA-reduced data to two dimensions using t-SNE.

4.0.1 2.1. PCA with and without transformations

Here we look at the influence of variance-stabilizing transformations on PCA. We will focus on the following transformations: - Square root (`sqrt(X)`): it is a variance-stabilizing transformation for the Poisson data. - Log-transform (`log2(X+1)`): it is also often used in the transcriptomic community.

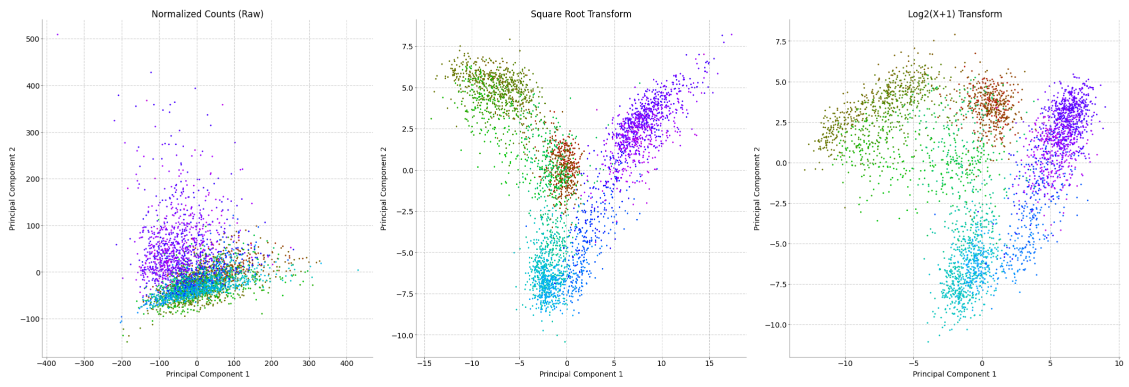
We will only work with the most important genes. For that, transform the counts into normalized counts (as above) and select all genes with normalized Fano factor above 3 and remove the rest. We will look at the effect that both transformations have in the PCA-projected data by visualizing the first two components. Interpret qualitatively what you see in the plot and compare the different embeddings making use of the ground truth clusters.

(3.5 pts)

[18]:
```
# --------------------------------
# Select important genes (0.5 pts)
# --------------------------------



highly_variable_mask = fano_normalized > 3
filtered_counts = normalized_counts[:, highly_variable_mask]

sqrt_transformed_counts = np.sqrt(filtered_counts)
log_transformed_counts = np.log2(filtered_counts + 1)

from sklearn.decomposition import PCA
```

[19]:
```
# --------------------------------------
# transform data and apply PCA (1 pt)
# --------------------------------------

from sklearn.decomposition import PCA

# perform PCA

# Initialize PCA to find the top 50 components
pca = PCA(n_components=50)

# Apply PCA to each of the three datasets
pca_raw = pca.fit_transform(filtered_counts)
pca_sqrt = pca.fit_transform(sqrt_transformed_counts)
pca_log = pca.fit_transform(log_transformed_counts)

pca_results = [pca_raw, pca_sqrt, pca_log]
titles = ["Normalized Counts (Raw)", "Square Root Transform", "Log2(X+1)␣
 ↪Transform"]
```

[20]:
```
# ------------------------------------------------
# plot first 2 PCs for each transformation (1 pt)
# ------------------------------------------------

fig, axs = plt.subplots(1, 3, figsize=(21, 7))
# add plot
for i, (data, title) in enumerate(zip(pca_results, titles)):
    # Scatter plot of the first two principal components
    # c=clusterColors[clusters] assigns the correct publication color to each␣
 ↪cell
    axs[i].scatter(data[:, 0], data[:, 1], c=clusterColors[clusters], s=5)

    axs[i].set_title(title)
    axs[i].set_xlabel("Principal Component 1")
    axs[i].set_ylabel("Principal Component 2")
    axs[i].grid(linestyle="--", alpha=0.6)

plt.savefig("../images/lab7-pca_transformations.png", dpi=300,␣
 ↪bbox_inches="tight")
plt.show()
```



Explanation (1 pt) …

These plots visualize the first two principal components (PCs) of the data after applying three different preprocessing steps: using the raw normalized counts, applying a square-root transform, and applying a log2(X+1) transform. The points are colored according to their ground-truth cluster identities to assess how well each method separates the different cell types.

- Normalized Counts (Raw): In this plot, the clusters are poorly separated. Most of the variance is captured along the first principal component (PC1), likely driven by a few highly expressed genes. This results in the clusters being smeared together without clear distinctions.
- Square Root Transform: This transformation shows some improvement. By reducing the influence of the most highly expressed genes, some clusters begin to emerge from the main group, but there is still significant overlap among them.
- Log2(X+1) Transform: This plot shows the clearest separation of clusters. The log-transform effectively balances the influence of genes across all expression levels, allowing PCA to identify the more subtle patterns that define different cell types. As a result, many distinct colored groups are visible and well-separated in the PC1/PC2 space.

Comparing the three embeddings, the log-transform and square root transform are more dispersed and disparate groups and thus provide better clustering than row counts.

4.0.2 2.2. tSNE with and without transformations

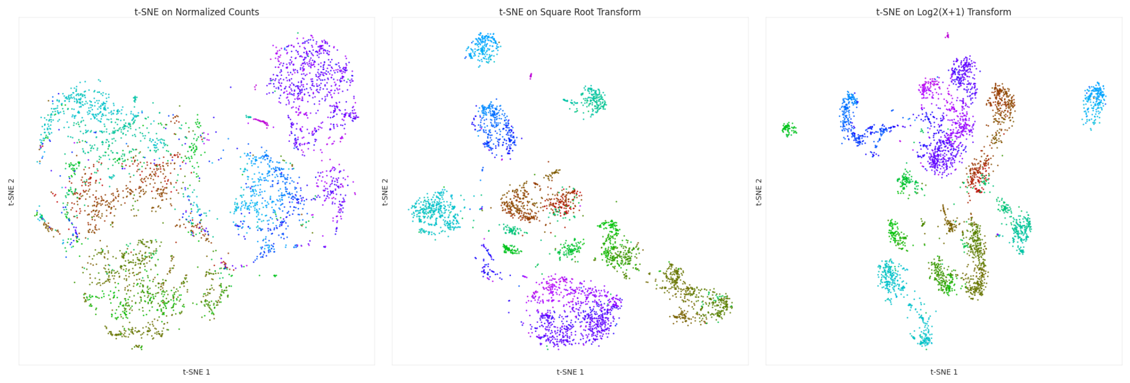
Now, we will reduce the dimensionality of the PCA-reduced data further to two dimensions using t-SNE. We will use only $n = 50$ components of the PCA-projected data. Plot the t-SNE embedding for the three versions of the data and interpret the plots. Do the different transformations have any effect on t-SNE?

(1.5 pts)

[21]:
```
# ------------------------
# Perform tSNE (0.5 pts)
# ------------------------
from tsnecuda import TSNE
```

```
tsne_raw = TSNE(n_components=2, perplexity=30, learning_rate=10).
 ↪fit_transform(pca_raw)
tsne_sqrt = TSNE(n_components=2, perplexity=30, learning_rate=10).fit_transform(
    pca_sqrt
)
tsne_log = TSNE(n_components=2, perplexity=30, learning_rate=10).
 ↪fit_transform(pca_log)
```

[27]:
```
# ----------------------------------------------
# plot t-SNE embedding for each dataset (1 pt)
# ----------------------------------------------
import seaborn as sns

sns.set_style("whitegrid")
# Create a list of t-SNE results and their corresponding titles
tsne_results = [tsne_raw, tsne_sqrt, tsne_log]
titles = [
    "t-SNE on Normalized Counts",
    "t-SNE on Square Root Transform",
    "t-SNE on Log2(X+1) Transform",
]

fig, axs = plt.subplots(1, 3, figsize=(21, 7))
# Loop through each t-SNE result and plot it
for i, (data, title) in enumerate(zip(tsne_results, titles)):
    # Create a scatter plot of the 2D t-SNE data
    # Color each point by its ground-truth cluster identity
    axs[i].scatter(data[:, 0], data[:, 1], c=clusterColors[clusters], s=5,␣
 ↪alpha=0.8)

    # Set the title and labels
    axs[i].set_title(title)
    axs[i].set_xlabel("t-SNE 1")
    axs[i].set_ylabel("t-SNE 2")

    # The axes on a t-SNE plot don't have a direct interpretation,
    # so we can remove the tick marks for a cleaner look.
    axs[i].set_xticks([])
    axs[i].set_yticks([])

plt.savefig("../images/lab7-tsne_transformations.png", dpi=300,␣
 ↪bbox_inches="tight")
plt.show()
```



Interpretation of t-SNE plots These plots show the 2D t-SNE embeddings for the three versions of the data: raw, square-root transformed, and log-transformed. The analysis addresses the question, "Do the different transformations have any effect on t-SNE?".

The answer is yes. The initial data transformation dramatically impacts the quality of the final t-SNE visualization:

- **Raw Counts**: The t-SNE embedding on the raw, untransformed data shows very poor separation. The distinct cell clusters are largely mixed together, failing to reveal the underlying structure of the data.
- **Square Root Transform**: This provides a much better result, with several major cell groups beginning to separate into distinct "continents" on the plot.
- **Log2(X+1) Transform**: This transformation also yeilds a good results. The clusters are well-separated and tightly grouped.

This comparison confirms that applying a strong variance-stabilizing transformation, like the log-transform, is a critical step for revealing the detailed structure of the data with t-SNE.

4.0.3 2.3. Leiden clustering

Now we will play around with some clustering and see whether the clustering methods can produce similar results to the original clusters from the publication. We will apply Leiden clustering (closely related to the Louvain clustering), which is standard in the field and works well even for very large datasets.

Choose one representation of the data (best transformation based in your results from the previous task) to use further in this task and justify your choice. Think about which level of dimensionality would be sensible to use to perform clustering. Visualize in the two-dimensional embedding the resulting clusters and compare to the original clusters.

(1.5 pts)

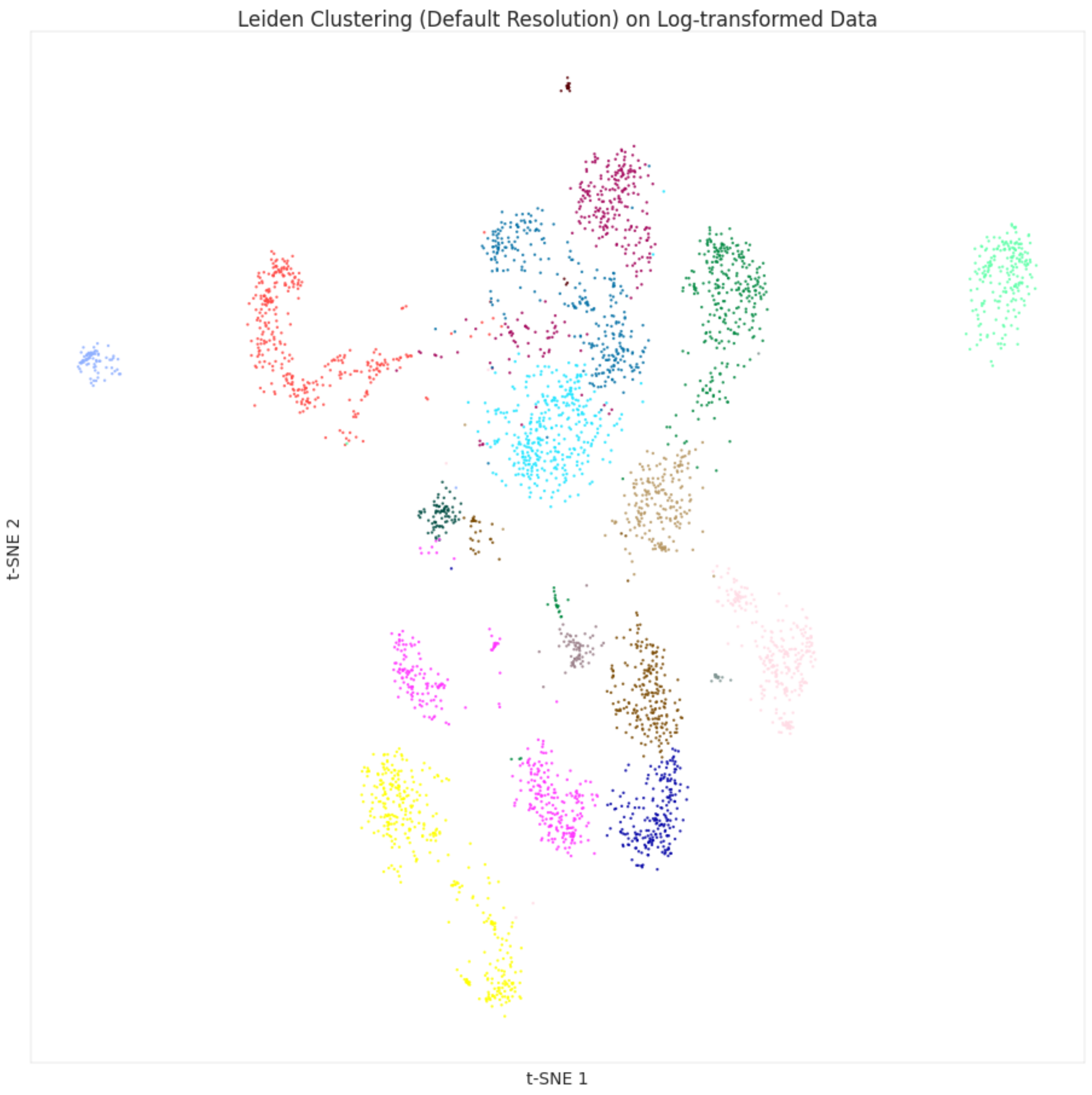
[28]:
```python
# To run this code you need to install leidenalg and igraph
# conda install -c conda-forge python-igraph leidenalg

import igraph as ig
```

```
from sklearn.neighbors import NearestNeighbors, kneighbors_graph
import leidenalg as la
```

[30]:
```
# Define some contrast colors

clusterCols = [
    "#FFFF00",
    "#1CE6FF",
    "#FF34FF",
    "#FF4A46",
    "#008941",
    "#006FA6",
    "#A30059",
    "#FFDBE5",
    "#7A4900",
    "#0000A6",
    "#63FFAC",
    "#B79762",
    "#004D43",
    "#8FB0FF",
    "#997D87",
    "#5A0007",
    "#809693",
    "#FEFFE6",
    "#1B4400",
    "#4FC601",
    "#3B5DFF",
    "#4A3B53",
    "#FF2F80",
    "#61615A",
    "#BA0900",
    "#6B7900",
    "#00C2A0",
    "#FFAA92",
    "#FF90C9",
    "#B903AA",
    "#D16100",
    "#DDEFFF",
    "#000035",
    "#7B4F4B",
    "#A1C299",
    "#300018",
    "#0AA6D8",
    "#013349",
    "#00846F",
    "#372101",
    "#FFB500",
    "#C2FFED",
    "#A079BF",
    "#CC0744",
    "#C0B9B2",
    "#C2FF99",
    "#001E09",
    "#00489C",
    "#6F0062",
    "#0CBD66",
    "#EEC3FF",
    "#456D75",
    "#B77B68",
    "#7A87A1",
    "#788D66",
    "#885578",
    "#FAD09F",
    "#FF8A9A",
    "#D157A0",
    "#BEC459",
    "#456648",
    "#0086ED",
    "#886F4C",
    "#34362D",
    "#B4A8BD",
    "#00A6AA",
    "#452C2C",
    "#636375",
    "#A3C8C9",
    "#FF913F",
    "#938A81",
    "#575329",
    "#00FECF",
    "#B05B6F",
    "#8CD0FF",
    "#3B9700",
    "#04F757",
    "#C8A1A1",
    "#1E6E00",
    "#7900D7",
    "#A77500",
    "#6367A9",
    "#A05837",
    "#6B002C",
    "#772600",
    "#D790FF",
    "#9B9700",
    "#549E79",
    "#FFF69F",
    "#201625",
    "#72418F",
    "#BC23FF",
    "#99ADC0",
    "#3A2465",
    "#922329",
    "#5B4534",
    "#FDE8DC",
    "#404E55",
    "#0089A3",
    "#CB7E98",
    "#A4E804",
    "#324E72",
    "#6A3A4C",
    "#83AB58",
    "#001C1E",
    "#D1F7CE",
    "#004B28",
    "#C8D0F6",
    "#A3A489",
    "#806C66",
    "#222800",
    "#BF5650",
    "#E83000",
    "#66796D",
    "#DA007C",
    "#FF1A59",
    "#8ADBB4",
    "#1E0200",
    "#5B4E51",
    "#C895C5",
    "#320033",
    "#FF6832",
    "#66E1D3",
    "#CFCDAC",
    "#D0AC94",
    "#7ED379",
    "#012C58",
]

clusterCols = np.array(clusterCols)
```

[32]:
```
# ------------------------------------------------------
# create graph and run leiden clustering on it (0.5 pts)
# hint: use `la?`, `la.find_partition?` and `ig.Graph?`
# to find out more about the provided packages.
# ------------------------------------------------------

# Construct kNN graph with k=15

A = kneighbors_graph(pca_log, n_neighbors=15, metric="cosine")

# Transform it into an igraph object

sources, targets = A.nonzero()
g = ig.Graph(list(zip(sources, targets)), directed=False)

# Run Leiden clustering
# you can use `la.RBConfigurationVertexPartition` as the partition type
partition = la.find_partition(g, la.RBConfigurationVertexPartition)
leiden_clusters = partition.membership
```

[39]:
```
# --------------------------
# Plot the results (1 pt)
# --------------------------



fig, ax = plt.subplots(figsize=(9, 9))
ax.scatter(
    tsne_log[:, 0], tsne_log[:, 1], c=clusterCols[leiden_clusters], s=2,␣
 ↪alpha=0.8
)
ax.set_title("Leiden Clustering (Default Resolution) on Log-transformed Data")
ax.set_xlabel("t-SNE 1")
ax.set_ylabel("t-SNE 2")
ax.set_xticks([])
ax.set_yticks([])
plt.savefig("../images/lab7-leiden_default.png", dpi=300, bbox_inches="tight")
plt.show()
```



4.0.4 2.4. Change the clustering resolution

The number of clusters can be changed by modifying the resolution parameter. How many clusters did we get with the default value? Change the resolution parameter to yield 2x more and 2x fewer clusters Plot all three results as t-SNE overlays (same as above).

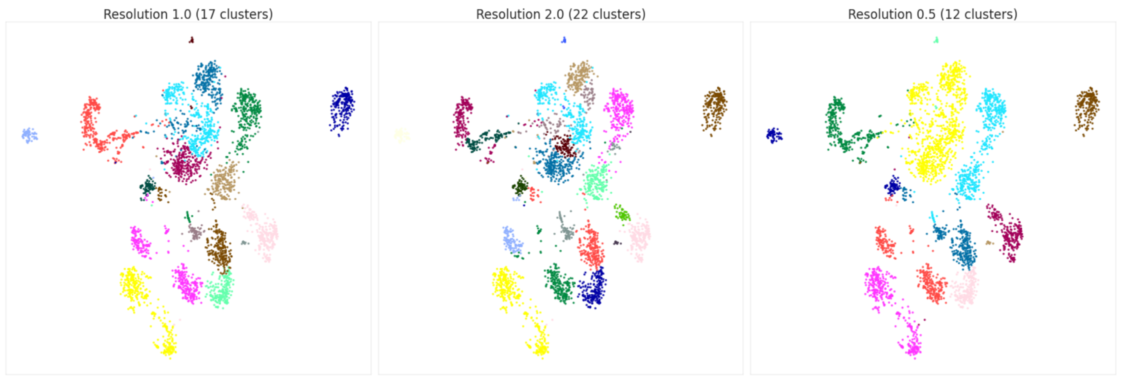
(1.5 pts)

[40]:
```
# ------------------------------------------------------------------
# run the clustering for 3 different resolution parameters (0.5 pts)
# ------------------------------------------------------------------
# Default resolution is 1.0
partition_default = la.find_partition(
    g, la.RBConfigurationVertexPartition, resolution_parameter=1.0
)
leiden_clusters_default = partition_default.membership
print(
    f"Number of clusters with default resolution (1.0): {len(np.
 ↪unique(leiden_clusters_default))}"
)

# 2x more clusters (resolution = 2.0)
partition_more = la.find_partition(
    g, la.RBConfigurationVertexPartition, resolution_parameter=2.0
)
leiden_clusters_more = partition_more.membership
print(f"Number of clusters with resolution 2.0: {len(np.
 ↪unique(leiden_clusters_more))}")

# 2x fewer clusters (resolution = 0.5)
partition_fewer = la.find_partition(
    g, la.RBConfigurationVertexPartition, resolution_parameter=0.5
)
leiden_clusters_fewer = partition_fewer.membership
print(
    f"Number of clusters with resolution 0.5: {len(np.
 ↪unique(leiden_clusters_fewer))}"
)
```

```
Number of clusters with default resolution (1.0): 17
Number of clusters with resolution 2.0: 22
Number of clusters with resolution 0.5: 12
```

[44]:
```
# --------------------------
# Plot the results (1 pt)
# --------------------------

fig, axs = plt.subplots(1, 3, figsize=(15, 5))
# add plot
axs[0].scatter(
    tsne_log[:, 0],
    tsne_log[:, 1],
    c=clusterCols[leiden_clusters_default],
    s=5,
    alpha=0.8,
)
axs[0].set_title(f"Resolution 1.0 ({len(np.unique(leiden_clusters_default))}␣
 ↪clusters)")
```

```
axs[0].set_xticks([])
axs[0].set_yticks([])

axs[1].scatter(
    tsne_log[:, 0], tsne_log[:, 1], c=clusterCols[leiden_clusters_more], s=5,␣
  ↪alpha=0.8
)
axs[1].set_title(f"Resolution 2.0 ({len(np.unique(leiden_clusters_more))}␣
  ↪clusters)")
axs[1].set_xticks([])
axs[1].set_yticks([])

axs[2].scatter(
    tsne_log[:, 0], tsne_log[:, 1], c=clusterCols[leiden_clusters_fewer], s=5,␣
  ↪alpha=0.8
)
axs[2].set_title(f"Resolution 0.5 ({len(np.unique(leiden_clusters_fewer))}␣
  ↪clusters)")
axs[2].set_xticks([])
axs[2].set_yticks([])

plt.savefig("../images/lab7-leiden_resolutions.png", dpi=300,␣
  ↪bbox_inches="tight")
plt.show()
```


Resolution 1.0 (17 clusters)
Resolution 2.0 (22 clusters)
Resolution 0.5 (12 clusters)