

Module 11 Assignment

585.751.81 Immunoengineering

1. Your company wants to understand more about how cancer cells influence immune cells in different environments. You have *in vitro* 2D culture, 3D organoid models, and *in vivo* mice models. Describe experiments/techniques (a, b, c below) that you could use with these models to learn about cancer and what you might learn from either: (30 points)
 - a. Conventional immunological
 - b. Microfluidic single cell assays
 - c. Imaging approaches

In vitro 2D cultures

Conventional assays like flow/mass cytometry could be used to identify and characterize immune cells cultured with cancer cells. It can identify novel immune cell subpopulations, or changes in immune cell type subsets, such as T cell or macrophages, and their activation states in response to cancer cell signals. ELISA/ELISPOT, Western Blot, or immunoprecipitation can help quantifying protein amounts, post-translational modifications, secreted cytokines and chemokines by cancer or immune cells, providing insights into the mechanisms of immune cell recruitment or suppression. RNA sequencing can be used to identify gene expression patterns linked to immune activation or evasion.

3D organoid models

These models can mimic the *in vivo* tumor context in replicating the spatial organization, cell-cell, and cell-matrix interactions of tumors and can help understanding the tumor microenvironment (TME). Conventional techniques like immunoprecipitation can be used to study protein-protein interactions within the TME, potentially helping to screen combinational therapies involving checkpoint inhibitors, to discover neoantigens for potential vaccines, or signaling pathways critical for tumor-immune interactions. Microscopy combined with fluorescent antibody to protein or receptor or genetic information, can help to visualize the spatial relationships and interaction between cancer cells and immune cells, providing insights into the tumor architecture and immune infiltration. Additionally, imaging techniques and questions, such as resemblance of human cancer organoid biobank to tumor tissue, tumor evolution, or improvement of treatment efficacy could be investigated in a 3D organoid model [1].

Microfluidic single cell arrays: can help to understand how T cells are recruited, reveal cell-cell over time interaction in mass between immune cells and cancer cells, help to examine the APC-T cell interaction dynamics and heterogeneity, how immune cells influence metastasis, provide a better understanding of cytotoxicity, evolution of enzymes and immune-based proteins, the control aspect of TME on immunosuppressants, or how cancer cells affect CAR T cells.



In vivo mice models

Tracking cancer-specific T-cells in vivo with gold nanoparticles, or radioisotopes and CT imaging could be performed ([1] and [2]) to understand design for instance of CAR T cells and off-target effects of cancer therapies. Imaging could be also used to understand tumor genesis, toxicity, and interactions with the immune system.

2. Your biopharmaceutical company wants to create a more reliable *in vitro* screening model. Design an organ-on-a-chip technology **to screen new vaccines**. In your design consider: (50 points)
 1. What are going to be your starting cells, where you will get them from
 2. What physical parameters would be important—e.g. flow rate, mechanical properties
 3. How do you get cells into the device and keep alive—e.g. density, hydrogels, nutrients, length of culture, etc.
 4. What is your output you will measure

Organ-on-a-chip or tumor-on-chip ([3], [4], [5], [6], [7], [8])

Vaccines based oncolytic viruses (OVs); have been developed to kill tumor cells while sparing healthy cells. Although OVs can induce immune infiltration into tumors, a strong immune response might also attack the virus itself, decreasing its efficacy and reducing its propagation. To further explore OV-based vaccine resistance mechanisms, we are designing a Tumor-on-a-Chip (ToC) which is a cell culture device stimulating the humanized tumor microenvironment. The chip is composed of 3 chambers; the central chamber contains the cells which are encapsulated within a collagen matrix that mimic the extra-cellular matrix and two lateral layers that are used to inject the virus. The setup allows the virus to diffuse gradually into the central chamber where the cancer cells are embedded, closely mimicking the interaction within the tumor and its surrounding stroma. The diffusion process permits a controlled exposure of the cancer cells to the virus, simulating a more natural vaccine action that might occur in a tissue or tumor microenvironment. We use soft lithography, a cheaper and more accessible alternative to traditional lithography, and PDMS polymer for the device fabrication.

The cancer cells used are A549 cells, derived from lung tissue of a lung cancer patient, cultured in high glucose DMEM (HyClone) with 10% fetal bovine serum. Depending on the cancer model and vaccine to screen, other cell lines must be identified.

These cells are prepared by trypsinization and centrifugation, and may be co-cultured with immune cells, for instance peripheral blood mononuclear cells (PMBCs), isolated using Lymphoprep (Stemcell) ([9]).

To enhance the virus's targeting capability against cancer cells, we use primary chicken embryo fibroblasts for virus amplification and titration. We prepare Collagen type I at a cool temperature to prevent premature polymerization. The A549 cells are then suspended in this collagen gel, either alone or mixed with PBMCs. This cell-collagen mixture is transferred into the central chamber of the ToC. Upon polymerization, the collagen forms a gel-like scaffold that supports a 3D cell culture structure. After the scaffold is set, the lateral chambers are filled with staining

solution, with caspase-3/7 green detection reagent alone for the control condition, or with the oncolytic vaccinia virus (OVV) at a calculated multiplicity of infection for the treatment condition, to avoid excessive killing of cancer cells. Additionally, we have engineered the OVV to include a gene encoding the mCherry protein, under the control of a viral promoter. This modification allows real-time monitoring of virus expression and infection dynamics through mCherry fluorescence in the infected cells.

We use the Caspase-3/7 green reagent to monitor and quantify apoptosis which upon activation, allows us to visualize in real-time cell death in response to viral infection. For this purpose, the 3D cell culture chips are mounted on the stage of an inverted widefield fluorescence video microscope.

Image acquisition is carefully scheduled at different resolutions to capture immune kinematics, cancer-immune interactions, and the progression of viral infection. We record metrics such as the percentage of dead cells, infected cells, and average intensities of apoptosis, and infection signals over time across 3 experimental setups: Control 1 (cancer cells with virus, no immune cells), Control 2 (cancer cells with immune cells, no virus) and Treatment (cancer cells with both immune cells and virus).

These experiments are designed to explore the synergistic interactions between OVV and immune cells in targeting cancer cells. Additional staining may be necessary to further identify the roles of different immune cell types in this interaction. While this organ-on-a-chip (OOC) primarily investigates the mechanisms by which OVVs affect cancer cells, it also has the potential to be adapted for screening other viral-based vaccines and their effects on cancer cells.

3. How can systems immunology transform how we investigate the immune system? In your response consider: (20 points)
 1. Tools and experiments we use
 2. Research team members
 3. Data visualization and interpretation

Systems immunology uses a repertoire of high-throughput and high-bandwidth technologies, which include various assays like flow cytometry, microfluidics, mRNA sequencing, as well as 3D cell cultures: organoids, organ-on-a-chip systems coupled with advanced imaging techniques and machine learning algorithms. These well-established methods enable large-scale analysis of cell type, cell states, their functions, and their products (e.g., cytokines, chemokines, metabolites); as well as protein-protein interactions [10].

The interdisciplinary nature of systems immunology requires collaboration of researchers with different skill sets. This includes biologists designing experiments, bioinformaticians developing data processing pipelines, genomic and immunologist experts, IT and ML engineers managing databases, computing infrastructure, implementing ML or Deep Learning algorithms, running statistical analysis, as well as mathematicians, and physicists who create new mechanistic models [10].

With the influx of complex and voluminous data sets, researchers have created innovative visualization and analysis tools which manage the high-dimensional nature and complexity of this data (PCA, least Square Analysis, Hierarchical Analysis, heatmaps, Oncotree, and many other tools in Data Science) but also derive meaningful insights that can lead to breakthroughs in understanding immune mechanisms from the molecular level to whole organ systems.

4. Practice PCA analysis with a sample data set from the paper (excel file):
Horton, Paul, and Kenta Nakai. "A probabilistic classification system for predicting the cellular localization sites of proteins." *Ismb*. Vol. 4. 1996.

The data is 336 x 7 (336 samples with 7 variables) with sample labels and 8 broad classifications. You can use a PCA function I created in matlab (called `pca`), or use Matlab's own `pca` function to do the analysis. Here is a helpful link to doing PCA in MatLab:

<https://www.mathworks.com/help/stats/quality-of-life-in-u-s-cities.html>

Generate a scatter plot with PC1 on the x axis and PC2 on the y axis and describe whether you see clustering of the data using this method and how you would go about further interpreting the results. (Extra Credit: 30 points)

Across the 7 variables, the data has similar medians and shows some outliers (Fig.1). The t^2 statistic indicates that NLPA_ECOLI is the furthest from the centroid of the average amino acid data across the 336 samples. The localization site for NLPA_ECOLI is 'imL'.

The first 3 principal components account for 90% of the variance (Fig. 2). The scatter plot with PC1 and PC2 reveals noticeable clustering by protein localization sites (Fig. 3), the clusters are not distinctly separated. This lack of clear separation could be attributed to the exclusion of PC3 and may also reflect that the underlying relationship between the 7 variables and the localization sites is not strongly linear.

All 7 variables are represented in the biplot (Fig. 4) by a vector, and the direction and length of the vector indicate how each variable contributes to the two principal components in the plot.

The largest coefficients in the first principal component are variable 6 and 7.

The second principal component, on the vertical axis, has positive coefficients for the variables 1,2,3,4 and negative coefficients for the remaining 3 variables. This indicates that the second component distinguishes among EColi proteins that have high values for the first set of variables and low for the second, and EColi proteins that have the opposite.

The next step will involve examining the correlations between each variable. Initial findings indicate that variables 7 and 6 are highly correlated and may be redundant (Fig. 5).

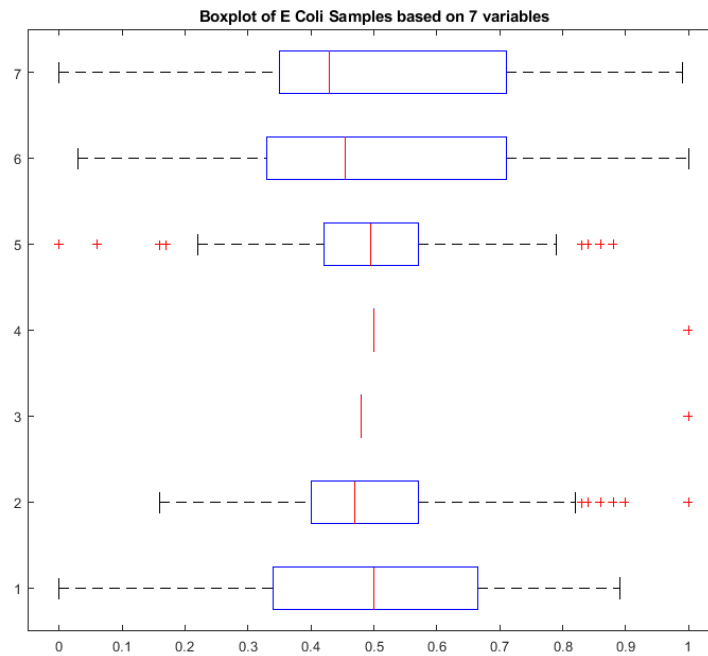


Figure 1 – Boxplot of EColi samples using 7 variables.

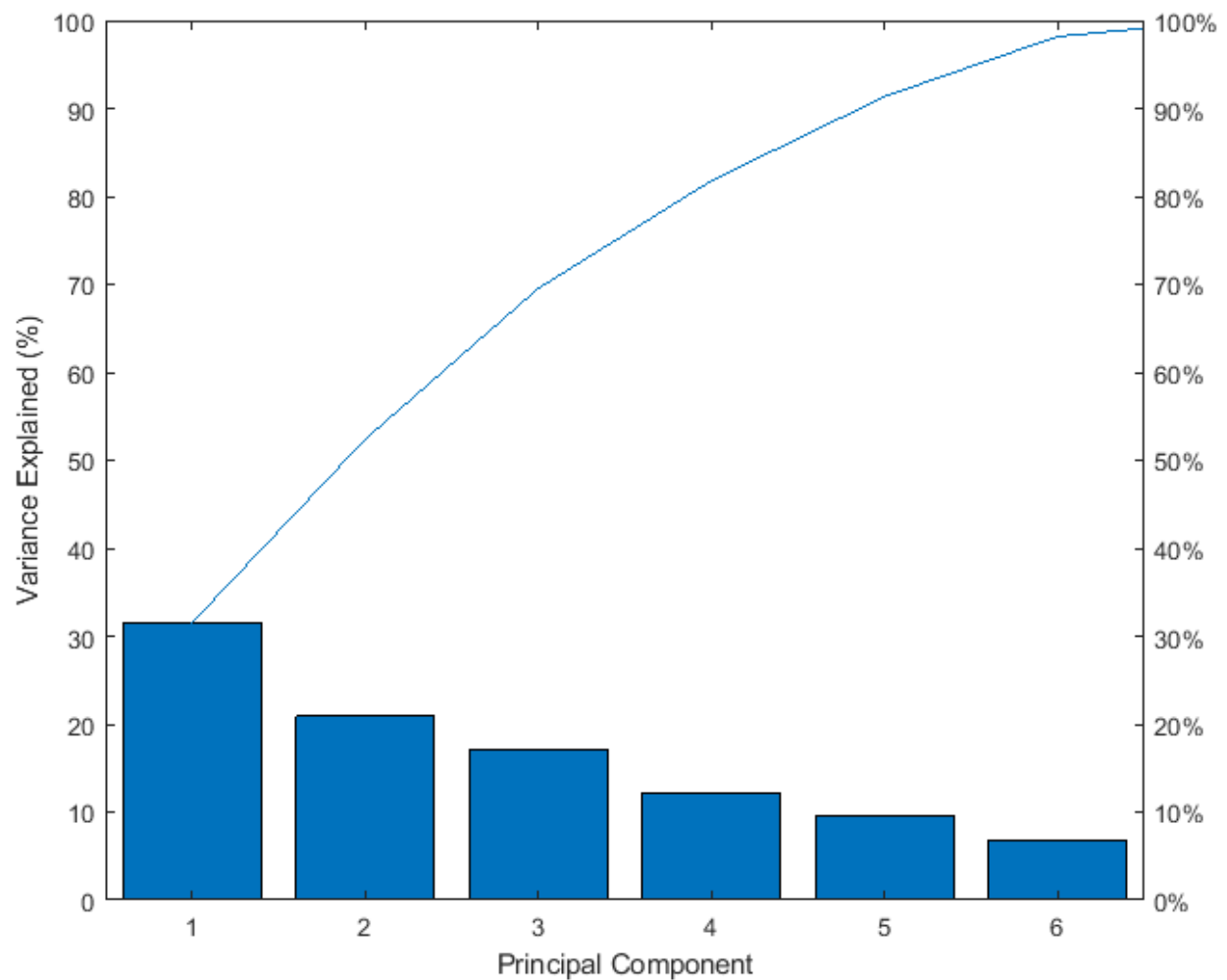


Figure 2 – Explained variance by the Principal Components.

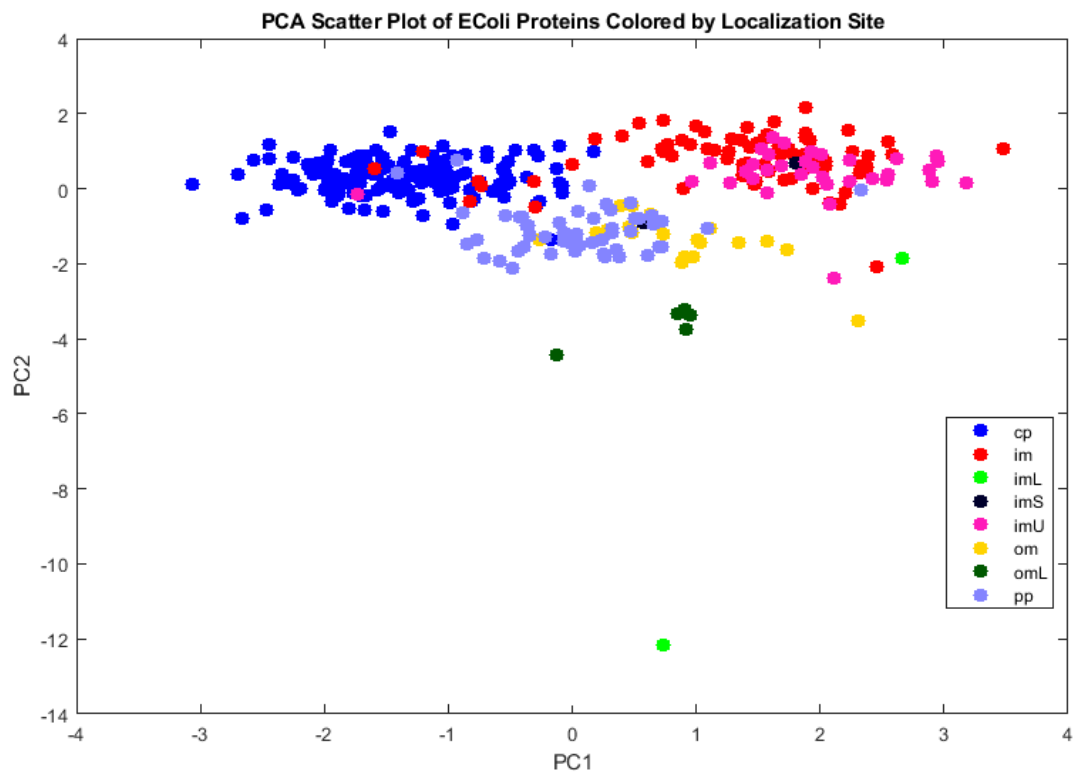


Figure 3 – PCA Scatter Plot.

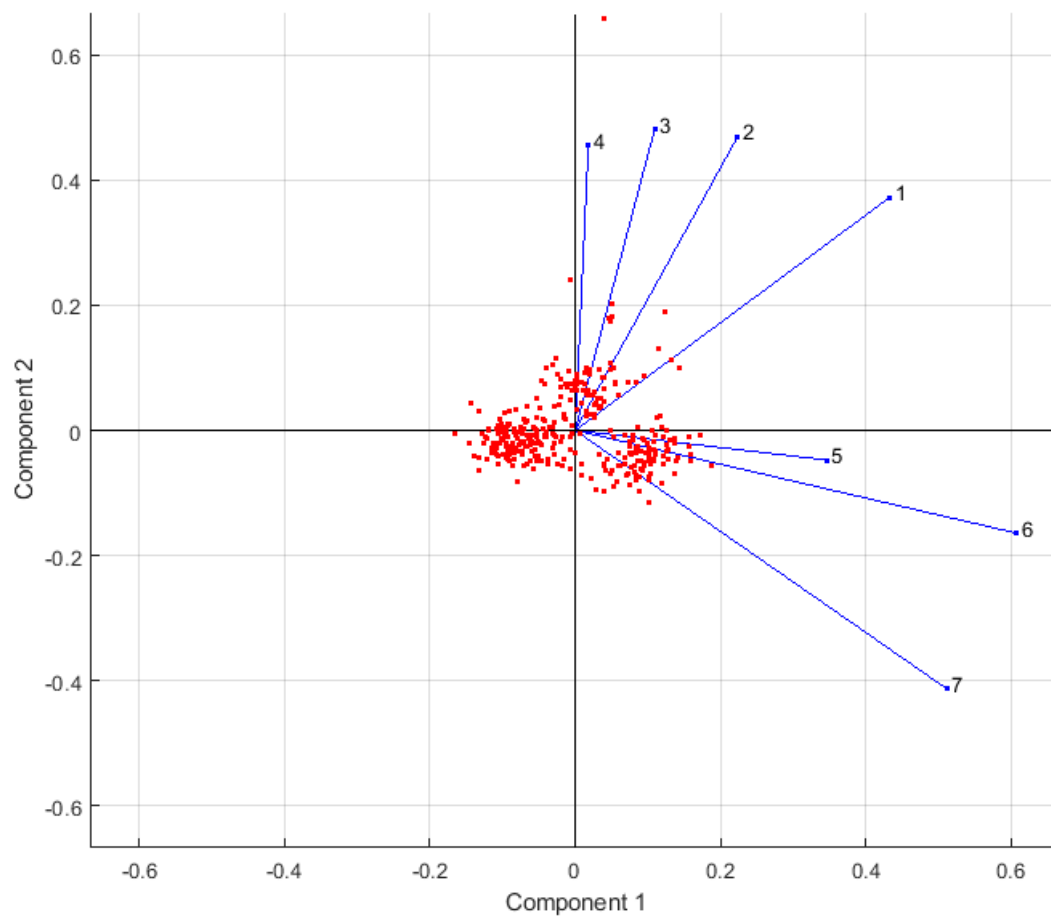


Figure 4 - Biplot

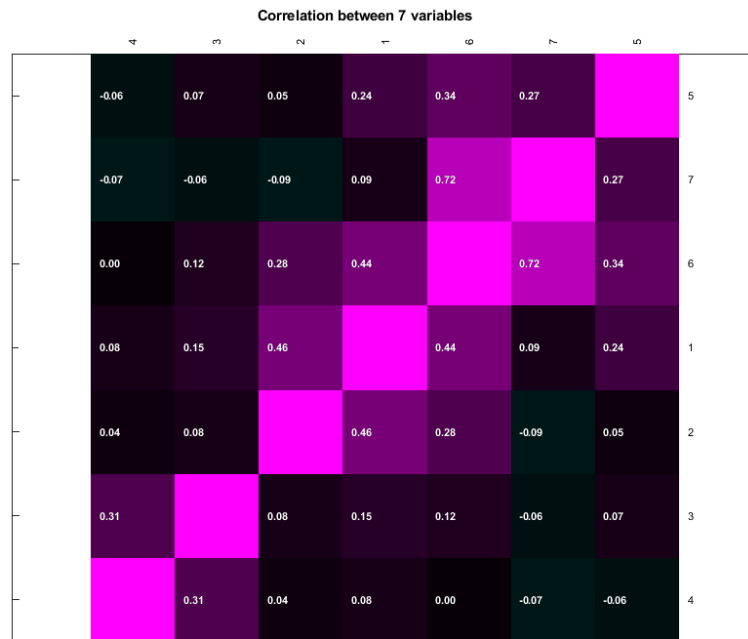


Figure 5 – Heatmap of Correlations between 7 variables

Next steps could be:

Cluster Analysis: Further cluster analysis could help identify distinct groups or patterns in the data that weren't previously apparent. This can be particularly useful if some variables are removed based on redundancy.

Regression Analysis: If the goal is to understand how these variables predict or relate to an outcome, regression models can be used. Here, we can explore how the remaining variables after removing redundancies affect the outcome.

Feature Engineering: we could also explore creating new variables from existing ones, especially from those that are not highly correlated. This process can uncover more useful features that improve the performance of predictive models.

More Visualization: Employing visualization techniques like heatmaps for correlation, pair plots, or three-dimensional scatter plots can provide deeper insights into the relationships between variables.

References

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