

Automating Flow Cytometry Data Analysis

USD M.Sc. Applied Data Science Capstone Project

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Date: 9Dec2024

Mount Google Drive

```
In [1]: # Uncomment if using Google Colaboratory
#from google.colab import drive
#drive.mount('/content/drive')
```

Import libraries:

```
In [2]: import FlowCal
import flowio
import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import seaborn as sns
import os
import pandas as pd
import matplotlib.ticker as ticker
import datetime
import klib

from scipy.signal import find_peaks
from sklearn.model_selection import train_test_split
from sklearn.decomposition import PCA
from sklearn.preprocessing import StandardScaler
from sklearn.metrics import silhouette_score
from sklearn.mixture import GaussianMixture
from sklearn.cluster import KMeans
from sklearn.cluster import DBSCAN
from openTSNE import TSNE
```

Handle Warnings

```
In [3]: import warnings
```

```
# Suppress warnings
warnings.filterwarnings('ignore')
```

Selected cellular surface markers to identify target Dendritic Cells (DC): Myeloid DC and Plasmacytoid DC.

Marker	Fluorochrome	Description
CD45RA	Ax700	An isoform of CD45, marking naïve T cells that haven't encountered their antigen
HLA-DR	BV786	An MHC class II molecule presenting antigens to CD4+ T cells; indicates antigen-presenting cells (APCs)
CD3	APC-H7	Part of the T-cell receptor complex, essential for T cell activation; found on all T cells
CD20	BUV805	A B cell marker involved in activation and proliferation; used to identify B cells
CD19	PE-Cy5	A co-receptor for B cell activation, primarily expressed on B cells
CD14	BV510	A co-receptor for LPS, marking monocytes and macrophages involved in innate immunity
CD123	BB660	The alpha chain of the IL-3 receptor, found on plasmacytoid dendritic cells and progenitor cells
CD11c	APC	An integrin marking dendritic cells and certain macrophages, important for cell adhesion and activation

Cellular Pathway:

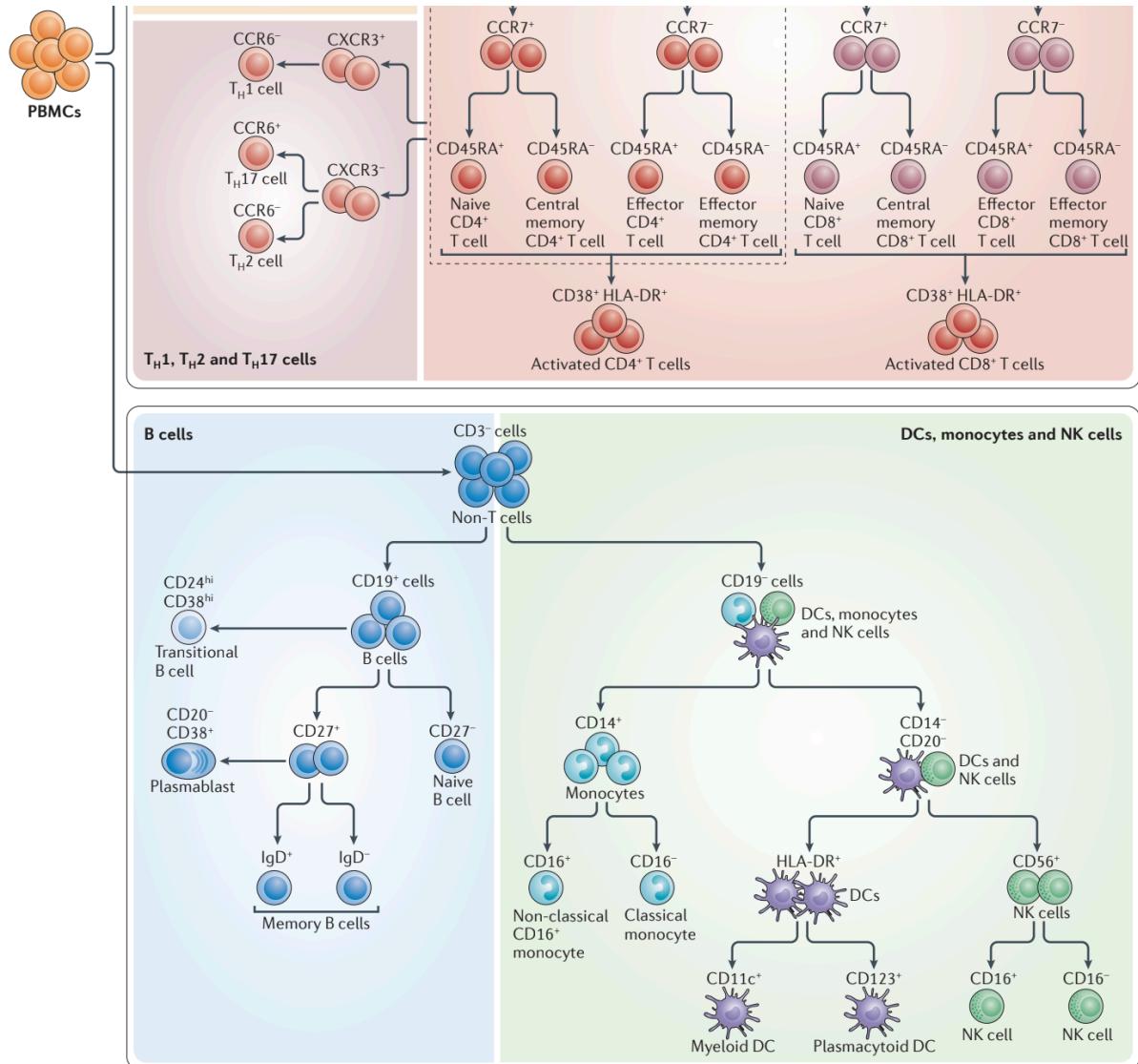
1. *PBMCs (Peripheral Blood Mononuclear Cells)*
Starting population
2. *CD3-*
Excludes T cells, focusing on B cells and myeloid cells
3. *CD19- & CD20-*
Excludes B cells, narrowing down to monocytes and dendritic cells
4. *CD14-*
Further excludes monocytes, leading to dendritic cell populations
5. *HLA-DR+*
Identifies antigen-presenting cells (APC)
6. *CD11c+ (Myeloid DC)*

Indicates myeloid dendritic cells \

OR

CD123+ (Plasmacytoid DC)

Indicates plasmacytoid dendritic cells



Flow Cytometry Data Extraction

```
In [4]: # User's MyDrive paths (JV for John Vincent Deniega; GR for Gabriella Rivera)
GR = "/content/drive/MyDrive/DC Marker Files/FCS"
```

```
# Defined only for JV's local Apple Mac Air M1 execution
home_dir = os.path.expanduser("~/")
```

```
JV = os.path.join(home_dir,
                  "Desktop",
                  "usd ADS",
                  "ADS599",
                  "Notebook",
                  "FlowRepository_FR-FCM-Z32U_files")
```

```
In [5]: # Define the path to the FCS file
fcs_folder = JV
pbmc_file = "PBMC_090120_1_DCs_A5_A05_031.fcs"
pbmc_path = os.path.join(fcs_folder, pbmc_file)
save_folder = JV

# Load the FCS data into an object
pbmc = FlowCal.io.FCSDData(pbmc_path)

# Inspect available attributes in the FCS file
print("Available attributes of FCSDData:")
for attr in dir(pbmc):
    if not attr.startswith('_'):
        print(attr)
```

Available attributes of FCSDData:

T
acquisition_end_time
acquisition_start_time
acquisition_time
all
amplification_type
amplifier_gain
analysis
any
argmax
argmin
argpartition
argsort
astype
base
byteswap
channel_labels
channels
choose
clip
compress
conj
conjugate
copy
ctypes
cumprod
cumsum

```
data
data_type
detector_voltage
diagonal
dot
dtype
dump
dumps
fill
flags
flat
flatten
getfield
hist_bins
imag
infile
item
itemset
itemsize
max
mean
min
 nbytes
ndim
newbyteorder
nonzero
partition
prod
ptp
put
range
ravel
real
repeat
reshape
resize
resolution
round
searchsorted
setfield
setflags
shape
size
sort
squeeze
std
strides
sum
swapaxes
take
```

```
text
time_step
tobytes
tofile
tolist
tostring
trace
transpose
var
view
```

```
In [6]: # Access the channel labels by calling the method
marker_labels = pbmc.channel_labels()

# Print the marker labels
print("Marker Labels:", marker_labels)
```

```
Marker Labels: [None, None, None, 'CADM1 FITC', 'CD141 BB630', 'CD123 BB66
0', 'FcER1a BB700', None, 'XCR1 BV421', 'CD14 BV510', 'CD16 BV570', None, 'C
D172a BV650', None, 'CD303 BV750', 'HLA-DR BV786', 'CD1c BUV395', 'Live Dead
UV Blue', None, 'CD56 BUV563', 'CD89 BUV661', 'CD163 BUV737', 'CD20 BUV805',
'CD11c APC', 'CD45RA Ax700', 'CD3 APC-H7', 'CD301 PE', None, 'CD19 PE-Cy5',
'CD5 PE-Cy55', 'CD88 PE-Cy7', None]
```

```
In [7]: %%time

# Convert pbmc to little-endian float32 to allow computational operations
if pbmc.dtype != '<f4':
    pbmc = np.array(pbmc, dtype='<f4')

# Rename the first three column labels with specified values lost during imp
marker_labels[0] = "FSC-A"
marker_labels[1] = "FSC-H"
marker_labels[2] = "SSC-A"
marker_labels[31] = "Time"

# Convert the FCS data from PBMC Sample 1 to a Pandas DataFrame
pbmc_s1_df = pd.DataFrame(pbmc)

# Assign marker labels to the DataFrame columns
pbmc_s1_df.columns = marker_labels

# Extract the 'Time' data and scale it to seconds
pbmc_s1_df['Time'] = pbmc_s1_df['Time'] * 0.01

# Convert all columns to float32 (little-endian), except 'Time'
numeric_columns = pbmc_s1_df.columns.difference(['Time'])

# Use astype to convert and force little-endian byte order for numeric colum
pbmc_s1_df[numeric_columns] = pbmc_s1_df[numeric_columns].astype('<f4')
```

```
# Save the formatted DataFrame as a CSV file
output_file = "pbmc_s1.csv"

# Uncomment this for Google Colab
#save_folder = "/content"

# # Uncomment this for local JV execution
save_folder = os.path.join(home_dir,
                           "Desktop",
                           "usd ADS",
                           "ADS599",
                           "Notebook",
                           "content")

output_path = os.path.join(save_folder, output_file)
pbmc_s1_df.to_csv(output_path, index=False)
```

CPU times: user 25.3 s, sys: 585 ms, total: 25.9 s
Wall time: 26.1 s

In [8]: # Verify PBMC DataFrame loaded correctly
pbmc_s1_df.head()

Out[8]:

	FSC-A	FSC-H	SSC-A	CADM1 FITC	CD141 BB630	CD123 BB660
0	87361.875000	79434.132812	598.278687	160.180847	59.948257	265.052338
1	126908.382812	108476.132812	1030.258667	202.591660	1682.306030	2094.691162
2	80773.437500	72883.585938	460.699219	63.809006	41.512314	187.331284
3	98579.289062	90491.320312	798.337646	180.572540	584.399658	4503.693359
4	81687.250000	74994.593750	586.497864	156.943649	-2.742810	89.636864

5 rows × 32 columns

In [9]: # Inspect General Dataframe Information
pbmc_s1_df.info()

```
<class 'pandas.core.frame.DataFrame'>
RangeIndex: 2088689 entries, 0 to 2088688
Data columns (total 32 columns):
 #   Column           Dtype  
 --- 
 0   FSC-A            float32
 1   FSC-H            float32
 2   SSC-A            float32
 3   CADM1 FITC      float32
 4   CD141 BB630     float32
 5   CD123 BB660     float32
 6   FcER1a BB700    float32
 7   None             float32
 8   XCR1 BV421      float32
 9   CD14  BV510      float32
 10  CD16  BV570      float32
 11  None             float32
 12  CD172a BV650    float32
 13  None             float32
 14  CD303 BV750     float32
 15  HLA-DR BV786    float32
 16  CD1c  BUV395    float32
 17  Live Dead UV Blue float32
 18  None             float32
 19  CD56  BUV563    float32
 20  CD89  BUV661    float32
 21  CD163 BUV737    float32
 22  CD20  BUV805    float32
 23  CD11c APC       float32
 24  CD45RA Ax700    float32
 25  CD3  APC-H7     float32
 26  CD301 PE        float32
 27  None             float32
 28  CD19  PE-Cy5    float32
 29  CD5  PE-Cy55    float32
 30  CD88  PE-Cy7    float32
 31  Time             float64
dtypes: float32(31), float64(1)
memory usage: 262.9 MB
```

Data Preprocessing: Domain-Based Filtering

```
In [10]: # Option 1: Uncomment if using GR's Google Colab
# Upload Dataset from Google Drive
#pbmc_s1_df = pd.read_csv("pbmc_s1.csv")

# Option 2: Uncomment if using local JV instance
pbmc_s1_df = pd.read_csv(os.path.join(save_folder, output_file))
```

```
# Round all columns to 4 decimal places
pbmc_s1_df = pbmc_s1_df.round(4)

# Display the shape of the DataFrame
print("Number of Original instances and features:", pbmc_s1_df.shape)
```

Number of Original instances and features: (2088689, 32)

In [11]: # Identify if there are any missing values
klib.missingval_plot(pbmc_s1_df)

No missing values found in the dataset.

In [12]: # Remove unnamed columns
pbmc_s1_df = pbmc_s1_df.loc[:, ~pbmc_s1_df.columns.str.contains('^Unnamed')]

Display the shape of the DataFrame after filtering
print("Number of instances and features after filtering:", pbmc_s1_df.shape)

Number of instances and features after filtering: (2088689, 27)

In [13]: # Remove unnecessary markers or features not relevant to dendritic cells of
pbmc_s1_df = pbmc_s1_df.drop(columns=['CADM1 FITC', 'CD141 BB630', 'FcER1a E
'XCR1 BV421', 'CD16 BV570', 'CD172a BV
'CD303 BV750', 'CD1c BUV395', 'CD56 BL
'CD89 BUV661', 'CD163 BUV737', 'CD301
'CD5 PE-Cy55', 'CD88 PE-Cy7'])

Display the shape of the DataFrame after filtering
print("Number of instances and features after filtering:", pbmc_s1_df.shape)

Number of instances and features after filtering: (2088689, 13)

Add compensation step to correct for spectral overlap between channels.

In [14]: # Define folder where FCS files are stored
fcs_folder = JV # SWITCH: Use JV or GR if user

Option 1: List of compensation files
comp_files = [
 "Comp_CD3 APC-H7_F3_F03_022.fcs",
 "Comp_CD11c APC_D3_D03_020.fcs",
 "Comp_CD14 BV510_G1_G01_007.fcs",
 "Comp_CD19 PE-Cy5_A3_A03_017.fcs",
 "Comp_CD20 BUV805_E1_E01_005.fcs",
 "Comp_CD45RA Ax700_E3_E03_021.fcs",
 "Comp_CD123 BB660_F2_F02_014.fcs",
 "Comp_HLA-DR BV786_C2_C02_011.fcs",
 "Comp_Live Dead UV Blue_F4_F04_030.fcs"
]

```
# Option 2: Uncomment for JV
# List of compensation files
comp_files = [
    "Comp_CD3(APC-H7_F3_F03_022.fcs", "Comp_CD11c(APC_D3_D03_020.fcs", "Comp"
]

# Function to load and extract relevant compensation column from FCS files to
def load_single_marker_compensation(comp_file):
    comp_path = os.path.join(fcs_folder, comp_file)
    comp_data = FlowCal.io.FCSDData(comp_path)

    # Extract the channel labels (protein markers)
    channel_labels = comp_data.channel_labels()

    # Find the first valid label (protein marker label)
    valid_labels = [label for label in channel_labels if label is not None]

    if not valid_labels:
        raise ValueError(f"No valid marker labels found in {comp_file}")

    marker_label = valid_labels[0]

    # Extract the column corresponding to the valid label (compensation value)
    marker_index = channel_labels.index(marker_label)
    comp_matrix = np.array(comp_data)

    # Create a DataFrame with the marker as the column header
    comp_df = pd.DataFrame(comp_matrix[:, marker_index], columns=[marker_label])

    return comp_df

# Initialize an empty DataFrame for the combined compensation matrix
combined_comp_df = pd.DataFrame()

# Iterate through all compensation files and append each one to the combined matrix
for comp_file in comp_files:
    try:
        comp_df = load_single_marker_compensation(comp_file)
        # Append the single marker column to the combined matrix
        combined_comp_df = pd.concat([combined_comp_df, comp_df], axis=1)
    except Exception as e:
        print(f"Error processing {comp_file}: {e}")

# Check the combined compensation DataFrame
print(f"Combined Compensation Matrix shape: {combined_comp_df.shape}")
print("First few rows of the combined matrix:")
print(combined_comp_df.head())
```

```
Combined Compensation Matrix shape: (20000, 9)
```

```
First few rows of the combined matrix:
```

	CD3 APC-H7	CD11c APC	CD14 BV510	CD19 PE-Cy5	CD20 BUV805	\
0	1299.212280	2243.835938	8174.851074	12725.849609	2599.364746	
1	3656.979736	4614.802734	6523.549805	11624.978516	3874.030273	
2	1322.079590	4660.074219	7912.099609	11610.429688	2736.321777	
3	6735.444824	5745.896484	7935.748047	10109.648438	166892.812500	
4	10459.347656	4029.746338	6292.143555	22367.416016	2053.884521	

	CD45RA Ax700	CD123 BB660	HLA-DR BV786	Live Dead UV Blue	
0	13199.549805	46082.457031	23752.394531	12288.229492	
1	5960.386719	32493.529297	23311.300781	9654.122070	
2	6686.757324	31992.738281	24991.388672	10087.193359	
3	6436.605957	41724.730469	27965.492188	313.156433	
4	6872.648926	35540.093750	17428.091797	11034.020508	

```
In [15]: # Define the list of markers that are present in the PBMC dataset and need compensation
compensation_markers = [
    'CD3 APC-H7', 'CD11c APC', 'CD14 BV510', 'CD19 PE-Cy5', 'CD20 BUV805',
    'CD45RA Ax700', 'CD123 BB660', 'HLA-DR BV786', 'Live Dead UV Blue'
]

# Extract the relevant columns from the PBMC data
pbmc_df = pbmc_s1_df[compensation_markers]

# Check if the shapes are aligned
print("PBMC DF shape:", pbmc_df.shape)
print("Compensation DF shape:", combined_comp_df.shape)

# Dynamically assign chunk_size based on sizes of PBMC and Comp so that it s
chunk_size = len(pbmc_df) // len(combined_comp_df)
compensated_data_chunks = []
```

PBMC DF shape: (2088689, 9)

Compensation DF shape: (20000, 9)

```
In [16]: chunk_size = len(pbmc_df) // len(combined_comp_df)

# Initialize an empty list to store compensated chunks
compensated_chunks = []

# Iterate through each chunk and perform matrix multiplication
# This has been scaled so that the smaller Compensation rows map for as many
for i in range(len(combined_comp_df)):
    start_idx = i * chunk_size
    end_idx = (i + 1) * chunk_size if i < len(combined_comp_df) - 1 else len(combined_comp_df)
    pbmc_chunk = pbmc_df.iloc[start_idx:end_idx]
    compensation_row = combined_comp_df.iloc[i]

    # Scale chunk by corresponding row, element-wise by column
```

```

compensated_chunk = pbmc_chunk * compensation_row.values
compensated_chunks.append(compensated_chunk)

# Combine all compensated chunks back into a single DataFrame
compensated_pbmc_df = pd.concat(compensated_chunks, ignore_index=True)

# Add any non-compensated columns back into the final DataFrame
for col in pbmc_s1_df.columns:
    if col not in compensation_markers:
        compensated_pbmc_df[col] = pbmc_s1_df[col]

# Check the final compensated DataFrame
print("Final Compensated DataFrame Shape:", compensated_pbmc_df.shape)
compensated_pbmc_df.tail()

```

Final Compensated DataFrame Shape: (2088689, 13)

Out[16]:

	CD3 APC-H7	CD11c APC	CD14 BV510	CD19 PE-Cy5	CD20 BUV805
2088684	4.576103e+06	1.099074e+06	1.313747e+06	1.283182e+06	2.188893e+05
2088685	3.779322e+06	3.529784e+07	3.180732e+07	1.503056e+07	3.476131e+06
2088686	3.181932e+05	2.576266e+06	1.532375e+06	3.440700e+06	8.648450e+04
2088687	5.538915e+06	9.764558e+05	6.389965e+05	1.356035e+06	1.166848e+06
2088688	2.252977e+06	1.495973e+06	9.412647e+05	3.893194e+06	4.777359e+05

In [17]:

```

# Save the formatted DataFrame as a CSV file
output_file = "pbmc_comp.csv"
output_path = os.path.join(save_folder, output_file) # save_folder defined in
compensated_pbmc_df.to_csv(output_path, index=False)

```

Apply preprocessing gating from EDA.

In [18]:

```

# Remove cells where Time is gated to exclude extreme or inconsistent flow rates
comp_pbmc_df = compensated_pbmc_df[(compensated_pbmc_df['Time'] > 3) &
                                    (compensated_pbmc_df['Time'] < 215)
                                    ]

# Display the shape of the DataFrame after filtering
print("Number of instances after filtering:", comp_pbmc_df.shape[0])

```

Number of instances after filtering: 2035056

In [19]:

```

# Remove instances where FSC is at a certain threshold
comp_pbmc_df = compensated_pbmc_df[(compensated_pbmc_df['FSC-A'] > 20000) &
                                    (compensated_pbmc_df['FSC-H'] < 200000) &
                                    (compensated_pbmc_df['FSC-A'] < 550000)
                                    ]

```

```
[1]:  
  
# Display the shape of the DataFrame after filtering  
print("Number of instances after filtering:", comp_pbmc_df.shape[0])
```

Number of instances after filtering: 2009588

```
In [20]: # Remove instances where SSC is at a certain threshold outside of typical range  
comp_pbmc_df = compensated_pbmc_df[(compensated_pbmc_df['SSC-A'] > 110) &  
                                      (compensated_pbmc_df['SSC-A'] < 20000)  
                                      ]
```

```
# Display the shape of the DataFrame after filtering  
print("Number of instances after filtering:", comp_pbmc_df.shape[0])
```

Number of instances after filtering: 2074179

```
In [21]: # Remove instances where Live Dead UV Blue is below a certain threshold as it is not useful  
comp_pbmc_df = compensated_pbmc_df[(compensated_pbmc_df['Live Dead UV Blue'] > 0)]  
  
# Display the shape of the DataFrame after filtering  
print("Number of instances after filtering:", comp_pbmc_df.shape[0])
```

Number of instances after filtering: 812331

```
In [22]: # Save the formatted DataFrame as a CSV file  
output_file = "pbmc_clean.csv" # Renamed to separate from raw data  
output_path = os.path.join(save_folder, output_file) # save_file defined in cell 1  
comp_pbmc_df.to_csv(output_path, index=False)
```

Exploratory Data Analysis

```
In [23]: # Upload Dataset from Google Drive  
pbmc_comp = pd.read_csv(os.path.join(save_folder, "pbmc_comp.csv"))  
  
# Select a random sample at set instances for computational compatibility  
sample_size = 100000  
pbmc_c = pbmc_comp.sample(n=sample_size, random_state=42)
```

```
In [24]: # Create the scatter plot  
plt.figure(figsize=(8, 5))  
sns.scatterplot(data=pbmc_c, x='Time', y='SSC-A', alpha=0.5)  
  
# Set plot labels and title  
plt.title('Acquisition')  
plt.xlabel('Time')  
plt.ylabel('SSC-A')  
  
# Set x and y axis limits
```

```

plt.xlim(-10, 230)
plt.ylim(0, 12500)

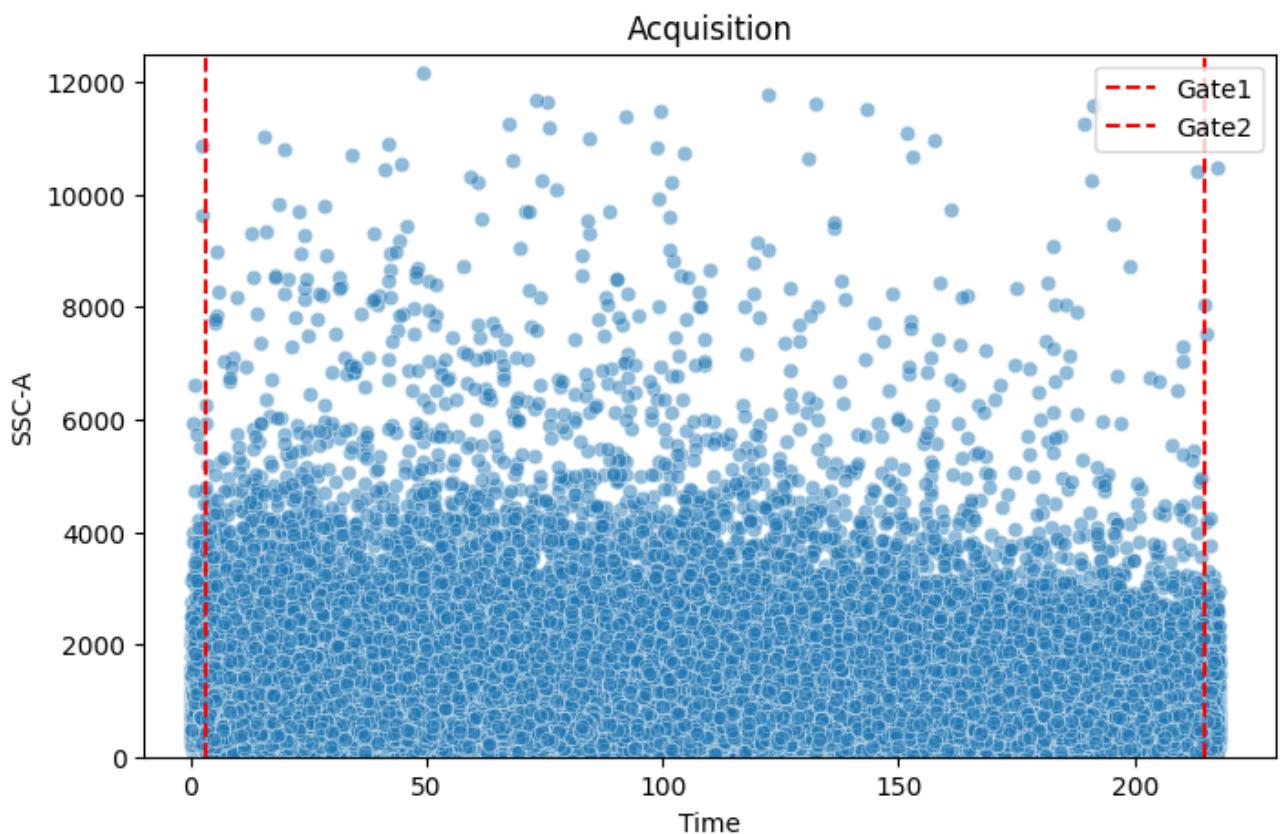
# Determine the x-value for the vertical lines
x_lim1 = 3    # Change this value based on the data
x_lim2 = 215  # Change this value based on the data

# Draw the vertical lines
plt.axvline(x=x_lim1, color='red', linestyle='--', label='Gate1')
plt.axvline(x=x_lim2, color='red', linestyle='--', label='Gate2')

# Add a legend to explain the lines
plt.legend()

# Show the plot
plt.show()

```



In [25]:

```

# Create a histogram to view FSC-A to filter out debris
plt.figure(figsize=(8, 5))
sns.histplot(pbmc_c['FSC-A'], bins=100000, kde=False)

# Set plot labels and title
plt.title('Frequency Distribution of FSC-A')
plt.xlabel('FSC-A')
plt.ylabel('Frequency')

```

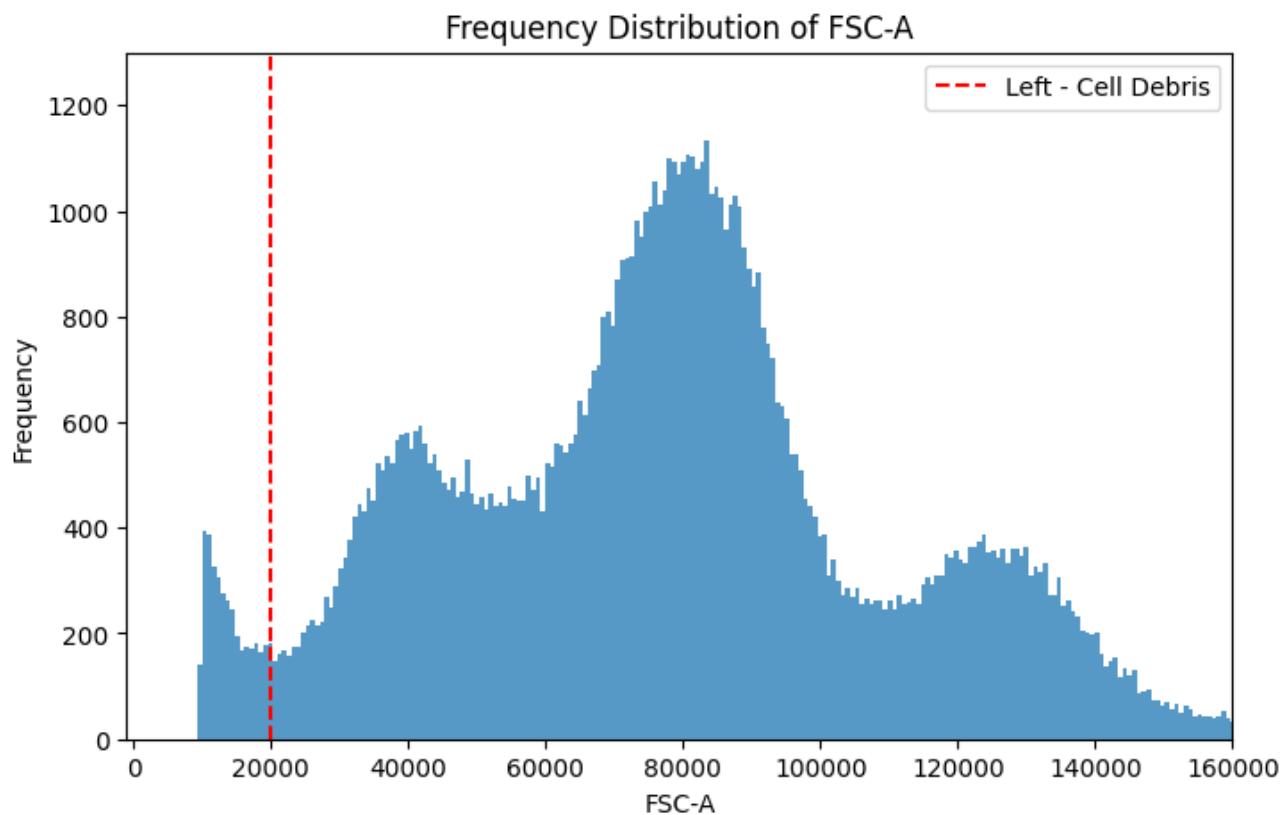
```
# Set x and y axis limits
plt.xlim(-1000, 160000)
plt.ylim(0, 1300)

# Determine the x-value for the initial debris gate
first_convex_x = 20000

# Draw the vertical line
plt.axvline(x=first_convex_x, color='red', linestyle='--', label='Left - Cell Debris')

# Add a legend to explain the line
plt.legend()

# Plot
plt.show()
```



```
In [26]: # Create the scatter plot to filter doublets or aggregated cells (high FSC-H)
plt.figure(figsize=(8, 5))
sns.scatterplot(data=pbmc_c, x='FSC-A', y='FSC-H', alpha=0.5)

# Set plot labels and title
plt.title('Single Cells')
plt.xlabel('FSC-A')
plt.ylabel('FSC-H')

# Set x and y axis limits
```

```

plt.xlim(0, 600000)
plt.ylim(0, 600000)

# Determine the y-value for the horizontal line
y_lim = 200000 # Change this value based on the data

# Determine the x-value for the vertical line
x_lim = 550000 # Change this value based on the data

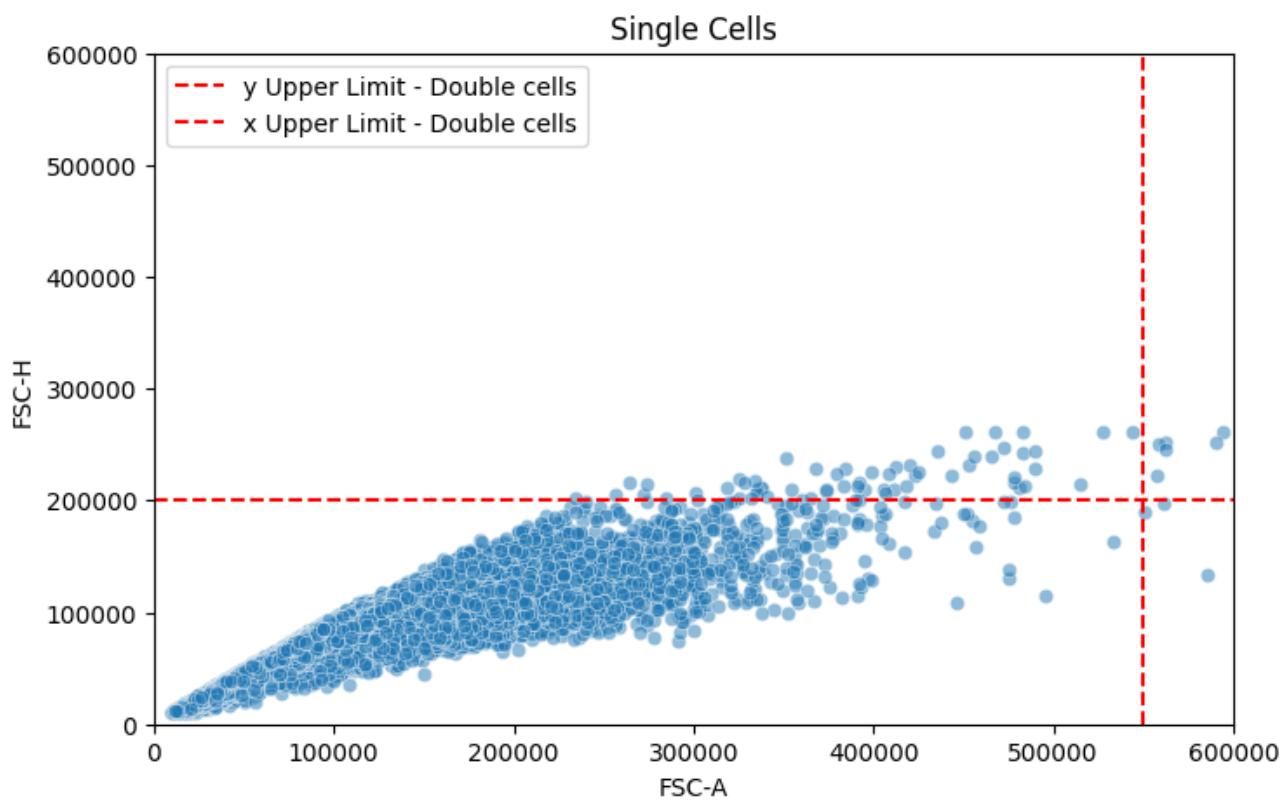
# Draw the horizontal line
plt.axhline(y=y_lim, color='red', linestyle='--', label='y Upper Limit - Double cells')

# Draw the vertical line
plt.axvline(x=x_lim, color='red', linestyle='--', label='x Upper Limit - Double cells')

# Add a legend to explain the lines
plt.legend()

# Show the plot
plt.show()

```



```

In [27]: # Create a histogram
plt.figure(figsize=(8, 5))
sns.histplot(pbmc_c['SSC-A'], bins=10000)

# Set plot labels and title
plt.title('Frequency Distribution of SSC-A')

```

```
plt.xlabel('SSC-A')
plt.ylabel('Frequency')

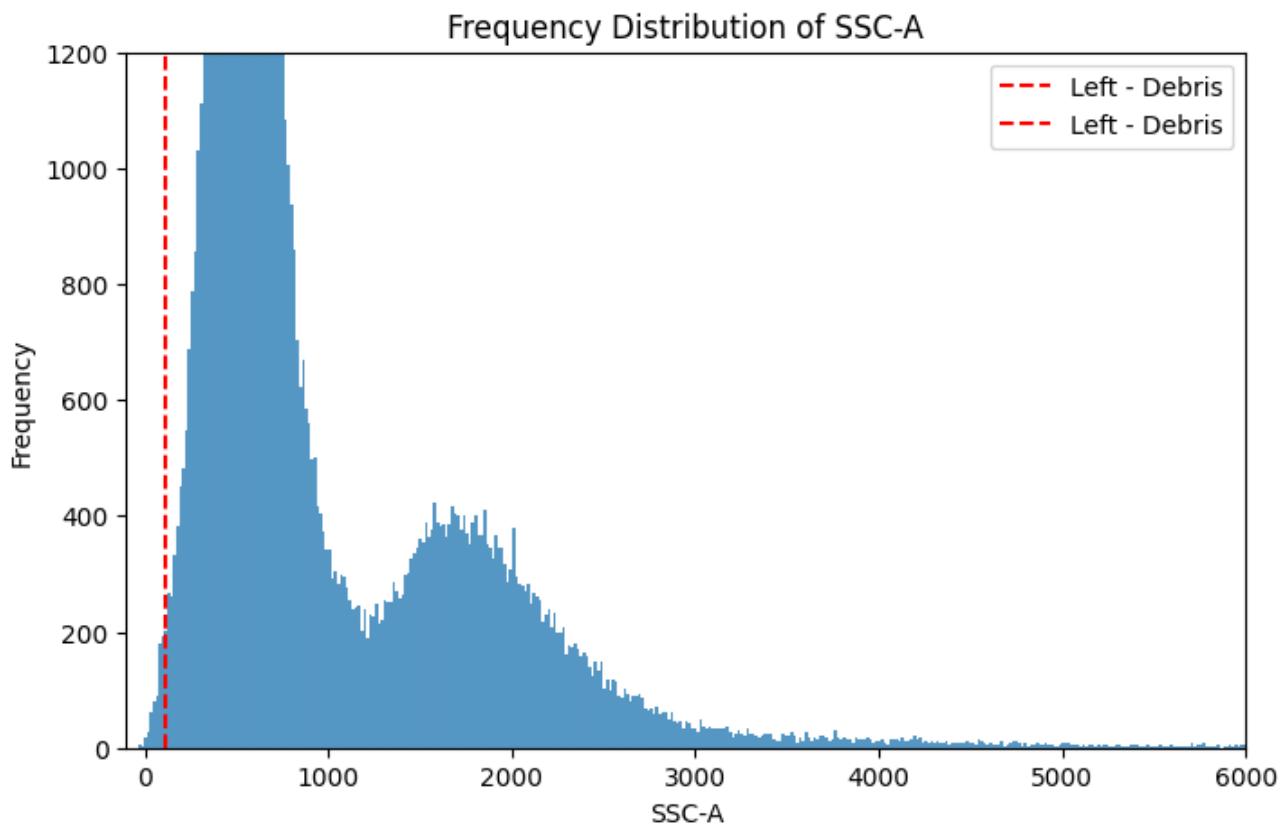
# Set x and y axis limits
plt.xlim(-100, 6000)
plt.ylim(0, 1200)

# Determine the x-value for the vertical line
x_lower = 110                         # Change this value based on the data
x_upper = 20000                         # Change this value based on the data

# Draw the vertical line
plt.axvline(x=x_lower, color='red', linestyle='--', label='Left - Debris')
plt.axvline(x=x_upper, color='red', linestyle='--', label='Left - Debris')

# Add a legend to explain the line
plt.legend()

# Plot
plt.show()
```



```
In [28]: # Create a histogram
plt.figure(figsize=(8, 5))
sns.histplot(pbmc_c['SSC-A'], bins=10000, log_scale=True)

# Set plot labels and title
```

```

plt.title('Frequency Distribution of Log(SSC-A)')
plt.xlabel('Log(SSC-A)')
plt.ylabel('Frequency')

# Set x and y axis limits
plt.xlim(100, 10000)
plt.ylim(0, 120)

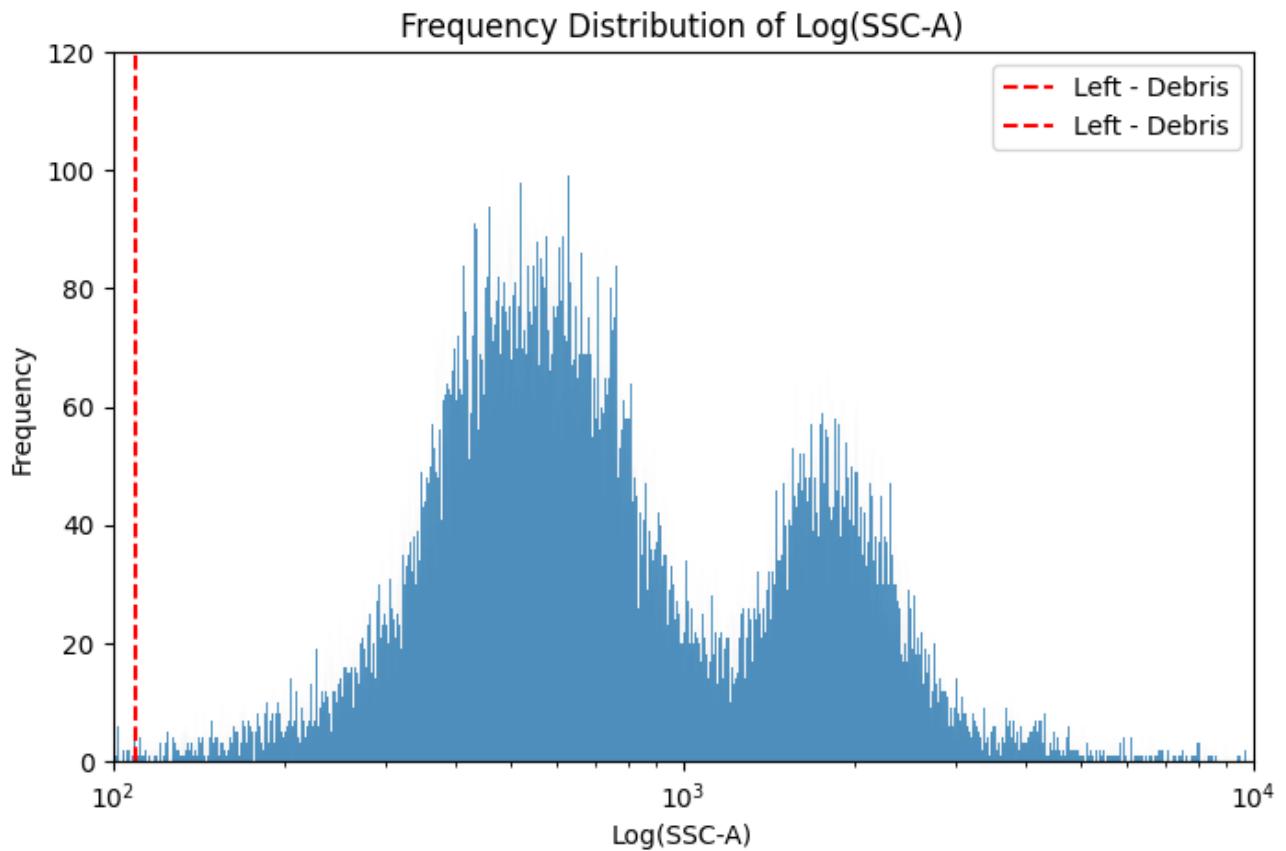
# Determine the x-value for the vertical line
x_lower = 110 # Change this value based on the data
x_upper = 20000 # Change this value based on the data

# Draw the vertical line
plt.axvline(x=x_lower, color='red', linestyle='--', label='Left - Debris')
plt.axvline(x=x_upper, color='red', linestyle='--', label='Left - Debris')

# Add a legend to explain the line
plt.legend()

# Plot
plt.show()

```



In [29]: # Create a histogram to screen non-viable cells (higher response due to open

```

plt.figure(figsize=(8, 5))
sns.histplot(pbmc_c['Live Dead UV Blue'], bins=30000)

```

```
# Set plot labels and title
plt.title('Frequency Distribution of Live Dead')
plt.xlabel('Live Dead')
plt.ylabel('Frequency')

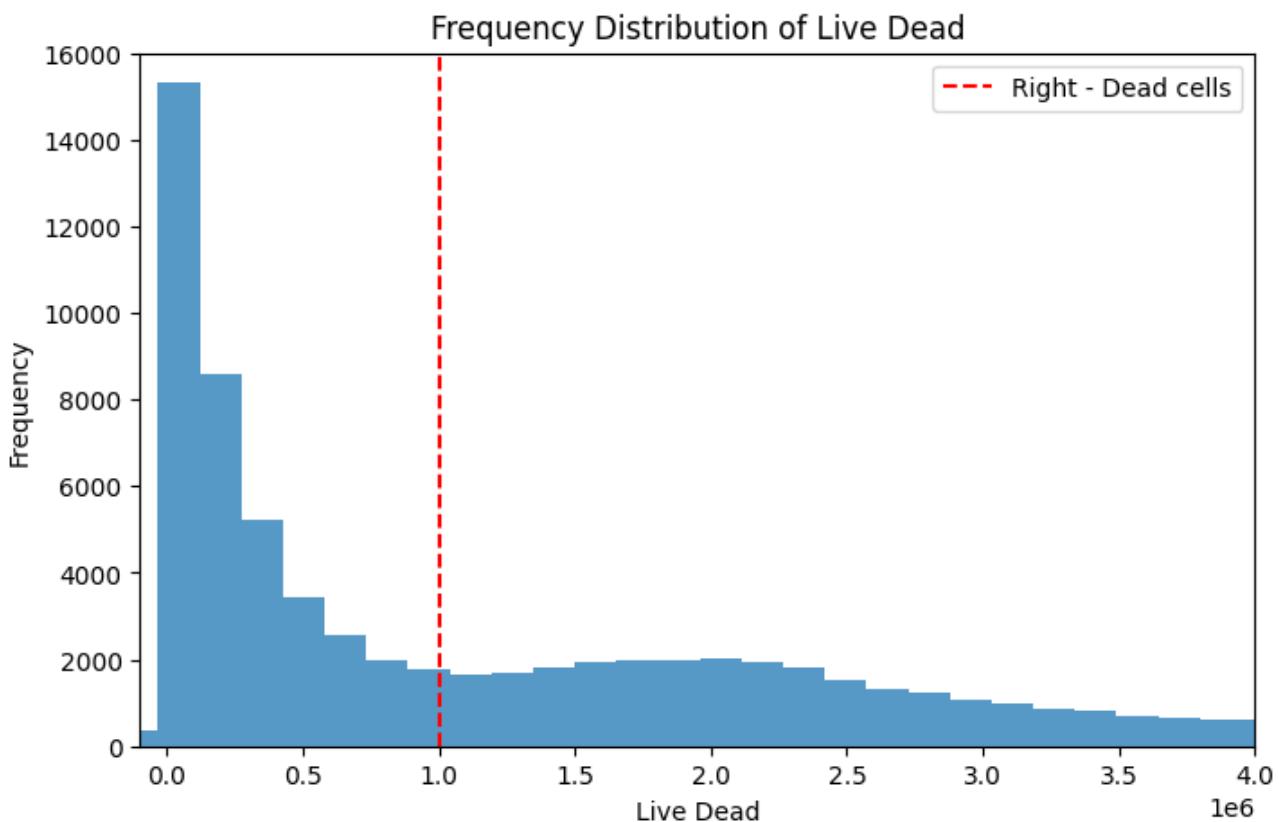
# Set x and y axis limits
plt.xlim(-100000, 4000000)
plt.ylim(0, 16000)

# Determine the x-value for the vertical line
x_lim = 1000000 # Change this value based on the data

# Draw the vertical line
plt.axvline(x=x_lim, color='red', linestyle='--', label='Right - Dead cells')

# Add a legend to explain the line
plt.legend()

# Plot
plt.show()
```



```
In [30]: # Create a histogram
plt.figure(figsize=(8, 5))
sns.histplot(pbmc_c['Live Dead UV Blue'], bins=1000, log_scale=True)
```

```

# Set plot labels and title
plt.title('Frequency Distribution of Log(Live Dead)')
plt.xlabel('Log(Live Dead)')
plt.ylabel('Frequency')

# Set x and y axis limits
plt.xlim(1000, 100000000)
plt.ylim(0, 600)

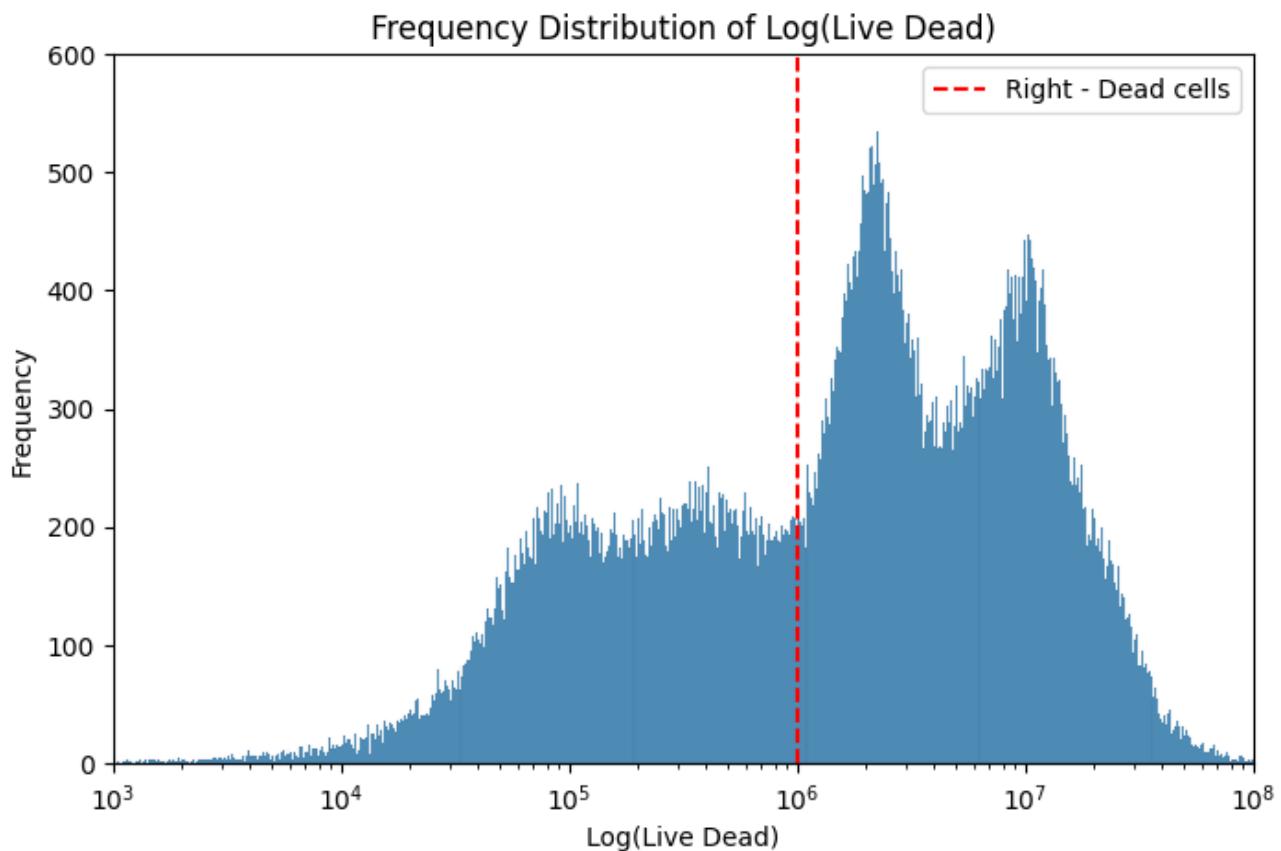
# Determine the x-value for the vertical line
first_convex_x = 1000000          # Change this value based on

# Draw the vertical line
plt.axvline(x=first_convex_x, color='red', linestyle='--', label='Right - Dead cells')

# Add a legend to explain the line
plt.legend()

# Plot
plt.show()

```

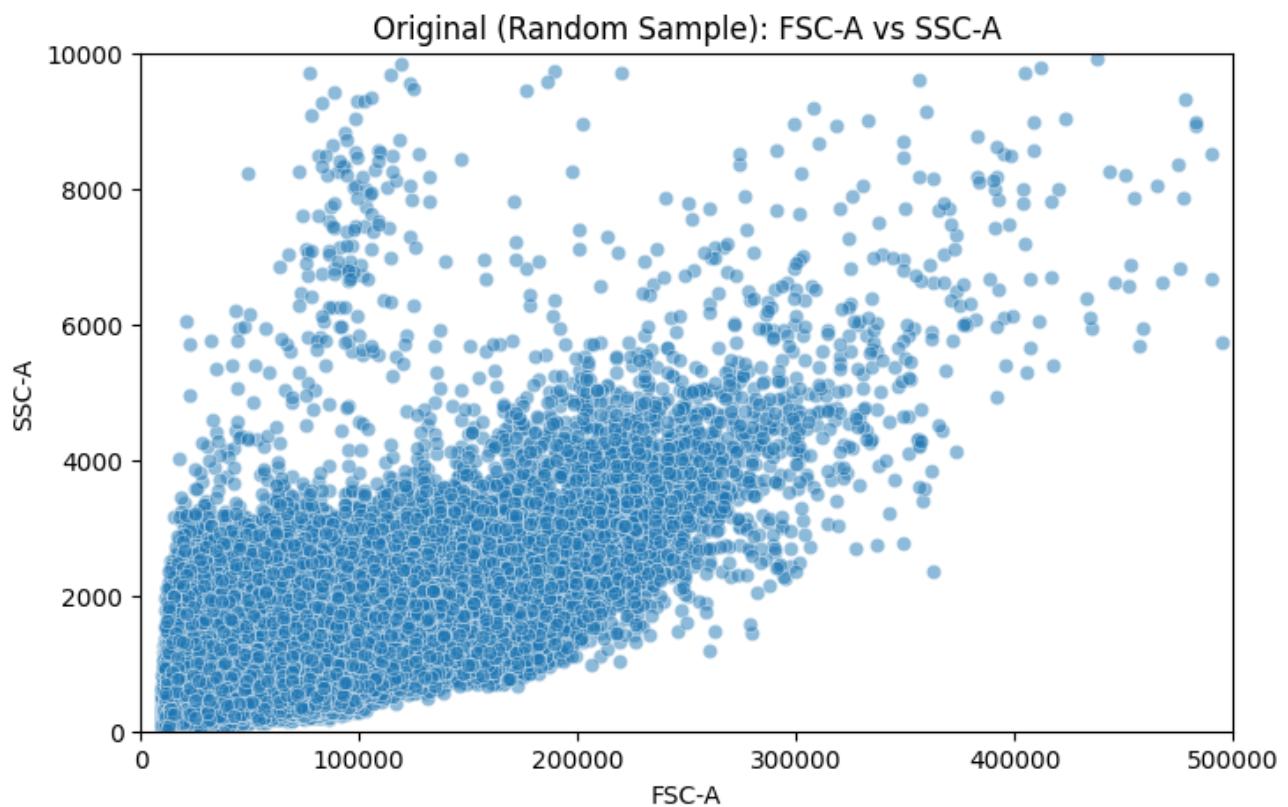


```
In [31]: # Create the scatter plot
plt.figure(figsize=(8, 5))
sns.scatterplot(data=pbmc_c, x='FSC-A', y='SSC-A', alpha=0.5)
```

```
# Set plot labels and title
plt.title('Original (Random Sample): FSC-A vs SSC-A')
plt.xlabel('FSC-A')
plt.ylabel('SSC-A')

# Set x and y axis limits
plt.xlim(0, 500000)
plt.ylim(0, 10000)

# Plot
plt.show()
```



EDA: Full Data Visualization

```
In [32]: # Upload Dataset from Google Drive
pbmc_clean = pd.read_csv("pbmc_clean.csv")

# Display the shape of the DataFrame
print("Number of instances and features:", pbmc_clean.shape)
```

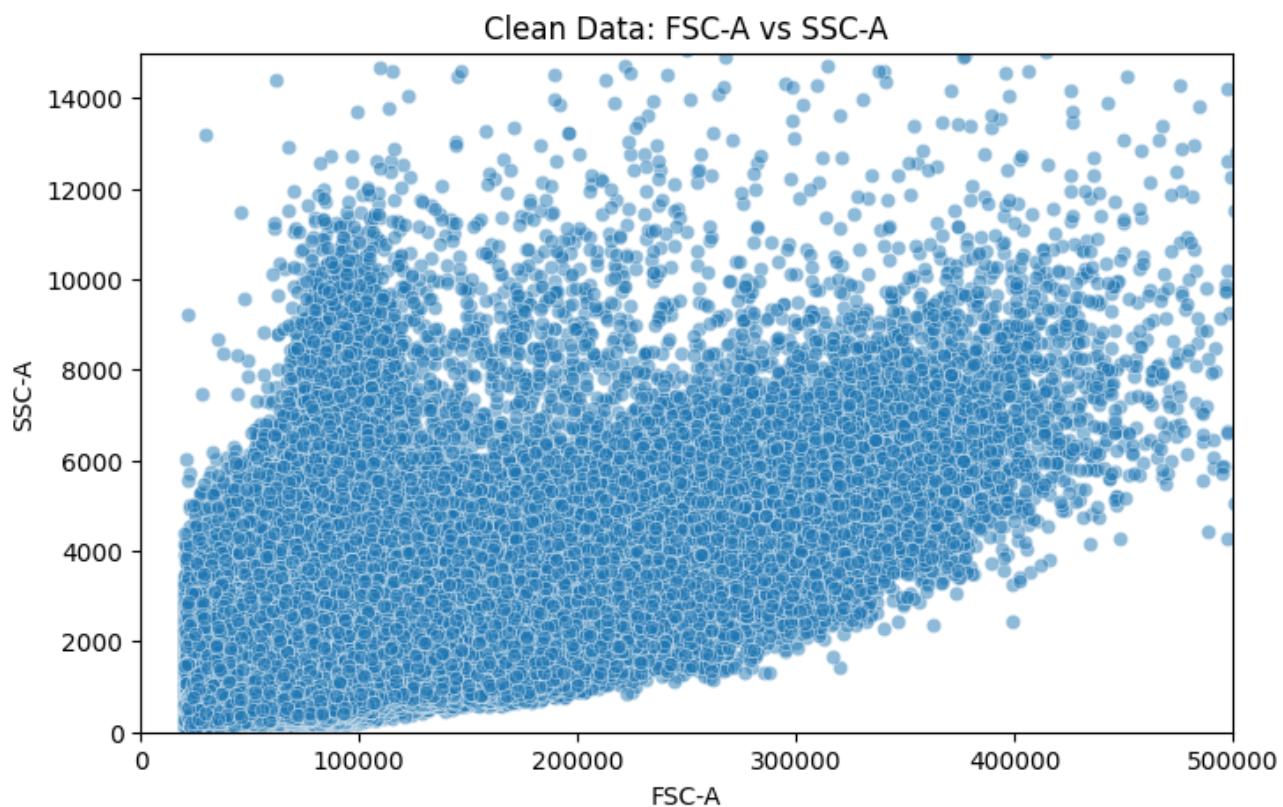
Number of instances and features: (1956288, 13)

```
In [33]: # Visualize data that has been cleaned with domain experience filters
plt.figure(figsize=(8, 5))
sns.scatterplot(data=pbmc_clean, x='FSC-A', y='SSC-A', alpha=0.5)
```

```
# Set plot labels and title
plt.title('Clean Data: FSC-A vs SSC-A')
plt.xlabel('FSC-A')
plt.ylabel('SSC-A')

# Set x and y axis limits
plt.xlim(0, 500000)
plt.ylim(0, 15000)

# Plot
plt.show()
```



Data Preprocessing: Transform Data for Efficient Computation and Memory Use

Option 1: Downsample data to 5% (or change `sample_fraction`, as needed)

Method: KMeans Cluster-based Stratified Sampling

Resulting Transformed DataFrame: `sampled_data`

Justification: Scale data proportionally while preserving maximum relative cluster density

```
In [34]: start_time = datetime.datetime.now()

x = 'CD3 APC-H7'
y = 'SSC-A'

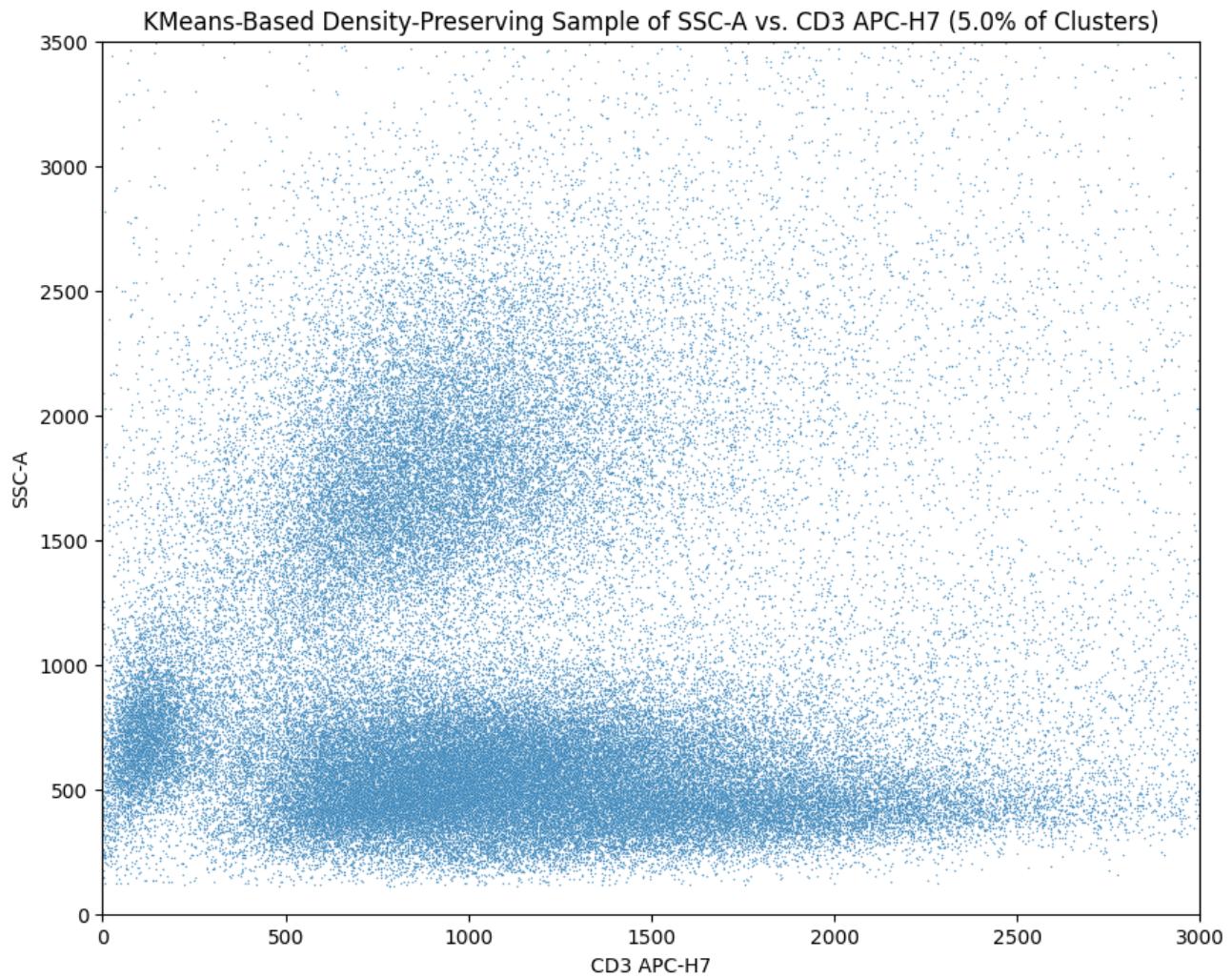
columns_to_use = ['FSC-A', 'FSC-H', 'SSC-A', 'CD123 BB660', 'CD14 BV510', 'H  
'Live Dead UV Blue', 'CD20 BUV805', 'CD11c APC', 'CD45RA A

# Apply KMeans to cluster the data
kmeans = KMeans(n_clusters=10, random_state=42) # Adjust the number of clusters
pbmc_clean['km_root_cluster'] = kmeans.fit_predict(pbmc_clean[columns_to_use])

# Perform stratified sampling based on clusters (larger clusters favored)
sample_fraction = 0.05 # Sample 10% of each cluster; change as needed
sampled_data = pbmc_clean.groupby('km_root_cluster', group_keys=False).apply
    lambda x: x.sample(frac=sample_fraction, random_state=42)).reset_index()

# Visualize the sampled data
plt.figure(figsize=(10, 8))
sns.scatterplot(data=sampled_data, x=x, y=y, s=1, alpha=0.7)
plt.title(f"KMeans-Based Density-Preserving Sample of {y} vs. {x} ({sample_fra  
plt.xlim(0, 3000)
plt.ylim(0, 3500)
plt.xlabel(x)
plt.ylabel(y)
plt.show()

end_time = datetime.datetime.now()
duration0 = end_time - start_time
print(f"Duration for this cell0: {duration0}")
```



Duration for this cell0: 0:00:01.966458

Apply Gaussian Mixture Model (GMM) for Clustering

Justification: GMM favors elliptical clusters, which may fit cellular shapes

```
In [35]: start_time = datetime.datetime.now()

# Define the columns to use for clustering
x = 'CD3 APC-H7'
y = 'HLA-DR BV786'
df = pbmc_clean.copy() # Use pbmc_clean.copy() or sampled_data.copy() as needed

# Apply GMM with 3 clusters
gmm = GaussianMixture(n_components=3, random_state=599)
gmm.fit(df[columns_to_use])

# Predict GMM cluster labels
df['gmm_cluster'] = gmm.predict(df[columns_to_use])
```

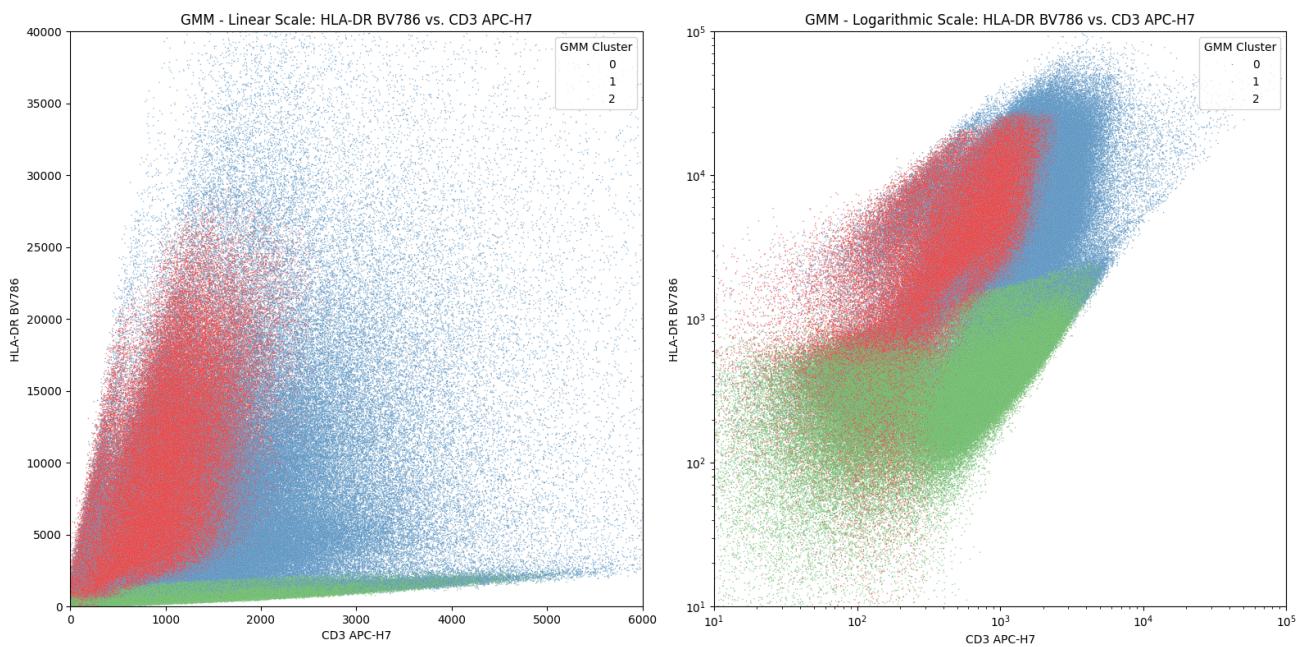
```
# Subplots for linear and logarithmic scales for easier viewing
fig, axes = plt.subplots(1, 2, figsize=(16, 8))

# Linear scale plot
sns.scatterplot(
    ax=axes[0],
    data=df,
    x=x,
    y=y,
    hue='gmm_cluster',
    palette='Set1',
    s=1,
    alpha=0.7
)
axes[0].set_xlim(0, 6000)
axes[0].set_ylim(0, 40000)
axes[0].set_title(f"GMM - Linear Scale: {y} vs. {x}")
axes[0].set_xlabel(x)
axes[0].set_ylabel(y)
axes[0].legend(title="GMM Cluster", loc='upper right')

# Logarithmic scale plot
sns.scatterplot(
    ax=axes[1],
    data=df,
    x=x,
    y=y,
    hue='gmm_cluster',
    palette='Set1',
    s=1,
    alpha=0.7
)
axes[1].set_xscale('log')
axes[1].set_yscale('log')
axes[1].set_xlim(10**1, 10**5)
axes[1].set_ylim(10**1, 10**5)
axes[1].set_title(f"GMM - Logarithmic Scale: {y} vs. {x}")
axes[1].set_xlabel(x)
axes[1].set_ylabel(y)
axes[1].legend(title="GMM Cluster", loc='upper right')

plt.tight_layout()
plt.show()

end_time = datetime.datetime.now()
duration1 = end_time - start_time
print(f"Duration for this cell1: {duration1}")
```



Duration for this cell1: 0:00:39.400164

Apply DBSCAN Algorithm

Justification: Number of clusters cannot be known apriori, which this algorithm automatically determines by density parameters (cluster radius and minimum data per cluster)

```
In [36]: start_time = datetime.datetime.now()

# Define the columns to use for clustering
x = 'CD3 APC-H7'
y = 'HLA-DR BV786'
df = sampled_data.copy() # Use pbmc_clean.copy() or sampled_data.copy() as raw data

columns_to_use = [x, y]

# Apply DBSCAN with specified parameters from manual search
dbscan = DBSCAN(eps=30, min_samples=110)
dbscan_labels = dbscan.fit_predict(df[columns_to_use])

# Add the cluster labels to the dataframe
df['DB_Cluster'] = dbscan_labels
db_df = df.copy()
# Subplots for linear and logarithmic scales for easier viewing
fig, axes = plt.subplots(1, 2, figsize=(16, 8))

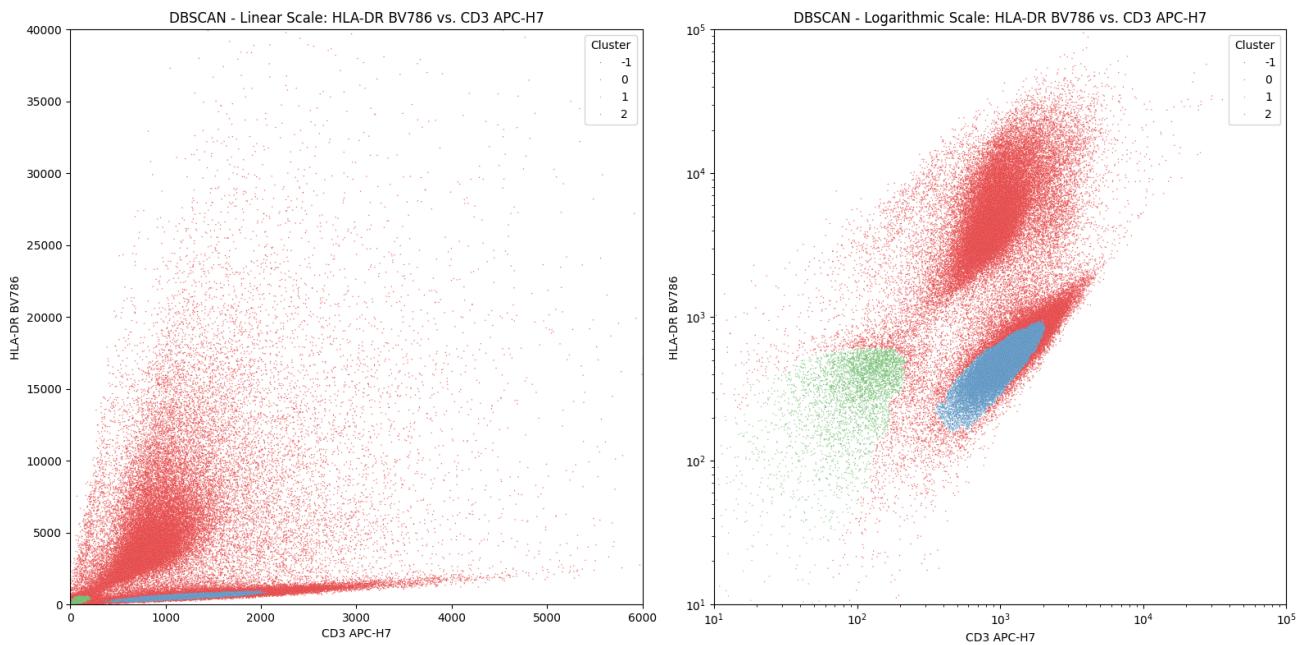
# Linear plot
sns.scatterplot(
    ax=axes[0],
```

```
    data=df,
    x=x,
    y=y,
    hue='DB_Cluster',
    palette='Set1',
    s=1,
    alpha=0.7
)
axes[0].set_xlim(0, 6000)
axes[0].set_ylim(0, 40000)
axes[0].set_title(f"DBSCAN - Linear Scale: {y} vs. {x}")
axes[0].set_xlabel(x)
axes[0].set_ylabel(y)
axes[0].legend(title="Cluster", loc='upper right')

# Logarithmic plot
sns.scatterplot(
    ax=axes[1],
    data=df,
    x=x,
    y=y,
    hue='DB_Cluster',
    palette='Set1',
    s=1,
    alpha=0.7
)
axes[1].set_xscale('log')
axes[1].set_yscale('log')
axes[1].set_xlim(10**1, 10**5)
axes[1].set_ylim(10**1, 10**5)
axes[1].set_title(f"DBSCAN - Logarithmic Scale: {y} vs. {x}")
axes[1].set_xlabel(x)
axes[1].set_ylabel(y)
axes[1].legend(title="Cluster", loc='upper right')

plt.tight_layout()
plt.show()

end_time = datetime.datetime.now()
duration2 = end_time - start_time
print(f"Duration for this cell2: {duration2}")
```



Duration for this cell2: 0:00:01.775456

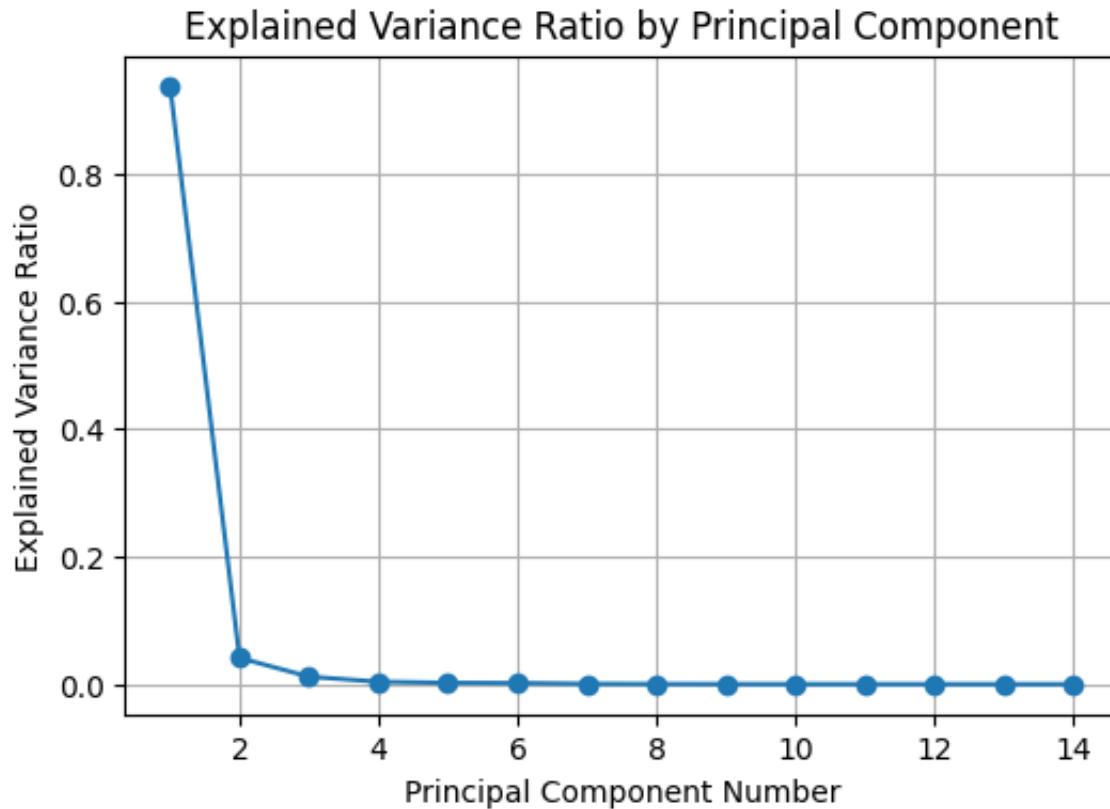
Option 2: Principal Component Analysis

Resulting Transformed DataFrame: **pbmc_clean_pca_df**

Justification: Dimensionality reduction to transform 14 columns into fewer components

```
In [37]: # Fit PCA on the sampling data
pca = PCA().fit(pbmc_clean)

# Plot the explained variance ratio
plt.figure(figsize=(6, 4))
plt.plot(range(1, len(pca.explained_variance_ratio_) + 1),
         pca.explained_variance_ratio_, marker='o')
plt.title('Explained Variance Ratio by Principal Component')
plt.xlabel('Principal Component Number')
plt.ylabel('Explained Variance Ratio')
plt.grid()
plt.show()
```



```
In [38]: start_time = datetime.datetime.now()

# Fit PCA on the sampling data
pca = PCA().fit(pbm_clean)

# Calculate cumulative explained variance
cumulative_variance = np.cumsum(pca.explained_variance_ratio_)

# Find the elbow point
# Calculate the difference in the cumulative variance to find the point of n
diff = np.diff(cumulative_variance)
elbow_point = np.argmax(diff) + 1

# Plot the cumulative explained variance
plt.figure(figsize=(6, 4))
plt.plot(range(1, len(cumulative_variance) + 1),
         cumulative_variance,
         marker='o',
         label='Cumulative Explained Variance')

# Add the elbow point to the plot
plt.scatter(elbow_point, cumulative_variance[elbow_point - 1], color='b', zorder=5)

# Add 95% threshold line
plt.axhline(y=0.95, color='r', linestyle='--', label='95% Threshold')
```

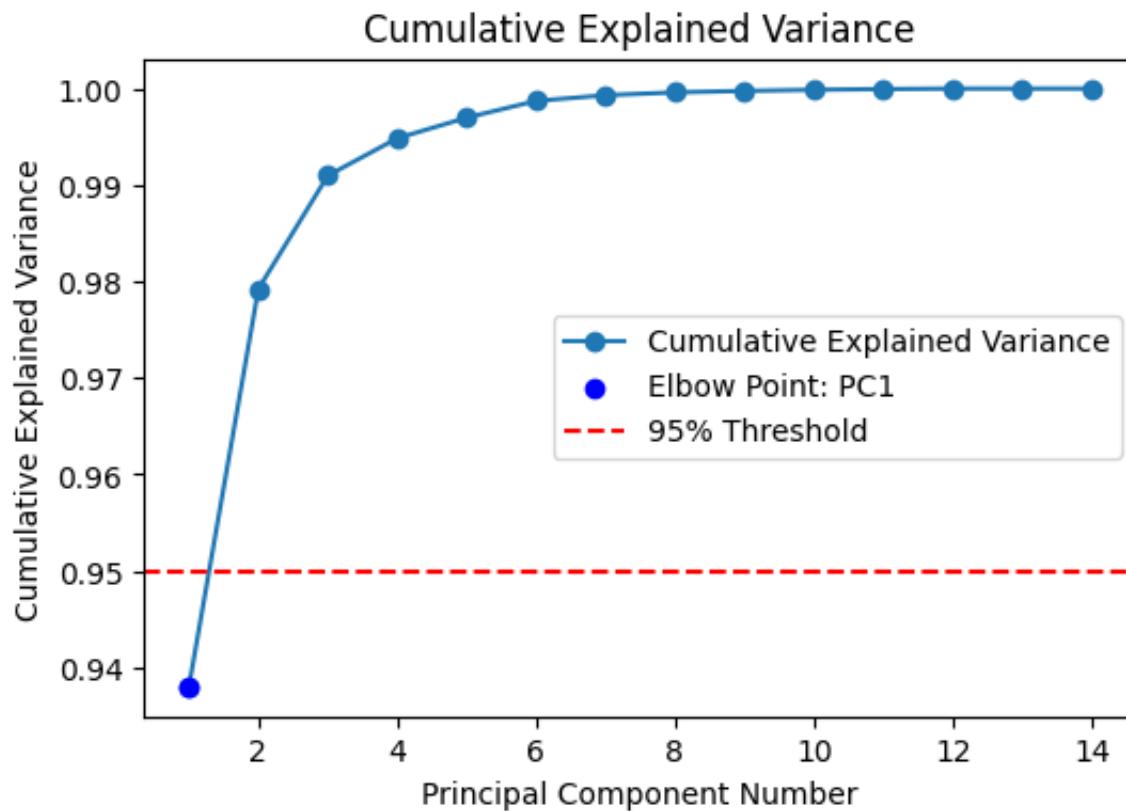
```

# Titles and labels
plt.title('Cumulative Explained Variance')
plt.xlabel('Principal Component Number')
plt.ylabel('Cumulative Explained Variance')

# Show the legend and grid
plt.legend()
plt.show()

end_time = datetime.datetime.now()
duration3 = end_time - start_time
print(f"Duration for this cell3: {duration3}")

```



Duration for this cell3: 0:00:00.131711

```

In [39]: start_time = datetime.datetime.now()

# # Define the columns to use for PCA
columns_to_use = ['FSC-A', 'FSC-H', 'SSC-A', 'CD123 BB660', 'CD14 BV510', 'H  
    'Live Dead UV Blue', 'CD20 BUV805', 'CD11c APC', 'CD45RA A  
    'CD3 APC-H7', 'CD19 PE-Cy5']

# PCA Transformation with 3 Components
pca = PCA(n_components=3, random_state=599) # Use 3 components to capture 9
pca_result = pca.fit_transform(pbmc_clean[columns_to_use])

```

```
# Create a new DataFrame with the PCA results
pbmc_clean_pca_df = pbmc_clean.copy()
pbmc_clean_pca_df['PCA1'] = pca_result[:, 0]
pbmc_clean_pca_df['PCA2'] = pca_result[:, 1]
pbmc_clean_pca_df['PCA3'] = pca_result[:, 2]

end_time = datetime.datetime.now()
duration4 = end_time - start_time
print(f"Duration for this cell4: {duration4}")
```

Duration for this cell4: 0:00:00.139518

Option 3: PCA+T-Distributed Stochastic Neighbor Embedding (Probabilistic)

Resulting Transformed DataFrame: `sample_tsne_df`

Justification: openTSNE is optimized for larger datasets than scikit-learn version

Wall Time: 2 min

```
In [40]: start_time = datetime.datetime.now()

open_tsne = TSNE(n_components=2, perplexity=30, n_jobs=-1, random_state=42)
sample_size = 100000
sample_tsne = open_tsne.fit(pca_result[:sample_size])
sample_tsne_df = pd.DataFrame(sample_tsne, columns=['TSNE1', 'TSNE2'])

end_time = datetime.datetime.now()
duration5 = end_time - start_time
print(f"Duration for this cell5: {duration5}")
```

Duration for this cell5: 0:01:57.404254

Modeling

Model 1 - Gaussian Mixture Model

Assumptions:

- Clusters are elliptical due to the multi-dimensional Gaussian distribution shape (complicated with higher dimensions)
- There is no noise / All cells belong to a cluster \

Wall Time:

From 5% Sampled Data

```
In [41]: start_time = datetime.datetime.now()

# Define the range of clusters to test
n_components_range = range(2, 6) # Testing from 2 to 5 components
silhouette_scores = []

data = sampled_data[columns_to_use].values

# Find the optimal number of components using Silhouette Score
for n_components in n_components_range:
    gmm = GaussianMixture(n_components=n_components, random_state=599,
                          reg_covar=1e-5)
    labels = gmm.fit_predict(data)

    # Calculate Silhouette Score
    score = silhouette_score(data, labels)
    silhouette_scores.append(score)
    print(f"n_components: {n_components}, Silhouette Score: {score:.4f}")

# Determine the optimal number of components
optimal_n_components = n_components_range[np.argmax(silhouette_scores)]
optimal_score = max(silhouette_scores)

print(f"\nOptimal number of components: {optimal_n_components}")
print(f"Optimal Silhouette Score: {optimal_score:.4f}")

end_time = datetime.datetime.now()
duration_down = end_time - start_time
print(f"Duration for this cell: {duration_down}")

n_components: 2, Silhouette Score: 0.1863
n_components: 3, Silhouette Score: 0.1159
n_components: 4, Silhouette Score: -0.0058
n_components: 5, Silhouette Score: -0.0153

Optimal number of components: 2
Optimal Silhouette Score: 0.1863
Duration for this cell: 0:05:12.810638
```

```
In [42]: start_time = datetime.datetime.now()

# Fit GMM with the optimal number of components
optimal_gmm = GaussianMixture(n_components=optimal_n_components, random_state=599)
optimal_labels = optimal_gmm.fit_predict(data)

# Add the labels back
```

```
sampled_data['Cluster'] = optimal_labels

# Calculate the percentage of points in each cluster
cluster_percentages = sampled_data['Cluster'].value_counts(normalize=True) *

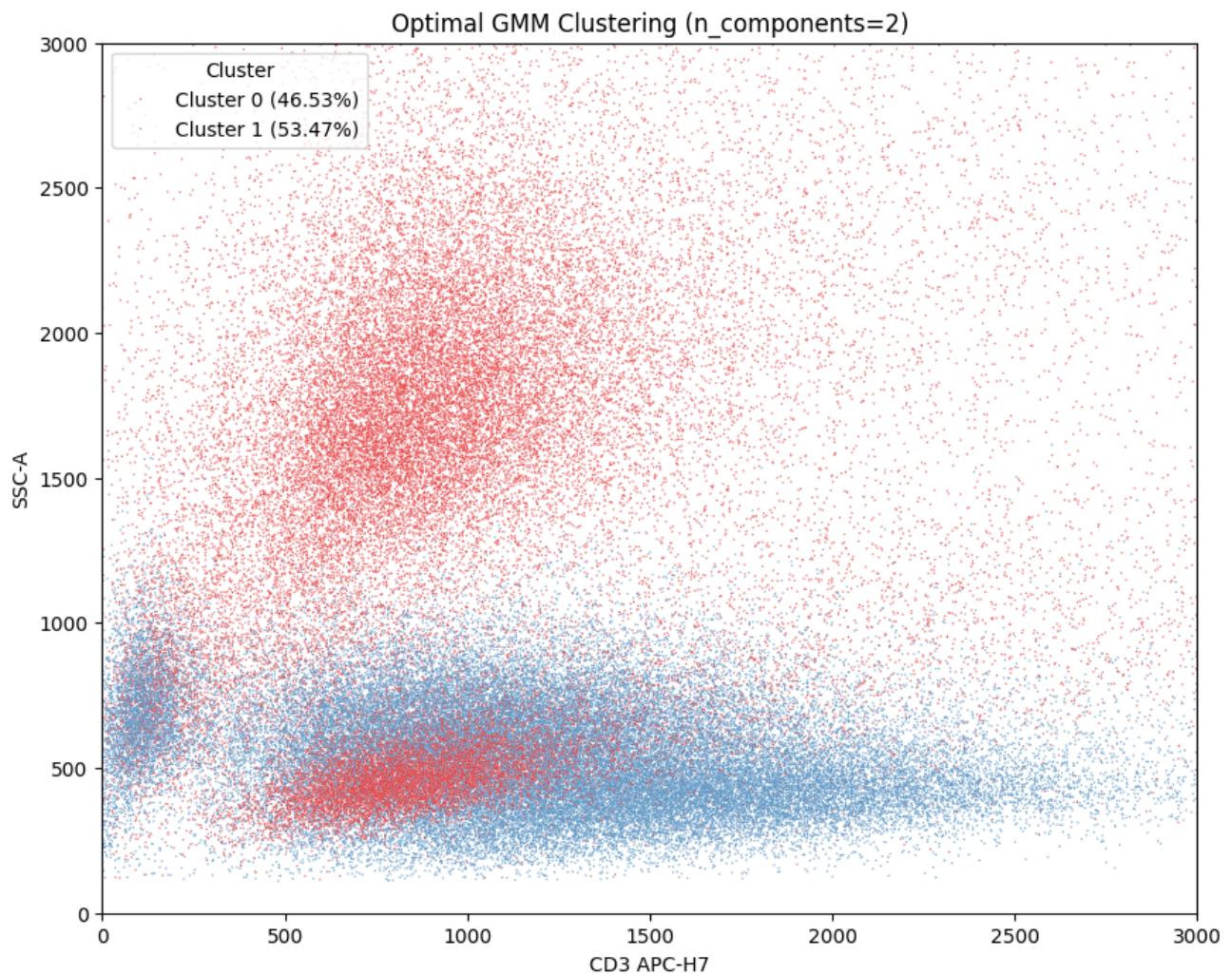
# Prepare cluster labels
legend_labels = {
    cluster: f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}

# Plot the clusters
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(data=sampled_data,
                           x='CD3 APC-H7',
                           y='SSC-A',
                           hue='Cluster',
                           palette='Set1',
                           s=1,
                           alpha=0.7)

# Update the legend labels to include percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [legend_labels[int(label)] for label in labels if label.isdigit()]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')

plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title(f"Optimal GMM Clustering (n_components={optimal_n_components})")
plt.show()

end_time = datetime.datetime.now()
duration6 = end_time - start_time
print(f"Duration for this cell6: {duration6}")
```



Duration for this cell6: 0:00:01.466652

```
In [43]: model = optimal_gmm
df = sampled_data.copy()
columns_to_use = ['FSC-A', 'FSC-H', 'SSC-A', 'CD123 BB660', 'CD14 BV510', 'H
    'Live Dead UV Blue', 'CD20 BUV805', 'CD11c APC', 'CD45RA A
    'CD3 APC-H7', 'CD19 PE-Cy5']
data = df[columns_to_use].values
labels = model.fit_predict(data)
sampled_gmm_score = silhouette_score(data, labels)
sampled_gmm_score
```

Out[43]: 0.18630547202212291

From PCA Only

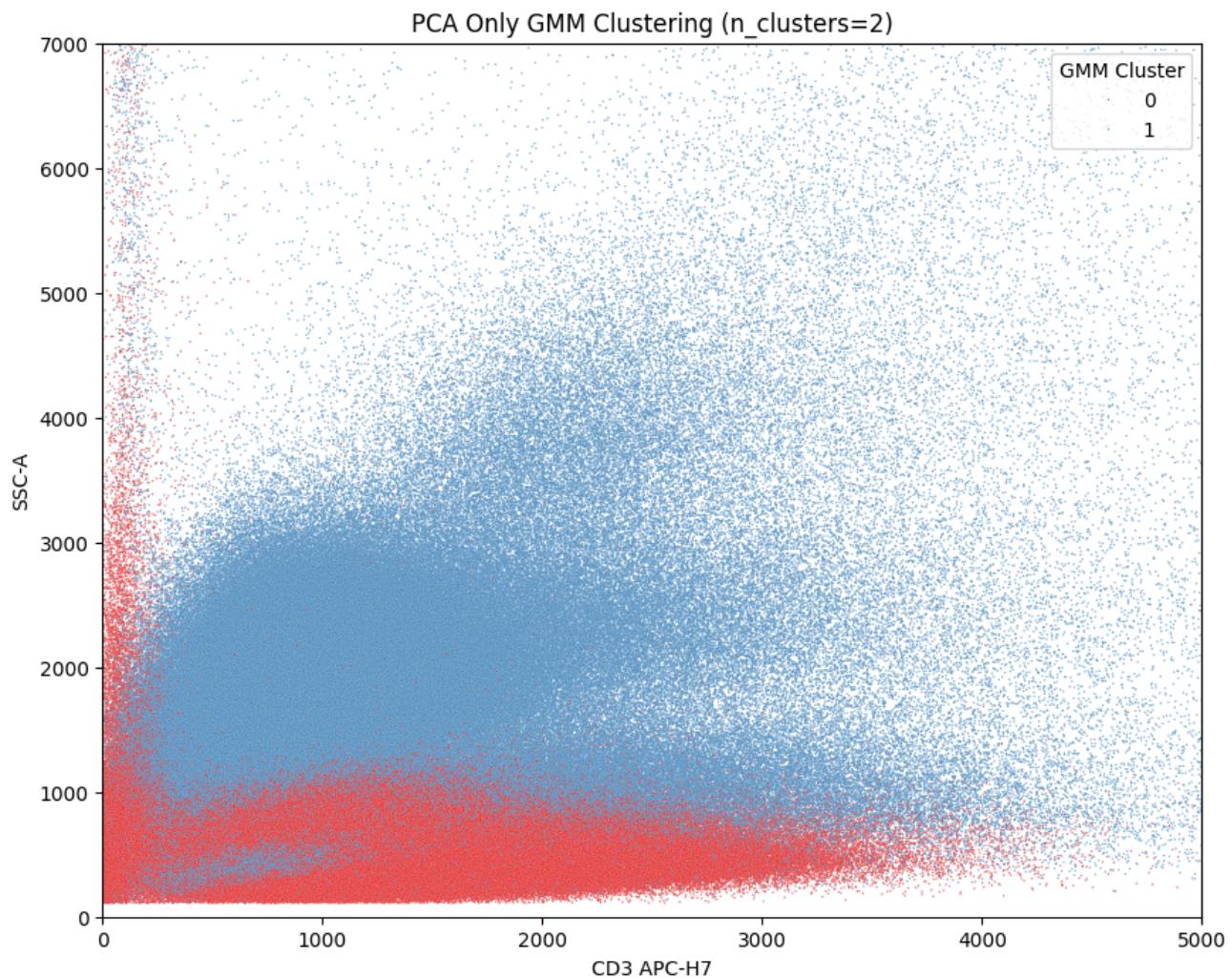
```
In [44]: start_time = datetime.datetime.now()

# GMM Clustering on the 3D PCA data
```

```
pca_gmm = GaussianMixture(n_components=optimal_n_components, random_state=59)
pbmc_clean_pca_df['gmm_cluster'] = pca_gmm.fit_predict(pca_result)

# Plotting the Original Features with GMM Clusters
plt.figure(figsize=(10, 8))
sns.scatterplot(
    data=pbmc_clean_pca_df,
    x='CD3 APC-H7',
    y='SSC-A',
    hue='gmm_cluster',
    palette='Set1',
    s=1,
    alpha=0.7
)
plt.title(f"PCA Only GMM Clustering (n_clusters={optimal_n_components})")
plt.xlim(0, 5000)
plt.ylim(0, 7000)
plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.legend(title="GMM Cluster", loc='upper right')
plt.show()

end_time = datetime.datetime.now()
duration8 = end_time - start_time
print(f"Duration for this cell8: {duration8}")
```



Duration for this cell8: 0:00:21.190579

In [45]: # Combine features and labels

```

model = pca_gmm
df = pbmc_clean_pca_df.copy()
data = pca_result
labels = model.fit_predict(data)

pca_with_labels = pd.DataFrame(data, columns=[f"PC{i+1}" for i in range(data.shape[1])])
pca_with_labels['cluster'] = labels

# Sample with replacement while maintaining stratification
''' Stratification is being performed by GMM cluster labels in order to best
the model's cluster distribution when performing Silhouette Score calculation
downsampled version of this PCA plot. Without downsampling, calculating the
Score would be excessively computationally expensive. This is different from
sampling performed on preprocessing since this is downsampled after GMM perf
and then stratified by that labeling.
'''
sample_size = 100000 # Desired total sample size

```

```

stratified_sample = pca_with_labels.groupby('cluster', group_keys=False).apply(
    lambda group: group.sample(
        n=int(sample_size * len(group) / len(pca_with_labels)), # Proportion
        replace=True,
        random_state=42
    )
)

# Extract sampled features and labels
sampled_features = stratified_sample.drop(columns=['cluster']).values
sampled_labels = stratified_sample['cluster'].values

# Calculate silhouette score
pca_gmm_score = silhouette_score(sampled_features, sampled_labels)
print(f"Silhouette Score on Stratified Sampled Data (With Replacement): {pca_gmm_score}")

```

Silhouette Score on Stratified Sampled Data (With Replacement): 0.2122198992
827778

From PCA+T-SNE

```

In [46]: start_time = datetime.datetime.now()

# Modeling PCA and T-SNE
tsne_gmm = GaussianMixture(n_components=optimal_n_components, random_state=42)
gmm_labels = tsne_gmm.fit_predict(sample_tsne_df[['TSNE1', 'TSNE2']])

sample_tsne_df['Cluster'] = gmm_labels

# Reset index of sample set to for merging
sample_reset = pbmc_clean.reset_index(drop=True)

# Merge the original sampling set data with the t-SNE cluster labels
sample_with_clusters = pd.concat([sample_reset, sample_tsne_df[['Cluster']]])


end_time = datetime.datetime.now()
duration9 = end_time - start_time
print(f"Duration for this cell9: {duration9}")

```

Duration for this cell9: 0:00:00.324118

```

In [47]: start_time = datetime.datetime.now()

# Reset index of sample set to for merging
sample_reset = pbmc_clean.reset_index(drop=True)

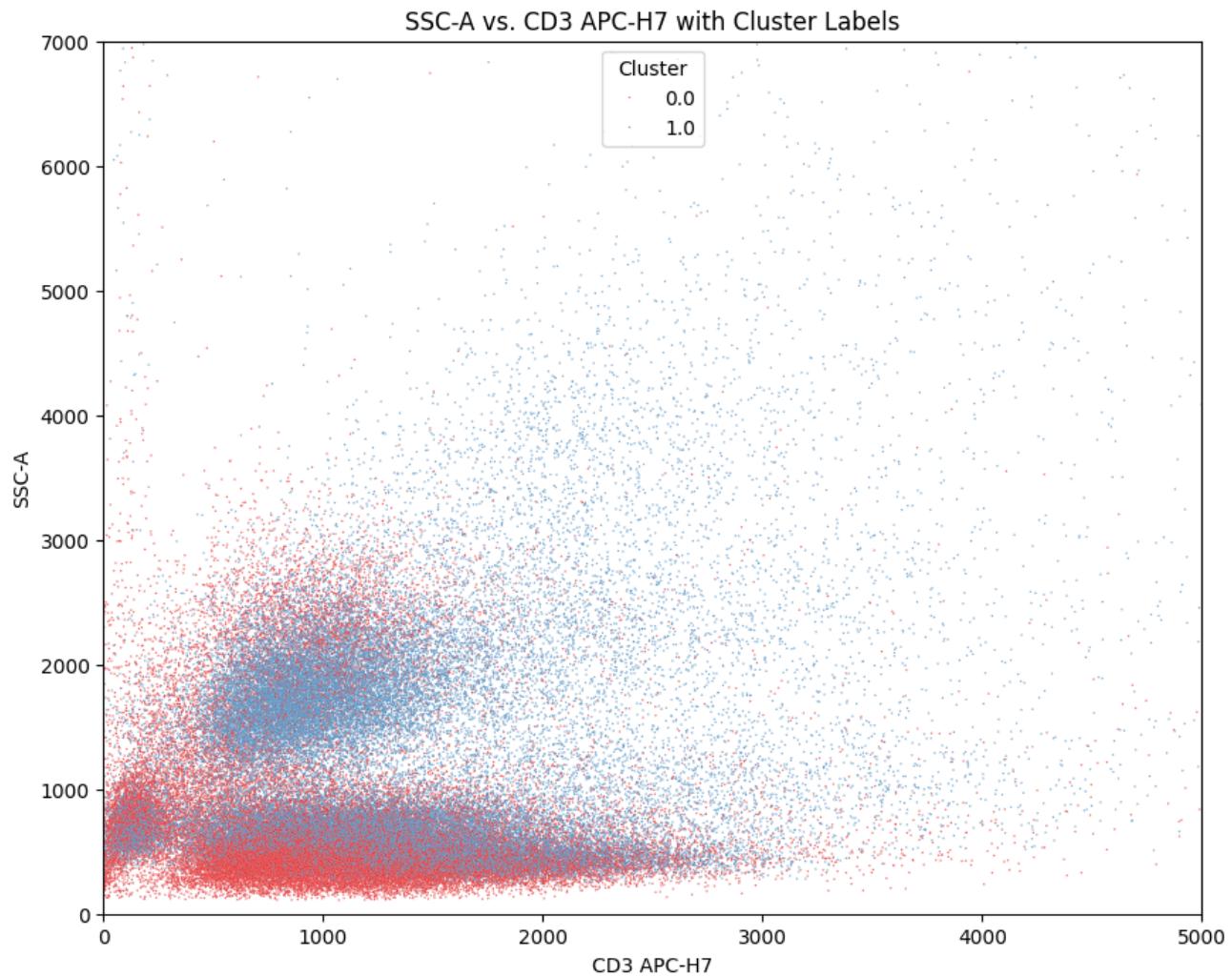
# Merge the original sampling set data with the t-SNE cluster labels
sample_with_clusters = pd.concat([sample_reset, sample_tsne_df[['Cluster']]])

```

```
# Plot "SSC-A" vs. "CD3 APC-H7" colored by cluster
plt.figure(figsize=(10, 8))
sns.scatterplot(data=sample_with_clusters,
                 x='CD3 APC-H7',
                 y='SSC-A',
                 hue='Cluster',
                 palette='Set1',
                 s=1,
                 alpha=0.7
)

plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 5000)
plt.ylim(0, 7000)
plt.title("SSC-A vs. CD3 APC-H7 with Cluster Labels")
plt.legend(title="Cluster", loc='best')
plt.show()

end_time = datetime.datetime.now()
duration10 = end_time - start_time
print(f"Duration for this cell10: {duration10}")
```



Duration for this cell10: 0:00:01.968034

```
In [48]: # Calculate Silhouette Score
model = tsne_gmm
df = sample_with_clusters.copy()
data = sample_tsne_df[['TSNE1', 'TSNE2']]
labels = model.fit_predict(data)
tsne_gmm_score = silhouette_score(data, labels)
tsne_gmm_score
```

Out[48]: 0.34371093073754183

Model 2 - K-Means Clustering

Assumptions:

- Clusters are spherically shaped since distance is measured from the cluster centroid \
- Not good against excessive noise since noise affects the cluster centroid location \

Wall Time: 9 min 41 sec

From Sampled Data

Perform Cross-Validation by Silhouette Score to find optimal k value

```
In [49]: start_time = datetime.datetime.now()

# Define the range of clusters to test
n_clusters_range = range(2, 6) # Testing from 2 to 5 clusters
silhouette_scores = []

data = sampled_data[['CD3 APC-H7', 'SSC-A']].values

# Find the optimal number of clusters by their Silhouette Score
for n_clusters in n_clusters_range:
    kmeans = KMeans(n_clusters=n_clusters, random_state=599)
    labels = kmeans.fit_predict(data)

    # Calculate Silhouette Score
    score = silhouette_score(data, labels)
    silhouette_scores.append(score)
    print(f"n_clusters: {n_clusters}, Silhouette Score: {score:.4f}")

# Store value of optimal number of clusters
optimal_n_clusters = n_clusters_range[np.argmax(silhouette_scores)]
optimal_score = max(silhouette_scores)

# Print to standard out for tracking purposes
print(f"\nOptimal number of clusters: {optimal_n_clusters}")
print(f"Optimal Silhouette Score: {optimal_score:.4f}")


end_time = datetime.datetime.now()
duration12 = end_time - start_time
print(f"Duration for this cell12: {duration12}")
```

n_clusters: 2, Silhouette Score: 0.4912
n_clusters: 3, Silhouette Score: 0.4335
n_clusters: 4, Silhouette Score: 0.4537
n_clusters: 5, Silhouette Score: 0.4153

Optimal number of clusters: 2
Optimal Silhouette Score: 0.4912
Duration for this cell12: 0:05:00.967114

```
In [50]: start_time = datetime.datetime.now()

# Fit k-means with the optimal number of clusters
```

```

optimal_kmeans = KMeans(n_clusters=optimal_n_clusters, random_state=599)
optimal_labels = optimal_kmeans.fit_predict(data)

# Add the labels back to the dataframe for plotting
sampled_data['Cluster'] = optimal_labels

# Calculate the percentage of points in each cluster
cluster_percentages = sampled_data['Cluster'].value_counts(normalize=True) *

# Store cluster labels
legend_labels = {
    cluster: f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}

end_time = datetime.datetime.now()
duration13 = end_time - start_time
print(f"Duration for this cell13: {duration13}")

```

Duration for this cell13: 0:00:00.189299

```

In [51]: start_time = datetime.datetime.now()

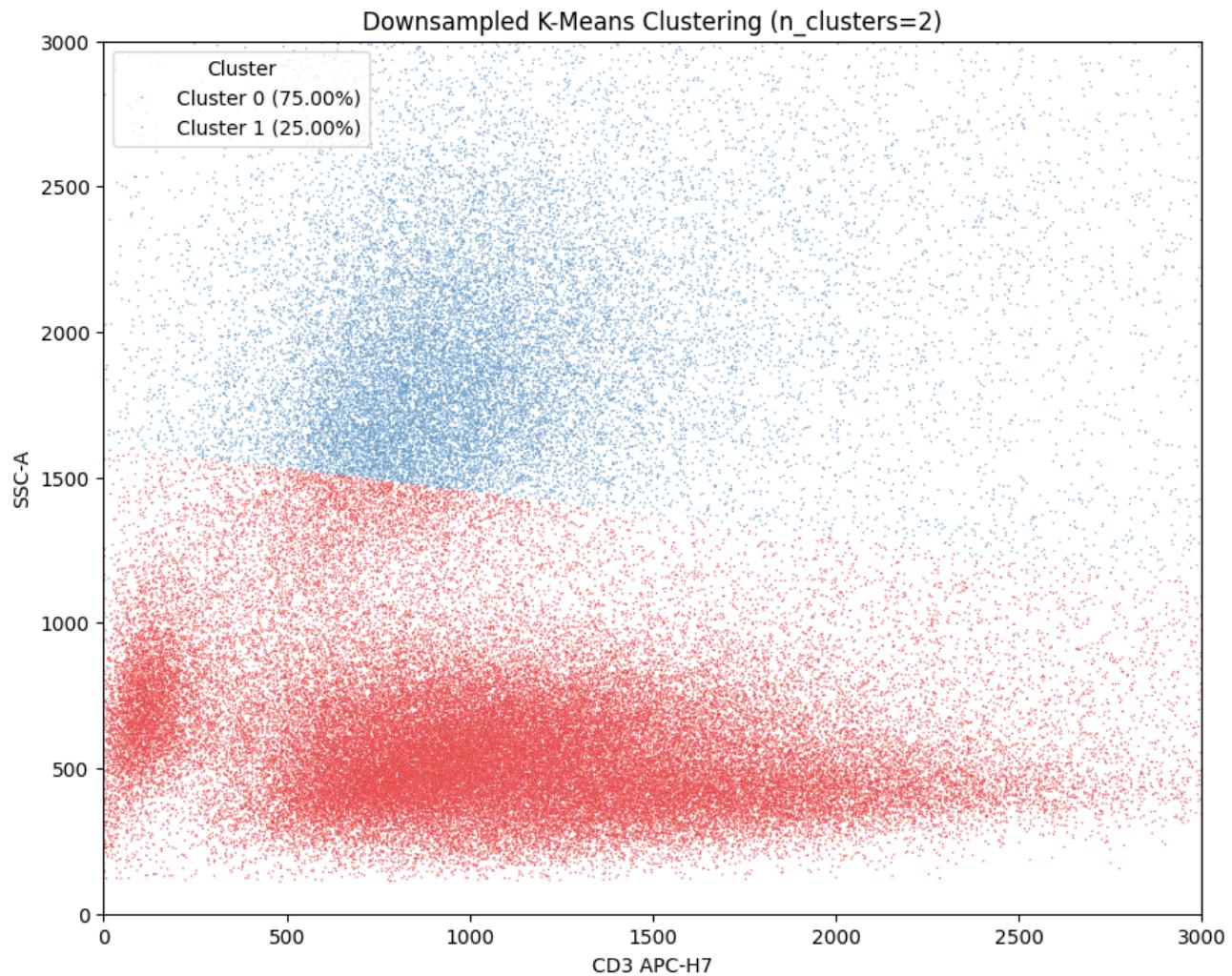
# Plot the clusters
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(data=sampled_data,
                           x='CD3 APC-H7',
                           y='SSC-A',
                           hue='Cluster',
                           palette='Set1',
                           s=1,
                           alpha=0.7)

# Update the legend labels to include percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [legend_labels[int(label)] for label in labels if label.isdigit()]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')

plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title(f"Downsampled K-Means Clustering (n_clusters={optimal_n_clusters})")
plt.show()

end_time = datetime.datetime.now()
duration14 = end_time - start_time
print(f"Duration for this cell14: {duration14}")

```



Duration for this cell14: 0:00:00.854669

```
In [52]: # Calculate Silhouette Score
model = optimal_kmeans
df = sampled_data.copy()
columns_to_use = ['FSC-A', 'FSC-H', 'SSC-A', 'CD123 BB660', 'CD14 BV510', 'H
    'Live Dead UV Blue', 'CD20 BUV805', 'CD11c APC', 'CD45RA A
    'CD3 APC-H7', 'CD19 PE-Cy5']
data = df[columns_to_use].values
labels = model.fit_predict(data)
sampled_km_score = silhouette_score(data, labels)
sampled_km_score
```

Out [52]: 0.5456452149840225

From PCA Only

```
In [53]: start_time = datetime.datetime.now()

# KMeans Clustering
```

```
pca_kmeans = KMeans(n_clusters=optimal_n_clusters, random_state=599)
pbmc_clean_pca_df['Cluster'] = pca_kmeans.fit_predict(pca_result)

# Calculate the percentage of points in each cluster
cluster_percentages = pbmc_clean_pca_df['Cluster'].value_counts(normalize=True)
legend_labels = [
    cluster: f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
]

# Plot the clusters
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(data=pbmc_clean_pca_df,
                           x='CD3 APC-H7',
                           y='SSC-A',
                           hue='Cluster',
                           palette='Set1',
                           s=1,
                           alpha=0.7)

# Update the legend labels to include percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [legend_labels[int(label)] for label in labels if label.isdigit()]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')

plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title(f"PCA Only KMeans Clustering (n_clusters={optimal_n_clusters})")
plt.show()

end_time = datetime.datetime.now()
duration15 = end_time - start_time
print(f"Duration for this cell15: {duration15}")
```



Duration for this cell15: 0:00:12.239447

```
In [54]: # Calculate Silhouette Score
model = pca_kmeans
df = pbmc_clean_pca_df.copy()
data = pca_result
labels = model.fit_predict(data)

# Combine features and labels
pca_with_labels = pd.DataFrame(data, columns=[f"PC{i+1}" for i in range(data.shape[1])])
pca_with_labels['cluster'] = labels

# Sample with replacement while maintaining stratification
''' Stratification is being performed by K-means cluster labels in order to
the model's cluster distribution when performing Silhouette Score calculation.
downsampled version of this PCA plot. Without downsampling, calculating the
Score would be excessively computationally expensive. This is different from
sampling performed on preprocessing since this is downsampled after K-means
and then stratified by that labeling.
'''
sample_size = 100000 # Desired total sample size
```

```

stratified_sample = pca_with_labels.groupby('cluster', group_keys=False).apply(
    lambda group: group.sample(
        n=int(sample_size * len(group) / len(pca_with_labels)), # Proportion
        replace=True,
        random_state=42
    )
)

# Extract sampled features and labels
sampled_features = stratified_sample.drop(columns=['cluster']).values
sampled_labels = stratified_sample['cluster'].values

pca_km_score = silhouette_score(sampled_features, sampled_labels)
print(f"Silhouette Score on Stratified Sampled Data (With Replacement): {pca_km_score}")

```

Silhouette Score on Stratified Sampled Data (With Replacement): 0.5510940292
625991

From PCA+T-SNE sample_tsne_df

```

In [55]: start_time = datetime.datetime.now()

# Apply KMeans to the t-SNE reduced data
tsne_kmeans = KMeans(n_clusters=optimal_n_clusters, random_state=42)
kmeans_labels = tsne_kmeans.fit_predict(sample_tsne_df[['TSNE1', 'TSNE2']])

# Add the KMeans cluster labels to the t-SNE DataFrame
sample_tsne_df['Cluster'] = kmeans_labels

# Reset index of pbmc_clean and merge with t-SNE cluster labels
sample_reset = pbmc_clean.reset_index(drop=True)
sample_with_clusters = pd.concat([sample_reset, sample_tsne_df[['Cluster']]])
```

Calculate the percentage of points in each cluster

```

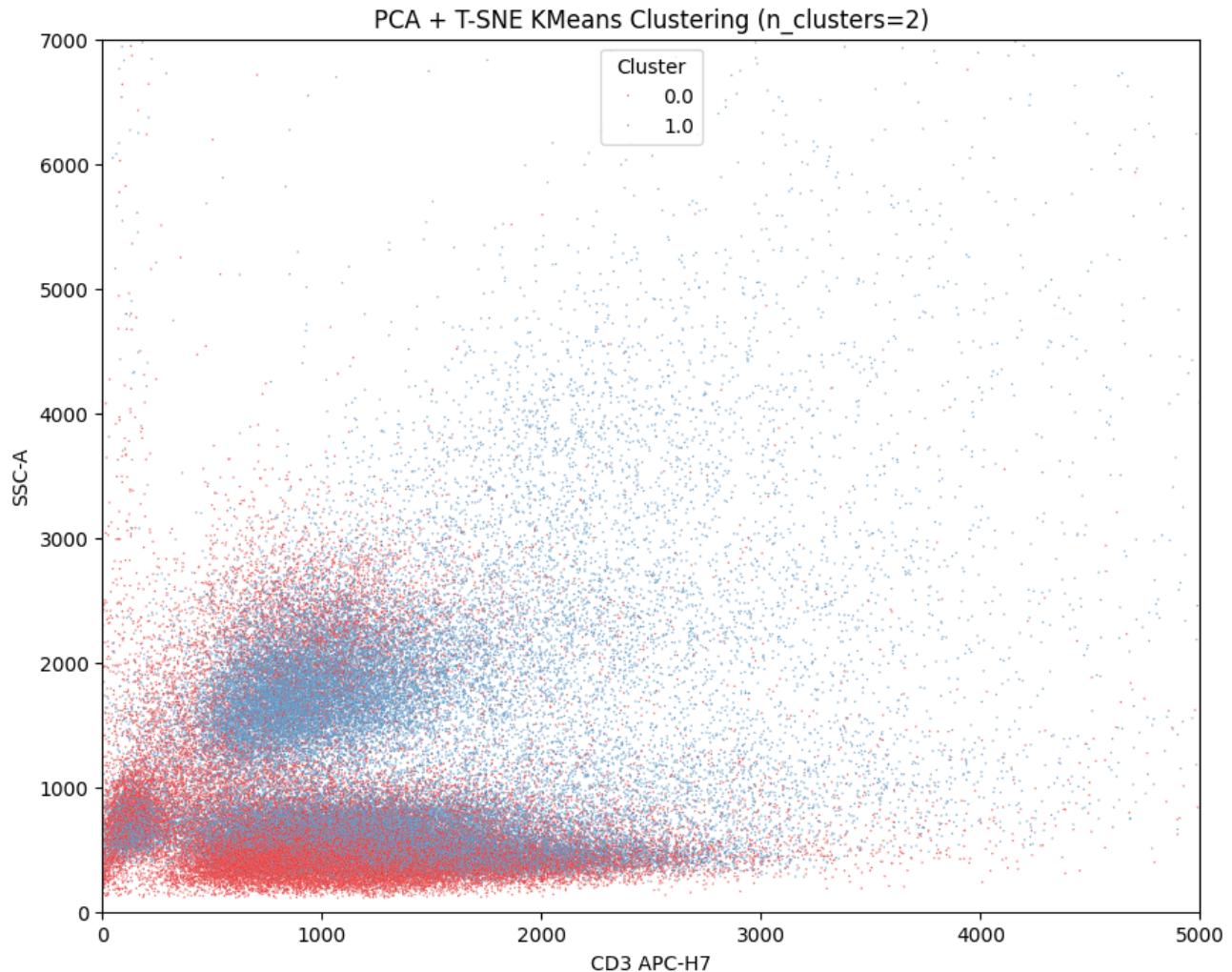
cluster_percentages = sample_tsne_df['Cluster'].value_counts(normalize=True)
legend_labels = {
    cluster: f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}
```

Plot "SSC-A" vs. "CD3 APC-H7" colored by cluster

```

plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(data=sample_with_clusters,
                           x='CD3 APC-H7',
                           y='SSC-A',
                           hue='Cluster',
                           palette='Set1',
                           s=1,
                           alpha=0.7)
```

```
)  
  
# Update the legend to include percentages  
handles, labels = scatter.get_legend_handles_labels()  
new_labels = [legend_labels[int(label)] for label in labels if label.isdigit()]  
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='best')  
  
plt.xlabel("CD3 APC-H7")  
plt.ylabel("SSC-A")  
plt.xlim(0, 5000)  
plt.ylim(0, 7000)  
plt.title(f"PCA + T-SNE KMeans Clustering (n_clusters={optimal_n_clusters})")  
plt.show()  
  
end_time = datetime.datetime.now()  
duration16 = end_time - start_time  
print(f"Duration for this cell16: {duration16}")
```



Duration for this cell16: 0:00:02.417748

```
In [56]: # Calculate Silhouette Score
model = tsne_kmeans
df = sample_with_clusters.copy()
data = sample_tsne_df[['TSNE1', 'TSNE2']]
labels = model.fit_predict(data)
tsne_km_score = silhouette_score(data, labels)
tsne_km_score
```

Out[56]: 0.34367338255618773

Model 3 - DBSCAN

Wall Time: 1 min 11 sec

From Sampled Data

```
In [57]: start_time = datetime.datetime.now()

# Modify Set1 to handle noise (-1) as gray and clusters with color
unique_clusters = db_df['DB_Cluster'].unique()
custom_palette = {
    cluster: color
    for cluster, color in zip(sorted(unique_clusters), sns.color_palette("Set1"))
}
# Ensure noise (-1) gets a consistent color
custom_palette[-1] = 'gray' # Use gray to de-emphasize from actual clusters

# Update the cluster percentages to include noise
cluster_percentages = db_df['DB_Cluster'].value_counts(normalize=True) * 100
if -1 not in cluster_percentages:
    cluster_percentages[-1] = 0.0 # Add noise with 0% if it doesn't exist

legend_labels = {
    cluster: f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}

# Replot with corrected labels and palette
plt.figure(figsize=(10, 8))
plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title("Downsampled DBSCAN SSC-A vs. CD3 APC-H7 Clustering (w/ Noise)")
scatter = sns.scatterplot(
    data=db_df,
    x='CD3 APC-H7',
```

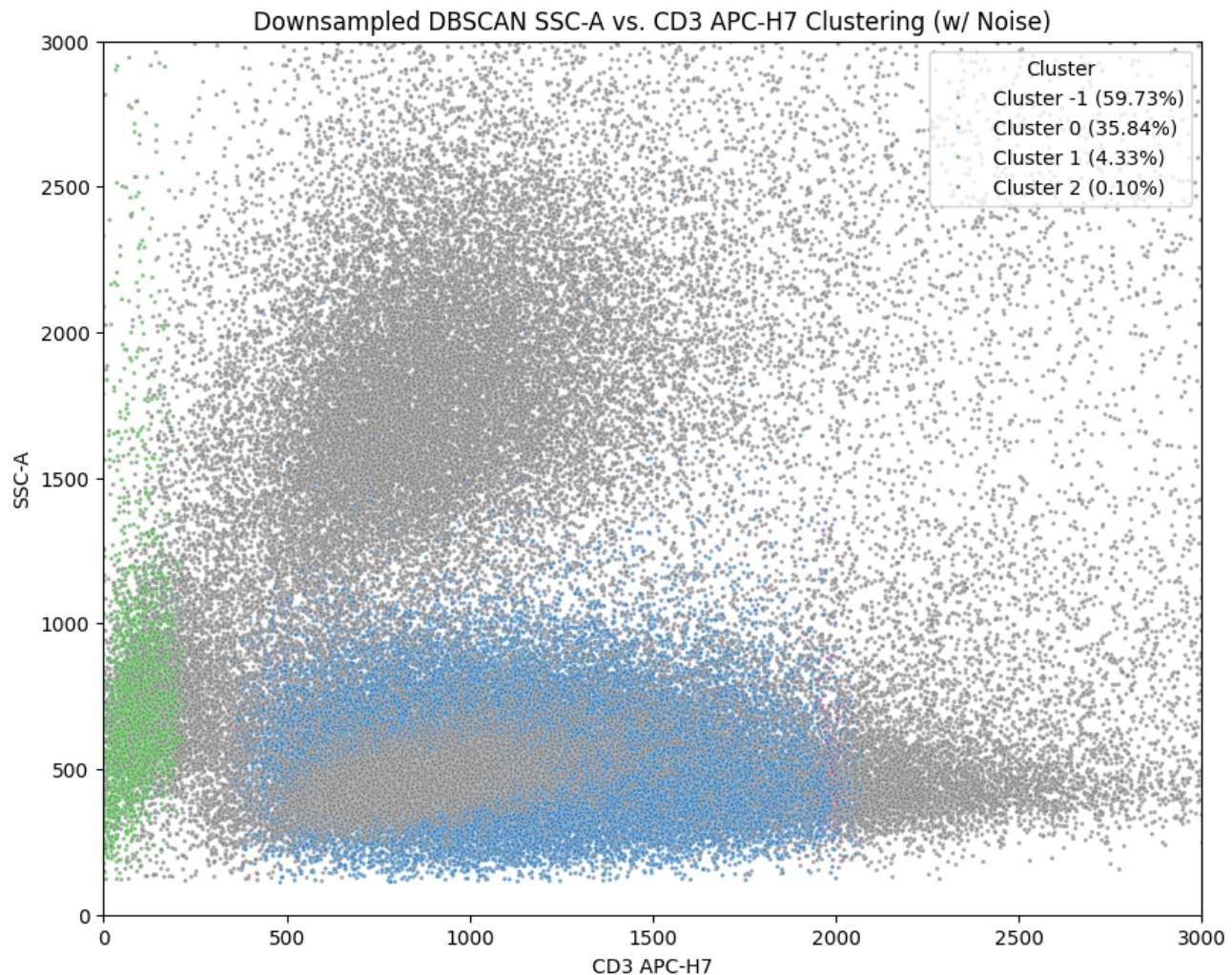
```

y='SSC-A',
hue='DB_Cluster',
palette=custom_palette,
s=5,
alpha=0.7
)

# Update the legend with percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [legend_labels[int(label)] for label in labels if label.isdigit()]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')
plt.show()

end_time = datetime.datetime.now()
duration17 = end_time - start_time
print(f"Duration for this cell17: {duration17}")

```



Duration for this cell17: 0:00:00.899955

In [58]: # Calculate Silhouette Score (excluding noise)

```

valid_labels = db_df[db_df['DB_Cluster'] != -1]['DB_Cluster']
valid_data = db_df[db_df['DB_Cluster'] != -1][['CD3 APC-H7', 'SSC-A']]
sampled_db_score = silhouette_score(valid_data, valid_labels)
sampled_db_score

```

Out[58]: 0.24956961783264517

From PCA Only

Wall Time: 2 min 14 sec

```

In [59]: start_time = datetime.datetime.now()

# Modify Set1 to handle noise (-1) as gray and clusters with color
unique_clusters = pbmc_clean_pca_df['Cluster'].unique()
custom_palette = {
    cluster: color
    for cluster, color in zip(sorted(unique_clusters), sns.color_palette("Set1"))
}
# Ensure noise (-1) gets a consistent color
if -1 in unique_clusters:
    custom_palette[-1] = 'gray' # Use gray to de-emphasize noise

# Calculate the percentage of points in each cluster, including noise
cluster_percentages = pbmc_clean_pca_df['Cluster'].value_counts(normalize=True)
legend_labels = {
    cluster: f"Noise ({percentage:.2f}%)"
    if cluster == -1
    else f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}

# Replot with corrected labels and palette
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(
    data=pbmc_clean_pca_df,
    x='CD3 APC-H7',
    y='SSC-A',
    hue='Cluster',
    palette=custom_palette,
    s=5,
    alpha=0.7
)

# Update the legend with percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [
    legend_labels[int(label)]
    if label.isdigit() or label == "-1"
    else label
    for label in labels
]

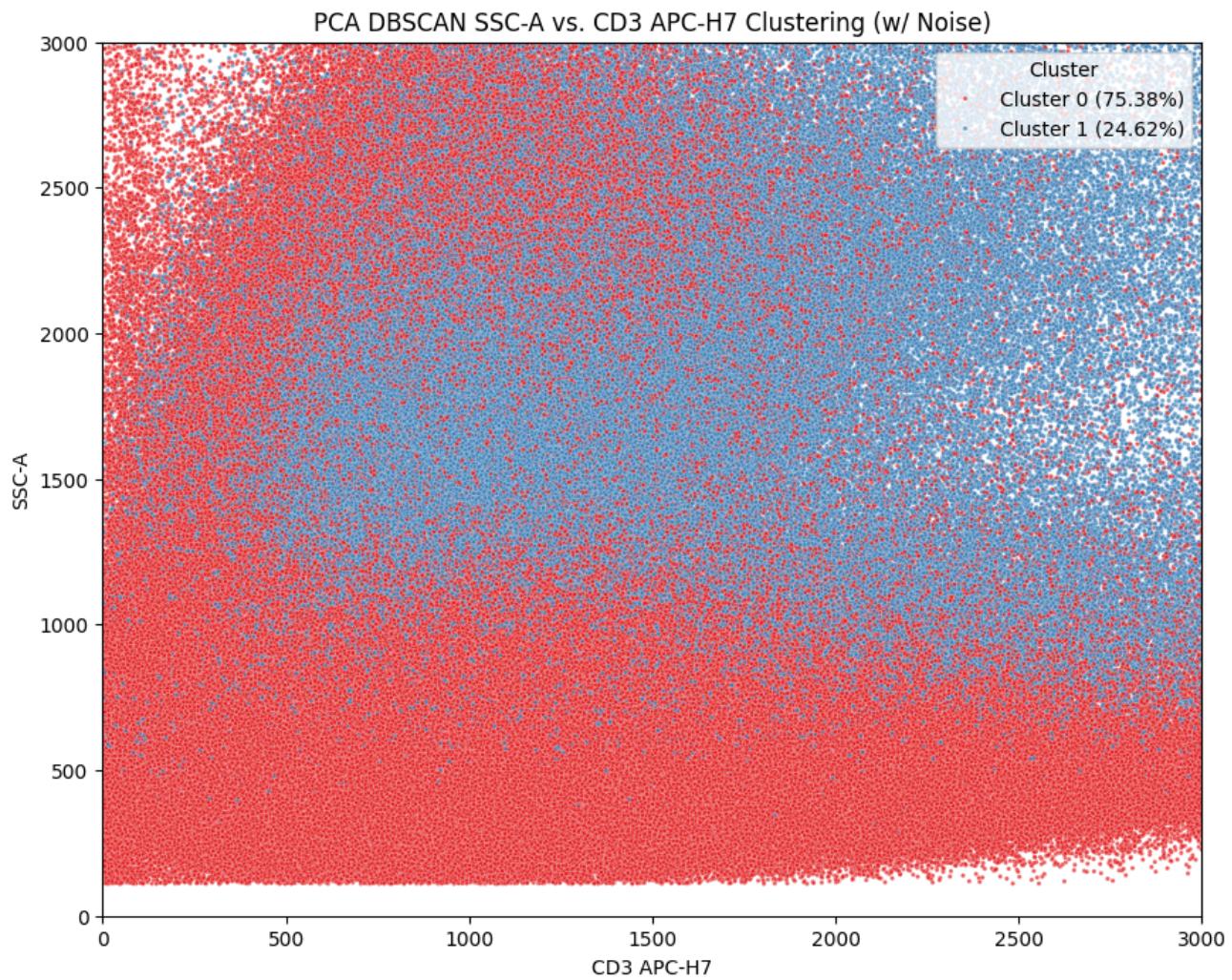
```

```

scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')
plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title("PCA DBSCAN SSC-A vs. CD3 APC-H7 Clustering (w/ Noise)")
plt.show()

end_time = datetime.datetime.now()
duration18 = end_time - start_time
print(f"Duration for this cell18: {duration18}")

```



Duration for this cell18: 0:00:13.015996

In [60]: # Only consider points that are part of a valid cluster (exclude noise label)
`valid_labels = pbmc_clean_pca_df[pbmc_clean_pca_df['Cluster'] != -1]['Cluster']
valid_data = pbmc_clean_pca_df[pbmc_clean_pca_df['Cluster'] != -1][['PCA1',`

In [61]: # Combine data and labels for easier manipulation
`valid_data_with_labels = pd.DataFrame(valid_data, columns=['PCA1', 'PCA2'])`

```

valid_data_with_labels['Cluster'] = valid_labels.values

# Sample with replacement while maintaining stratification
''' Stratification is being performed by DBSCAN cluster labels in order to b
the model's cluster distribution when performing Silhouette Score calculatio
downsampled version of this PCA plot. Without downsampling, calculating the
Score would be excessively computationally expensive. This is different from
sampling performed on preprocessing since this is downsampled after DBSCAN p
and then stratified by that labeling.
'''

sample_size = 100000 # Desired total sample size
stratified_sample = valid_data_with_labels.groupby('Cluster', group_keys=False)
    lambda group: group.sample(
        n=min(len(group), int(sample_size * len(group)) / len(valid_data_with_
replace=True,
        random_state=42
    )
)

# Extract sampled features and labels
sampled_features = stratified_sample[['PCA1', 'PCA2']].values
sampled_labels = stratified_sample['Cluster'].values

# Calculate silhouette score
pca_db_score = silhouette_score(sampled_features, sampled_labels)
print(f"Silhouette Score on Stratified Sampled Data (With Replacement): {pca

```

Silhouette Score on Stratified Sampled Data (With Replacement): 0.5618682551
325294

Not sure why there is no noise, but that there's three clusters. This is likely due to how the transformed dataframe used PCA to assign values back to the dataframe.

From PCA+T-SNE

```

In [62]: start_time = datetime.datetime.now()

# Modify Set1 color to handle noise (-1) as gray and clusters with color
unique_clusters = sample_tsne_df['Cluster'].unique()
custom_palette = {
    cluster: color
    for cluster, color in zip(sorted(unique_clusters), sns.color_palette("Set1"))
}
# Ensure noise (-1) gets a consistent color
if -1 in unique_clusters:
    custom_palette[-1] = 'gray' # Use gray to de-emphasize noise

# Calculate the percentage of points in each cluster, including noise
cluster_percentages = sample_tsne_df['Cluster'].value_counts(normalize=True)

```

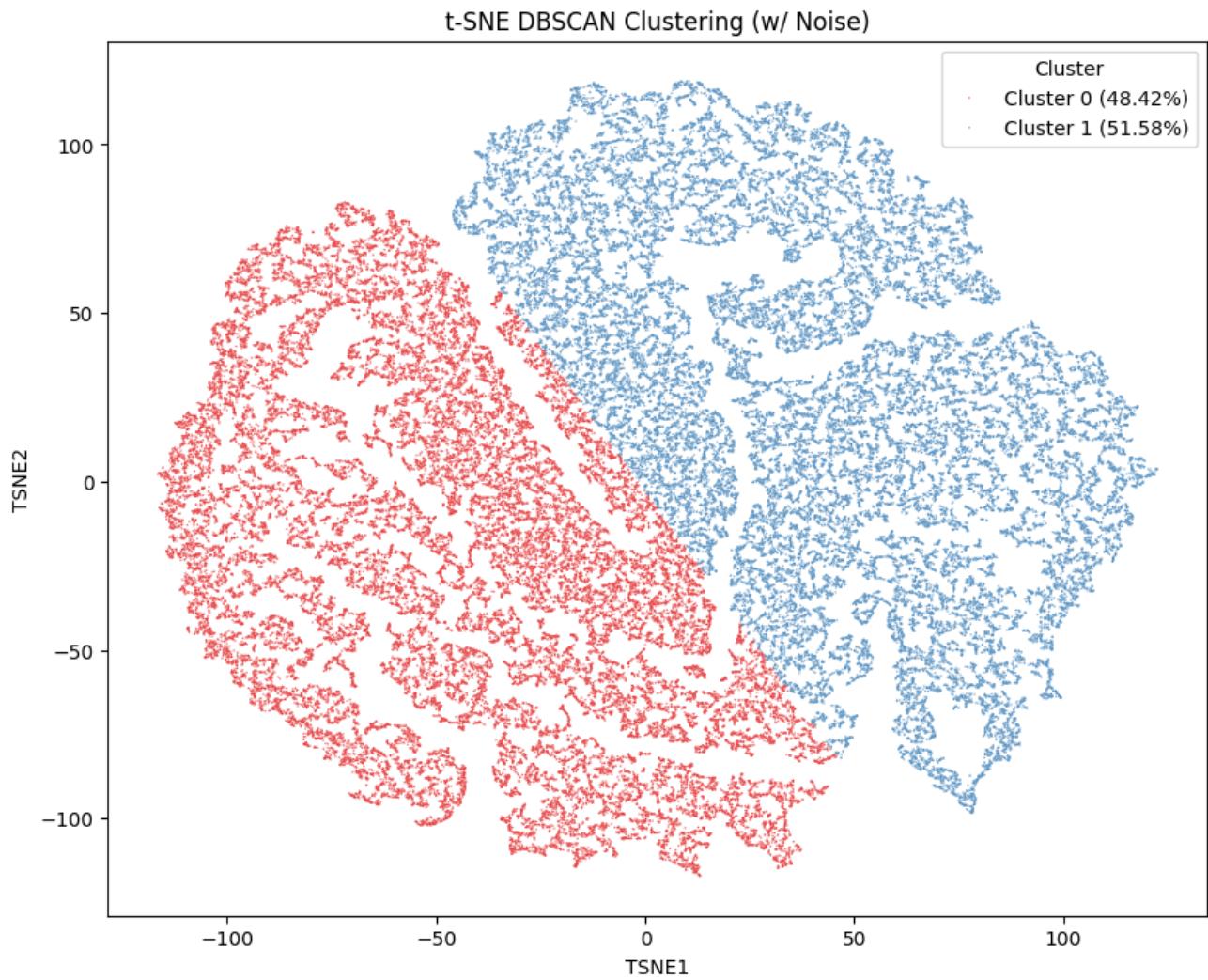
```
legend_labels = {
    cluster: f"Noise ({percentage:.2f}%)" if cluster == -1 else f"Cluster {cluster}"
    for cluster, percentage in cluster_percentages.items()
}

# Replot with corrected labels and palette
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(
    data=sample_tsne_df,
    x='TSNE1',
    y='TSNE2',
    hue='Cluster',
    palette=custom_palette,
    s=1,
    alpha=0.7
)

# Update the legend with percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [
    legend_labels[int(label)] if label.isdigit() or label == "-1" else label
    for label in labels
]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper left')

plt.xlabel("TSNE1")
plt.ylabel("TSNE2")
plt.title("t-SNE DBSCAN Clustering (w/ Noise)")
plt.show()

end_time = datetime.datetime.now()
duration19 = end_time - start_time
print(f"Duration for this cell19: {duration19}")
```



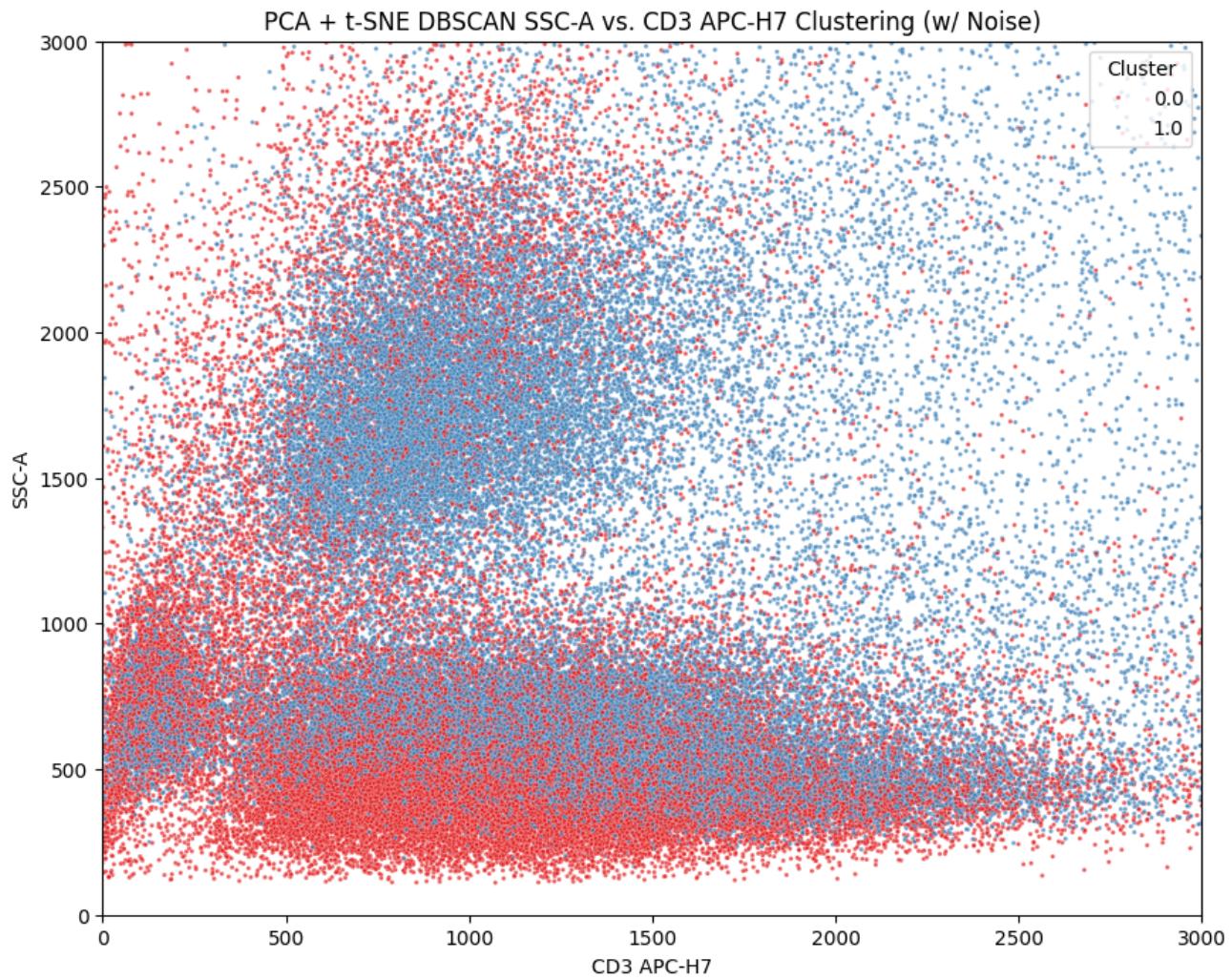
Duration for this cell19: 0:00:00.809779

```
In [63]: start_time = datetime.datetime.now()

# Modify Set1 color to handle noise (-1) as gray and clusters with color
unique_clusters = sample_tsne_df['Cluster'].unique()
custom_palette = {
    cluster: color
    for cluster, color in zip(sorted(unique_clusters), sns.color_palette("Set1"))
}
# Ensure noise (-1) gets a consistent color
if -1 in unique_clusters:
    custom_palette[-1] = 'gray' # Use gray to de-emphasize noise

# Calculate the percentage of points in each cluster, including noise
cluster_percentages = sample_tsne_df['Cluster'].value_counts(normalize=True)
legend_labels = {
    cluster: f"Noise ({percentage:.2f}%)"
    if cluster == -1
    else f"Cluster {cluster} ({percentage:.2f}%)"
    for cluster, percentage in cluster_percentages.items()
}
```

```
# Merge SSC-A and CD3 APC-H7 data from original data into sample_tsne_df
sample_reset = pbmc_clean.reset_index(drop=True)
sample_with_clusters = pd.concat([sample_reset, sample_tsne_df[['Cluster']]])  
  
# Replot with corrected labels and palette
plt.figure(figsize=(10, 8))
scatter = sns.scatterplot(
    data=sample_with_clusters,
    x='CD3 APC-H7',
    y='SSC-A',
    hue='Cluster',
    palette=custom_palette,
    s=5,
    alpha=0.7
)  
  
# Update the legend with percentages
handles, labels = scatter.get_legend_handles_labels()
new_labels = [
    legend_labels[int(label)] if label.isdigit() or label == "-1" else label
    for label in labels
]
scatter.legend(handles=handles, labels=new_labels, title="Cluster", loc='upper right')
plt.xlabel("CD3 APC-H7")
plt.ylabel("SSC-A")
plt.xlim(0, 3000)
plt.ylim(0, 3000)
plt.title("PCA + t-SNE DBSCAN SSC-A vs. CD3 APC-H7 Clustering (w/ Noise)")
plt.show()  
  
end_time = datetime.datetime.now()
duration20 = end_time - start_time
print(f"Duration for this cell20: {duration20}")
```



Duration for this cell20: 0:00:01.516789

```
In [64]: # Calculate Silhouette Score
valid_labels = sample_tsne_df[sample_tsne_df['Cluster'] != -1]['Cluster']
valid_data = sample_tsne_df[sample_tsne_df['Cluster'] != -1][['TSNE1', 'TSNE2']]
tsne_db_score = silhouette_score(valid_data, valid_labels)
```

Model Selection

Model Performance Evaluation and Model Selection

```
In [65]: # Create labels and the corresponding durations for each model
labels = ['Sampled_GMM',
          'PCA_GMM',
          'TSNE_GMM',
          'Sampled_KMeans',
          'PCA_KMeans',
```

```
'TSNE_KMeans',
'Sampled_DBSCAN',
'PCA_DBSCAN',
'TSNE_DBSCAN'
]

scores = [sampled_gmm_score,
          pca_gmm_score,
          tsne_gmm_score,
          sampled_km_score,
          pca_km_score,
          tsne_km_score,
          sampled_db_score,
          pca_db_score,
          tsne_db_score
]

# Pair labels and scores, then sort by scores in descending order
sorted_pairs = sorted(zip(scores, labels), reverse=True, key=lambda x: x[0])
sorted_scores, sorted_labels = zip(*sorted_pairs)

# Convert tuples back to lists
sorted_scores = list(sorted_scores)
sorted_labels = list(sorted_labels)

# Set Seaborn style and color palette
sns.set(style="whitegrid")
colors = sns.color_palette("Set2", n_colors=9)

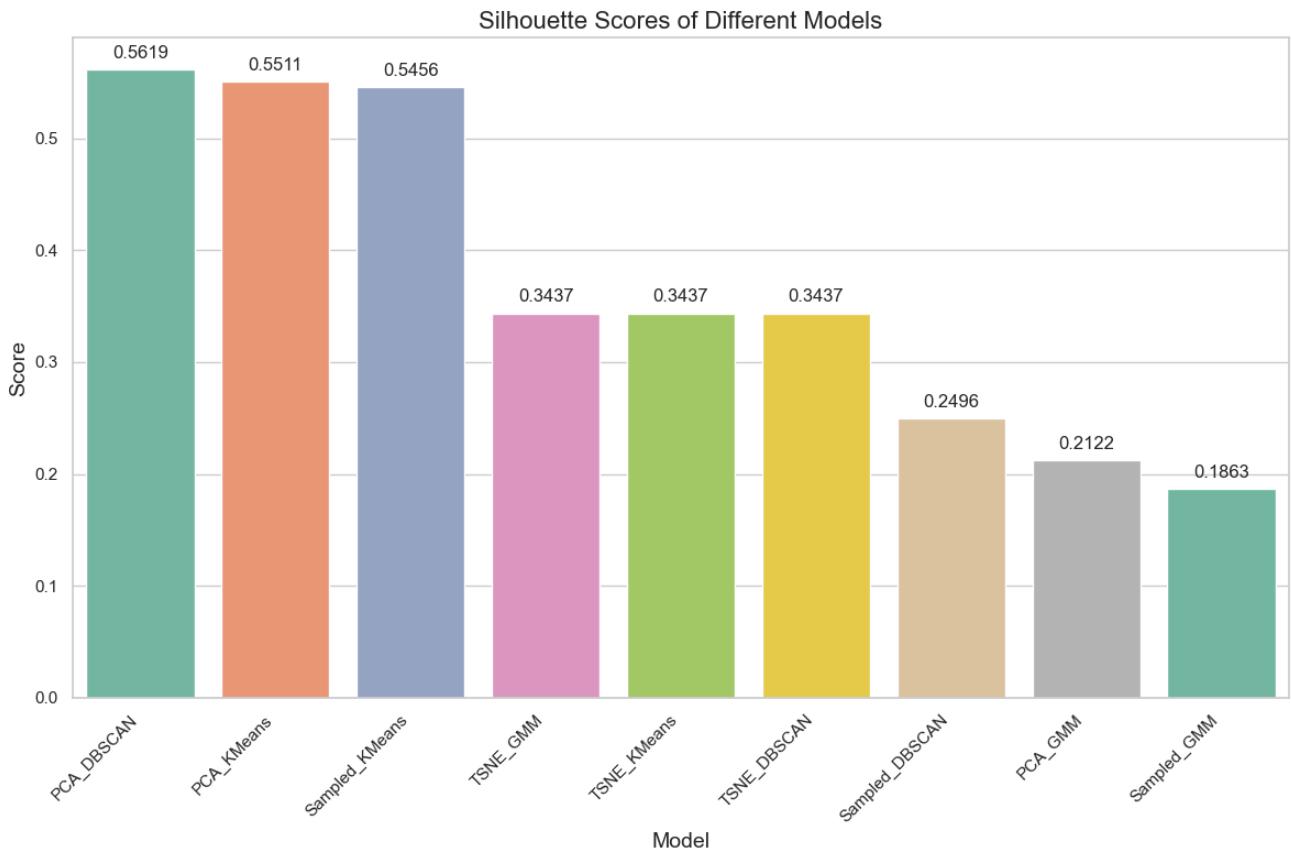
# Plot bar plot
fig, ax = plt.subplots(figsize=(12, 8))
sns.barplot(x=sorted_labels, y=sorted_scores, ax=ax, palette=colors)

# Adding titles and labels
ax.set_title('Silhouette Scores of Different Models', fontsize=16)
ax.set_ylabel('Score', fontsize=14)
ax.set_xlabel('Model', fontsize=14)

# Adding value annotations on top of bars
for i, score in enumerate(sorted_scores):
    ax.text(i, score + 0.01, f'{score:.4f}', ha='center', fontsize=12)

# Rotate x-axis labels to fit
plt.xticks(rotation=45, ha='right')

plt.tight_layout()
plt.show()
```



```
In [66]: # Convert all durations to total minutes for each step
def to_minutes(td):
    return td.total_seconds() / 60

gmm_time = to_minutes(duration_down + duration6 + duration8 + duration9 + du
kmeans_time = to_minutes(duration12 + duration13 + duration14 + duration15 +
dbSCAN_time = to_minutes(duration17 + duration18 + duration19 + duration20)

# Create labels and the corresponding durations for each model
labels = ['GMM', 'K-Means', 'DBSCAN']

durations = [gmm_time, kmeans_time, dbSCAN_time]

# Set Seaborn style and a larger color palette
sns.set(style="whitegrid")
colors = sns.color_palette("Set2", n_colors=9)

# Plotting the bar plot using Seaborn
fig, ax = plt.subplots(figsize=(12, 8))
sns.barplot(x=labels, y=durations, ax=ax, palette=colors)

# Adding titles and labels
ax.set_title('Duration of Different Models', fontsize=16)
```

```

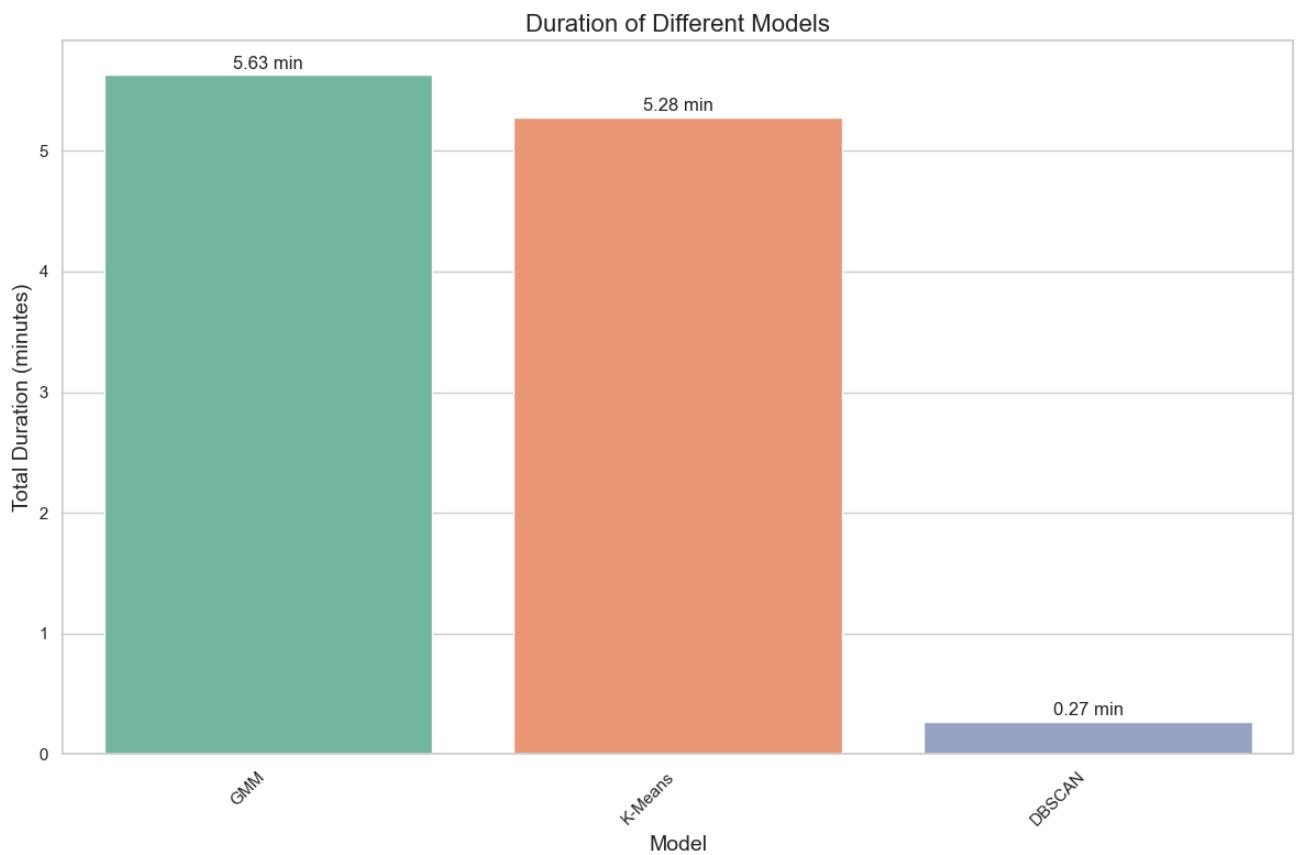
ax.set_ylabel('Total Duration (minutes)', fontsize=14)
ax.set_xlabel('Model', fontsize=14)

# Adding value annotations on top of bars
for i, duration in enumerate(durations):
    ax.text(i, duration + 0.05, f'{duration:.2f} min', ha='center', fontsize=14)

# Rotate x-axis labels for readability
plt.xticks(rotation=45, ha='right')

# Show the plot
plt.tight_layout()
plt.show()

```



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