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by

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Eidesstattliche Erklärung

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Wien, am 8. Juli 2024

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Contents

1 Introduction

Research questions:

- Which criteria can distingish between unactivated, activated and pre-activated cells?
- Do different types of activated cells exists? How are they different?
- With which frequencies does the Calcium concentration repeat after activation?
- Is there a difference in frequencies between mouse and human cells?

2 T Cells, Calcium Concentration

Lymphocytes form a key component of the immune system. T cells are a type of lymphocyte and are responsible for responding to viruses, fungi, allergens and tumors. Different subtypes of t cells exist, that fulfill various responsibilities. They are transported throughout the body via the lymphatic system and blood.[KCF18]

Precursor cells are formed in the bone marrow. Once they are transported to the thymus they undergo maturation and selection to become t cells. Each cell forms receptors, called t cell receptors (TCR), that respond to one perticular out of many ($10^6 - 10^9$) possible short pieces of proteins, called peptides. These peptides are attached to the major histocompatibility complex (MHC) present on antigens and antigen presenting cells (APC). Important aspects of the selection are ensuring that the t cells react to foreign peptides, but not to those present on the body's own cells.[AH24]

In positive selection cells in the thymus present peptides on their MHC. If a t cell is unable to bind, it will undergo apoptosis, a type of cell death. T cells which were able to bind recieve survival signals. Negative selection verifies that t cells will not attack the body's own cells. This is done by only selecting t cells which only bind moderatly to the peptides presented, as a strong bond sugessts that these t cells would have a high likelihood of being reactive to own cells.[Hag18] If a t cell passed both the positive and negative selection it is transported to the periphery.

There are multiple types of peripheral t cells. Native t cells respond to new antigens. Cytotoxic t cells kill cells which present peptides on their MHC compatible with the t cells TCR. Helper T cells activate other parts of the immune response. Memory t cells shorten the reaction time when the same antigen is encountered again at a later point in time. Suppressor t cells moderate the immune response.[Gan97]

2.1 Components of a T Cell

T cell components relevant in activation and subsequent changes in intracellular Ca^{2+} are listed below and schematically shown in figure 2.1.

- **T cell receptor (TCR):** Receptor on the cell surface that can recognize peptides. By the simultanious triggering of the TCR and co-stimulator signaling is induced that leads to activation.
- **Co-stimulator:** A stimulation of co-stimulatory molecules is necessary in order for signaling to occur as part of activation.
- **Endoplasmic reticulum (ER):** A series of connected sacs in the cytoplasm that is attached to the nucleus. Important functions are folding, modification and transportation of proteins.[Rog24]

- **Ca^{2+} permiable ion channel on the ER:** There are several Ca^{2+} channels present on the ER. Some receptors are responsible for releasing Ca^{2+} into the cytoplasm, when the intracellular Ca^{2+} concentration is low. [SB16]
- **Ca^{2+} storage in the ER:** Ca^{2+} is stored in the ER and can be released by Ca^{2+} periable ion channels on the ER.
- **Cytoplasm:** The semifluid substance enclosed in the plasm membrane. It contains organells, ions, proteins and molecules.
- **Stromal interaction molecule (STIM):** If the Ca^{2+} storage in the ER is depleted STIM proteins cluster where the ER is in the visinity of the plasm membrane and assembles CRAC, which then leads to uptake in extracellular Ca^{2+}. [SB16]
- **Plasm membrane:** A semipermiable structure forming the wall of the cell made up of lipids and proteins. Ion channels and transport proteins allow certain substances to move through.[Gan12]
- **Ca^{2+} release activated Ca^{2+} channel (CRAC):** Opened after a decrease in ER stored Ca^{2+} is sensed by STIM, these channels intake Ca^{2+} from outside the cell.[SI13]
- **Cytoskeleton:** A system of fibers within the cell, that allows it to change shape and move.[Gan12]
- **Nucleus:** An organelle that stores most of the DNA, controls cell growth and cell division. A double membrane separates it from the cytoplasm.[CA22]

Relevant components of APC are the

- **Major histocompatibility complex (MHC)**, which can present peptides, and the
- **Co-stimulator**, which can form a bond with the co-stimulator on a t cell.

Both are present on the surface of the APC.

2.2 Activation

Activation is necessary for t cells to divide and perform their functions.[Gan97]

When a native t cell encounters a peptide on an APC that is compatible, a bond is formed between the TCR on the t cell and the peptide-MHC complex on the APC. This recognition can be triggered by less than ten molecules of foreign substance and is therefore described as near perfect. Sufficiently long contact is nececcary between the APC and the t cell in order for the t cell to activate. The role of contact time in t cell activation is modelled by Morgan et.al.[ML23].

The presence of co-stimulatory molecules is needed for proper activation. The bond between the co-stimulatory molecules on the t cell and APC plays a role in signaling. Ca^{2+} signals play a vital part in t cell activation.

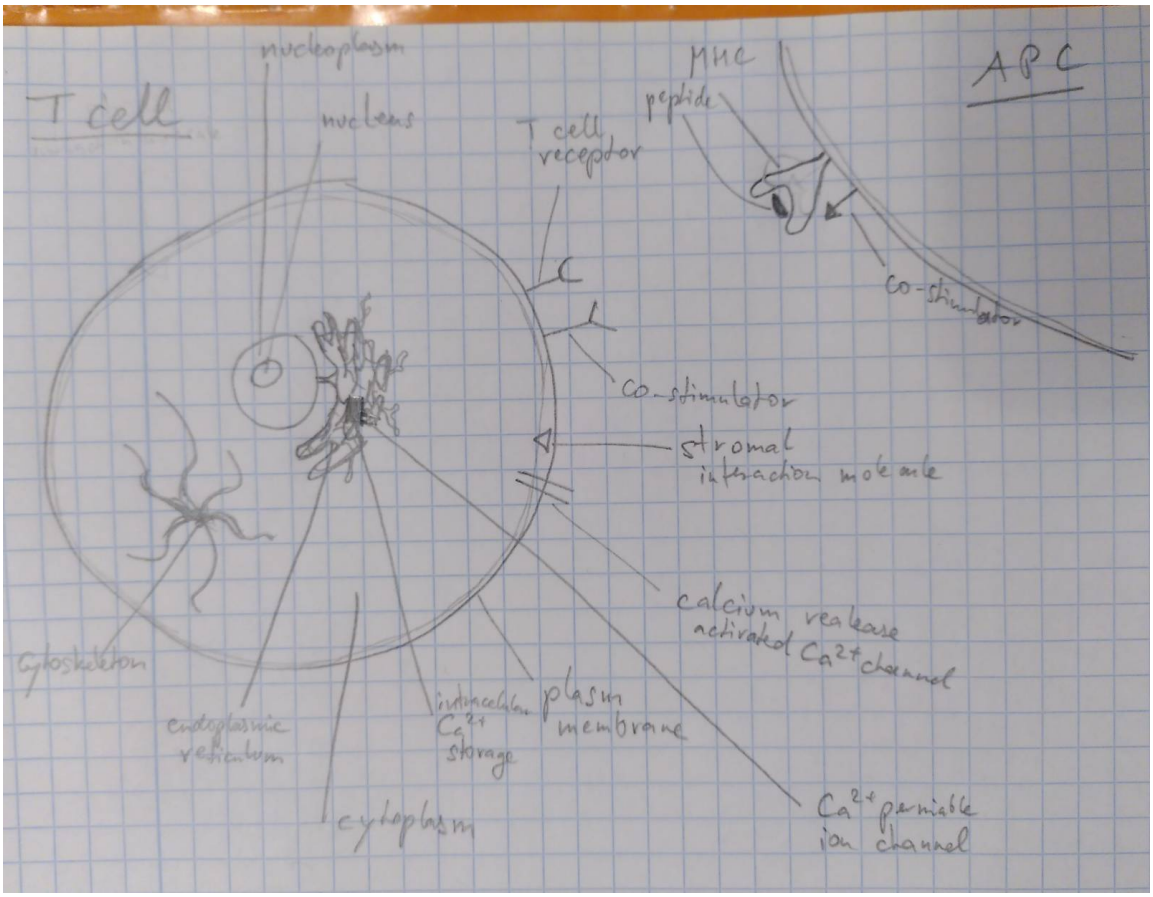

Figure 2.1: Skematic view of a t cell and antigen presenting cell, with all relevant components.

An increase of Ca^{2+} in t cells during activation is caused by the stimulation of Ca^{2+} permiable ion channel receptors on the ER membrane. Ca^{2+} is released from the ER into the cytoplasm. Additionally this decrease in Ca^{2+} is sensed by STIM, which leads to an influx of Ca^{2+} through plasma membrane CRAC channels.[SKJ09]

As the intracellular Ca^{2+} concentration is dependent on the interaction between Ca^{2+} sources and sinks, a variety of different forms in Ca^{2+} concentration have been observed. Examples are infrequent spikes, sustained oscilations and plateaus.[Lew01]

Intercellular Ca^{2+} increase together with other signals lead to a redistribution of receptors, signaling molecules and organelles.[JRB14]

3 Data

From section 2.2 we gather that analysing the intracellular Ca^{2+} concentration gives us good insight in wheter and when a cell activates. Additionally it can be measured relatively easily by the method described in this chapter.

3.1 Structure of Data

The data matrix has a row per tracked cell and frame. The information stored for each cell and frame combination is described in detail in table 3.1.

Name	Data Type	Description
x	float64	Position of cell in pixels along the horizontal axis
y	float64	Position of cell in pixels along the vertical axis
frame	int32	Number of frame, with frame rate of 1 frame per second
mass short	float64	Brightness of cell in 340nm channel
bg short	float64	Background in 340nm channel
mass long	float64	Brightness of cell in 380nm channel
bg long	float64	Background in 380nm channel
ratio	float64	Calculated as mass short divided by mass long
particle	int32	Identification for each particle

Table 3.1: Description and data type of all columns present in the data matrix.

One recording can have between 500 and 10000 cells and is between 700 and 1000 frames long, which corresponds to between about 11 and 17 minutes. The ratio is typically between 0 and 5.

3.2 How it was generated

exprimental setup, what types of t cells where used?, apc layer, explain steps in experiment

3.2.1 Jurkat Cells, 5c.c7 primary mouse T cells and Fura-2

The prototypical cell line to study T cell signaling is the Jurkat cell line.[ML23] It was obtained from the blood of a boy with T cell leukemia.[SSB77] Different cell lines within the Jurkat family are described by Abraham and Weiss.[AW04] They provide a timeline of discoveries linked to Jurkat cells and t cell receptor signalling.

Another type of T cells used in signaling studies are gathered from mice. [Additional information]

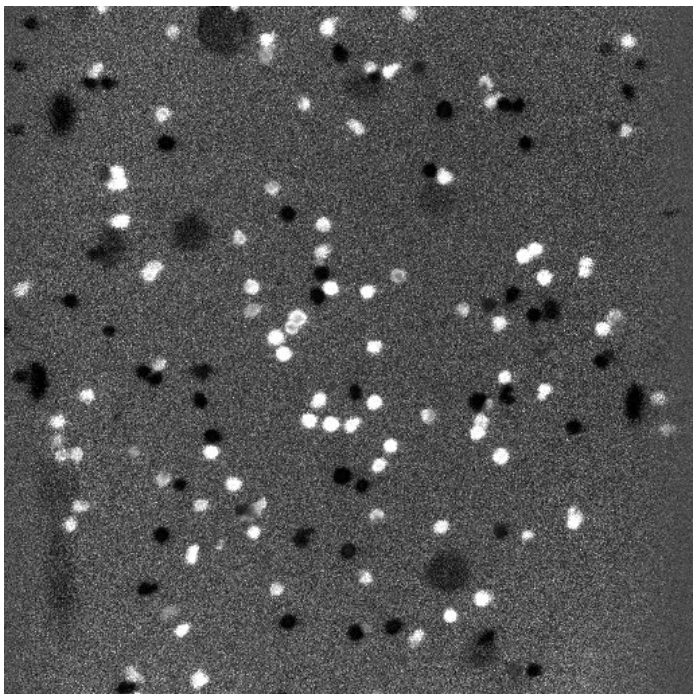

Figure 3.1: Single frame showing the ratio of the 340nm and 380nm images from a recording of human Jurkat cells. Activated cells apear lighter, unactivated cells darker than the background. Big dark circles are out of focus cells that have not yet settled on to the plate.

In order to be able to measure the intracellular Ca^{2+} concentration of cells they can be labelled with Fura-2. This method provides a way to record the Ca^{2+} concentration of multiple cells over a time period.[MMS17] Challenges encountered when using Fura-2 on certain cell types are described by Roe, Lemasters and Herman along with their respective solutions.[RLH90]

3.2.2 Measuring Calcium Concentration

After the cells have been labeled with Fura-2 an recording of up to 15 minute can be generated. To achieve this the cells and stimulant are photographed at both 340nm and 380nm wavelength once per second. The resolution of the images are 1.6um per pixel. By calculating the ratio of the two images at each point the Ca^{2+} concentration can be observed. An examplary resulting image showing the ratio is shown in figure 3.1. The T cells are appear a lighter shade than the background.

To activate the cells in the duration of the recording they are transfered to a plate covered with replicas of the MHC-peptide complex normally present on APCs. For a negative control the plate is not covered with peptides, while for the positive control the plate is covered very densly. Recordings of different densities in peptides lead to activation of only some of the t cells.

3.2.3 Processing

To track single t cells moving around during the video the sum of the 340nm and 380nm image for each second is calculated. In this image it is easier to separate t cells from the background. Therefore it is used to track the movement of cells. Each cell is numbered, such that the same cell will have the same number during as much of the video as possible. The position and shade during both 340nm and 380nm as well as the ratio of each particle and each frame is then recorded into the data structure used in this work. The first 50 frames at the start of the recording are discarded due to the video being out of focus. Additionally cells only appearing in fewer than 20 frames are discarded as they most likely represent trackactories incorrectly tracked.

4 Optimization Algorithm

objective, mathematical formulation of problem

4.1 Algorithm Name

algorithm description
  pseudo code for algorithm
  [proof of convergence, if applicable]

5 Results

6 Conclusion

Bibliography

[AH24] K Maude Ashby and Kristin A Hogquist. "A guide to thymic selection of T cells". In: *Nature Reviews Immunology* 24.2 (2024), pp. 103–117.

[AW04] Robert T Abraham and Arthur Weiss. "Jurkat T cells and development of the T-cell receptor signalling paradigm". In: *Nature reviews immunology* 4.4 (2004), pp. 301–308.

[CA22] Geoffrey M Cooper and Kenneth Adams. "The Nucleus". eng. In: *The cell: a molecular approach*. 19. edition. Oxford University Press, 2022, pp. 336–364. ISBN: 9780197583722.

[Gan12] William F. Ganong. "Overview of Cellular Physiology in Medical Physiology". eng. In: *Review of medical physiology*. 24. edition. Stamford, Conn: McGraw-Hill, 2012, pp. 35–66. ISBN: 9780071780032.

[Gan97] William F. Ganong. "Circulating Body Fluids". eng. In: *Review of medical physiology*. 18. ed. Stamford, Conn: Appleton & Lange, 1997, pp. 486–488. ISBN: 9780838584439.

[Hag18] Kimberly Hagel. *Positive and Negative Selection of T Cells*. 2018. URL: https://immunobites.com/2018/08/20/positive-and-negative-selection-of-t-cells/ (visited on 06/21/2024).

[JRB14] Noah Joseph, Barak Reicher, and Mira Barda-Saad. "The calcium feedback loop and T cell activation: how cytoskeleton networks control intracellular calcium flux". In: *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1838.2 (2014), pp. 557–568.

[KCF18] Brahma V Kumar, Thomas J Connors, and Donna L Farber. "Human T cell development, localization, and function throughout life". In: *Immunity* 48.2 (2018), pp. 202–213.

[Lew01] Richard S Lewis. "Calcium Signaling Mechanisms in T Lymphocytes". In: *Annual Review of Immunology* 19.Volume 19, 2001 (2001), pp. 497–521. ISSN: 1545-3278. DOI: https://doi.org/10.1146/annurev.immunol.19.1.497. URL: https://www.annualreviews.org/content/journals/10.1146/annurev.immunol.19.1.497.

[ML23] Jonathan Morgan and Alan E Lindsay. "Modulation of antigen discrimination by duration of immune contacts in a kinetic proofreading model of T cell activation with extreme statistics". In: *PLOS Computational Biology* 19.8 (2023), e1011216.

[MMS17] Magdiel Martínez, Namyr A Martínez, and Walter I Silva. "Measurement of the intracellular calcium concentration with Fura-2 AM using a fluorescence plate reader". In: *Bio-protocol* 7.14 (2017), e2411–e2411.

[RLH90] MW Roe, JJ Lemasters, and B Herman. "Assessment of Fura-2 for measurements of cytosolic free calcium". In: *Cell calcium* 11.2-3 (1990), pp. 63–73.

[Rog24] Kara Rogers. *endoplasmic reticulum*. 2024. URL: https://www.britannica.com/science/endoplasmic-reticulum (visited on 06/23/2024).

[SB16] Dianne S. Schwarz and Michael D. Blower. "The endoplasmic reticulum: structure, function and response to cellular signaling". In: *Cellular and Molecular Life Sciences* 73 (2016), pp. 79–94. DOI: https://doi.org/10.1007/s00018-015-2052-6. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4700099/.

[SI13] Peter B Stathopulos and Mitsuhiko Ikura. "Structural aspects of calcium-release activated calcium channel function". In: *Channels* 7.5 (2013). PMID: 24213636, pp. 344–353. DOI: 10.4161/chan.26734. eprint: https://doi.org/10.4161/chan.26734. URL: https://doi.org/10.4161/chan.26734.

[SKJ09] Jennifer E Smith-Garvin, Gary A Koretzky, and Martha S Jordan. "T cell activation". In: *Annual review of immunology* 27 (2009), pp. 591–619.

[SSB77] Ulrich Schneider, Hans-Ulrich Schwenk, and Georg Bornkamm. "Characterization of EBV-genome negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma". In: *International journal of cancer* 19.5 (1977), pp. 621–626.