



TECHNISCHE  
UNIVERSITÄT  
WIEN

B A C H E L O R ' S   T H E S I S

(**Arbeitstitel**)

submitted to the

Institute of  
Analysis and Scientific Computing  
TU Wien

under the supervision of

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## Acknowledgement



# Eidesstattliche Erklärung

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Wien, am 30. Juni 2024

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# 1 Introduction



## 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 particular 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 receive 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 moderately to the peptides presented, as a strong bond suggests 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  $\text{Ca}^{2+}$  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 simultaneous 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<sup>2+</sup> permeable ion channel on the ER:** There are several Ca<sup>2+</sup> channels present on the ER. Some receptors are responsible for releasing Ca<sup>2+</sup> into the cytoplasm, when the intracellular Ca<sup>2+</sup> concentration is low. [SB16]
- **Ca<sup>2+</sup> storage in the ER:** Ca<sup>2+</sup> is stored in the ER and can be released by Ca<sup>2+</sup> permeable 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<sup>2+</sup> 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<sup>2+</sup>. [SB16]
- **Plasm membrane:** A semipermeable 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<sup>2+</sup> release activated Ca<sup>2+</sup> channel (CRAC):** Opened after a decrease in ER stored Ca<sup>2+</sup> is sensed by STIM, these channels intake Ca<sup>2+</sup> 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<sup>2+</sup> signals play a vital part in t cell activation.

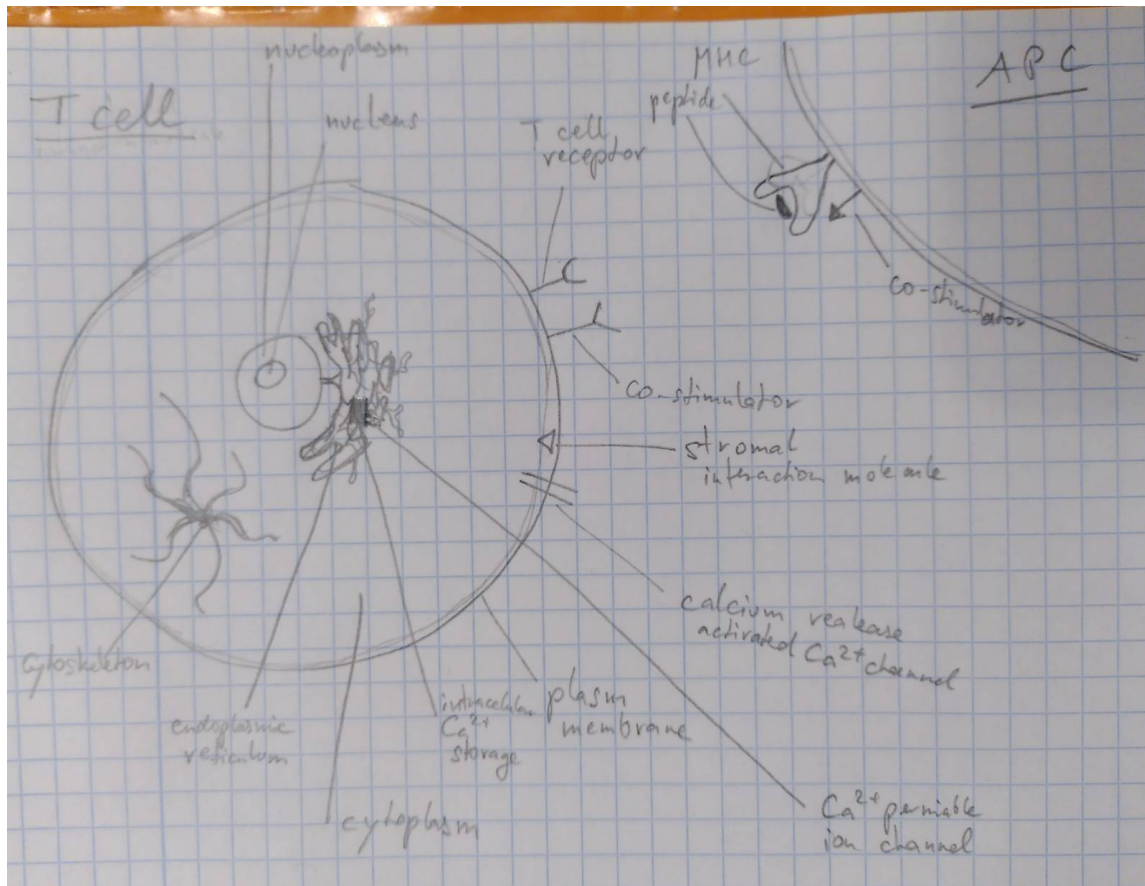


Figure 2.1: Schematic view of a t cell and antigen presenting cell, with all relevant components.

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

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

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

### 2.3 Recording Calcium Concentration during Activation

## 3 Data

calcium concentration shows activatedness of t cells (reference chapter t cells), relatively easy to measure

### 3.1 Structure of Data

what format is the data in? which columns are present + datatypes

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.

### 3.2 How it was generated

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

- Date: 18/12/23
- Cells: Jurkat wt labelled with Fura-2
- Sample: PDMS coated with OKT3 (positive control)
- Imaging: SDT3, ratiometric Ca imaging, 340nm & 380 nm, Total cycle time 1000ms (-1 1 frame per sec in sum/ratio image)
- pixel size: 1.6 um / px

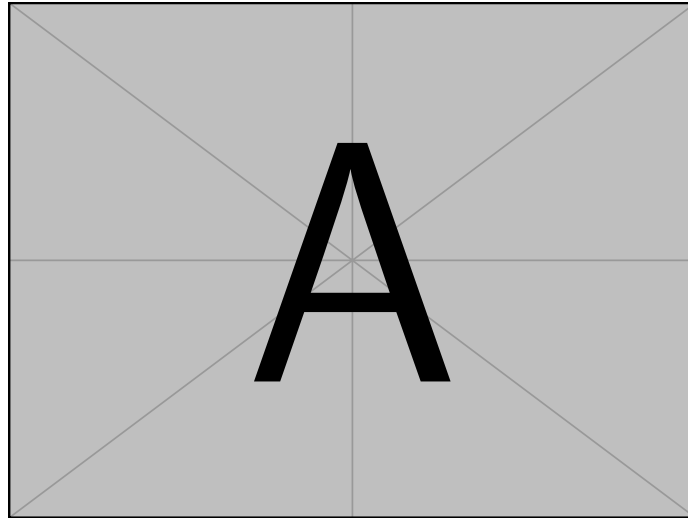


Figure 3.1: Single frame showing the ratio of the 340nm and 380nm images.

#### 3.2.1 Jurkat 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.

In order to be able to measure the intracellular  $\text{Ca}^{2+}$  concentration of cells they can be labelled with Fura-2. This method provides a way to record the  $\text{Ca}^{2+}$  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 up to 15 minute recording 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  $\text{Ca}^{2+}$  concentration can be observed. An exemplary resulting image showing the ratio is shown in figure 3.1. The T cells appear a lighter shade than the background.

#### 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**





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