

# **Dynamic HIV/AIDS Parameter Estimation with Applications**

by

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## Summary

### Dynamic HIV/AIDS Parameter Estimation with Applications

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This dissertation is primarily concerned with dynamic HIV/AIDS parameter estimation, set against the background of engineering, biology and medical science. The marriage of these seemingly divergent fields creates a dynamic research environment that is the source of many novel results and practical applications for people living with HIV/AIDS.

A method is presented to extract model parameters for the three-dimensional HIV/AIDS model in situations where an orthodox LSQ method would fail. This method allows information from outside the dataset to be added to the cost functional so that parameters can be estimated even from sparse data. Estimates in literature were for at most two parameters per dataset, whereas the procedures described herein can estimate all six parameters.

A standard table for data acquisition in hospitals and clinics is analyzed to show that the table would contain enough information to extract a suitable parameter estimate for the model.

Comparison with a published experiment validates the method, and shows that it becomes increasingly hard to coordinate assumptions and implicit information when analyzing real data.

Parameter variations during the course of HIV/AIDS are not well understood. The results show that parameters vary over time. The analysis of parameter variation is augmented with a novel two-stage approach of model identification for the six-dimensional model. In this context, the higher-dimensional models allow an explanation for the onset of AIDS from HIV without any variation in the model parameters.

The developed estimation procedure was successfully used to analyze the data from forty four patients of Southern Africa in the HIVNET 28 vaccine readiness trial. The results are important to form a benchmark for the study of vaccination. The results show that after approximately 17 months from seroconversion, oscillations in viremia flattened to a  $\log_{10}$  based median set point of 4.08, appearing no different from reported studies in subtype B HIV-1 infected male cohorts. Together with these main outcomes, an analysis of confidence intervals for set point, days to set point and the individual parameters is presented. When estimates for the HIVNET 28 cohort are combined, the data allows a meaningful first estimate of parameters of the three-dimensional HIV/AIDS model for patients from southern Africa.

The theoretical basis is used to develop an application that allows medical practitioners to estimate the three-dimensional model parameters for HIV/AIDS patients. The program demands little background knowledge from the user, but for practitioners with experience in mathematical modeling, there is ample opportunity to fine-tune the procedures for special needs.

### Keywords

HIV/AIDS model parameters, dynamic systems, dynamic parameter estimation, parameter variation, viral set point estimation, bioengineering, optimization and medical systems.

## Opsomming

Hierdie verhandeling se primêre fokus is 'n dinamiese MIV/VIGS parameter beraming, wat op die agtergrond van ingenieurswese, biologiese- en mediese wetenskappe baseer is. Die samevloei van hierdie diverse navorsingsvelde skep nuwe geleenthede van waar 'n veelvoud nuwe resultate en praktiese toepassings gevind is vir mense wat met die MI-virus leef.

'n Metode is voorgestel wat model parameters van die driedimensionele MIV/VIGS model kan beraam in situasies waar 'n tradisionele metode sou vaal. Hierdie nuwe metode maak voorsiening om van informasie, wat van externe bronne verkry is, gebruik te maak om te verseker dat parameters gevind kan word, selfs met 'n tekort aan data. In die literatuur word op die meeste twee parameters per dataset beraam, waar hierdie prosedure al ses parameters kan vind.

'n Standaard tabel wat in hospitale en klinieke gebruik word om data in te samel is hier getoets om te verseker dat die tabel genoeg informasie bevat vir die beraming van parameters.

Die prosedure word gebruik om data van 'n gevestigde experiment te analiseer en om so-doende die nuwe prosedure in perspektief te stel. Die vergelyking van resultate maak dit duidelik dat aannames en gempliseerde informasie fyn bestudeer moet word om te verseker dat resultate met die realiteit klop.

Die verandering van parameters met die verloop van MIV/VIGS is steeds 'n vraagstuk. Resultate in hierdie verhandeling dui daarop dat daar wel veranderings in die parameters is. Die analise van die verandering van parameters word gesteun deur 'n nuwe tweefase benadering tot parameter estimasie vir die sesdimensionele MIV/VIGS model. Die aanslag tot die probleem in hoër dimensies verduidelik die progressie van MIV tot VIGS, sonder enige verandering in parameters.

Die ontwikkelde metode is suksesvol gebruik om die data van vier en veertig pasiënte van die Suid-Afrikaanse HIVNET 28 inentingsstudie te analiseer. Die resultate is belangrik om 'n standaard te stel waarteen die effektiwiteit van 'n entstof gemeet kan word in toekomstige studies. 'n Analiese van statistiese intervalle vir die ekwilibrium, die dae tot ekwilibrium en individuele parameters is gedoen en 'n eerste braming van parameters vir die driedimensionele model vir Suid-Afrikaanse pasiënte is gemaak.

Die teoretiese grondslag is gebruik om 'n rekenaar program te ontwikkel wat medici in staat stel om die parameters van die driedimensionele model vir individuele pasiënte te bepaal. Die program benodig minimale rekenaar kennis van die gebruiker en gebruikers met 'n agtergrond in wiskundige modellering kan die program vir spesiale behoeftes aanpas.

### Sleutelwoorde

MIV/VIGS model parameters, dinamiese parameter estimasie, parameter variasie, virale gesadigde toestand estimasie, bioingenieurswese, optimisasie and mediese sisteme.

## Abbreviations

HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
HAART	Highly active antiretroviral therapy
LSQ	Least square
ODE	Ordinary differential equation
HIVNET 28	A vaccine readiness trial in southern Africa

## Nomenclature for the Basis Model

### Variables

$T$	Healthy CD4 <sup>+</sup> T cells
$T^*$	Infected CD4 <sup>+</sup> T cells
$v$	Free virus

### Parameters

$s$	Source constant for healthy CD4 <sup>+</sup> T cells
$d$	Death rate constant of healthy CD4 <sup>+</sup> T cells
$\beta$	Infection rate constant
$\delta$	Death rate constant for infected CD4 <sup>+</sup> T cells
$c$	Clearance rate constant of virus particles
$k$	Virus production rate constant

### Notational

$\chi$	The vector of model parameters $[s \ d \ \beta \ \delta \ c \ k]^T$
$\hat{\chi}$	The vector of model parameter estimates $[\hat{s} \ \hat{d} \ \hat{\beta} \ \hat{\delta} \ \hat{c} \ \hat{k}]^T$
$\vec{x}(t)$	The state vector $[T(t) \ T^*(t) \ v(t)]^T$
$\vec{x}_0$	The initial state vector $[T_0 \ T_0^* \ v_0]^T$

## Acknowledgements

*Lord remind us that we must die, so that we shall become wise.*

I am truly thankful for the opportunity I have to do research in an amazing field, where people from diverse backgrounds find a common denominator. I consider myself to be blessed. Throughout the past years I have come to know death as a companion and in this research area one is always reminded of the human struggle to extend life. I want to express my utmost gratitude towards Jesus Christ, who lived a life of true love, died for me and claims to be alive today. Through him I find comfort in the face of death and he allows me to live my life in hope.

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Mom: thank you for keeping me in your prayers and for living by example. I appreciate your love, faith and commitment.

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Van Zyl: you are family! Thanks buddy, jy's 'n staatmaker.

To all friends, fellow students and acquaintances who have reminded me throughout my studies that there is more to life than academics: thank you! You allowed me to gain valuable perspective to ensure that my work could flow from my life and not the other way round.

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### **My prayer**

God, if you are listening, please be with all those who live with HIV/AIDS. Thank you for the opportunities that I have to do research in such an interesting field. Remind me every day that I am only human and I will die. Lord, I appreciate the research opportunities that I have, yet I would rather have seen solutions to HIV/AIDS. At the moment it seems that things are different. Please, let there be light. I am afraid of a future where AIDS is rampant. Lord, let there be cures. Father, if you are behind the wonderful discoveries that we make throughout research, I thank you!

Dedicated to my father and friend:  
Oswald Heinrich Filter  
(1948–2003)

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# **Part I.**

## **Background and Methods**

# 1. Introduction

## 1.1. Background

**H**UMAN IMMUNODEFICIENCY VIRUS (HIV) and the associated Acquired Immunodeficiency Syndrome (AIDS) are the focus of active research in numerous medical institutions. The grave future of millions of people living with HIV/AIDS is one of the driving forces behind the near frantic effort to find effective ways to combat HIV. The economic impact on countries like South Africa will be extreme, with difficulties that are compounded by an increasing number of AIDS orphans in need of care, together with AIDS patients who need medical attention [1].

In an effort to curb AIDS, several programs have been initiated and new institutions have come to life. Programs that promote AIDS education are a high priority, since there is no cure for HIV/AIDS [2].

On the medical side there are two main fronts of research, namely prevention and treatment of HIV/AIDS. Significant progress in the area of antiretroviral medication has been made in the past decade, leading to the ability to suppress HIV particles below detectable levels for standard tests [1, 3–5]. This medication is used to treat HIV/AIDS patients and to lower the probability of mother-child virus transmission. The final step to eradicate the virus, though, has not been achieved.

In the past, vaccination was an effective tool to fight and eradicate virulent diseases. Vaccination is thus an actively researched area of HIV/AIDS [6]. Even though a vaccine might be found, there are still millions of people living with HIV, underlining the importance of research in the treatment of infected individuals.

The combination of mathematical modeling with highly active antiretroviral therapy (HAART) has helped to reshape the perception of the disease. HAART therapy is extremely effective compared to earlier therapy and a rapid decrease of virus particles was noted after initiation of therapy in trials. When these findings were interpreted in the context of mathematical models, key parameters were extracted. Half-lives for infected cells and virus clearance were estimated to be in the order of days and even hours,

which highlighted the dynamic nature of HIV. This discovery changed the perception that HIV/AIDS is a slow disease and indicated that more frequent blood samples are necessary in monitoring the natural history of the disease. Before this discovery samples were typically taken every six to twelve months [7–10].

Despite many advances and continuous research in HIV/AIDS, a vast amount of topics remain to be explored [5]. The latest addition of experts involved in HIV/AIDS research are control systems engineers. This addition resulted from the opportunity to apply established results from the control theoretic field to the mathematical models of HIV/AIDS. It is in the context of modeling, that control engineers can apply their knowledge and use established techniques to help scientific discovery and formulate guidelines for clinical tests and practical measurements [1].

As indicated in [5], there are still many uncertainties in the area of HAART. The best time to initiate therapy, dosage levels and the optimal combination of drugs are areas currently demanding further research. Together with these problems there is the dilemma of side effects of the different drugs. There is thus a need to determine the influence of HAART on the virus and on the immune system on an individual basis. This is necessary to insure that each patient can be treated according to personal needs.

A model that describes the interaction of HIV with the patient's immune system and the influence of drugs on the virus can be a helpful tool to decide on treatment strategies in HIV/AIDS patients. The dynamic three-dimensional model as described in [11] forms the basis of this research. Even though there are general observations that can be made from biological models and their structure, clear benefits in the treatment strategy arise only when a model is tailored to each patient's individual parameters. Furthermore, the viral load in HIV patients may be a critical endpoint in vaccine trials by which to judge efficacy [6]. In this context, models are used to estimate future viral load levels for patients with early HIV infection.

In order to use a mathematical model in practical situations, its parameters must be determined from measurements that are accessible to local health services. Ideally, the model should also allow for generalizations of parameters from other estimates and research.

The dynamic three-dimensional HIV/AIDS model is the basis of active research [1]. Since all parameters of this model can be determined from  $CD4^+$  T cell levels and viral load in blood [12]—as opposed to higher-dimensional models [13]—, the three-dimensional model is a good starting point for practical applications.

This dissertation is primarily concerned with dynamic HIV/AIDS parameter estimation, set against the background of engineering, biology and medical science. As will become clear in the following pages, the marriage of these seemingly divergent fields creates a dynamic research environment and is the source of many novel results.

## 1.2. Problem statement

Currently there are numerous approaches to modeling HIV/AIDS [4]. A common basis for dynamic models is the three-dimensional model as described in [4, 11, 14]. Since this is the proposed basis model for this research, the model must be considered within the context of other models to determine its strengths and weaknesses. The model choice should be substantiated with theoretical, simulated and practical results [15].

Until now, much of the research into dynamic HIV/AIDS models has included simplifications and assumptions in order to estimate up to two parameters of the three-dimensional model for groups of patients. In this research a procedure is developed to estimate all six parameters of individual patients, subject to the constraints in [12]. Innovative use of optimization methods is called for in this context, where the nonlinear nature of the problem necessitates the implementation of state-of-the-art procedures.

At the same time, the input from medical experts and practical experience has to be incorporated in the theoretical field of parameter estimation. Here the ability to translate medical diagnosis and symptomatic information into tangible guidance mechanisms for the estimation routines is required.

The three- and four-dimensional basis models cannot account for all observations in the pathogenesis of HIV/AIDS. Unfortunately, when the model is expanded to broaden its applicability, each added dimension increases the complexity of the model and its analysis. Consequently, additional measurements are required to determine patient specific parameters.

Since the three-dimensional model alone cannot account for the progression from HIV to AIDS, the variation of parameters must be analyzed to find possible dependencies between parameters and allow for the refinement of the basis model [15]. There is a trade-off between model accuracy, model complexity, and the amount of measurements needed to determine model parameters; in practical situations it is often impossible to adhere to schedules where frequent blood samples are needed over extended periods. This

research must allow for simplification and adaption of established estimation routines to help with the analysis of higher-dimensional models.

The relevance of the three-dimensional model in the medical decision making process should be reviewed. The interpretations of model parameters and recommendations that can be made from such interpretations must be considered, and those properties that can be used to support the key decisions in HIV/AIDS treatment must be highlighted. This will ensure that the medical community can benefit from research conclusions.

The availability of data that can be used to test and analyze results is a key consideration in this research. For each patient there is a set of parameters that must be determined before the simulation of different treatment scenarios can begin. In order to determine these parameters, it is necessary that the correct measurements are available. Medical guidelines are often in disagreement with the requirements for successful identification of parameters [5, 14]. Also, many data sets are minimal due to the cost of measurement, or the lack of knowledge about the requirements for HIV/AIDS parameter identification. Procedures need to be adaptable to make effective use of results from established research and information from sources outside the direct measurement set. This is done with the aim to help practitioners who have symptomatic evidence for patients to refine the results and help with treatment decisions for individual patients.

For both the three-dimensional and four-dimensional HIV/AIDS models, a thorough analysis of the minimal set of necessary measurements was performed in [12]. This analysis was extended to a six-dimensional model in [13]. From the results in [12, 13], it is clear that the theoretical minimal set of measurements is not always applicable to practical situations where uncertainties in measurements are present together with external influences. This highlights the need to integrate theoretical knowledge into practical procedures.

Even though it was shown that the model parameters for the basis model can be determined from specific measurement sets, the procedures to do so were left for future development [14]. This research must rely on results from control theory, numerical methods and the wealth of information in mathematical modeling, to synthesize a procedure that is applicable to the identification problem at hand. At the same time, dynamic HIV/AIDS parameter estimation should be accessible to a wide audience, through the use of current technology.

Data for the HIVNET 28 vaccine readiness trial [6] conforms to the minimum identification requirements in [12]. Analysis of this data in a group context calls for the

application of parameter identification and the interpretation of results. In addition to the basic analysis, the results must be analyzed from a statistical perspective in order to find confidence intervals on the estimate that allow a benchmark to be set for future comparison.

### 1.3. Research contribution

A parameter estimation routine with refinable cost function has been synthesized that can be used to estimate all parameters of the dynamic three-dimensional HIV/AIDS model [16,17]. This is the first procedure that endeavors to estimate all six parameters of the basis model from CD4<sup>+</sup> T cell and viral load data for individual patients, following the findings in [12].

The estimation procedure was used successfully to analyze the data from the HIV-NET 28 vaccine readiness trial. Even though the data per patient is sparse, the extraction of the average set point, the time from seroconversion until this set point is reached and a group estimate of parameters was possible [6,17]. The results are used to form a benchmark against which the effectivity of vaccines can be measured [6].

It has been noted that patients' parameters may change during the course of the disease [18]. To confirm this, a novel approach is followed to analyze the variation of parameters for the three-dimensional model over disease progression from HIV infection to the onset of AIDS. The results also give a phenomenological indication of the parameter variations [16,17].

The analysis of parameter variation is used as a foundation to examine the data of [10] from the perspective of a multi-compartmental model. It is shown how the results in [17] can be interpreted to allow parameter identification in such models. The results from the analysis are linked to an established multi-compartmental model and allow a first explanation for disease progression from HIV to AIDS, based solely on dynamical compartmental models. This addresses a major concern about earlier models [15].

The estimation routine forms the basis for a computer application that allows patients, practitioners and researchers to use the developed routine in practical situations [19].

By initially focussing on the problem of parameter identification for the dynamic three-dimensional HIV/AIDS model, it was possible to implement a procedure that has



proven applicable to a wide range of problems. The field of biological modeling, and specifically HIV/AIDS modeling, is riddled with unsolved questions. This document presents solutions to some of these problems – solutions that generate exciting opportunities and, in turn, pose many new questions for the interested researcher. As numerous researchers have come to know: A good question is worth a thousand answers [42].

## 1.4. Organization of document

This document is organized in the following manner:

Chapter 1, of which this is a subsection, introduces the background against which the dissertation is set. The problem statement is laid out, followed by a description of the contribution that this study has made to research. The introductory chapter is concluded with a description of the organization of the document.

Chapter 2 gives an overview of dynamic HIV/AIDS models, with specific attention to the basis model used in the research. The overview of these models is taken from literature and combined with the description of some fundamental decisions that were made to establish the model type and scope for this research. The chapter starts with an introductory overview of biological modeling principles and the role of control engineers in this field. From here, dynamic HIV/AIDS models are introduced, with a detailed description of the three-dimensional model, its equations, governing dynamics and steady state. This is followed by a description of higher dimensional models that are based on, and extend, the three-dimensional model. Finally the model choice for this research is discussed in the context of identifiability and the influence of proliferation terms on the basis model.

Chapter 3 presents different parameter estimation procedures, focussing on the methods that are implemented for this research. The chapter starts out by discussing a standard least square estimate for the parameters of the basis model as described in literature. The drawbacks and limitations of the standard method are considered and an estimation procedure is presented that is based on least square distance but includes additional customizable penalty terms. Modifications and variants of this method are discussed in the context of the basis model and additional refinements of the procedure are presented. Finally, the search method used in this research is discussed together with some alternatives.

Chapter 4 is the first of three chapters discussing the results of this research. Results are grouped together with their relevant discussions and preliminaries to the conclusions

to facilitate continuity in the document. This allows for a more concise presentation of the conclusions in chapter 7, with the relevant information grouped together for the interested reader.

Chapter 4 gathers the general results of this research. The first part of the chapter contains simulation results that depict the influence of a proliferation term on the three-dimensional model. This is followed by results from validating the estimation routine against generated data that show the influence of additional penalty terms in the cost function. The applicability of data-collection tables with a reduced number of data-points is investigated in the context of practical data collection at hospitals. Next, a published experiment [4,8] is reproduced to validate the estimation procedure in the context of accepted results from literature. In this experiment the influence of assumptions and outside information are exemplified. The chapter is concluded with an investigation into the variation of parameters of the basis model throughout the pathogenesis of HIV/AIDS. First a phenomenological approach is followed to find possible variations in parameters. These results are then interpreted to explore dual compartmental dynamics as an alternative to blind variation of parameters. This exploration exemplifies the adaption of the estimation procedure to investigate complex systems from the foundation of the basis model. Finally, the results of the dual compartmental dynamics are linked back to an established model to allow additional perspective on the estimated parameters.

Chapter 5 presents the results from the HIVNET 28 vaccine readiness study in southern Africa. First the context of the study is described. Since the HIVNET 28 study has a slightly different emphasis than the primary aim of this research, the procedure is tailored to accommodate the given situation, exemplifying the flexibility of the estimation procedure. The adaption of the procedure for the application to a cohort of patients is presented. This includes a strategy to include patients in the study that would not meet the identifiability requirements under normal circumstances. Next, the necessary assumptions are laid out for parameter estimation in the HIVNET 28 context. This is followed by a presentation of the actual set point estimations and other results. Finally the confidence intervals and sensitivity analysis of estimations for the HIVNET 28 study are presented.

Chapter 6 introduces a computer program as a practical application of the research. The link between theory and practice is discussed, and a summary of the key features of the program are given. Finally, example figures detailing key aspects of the program are presented with a discussion of necessary additions and possible expansions.

Chapter 7 exhibits the conclusions for this research. In addition to these, impetus for further research is presented and open questions are posed.

Appendices at the end of the document contain supplemental information pertaining to the program code, application usage and other material.

Each chapter is concluded with a short summary, highlighting the main results and conclusions contained therein.

## 2. Model Review

### 2.1. Biological modeling

**B**EFORE delving into the models specific to HIV/AIDS, it is fitting to give some preliminary background on biological models in general. In the preface of [20], the authors give a brief overview of the state of biological modeling:

In the past few decades, biology has developed from a mostly observational discipline of classification and description into a branch of science that involves complex theories and mathematical models. There is an increasing need for today's research biologist to formulate, and to study the behavior of, a wide range of mathematical models. Some models are stochastic and others deterministic, describing a cross-section of a population at a fixed time, or the governing dynamic processes. Some models are simply phenomenological descriptions while others involve detailed mechanisms and sub-mechanisms, even down to the level of the genetic code.

As well as formulating theories in precise mathematical form, and studying them, there is an important requirement to put the models to empirical test. This involves making observations and using the resulting data either to estimate aspects of the mathematical modes, or to test the theory. Even though many biological models are at present deterministic, biological phenomena differ from their physical counterparts by being subject to (seemingly) random influences. Thus the process of matching or comparing data with model predictions usually involves statistical methods. It is therefore important to understand and to use methods of formulating deterministic and stochastic models and fitting and testing them against real data [20].

In the field of modeling, and in this case biological modeling, the control engineer can make decisive contributions [1, 21, 22]. Where biology has its roots as an observational discipline, engineers have long strived to create new concepts and ideas to implement in ever more elaborate artifacts. The marriage of these seemingly divergent fields is bound to be fertile ground for creativity and exciting research.

In the spirit of balancing creativity, research and verification, John Skellam offers this

excellent advice to modelers in biology [23]:

“Roughly speaking, a model is a peculiar blend of fact and fantasy, of truth, half-truth and falsehood. In some ways a model may be reliable, in other ways only helpful and at times and in some respects thoroughly misleading. The fashionable dogma that hypothetical schemes can be tested in their totality in some absolute sense, is hardly conducive to creative thinking. It is indeed, just as great a mistake to take the imperfections of our models too seriously as it is to ignore them altogether. ...

Mathematical model-making in Population Dynamics looks easy, and so it is if we do not care about the uses and abuses to which our models may be put. In theory all that we have to do is to incorporate those things that matter and to ignore those things that do not matter, if we can, or if we can't, treat them in any arbitrary way that suits our mathematical convenience. By following the reassuring principle that conclusions hold more often than do the detailed premises, we can always entertain the prospect that results of wider applicability will emerge.

The snag is that it is not given to anyone to know beforehand which features matter and which do not, and in a real life context all sorts of things matter in varying degrees. The best we can do is to begin with schemes that may not be entirely realistic but which are at least meaningful to others as well as to ourselves.

It is important to scrutinize the models carefully and to explore numerous variants in order to allay fears and doubts created by the many artifacts we invariably introduce and glorify in the name of abstraction.

Finally we must seek empirical verification wherever and whenever possible, and in particular determine by empirical means the true range of applicability of the theoretical schemes and speculations that we have been bold enough to propose.”

It is hoped that this research reflects the great potential that can be unlocked when his advice is followed.

## 2.2. HIV/AIDS models

One of the key markers of HIV/AIDS progression is the decline of CD4<sup>+</sup> T cells in patients [5]. Many models have been developed to describe the immune system, its interaction with HIV, and the decline in CD4<sup>+</sup> T cells. Stochastic models can be used to account for events early in the disease, when the viral level is low and few cells are infected, or where the variability among individuals is of interest. One class of stochastic models has looked at the effects of increasing variability among viral strains in the progression to AIDS.

Deterministic models that examine the changes in mean cell numbers, have been developed by many authors. These models are more applicable to the later stages of the disease, where the population sizes are large. The models typically consider the dynamics of the CD4<sup>+</sup> T cell and virus populations as well as the effects of therapy. Other immune system populations, such as macrophages or CD8<sup>+</sup> cells, have been included in some of these models [24]. Many of these models, and especially those developed before 1995, have tended to focus on the kinetics of T cell decline. Unfortunately, many different models can mimic this aspect of HIV infection without providing additional insight into the disease [4]. For further details on these and other model types and their application, see [4, 12, 25] and the references therein.

The development of rapid, sensitive, and accurate methods for measuring the number of virus particles in blood provided the impetus for further dynamic modeling [8].

Because of the difficulties of doing experiments in humans, fundamental information was lacking about the dynamics of HIV infection. As an example, since the disease takes ten years, on average, to develop, it was assumed that the components of the disease process were slow as well. This turned out to be incorrect. Modeling, together with the appropriate experiments has revealed that HIV is a dynamic disease that encompasses time scales of hours, days, weeks and months [4, 7].

The focus of this chapter is on the dynamic models that have made a substantial impact on the understanding of HIV infection. These models follow a deterministic approach, and the three-dimensional version of these models forms the basis for analysis in the following chapters.

### 2.3. The three-dimensional model

A model that entails these faster virus dynamics is based on the interaction between healthy  $CD4^+$  T cells, infected  $CD4^+$  T cells and free virus particles in the bloodstream. The model has three variables representing these three concentrations in the bloodstream. This model is a good starting point for analysis of viral dynamics, especially in situations where steady state values are perturbed by means of effective treatment strategies, as for instance highly active antiretroviral therapy (HAART).

The background to this model is set out in [11]. The model describes the quantities of uninfected cells, infected cells, and free viruses in a system of three differential equations. It is assumed that uninfected cells encounter free virus to become infected cells. The rate of production of new infected cells is proportional to the product of the density of uninfected cells times the density of free virions. Free virions are produced by infected cells. All three populations die, and are cleared, at their respective rates, and it is assumed that the system constantly replaces uninfected cells. With these governing assumptions, a model of virus dynamics can be defined.

Suppose the system is initially uninfected and uninfected cells are at an equilibrium value. When a small amount of virus particles or infected cells are added to the system, invading virions might infect a number of healthy cells, which will produce new virions, which in turn can infect healthy cells. A chain reaction starts that can result in two scenarios: either the initial infection dies out, or it leads to an explosion of free virions and infected cells in the system. The critical value that determines whether or not the chain reaction is sustained, depends on the basic reproductive ratio of the infection, denoted by  $R_0$ . For viral infection,  $R_0$  is the average number of infected cells that derive from any one infected cell in the beginning of the infection. If, on average, every infected cell produces less than one newly infected cell, i.e.  $R_0 < 1$ , then the infection will not be established. If infected cells do produce more than one newly infected cell, i.e.  $R_0 > 1$ , the infection will take off.

In the latter case, virions and infected cells will grow exponentially at first, while uninfected cells stay roughly constant. As time progresses, the uninfected cells will decline and the exponential growth of virus will slow down. The abundance of virus will reach a maximum and subsequently decline, while at this same time, the uninfected cells will pass through a minimum and increase again. The system will display damped oscillation towards an equilibrium point.

At equilibrium, the virus is controlled by a limited supply of uninfected cells. On

average, every infected cell would give rise to one newly infected cell. The equilibrium level of uninfected cells is thus given by the number of uninfected cells prior to infection, divided by  $R_0$ . A large value for  $R_0$  will thus result in a greatly reduced level of uninfected cells.

An interesting feature of the model is that a cytopathic virus, which rapidly kills infected cells, will only achieve a small equilibrium abundance of infected cells, irrespective of the amount of virions produced by any one infected cell. In contrast, a non-cytopathic virus will lead to an equilibrium abundance of infected cells at a similar level to the original level of uninfected cells prior to infection. In both cases, the rate at which virions can infect target cells has only a minor effect on the equilibrium abundance of infected cells. This feature of the model may run counter to intuition, since one might be tempted to conclude that a high rate of infection will always result in a high level of infected cells, which is not the case.

Even with its relative simplicity the three-dimensional model can be used effectively to describe the primary stages of HIV infection (or SIV and other viral infections for that matter). The peak in virus load during the first weeks of infection with a subsequent decline to relative stability, is inherent in the model dynamics. The model helps to estimate rate constants of the initial virus expansion and decline. It is also possible to get an idea of the reproductive ratio,  $R_0$  during this stage. Extensive data from SIV infection show a strong correlation between the virus growth rate during the first week of infection and the steady-state virus load, which in turn is related to the rate of progression to disease and death.

### 2.3.1. Equations for the three-dimensional model

A three-dimensional model of HIV/AIDS consists of three variables: the population sizes of uninfected cells ( $T$ ), infected cells ( $T^*$ ), and free virus particles ( $v$ ). For this model it is assumed that free virus particles infect uninfected cells at a rate proportional to the product of their abundances,  $\beta vT$ . The rate constant,  $\beta$ , describes the efficacy of this process. Infected cells produce free virus particles at a rate proportional to their abundance,  $kT^*$ . Infected cells die at a rate  $\delta T^*$ , and free virus particles are removed from the system at a rate  $cv$ . Therefore, the average lifetime of an infected cell is  $1/\delta$  whereas the average lifetime of a free virus particle is  $1/c$ . The total amount of virus particles produced from one infected cell, the “burst size”, is  $k/\delta$ . If the production of CD4<sup>+</sup> T cells is considered to be constant,  $s$ , for the modeled period and the death rate is  $dT$  for the uninfected cells (average lifetime of an uninfected cell is thus  $1/d$ ), the



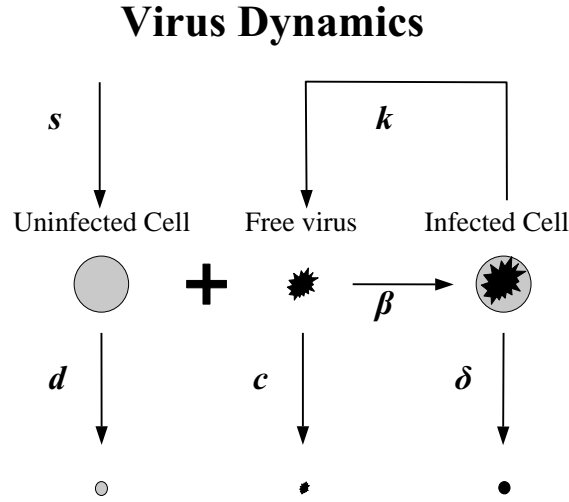


Figure 2.1.: Schematic illustration of the three-dimensional model of virus dynamics [11].

three-dimensional model of virus dynamics is obtained as in [4, 11]:

$$\begin{cases} \dot{T} &= s - dT - \beta T v, \\ \dot{T}^* &= \beta T v - \delta T^*, \\ \dot{v} &= k T^* - c v. \end{cases} \quad (2.1)$$

A schematic of this model can be seen in Fig. 2.1.

For ease of notation in this document, the following notation is defined for the three-dimensional model (2.1):  $\chi = [s \ d \ \beta \ \delta \ c \ k]^T$  is the vector of model parameters, and  $\hat{\chi} = [\hat{s} \ \hat{d} \ \hat{\beta} \ \hat{\delta} \ \hat{c} \ \hat{k}]^T$  the estimate of these parameters. The state vector  $\vec{x}(t) = [T(t) \ T^*(t) \ v(t)]^T$ , and the initial state vector  $\vec{x}_0 = [T_0 \ T_0^* \ v_0]^T$ .

### 2.3.2. Model dynamics

Before infection the viral load and infected  $CD4^+$  T cell load are fixed at zero with the uninfected  $CD4^+$  T cells at the steady state  $T = s/d$ . If an infection occurs at  $t_0 = 0$ , with a certain amount of virus particles,  $v_0$ , then the initial conditions are  $T_0 = s/d$ ,  $T_0^* = 0$  and  $v_0$ .

The infection is only established when a specific condition is met. The crucial quantity in this case is the basic reproductive ratio of the virus,  $R_0$ , which is defined as the

## Basic reproductive ratio

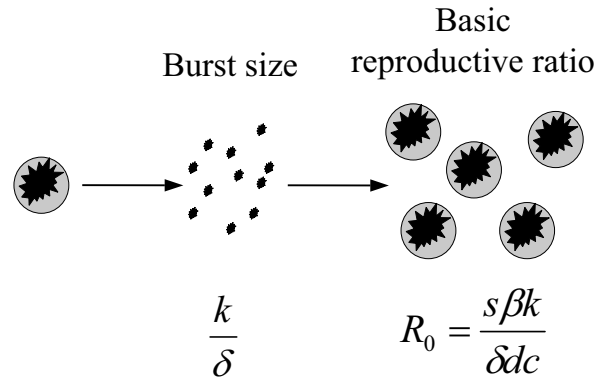


Figure 2.2.: Schematic illustration of the burst size and basic reproductive ratio [11].

number of newly infected cells that arise from any one infected cell when almost all cells are uninfected [11]. The burst size, which is the total number of virions produced from any one infected cell, and the basic reproductive ratio are illustrated in Fig. 2.2.

The rate at which one infected cell gives rise to new infected cells is given by  $\beta kT/c$ . Since the uninfected cell level is initially  $T = s/d$  and the lifetime of an infected cell is  $1/d$ , the basic reproductive ratio is given by [11]

$$R_0 = \frac{\beta s k}{\delta d c}. \quad (2.2)$$

If  $R_0 < 1$  then the virus will not spread, since infected cells will on average produce less than one newly infected cell and the chain reaction is sub-critical. If there are  $N$  infected cells at infection, then roughly  $\ln N / \ln(1/R_0)$  rounds of replication can be expected before the virus population dies out.

If  $R_0 > 1$  then every infected cell produces, on average, more than one newly infected cell and an explosive multiplication of virus will result with an initial growth of

$$v(t) = v_0 e^{r_0 t},$$

where the growth rate of the virus population,  $r_0$ , is given by the larger root of the equation  $r_0^2 + (\delta + c)r_0 + \delta c(1 - R_0) = 0$  [11]. Virus growth will not continue indefinitely,

since the supply of uninfected cells is limited. There will be a peak in viral load and subsequently damped oscillation towards an equilibrium.

### 2.3.3. Steady state

The steady state of the model is also called the equilibrium state or, the variables have reached a set point. The steady state values of uninfected CD4<sup>+</sup> T cells, infected CD4<sup>+</sup> T cells and viral load in (2.1) are given by [11]

$$T_s = \frac{\delta c}{\beta k} = T_0/R_0, \quad (2.3)$$

$$T_s^* = \left(\frac{ks\beta}{\delta dc} - 1\right) \frac{dc}{\beta k} = (R_0 - 1) \frac{dc}{\beta k}, \quad (2.4)$$

$$v_s = \left(\frac{ks\beta}{\delta dc} - 1\right) \frac{d}{\beta} = (R_0 - 1) \frac{d}{\beta}. \quad (2.5)$$

At steady state, one infected cell will on average give rise to one newly infected cell. The probability that a cell remains uninfected during its lifetime is  $1/R_0$ .

The ratio of uninfected cells before infection and at steady state is  $T_0/T_s = R_0$ . If  $R_0 \gg 1$ , then  $T_s$  will be much less than  $T_0$ , which means that the uninfected cell level is much smaller at steady state than it was before infection. Also in this case, the steady state abundance of infected cells and free virus is approximately given by  $T_s^* \approx s/\delta$  and  $v_s \approx (sk)/(\delta c)$ . Note that both quantities do not depend on the infection parameter  $\beta$ . Intuitively this is because a highly infectious virus (large  $\beta$ ) will rapidly infect uninfected cells, but at steady state there will only be few uninfected cells in the system, whereas a less infectious virus (smaller  $\beta$ ) will take longer to infect uninfected cells, but at steady state the abundance of uninfected cells is higher. For both viruses the product  $\beta T_s$  will be similar at steady state and result in a constant rate of production of infected cells and similar steady state abundances of infected cells and free virus.

For a highly cytopathic virus ( $\delta \gg d$ ), the steady state abundance of infected cells will be small compared to the abundance of cells prior to infection. Thus larger values of  $\delta$  result in lower levels of both infected cells and free virus.

For a non-cytopathic virus ( $\delta \approx d$ ), the steady state level of infected cells will be close to the total abundance of susceptible cells prior to infection.

## 2.4. Higher-dimensional models

As described in the survey paper [1], there are some refinements made to the model (2.1) by different authors.

The pool of virus producing CD4<sup>+</sup> T cells has been estimated to be very small ( $3 \times 10^7$  [26]). If there would be no other source of virus and the death rate of infected cells is taken to be 0.45 (see section 4.3), it is estimated that the CD4<sup>+</sup> T cell pool could have been eradicated in about 25 days ( $3 \times 10^7 \exp(-0.45 \times 25) < 1$ ).

There are additional cellular reservoirs of virus. A quantitative image analysis technique was used to reveal the viral burden in lymphoid tissues [27], particularly on the surface of follicular dendritic cells (FDC). Perelson *et al* observed that, after the rapid first phase of decay during the initial 1–2 weeks of antiretroviral treatment, plasma virus levels declined at a considerably slower rate [4]. This second phase of viral decay was attributed to the turnover of a longer-lived virus reservoir of infected cell population, which was determined to have a half-life of 1–4 weeks. This means that on average it would take between two and a half, and three years of perfectly effective treatment to eradicate the virus. This possibility generated optimism for the eradication of HIV in 1996 [3].

The long-lived cell model takes the following form

$$\begin{cases} \dot{T} &= s - dT - \beta T v, \\ \dot{T}^* &= \beta T v - \delta T^*, \\ \dot{M} &= s_M - d_M M - \beta_M v M, \\ \dot{M}^* &= \beta_M v M - \delta_M M^*, \\ \dot{v} &= k T^* + p_M M^* - c v. \end{cases} \quad (2.6)$$

Here  $M^*$  denotes the concentration of productively infected long-lived cells. While the long-lived cell model fits the patient data, it is not the only reasonable biological model. The source underlying the second-phase kinetics might be the release of virions trapped in the lymphoid tissues. It might be linked to infected macrophage cells [28], or it could also be due to the activation of latently infected cells. The latently infected cells are cells that harbor HIV DNA as a provirus, but are not producing virus. These cells, when activated into cell division, reproduce their DNA, and in the process read the viral DNA. The latently infected cell model will be described below.

Both the latently infected cell model and the long-lived cell model make identical predictions for the course of second-phase decay, and the primary source of second-

Table 2.1.: Virus reservoirs and life spans

Cell	Size	Half-live	Eradication
Active CD4	$3 \times 10^7$	1 day	25 days
FDC	$3 \times 10^8 - 10^{11}$	1–4 wks	0.5–2.8 yrs
Macrophage	n.a.	1–4 wks	n.a.
Memory CD4	$10^5 - 10^6$	6–44 mon	9–72 yrs

phase virus is linked to long-lived cells in [4]. This prediction is based on an improved expression obtained from (2.6) by assuming perfect inhibition ( $\beta = 0$  and  $\beta_M = 0$ ) [4],

$$v(t) = A \exp(-\delta t) - B \exp(-ct) + C \exp(-\delta_M t).$$

Continued monitoring of patients on HAART for extended periods has provided evidence for the existence of a possible reservoir of long-lived CD4<sup>+</sup> memory T lymphocytes, resulting in a third phase of HIV decay being observed during HAART. The kinetics of this decay are extremely slow, and the half-life of the memory cell reservoir has been estimated at between 6 and 44 months. As a result the predicted time required for effective antiretroviral therapy to eradicate HIV from the body could range anywhere between 9 and 72 years. This suggests that a true virologic cure is unattainable using the conventional antiretroviral regimens.

These findings are described in [29] and the results summarized in Table 2.1.

### 2.4.1. Model with latently infected cells

In [1] the following model is considered to be one of the most popular models in current use:

$$\dot{T} = s + r\phi(T) - dT - \beta v_1 T, \quad (2.7)$$

$$\dot{T}_1 = q_1 \beta v_1 T - \delta_1 T_1 - k_1 T_1, \quad (2.8)$$

$$\dot{T}_2 = q_2 \beta v_1 T + k_1 T_1 - \delta_2 T_2, \quad (2.9)$$

$$\dot{v}_1 = k_2 T_2 - cv_1, \quad (2.10)$$

$$\dot{v}_2 = k_3 T_2 - cv_2. \quad (2.11)$$

In this model, (2.7) describes the population dynamics of the uninfected CD4<sup>+</sup> T cells. It is a one-compartment model, and  $T$  is identified with the CD4<sup>+</sup> T cell counts

in blood.  $s$  is the rate at which new  $CD4^+$  T cells are created from sources within the body, such as the thymus.  $CD4^+$  T cells can also be created by proliferation of existing T cells. The second term,  $r\phi(T)$ , denotes the rate of proliferation according to the rate constant  $r$  and function  $\phi$ . The death rate constant of uninfected cells is denoted by  $d$ . HIV infection is represented by the “mass-action” term of  $\beta v_1 T$ , with  $\beta$  being the infection rate constant and  $v_1$  the concentration of infectious virions.

A common assumption among authors is that the source term,  $s$  is constant, and the proliferation effect may be lumped into the death rate constant,  $d$  [11]. This assumption reduces the timescale over which the model can be applied. Since proliferation is lumped with the death rate constant, one must also accept that  $d$  might be negative.

The thymus and bone marrow can be infected by HIV, thus it is believed that the source term is a decreasing function of viral load. For example,  $s(v) = \theta s / (\theta + v)$  [30] or  $s(v) = \theta_1 s + \theta_2 s / (B_s + v)$  [31].

There are suggestions that the proliferation rate is density-dependent, and that proliferation slows with increase in  $CD4^+$  T cell count. The most common form for  $\phi$  is taken as the following logistic function [4],

$$\phi(T) = T \left( 1 - \frac{T}{T_{max}} \right), \quad (2.12)$$

in which  $T_{max}$  is the  $CD4^+$  T cell population density at which proliferation shuts off. In the presence of HIV, there are three types of  $CD4^+$  T cells: uninfected cells  $T$ , latently infected cells  $T_1$  and productively infected cells  $T_2$ . It would thus be reasonable to change the logistic proliferation term to  $rT \left( 1 - \frac{T+T_1+T_2}{T_{max}} \right)$ . However the proportion of infected cells is small and this correction is ignored [4].

In (2.8), the population dynamics of latently infected cells is described.  $\delta_1$  is the death rate constant of latently infected cells,  $k_1$  is the rate constant linked to the conversion from latent to productively infected cells. Latently infected cells are produced with a rate constant of  $q_1\beta$  with  $q_1 \leq 1$ .

(2.9) describes the population dynamics of the productively infected cells. These cells produce virus particles and are also called actively infected cells.  $\delta_2$  is the death rate constant of actively infected cells. Productively infected cells are produced with a rate constant of  $q_2\beta$  with  $q_2 \leq 1$ . Since some cells may harbor defective provirus it is assumed that  $q_1 + q_2 \leq 1$ .

(2.10) represents the dynamics in the concentration of infectious virions. When one considers the control effect of protease inhibitors,  $v_1$  includes the population of virus

that is not influenced by the protease inhibitor. The infectious virions are produced by the actively infected  $T_2$  cells with a rate constant  $k_2$ , and  $c$  is the death rate constant of infectious virions. In this equation, the loss of virus due to infection of a cell is ignored. Each time a cell is infected, at least one virion must enter and thus one might add the term  $\beta v_1 T$  to (2.10), but the effect of this term is not statistically significant [4].

The dynamics of the concentration of non-infectious virion is represented by (2.11). The population of defective virus particles is denoted by  $v_2$ . These non-infectious particles are produced by the actively infected cells,  $T_2$ , at a rate constant  $k_3$ , and the non-infectious particles are assumed to be removed from the system at the same rate constant  $c$ , as the infectious virions.  $\bar{k} = k_2 + k_3$  is the rate constant of total virion production. At times  $\bar{k}$  is denoted as  $\bar{k} = N\pi$ , in which case  $N$  is the total number of virions produced by an actively infected cell during its lifetime.  $N$  is hard to measure accurately [15], and thus  $k_2$  and  $k_3$  are kept in the model.

### Derived models

The model described by (2.7)–(2.11) is the basis for several classes of models. For instance, if it is assumed that the non-infectious virus is negligible then  $k_3 = 0$ , and (2.11) becomes  $\dot{v}_2 = -cv_2$ . This is especially true in the case when no treatment by protease inhibitors is present. In this case  $v_2$  tends to zero, and the system becomes

$$\begin{aligned}\dot{T} &= s + r\phi(T) - dT - \beta vT, \\ \dot{T}_1 &= q_1\beta vT - \delta_1 T_1 - k_1 T_1, \\ \dot{T}_2 &= q_2\beta vT + k_1 T_1 - \delta_2 T_2, \\ \dot{v} &= k_2 T_2 - cv.\end{aligned}\tag{2.13}$$

A variation of this fourth order model was proposed in [4] in the following form

$$\begin{aligned}\dot{T} &= s + r\phi(T) - dT - \beta vT, \\ \dot{T}_1 &= q\beta vT - \delta_1 T_1 - k_1 T_1, \\ \dot{T}_2 &= \beta vT + k_1 T_1 - \delta_2 T_2, \\ \dot{v} &= k_2 T_2 - cv.\end{aligned}\tag{2.14}$$

Here it is assumed that  $q < 1$ . This model corresponds to the general case when  $q_2 = 1$  and the restriction  $q_1 + q_2 \leq 1$  becomes  $q_1 + q_2 \leq 2$ .

In some of the earlier variations of this model it was assumed that, upon infection,  $CD4^+$  T cells are by default latently infected ( $T_1$ ) cells. Actively infected cells are

presumed to be generated from latently infected cells with a rate constant  $k_1$ . Thus in the general form, the infection term  $q_2\beta v_1T$  is not present in (2.9), and  $q_1 = 1$  in (2.8). Thus the system is simplified to

$$\begin{aligned}\dot{T} &= s + r\phi(T) - dT - \beta vT, \\ \dot{T}_1 &= \beta vT - \delta_1T_1 - k_1T_1, \\ \dot{T}_2 &= k_1T_1 - \delta_2T_2, \\ \dot{v} &= k_2T_2 - cv.\end{aligned}\tag{2.15}$$

The identifiability of these three variations of the fourth order model were investigated in [12], with interesting and informative results. These will be discussed later.

### Connections to the three-dimensional model

If the distinction between actively infected cells and latently infected cells is not necessary, one could denote the total CD4<sup>+</sup> T cell population ( $T_1 + T_2$ ) as  $T^*$ . If the average death rate constant of infected cells is assumed to be  $\delta$ , then one has the following model [4, 11, 24, 32],

$$\begin{aligned}\dot{T} &= s + r\phi(T) - dT - \beta vT, \\ \dot{T}^* &= \beta vT - \delta T^*, \\ \dot{v} &= k_2T^* - cv.\end{aligned}\tag{2.16}$$

If the proliferation term,  $r\phi(T)$  is lumped with the parameter  $d$ , this model reduces to the three-dimensional model considered at the beginning of this chapter.

#### 2.4.2. Extended compartmental HIV/AIDS model

The different compartments that harbor HIV, can be combined into a single model. Thus, for instance the long-lived cell model can be combined with the model incorporating latent infection to synthesize a model that captures the dynamics of both compartments. In [13], the identifiability properties of such an extended compartmental HIV/AIDS model are discussed. The model is outlined in [4], but, for identifiability



analysis, the proliferation term was dropped. The resulting model is

$$\begin{aligned}
 \dot{T} &= s_T - d_T T - \beta_T T v, \\
 \dot{T}_1 &= q_1 \beta_T T v - k T_1 - \delta_1 T_1, \\
 \dot{T}_2 &= q_2 \beta_T T v + k T_1 - \delta_2 T_2, \\
 \dot{M} &= s_M - d_M M - \beta_M v M, \\
 \dot{M}^* &= \beta_M v M - \delta_M M^*, \\
 \dot{v} &= \pi_T T_2 + \pi_M M^* - c v.
 \end{aligned} \tag{2.17}$$

The state variables  $T$ ,  $T_1$ ,  $T_2$ ,  $M$ ,  $M^*$  and  $V$  are the concentrations of the uninfected CD4<sup>+</sup> T cells, the latently infected CD4<sup>+</sup> T cells, the actively infected CD4<sup>+</sup> T cells, the uninfected macrophages, the infected macrophages and the free virus particles respectively.  $s_T$  and  $s_M$  are the source rate constants for uninfected CD4<sup>+</sup> T cells and macrophages respectively, while  $d_T$  and  $d_M$  are their respective death rate constants. Similarly,  $\beta_T$  and  $\beta_M$  are the infectivity rate constants and are an indication of the effectiveness of the infection process of the respective cells by the virus.  $q_1$  and  $q_2$  are the probabilities that upon infection, a CD4<sup>+</sup> T cell will become either latent or actively produce virus, while  $k$  is the conversion rate constant from latent to active virus producer.  $\delta_1$ ,  $\delta_2$  and  $\delta_M$  are the death rate constants for the infected CD4<sup>+</sup> T cells and macrophages respectively.  $\pi_T$  and  $\pi_M$  are the rate constants at which the respective infected cells produce virus and the virus dies with a rate constant  $c$ .

The combination of the models is fairly intuitive, yet complex dynamics can be expected from this system.

## 2.5. Model choice for parameter identification

For this research, the main aim is to identify model parameters for individual patients. With this aim in mind, it is important to decide on structures that will allow parameter identification within the prevailing circumstances of patients.

Furthermore, for the purpose of estimating model parameters, it is assumed that plasma viral load,  $v$  and CD4<sup>+</sup> T cell count,  $T$  are measured, in accordance with the current prevailing medical practice [5].

The choice of basis model is important and will be discussed in more detail here.

### 2.5.1. Identifiability

The first identifiability study on HIV models from an engineering perspective was performed in [12].

Before identification of HIV/AIDS model parameters, it is necessary to ask which variables can be measured. Clinically, many variables can be determined, though some of them with low accuracy and high cost. The recommendation by the panel on clinical practices for treatment of HIV infection [5] and the WHO [33] is to measure the viral load and the CD4<sup>+</sup> T cell counts in HIV patients. Since CD4<sup>+</sup> T cells are predominantly healthy cells [26], and for technical reasons, the viral load and healthy CD4<sup>+</sup> T cells are considered to be the measured output [12].

The identifiability study addresses two main questions: How many measurements of CD4<sup>+</sup> T cells and viral load are needed for parameter identification? and when should these measurements be done? Only when these questions have been answered can one progress with confidence to the problem of accurately estimating model parameters from available measurements.

#### Identifiability of the three-dimensional model

Identifiability (observability) is a system property of whether all parameters (state variables) can be calculated from the measured output. The precise meaning of observability and identifiability is defined in [34,35]. When the outputs to the three-dimensional model are taken as

$$\begin{aligned} y_1 &= T, \\ y_2 &= v, \end{aligned} \tag{2.18}$$

the system (2.1) is both observable and identifiable, as was shown in [12].

An overview of the reasoning in [12] is as follows: Identifiability (more precisely, algebraic identifiability), means that all parameters of the model can be determined from the measured output. To find the conditions under which these parameters can be determined, higher order differential equations are computed as,

$$\dot{y}_1 = \theta_1 + \theta_2 y_1 + \theta_3 y_1 y_2, \tag{2.19}$$

$$\ddot{y}_2 = \theta_4 \dot{y}_2 + \theta_5 y_2 + \theta_6 y_1 y_2, \tag{2.20}$$

where

$$\begin{aligned}\Theta &= (\theta_1, \theta_2, \theta_3, \theta_4, \theta_5, \theta_6)^T \\ &= (s, -d, -\beta, -\delta - c, -\delta c, k\beta)^T.\end{aligned}$$

$\Theta$  defines a one-to-one map of parameters for  $\beta \neq 0$  and  $c > \delta$  [11]. Thus, identification of  $\Theta$  is equivalent to the identification of the original parameters of (2.1). Since the system described by (2.19)–(2.20) is identifiable from measurements (2.18), all the original parameters of the three-dimensional model are identifiable from the measurement of the viral load and the CD4<sup>+</sup> T cell counts in the blood of an HIV patient.

Based on (2.19) and (2.20) one can see that it is necessary to generate a minimum of six equations, three from each equation, in order to solve for the parameters. This is achieved by differentiating (2.19) and (2.20) twice, resulting in derivatives of  $y_1$  and  $y_2$  up to the order of 3 and 4 respectively. To cope with these derivative orders, it is necessary to have at least five measurements of the viral load,  $y_2$ , and four measurements of the CD4<sup>+</sup> T cell count,  $y_1$ , for a complete determination of all the parameters in (2.1)<sup>1</sup>.

### Identifiability of the latently infected cell model

The four-dimensional model of (2.7)–(2.11) exhibits additional identifiability properties. If the proliferation term is lumped into the parameters  $s$  and  $d$ , then, in general, ten parameters are to be identified:  $s, d, \beta, q_1, q_2, \delta_1, \delta_2, k_1, k_2, c$ .

The identifiability theory of [12] shows that the system is not algebraically identifiable if the outputs are still taken as (2.18). Besides the identification of  $s, d$  and  $\beta$ , five of the seven remaining parameters can be computed from these measured outputs and the remaining two parameters only if

$$q_1 q_2 \beta k_2 (\delta_1 + k_1 - \delta_2) (\delta_2 - c) (\delta_1 + k_1 - c) \neq 0. \quad (2.21)$$

Some interesting conclusions are drawn from this equation in different situations [12].

$q_1 = 0$

In this case  $\delta_1$  and  $k_1$  cannot be separated from  $\delta_1 + k_1$ . This is interpreted as a blockage of the channel for infected cells to become latent. As a result one cannot tell from CD4<sup>+</sup> T cell and viral load alone how many latently infected cells die naturally and how many turn into actively infected cells.

<sup>1</sup>Later on, in chapter 5, this is referred to as the (5+4) sample condition.

$$q_2 = 0$$

In this case the system is not algebraically identifiable. Observe that this corresponds to the model in (2.15), which has only eight parameters. Despite having two less parameters, not all eight parameters can be determined for (2.15).

$$k_2 = 0$$

This means that infected cells do not produce virus and no information about the CD4<sup>+</sup> T cells can be extracted from the viral load. The model predicts that the infection would be cleared in this case.

$$\delta_1 + k_1 = \delta_2$$

(2.21) would be violated and the system is not algebraically identifiable. Practically this means that if active and latent cells disappear at the same rate, then the dynamics of these two kinds of cells are indistinguishable.

$$\delta_2 = c$$

(2.21) is violated and the system is not algebraically identifiable. This corresponds to the situation where actively infected cells and the virus disappear at the same rate and it is impossible to distinguish their corresponding dynamics.

$$\delta_1 + k_1 = c$$

(2.21) is violated and the system is not algebraically identifiable. This is similar to the previous case. Since latently infected cells and the virus disappear at the same rate, it is impossible to distinguish the corresponding dynamics.

It is clear that the latently infected cell model has more stringent restrictions for successful identification of parameters. Some of the parameters can be determined by different methods, as in the experiment of [26], where  $q_1$  is found to be  $q_1=0.01$  and in [36], where  $q_2$  is estimated at  $q_2 = 0.02$ . When these parameters are known, it is interesting to see if the other parameters are still algebraically identifiable. An exhaustive list of cases is discussed in [12].

When patients do a *comprehensive test* before initiation of HAART then the initial state of the patient is known. In identifiability theory, the knowledge of initial states is characterized by geometrical identifiability and in [12] it is shown that the system is fully geometrically identifiable. This means that for the latently infected cell model, the parameters can be estimated if we know the initial levels of active and latent CD4<sup>+</sup> T cells in addition to the viral load and CD4<sup>+</sup> T cell levels measured after treatment.

Basic model					
$T$	$v$				
4	5				
Latently infected cell model					
$T$	$T_1$	$T_2$	$v$		
6	1	1	8		
Extended Model, option 1					
$T$	$T_1$	$T_2$	$M$	$M^*$	$v$
11	0	0	11	0	13
Extended Model, option 2					
$T$	$T_1$	$T_2$	$M$	$M^*$	$v$
5	1	6	1	0	10

Table 2.2.: Models and their minimal measurement strategies

### Identifiability of higher order systems

The identifiability of the extended model is addressed in [13]. It will suffice to note that in order to identify parameters of multi-compartmental models, one needs measurements from the other compartments as well. Also, as the complexity of models increases, the accuracy of measurements and the number of samples needed, increases accordingly. In [13] the conclusion is drawn that it is possible to obtain parameters for the higher order model from viral load and uninfected  $CD4^+$  T cell counts only, but that this would require too many viral load measurements to be practical.

A summary of the models and their respective minimal measurement strategies is given in Table 2.2. It is clear that, from a practical perspective, the first aim should be to identify the parameters of patients for the three-dimensional model.

### 2.5.2. Proliferation terms in the three-dimensional model

The identifiability analysis gives a clear indication that the three-dimensional model of (2.1) should be used as the starting point for analysis of individual patient data. Despite the choice of model for practical reasons, there is still the question whether the proliferation term should be included in  $CD4^+$  T cell generation, or if it can be lumped with the source term and death rate of cells,  $s$  and  $d$ . As described earlier, the most common proliferation term in current use is (2.12).

One obvious drawback when this term is included in the model for parameter identification, is that two additional parameters,  $p$  and  $T_{max}$ , must be determined together with the other six parameters of (2.1). This would increase the minimum amount of

measurements needed to estimate all parameters of the model. If the analysis must be done with the minimal set of measurements, the basis model should be used. An alternative would be to fix  $p$  and  $T_{max}$  to the standard values found in [22, 30] and continue with the estimation of the other parameters as before.

Since the second option would result in fixed values for  $p$  and  $T_{max}$  for all parameter estimates, the question arises if there is any gain in estimation of the other parameters to show for the addition of this term. To answer this question, the influence of the proliferation term is quantified.

In order to estimate the influence of the proliferation term, the rates of change for the CD4<sup>+</sup> T cells in both versions of the model are equated

$$s_1 - d_1T - \beta_1v_1T = s_2 + pT\left(1 - \frac{T}{T_{max}}\right) - d_2T - \beta_2v_1T. \quad (2.22)$$

If the proliferation is considered to be lumped into  $d$  only, then  $s_1 = s_2$  and  $\beta_1 = \beta_2$ , giving

$$d_1 = d_2 - p\left(1 - \frac{T}{T_{max}}\right). \quad (2.23)$$

Thus the lumped death rate would vary from a maximum variation at  $T = 0$  to no variation at  $T = T_{max}$ . One can easily see that it would be possible for the lumped parameter  $d_1$  to become negative in some situations.

For a standard set of parameters  $s = 10$ ,  $d = 0.01$ ,  $\beta = 5 \times 10^{-6}$ ,  $\delta = 0.5$ ,  $c = 3$ ,  $k = 1000$ ,  $p = 0.03$  and  $T_{max} = 1500$ , the equilibrium value of  $T$  is  $T_e = \frac{\delta c}{\beta k} = \frac{0.5 \times 3}{5 \times 10^{-6} \times 1000}$ , and  $d_1$  can be computed as  $d_1 \approx -0.014$ .

In Chapter 4, section 4.1, the two models are compared by means of simulation.

In [37] the choice of models is discussed:

“In modeling a complex process we can try to incorporate as many of the facts as possible, which necessarily involves many parameters, estimations of which are difficult to obtain with extant data. An alternative approach is to start with as simple a model as possible but which captures the key elements and for which it is possible to determine estimates for the fewer parameters. There is a trade-off between comprehensiveness and thus complexity, and the difficulty of estimating many parameters and a simpler approach in which parameter values can be reasonably assessed.”

Based on the presented simulations, taken together with this advice, the latter ap-

proach is chosen for this research. In the following sections, the model (2.1) constitutes the basis of analysis.

## 2.6. Summary of chapter

Chapter 2 gives an overview of dynamic HIV/AIDS models, with specific attention to the basis model used in this research. Models from literature are analyzed in combination with the description of some fundamental decisions that were made to establish the model type and scope for this research.

The chapter starts with an introductory overview of biological modeling principles and the role of control engineers in this field in section 2.1.

From here section 2.2 introduces dynamic HIV/AIDS models, with particular attention given to the three-dimensional model, its equations, governing dynamics and steady state in section 2.3.

Higher dimensional models that are based on and extend the three-dimensional model are described in section 2.4. The models and their derivatives are linked back to the basis model to allow additional perspective on their relationship.

Finally section 2.5 investigates the model choice for this research. Models are discussed in the context of identifiability and the section looks into the influence of proliferation terms on the basis model.



## 3. Procedures

**T**HE LINK between dynamic HIV/AIDS models and their practical applications lies with the estimation of model parameters. In the previous chapter, various models that describe the dynamics of HIV within a patient were considered. When a model is used as a tool for treatment decisions, its parameters must be determined from available measurements. Ideally, the procedure that is used should also allow for situations where it is impossible to extract all parameters and accommodate generalizations of parameters that can be taken from other estimates and research.

This chapter describes the procedures that are used to extract model parameters and the specific additions and modifications to standard methods that are necessary to allow parameter estimation in situations where pure least square (LSQ) methods would fail.

### 3.1. Pure least square method

First a pure LSQ estimation is presented. In this section it is shown how the six parameters of (2.1) can be extracted from clinical measurements using classical LSQ data fitting.

Recall the three-dimensional model of HIV/AIDS (2.1), of section 2.3.1:

$$\begin{cases} \dot{T} &= s - dT - \beta T v, \\ \dot{T}^* &= \beta T v - \delta T^*, \\ \dot{v} &= k T^* - c v. \end{cases}$$

For the purpose of estimating model parameters, it is assumed that plasma viral load and CD4<sup>+</sup> T cell count are measured in accordance with the current prevailing medical practice [5]. That is, the measurement outputs are  $y_1 = T$ , and  $y_2 = v$ .

Following the strategy in [12], it can be shown that the system (2.1) is identifiable

and can be transformed into the system

$$\dot{y}_1 = \theta_1 + \theta_2 y_1 + \theta_3 y_1 y_2, \quad (3.1)$$

$$\ddot{y}_2 = \theta_4 \dot{y}_2 + \theta_5 y_2 + \theta_6 y_1 y_2, \quad (3.2)$$

with the following parameter mapping:

$$\begin{bmatrix} s \\ d \\ \beta \\ \delta \\ c \\ k \end{bmatrix} = \begin{bmatrix} \theta_1 \\ -\theta_2 \\ -\theta_3 \\ \frac{-\theta_4 - \sqrt{\theta_4^2 + 4\theta_5}}{2} \\ \frac{-\theta_4 + \sqrt{\theta_4^2 + 4\theta_5}}{2} \\ -\frac{\theta_6}{\theta_3} \end{bmatrix}. \quad (3.3)$$

Now suppose that  $y_1$  and  $y_2$  are measured at time instants  $t = t_0, t_1, \dots, t_N$ , then

$$\begin{aligned} \dot{y}_1(t_0) &= \theta_1 + \theta_2 y_1(t_0) + \theta_3 y_1(t_0) y_2(t_0), \\ &\dots \\ \dot{y}_1(t_N) &= \theta_1 + \theta_2 y_1(t_N) + \theta_3 y_1(t_N) y_2(t_N). \end{aligned}$$

Denote

$$\begin{aligned} A_N &= \begin{bmatrix} 1 & y_1(t_0) & y_1(t_0)y_2(t_0) \\ \vdots & \vdots & \vdots \\ 1 & y_1(t_N) & y_1(t_N)y_2(t_N) \end{bmatrix}, \\ B_N &= \begin{bmatrix} \dot{y}_1(t_0) & \dots & \dot{y}_1(t_N) \end{bmatrix}, \\ \theta^1 &= \begin{bmatrix} \theta_1 & \theta_2 & \theta_3 \end{bmatrix}^T, \end{aligned} \quad (3.4)$$

then the least-square estimation of  $\theta^1$  is

$$\hat{\theta}^1 = (A_N^T A_N)^{-1} A_N^T B_N.$$

The least-square estimations exist if  $A_N^T A_N$  is invertible. Similarly, one can generate a least-square estimation of  $\theta^2 = [\theta_4 \ \theta_5 \ \theta_6]^T$ .

One could estimate the differential terms in (3.1) by means of  $\Delta y_n / \Delta t$ . Thus the

derivative is estimated by a direct comparison between two adjacent data points. The estimation for  $y_1$ , for instance, is then given by

$$\dot{y}_1 \approx \frac{y_1(t_0 + d_1) - y_1(t_0)}{d_1}.$$

Clearly this method is prone to erratic estimations of the derivative, especially when a random component is present in the measurement. When data points are significantly separated in time, this estimation will become even more imprecise.

Because of the erratic behavior of the derivative estimation, this approach is bound to fail, even with seemingly insignificant measurement noise. Before any progress can be made in the presence of noise, a method has to be followed that does not rely on an estimation of the derivative.

In [14] these deficiencies are noted, and the problem is addressed through the use of adaptive observers. The first step to achieve even higher accuracy is to increase the accuracy of the derivative estimation. Also, the method should be immune to random deviations in the measurements as far as possible. With these goals in mind, a search method is used that is based on a customizable cost function.

The idea to customize the cost function is partly inspired by [38]. A customizable cost function allows for additional flexibility together with the LSQ fit. Plausible assumptions can be added in a bid to increase the accuracy of estimations. In addition to this, limits on the parameters can be enforced to ensure that outlier points do not draw the parameters into unreasonable ranges.

## 3.2. Estimation with LSQ-based penalty function

### 3.2.1. Cost function overview

As with the method considered in [14], this method is in essence least square (LSQ) based, but with two important differences. Firstly, derivative estimation is only present in the controlled environment of the numerical ordinary differential equation (ODE) solver. Secondly, the cost function is not limited to the LSQ distance, but can be expanded with penalty terms to increase the accuracy of parameter estimation.

The Nelder-Mead Simplex search method is used as the optimization routine<sup>1</sup> and the

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<sup>1</sup>Successful estimation is not dependent on the chosen search method but, due to abrupt changes in the cost landscape, it is desirable to start with a search method that does not rely on a smooth cost function. At the solution point of the initial search, one can switch to a different algorithm to refine the solution.

steps in the cost function are as follows:

- i. The function receives a list of data points for the CD4<sup>+</sup> T cell and the virus count with their respective time points. Together with these,  $\hat{\chi}$  is also passed to the function.
- ii. When constraints are specified for  $\chi$ , they are enforced at this point. If constraints are not met, a simplified cost is returned (by default only positive values are allowed for the parameters).
- iii. The function uses  $\hat{\chi}$  and solves the dynamic model of (2.1). This is done within the framework of a pre-existing numerical ODE solver.
- iv. The differences between each data point and its predicted value are squared and summed.
- v. Any additional penalty terms are calculated and added to the total cost, which is returned to the optimizing function.

At each iteration of the search, the cost function is called by the optimization routine, until the pre-set tolerance is met. Since the numerical solution of the ODE can be time consuming, it is desirable to keep its evaluation to a minimum, thus a simplified cost is returned at step (ii), if any of the constraints are not met. Clearly this method is not prone to the derivative estimation error, as is the LSQ method considered in [14].

Apart from the problem of derivative estimation, a second drawback in the pure LSQ method is that the equations that are fitted to the data, contain product terms of CD4<sup>+</sup> T cell and virus counts. This forces the data vectors to be of equal length for proper estimation. Since the cost function does not require any product terms, this constraint on the length of CD4<sup>+</sup> T cell and virus data vectors can be dropped.

The basic cost for a nominal set of parameters,  $\hat{\chi}$ , and initial conditions,  $\hat{x}_0$ , is computed by generating  $\hat{T}$  and  $\hat{v}$  with a numerical ODE solver. Together with  $N$  measurements of  $T$  and  $K$  measurements of  $v$ , at time  $t_1, \dots, t_N$ , and  $\tau_1, \dots, \tau_K$  respectively, the basic cost function is defined as,

$$J_w = \sum_{n=1}^N \frac{(\hat{T}(t_n) - T_n)^2}{N \text{mean}(T_n)} + \sum_{k=1}^K \frac{(\hat{v}(\tau_k) - v_k)^2}{K \text{mean}(v_k)}. \quad (3.5)$$

From [39] it is known that the viral load tests are log based. Also for the experiments performed in [4], the virus data is fitted to the least square of logarithmic distance. Thus, following the scheme in [20, p. 444], the basic cost is modified to incorporate

logarithmic distance for the virus data points:

$$J_l = \sum_{n=1}^N \frac{(\hat{T}(t_n) - T_n)^2}{\text{mean}(T_n)N} + \sum_{k=1}^K \frac{(\log \hat{v}(\tau_k) - \log v_k)^2}{\text{mean}(\log v_k)K}. \quad (3.6)$$

In both cases data vectors are weighted by their mean value and length. For group analysis it is useful to use the means of the cohort data to allow better comparison between patients. Standard procedures apply when adding individual weights to samples. If detailed information is available about the variances in data points, this information can be incorporated as in [20, pp. 448–449].

### 3.2.2. Penalty terms and refinements

When a dataset does not contain enough information for parameter estimation on its own, it might yet be possible to extract key parameters when prevailing circumstances are known. In these situations the addition of custom penalty terms to the cost function is helpful, since the penalty terms allow outside knowledge of the dataset to be incorporated into the parameter estimation cycle. Refinements in the cost function are incorporated at steps (ii) and (v) above. Common refinements for this research are:

#### **Enforcing limits.**

This is done at step (ii), before the intensive calculations in the cost function, by checking parameters against a predefined range and correcting any parameters that do not fall within the specifications<sup>2</sup>. A second option is to add parameter limits to the cost value at step (v). This allows for weighted penalties where parameter limits are not well-defined. The second option is not desirable for hard limits, since the computationally intensive ODE will be solved even when the parameters fall outside the prescribed limits.

#### **Prior knowledge of parameters.**

When a parameter is known from another source (*e.g.*, experiment, assumption or literature), this knowledge is incorporated at step (ii). At the same time, the optimization routine can be instructed not to search for fixed parameters.

#### **Prevailing conditions.**

When prevailing conditions (*e.g.*, a patient is in steady state before initiation of

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<sup>2</sup>In contrast to `fminsearch` in Matlab, some solvers allow constraints to be placed separately from the main cost function. When available, this option should be used to place hard limits on the parameters.

therapy) for a dataset are known, these conditions are added by means of an additional term in step (v). This term must be scaled to ensure its proper influence.

As an example, consider the experiment described in [4, pp. 16–19] (originally published in [8]). In this experiment key assumptions were made to extract two of the six parameters. Firstly each patient was assumed to be at steady state (set point has been reached) before initiation of therapy. This is a prevailing condition for that experiment, since the author had access to viral load data before the experiment, which indicated that the viral loads were in steady state. Secondly in [11, p.32] it is stated that infected cells live longer than free virus. This information is reflected in  $J_r$  by adding two terms to the basic cost.

$$J_r = J + k_1 \max\left(\frac{d\hat{v}_s}{dt}, 0\right) + k_2 \max(\hat{\delta} - \hat{c}, 0), \quad (3.7)$$

where  $J$  is either  $J_w$  or  $J_l$ ,  $\hat{v}_s$  is the vector of computed viral loads returned by the ODE solver and truncated after a few days, and the scaling constants  $k_1$  and  $k_2$  are chosen such that any violation of the prevailing conditions would result in a marked increase of the cost function.

The first refinement term is the maximum numerical derivative of  $\hat{v}_s$ , which corresponds to the knowledge that the patient is in steady state before initiation of therapy. As alternative for this term, the positive numerical derivatives can be summed, subject to the proper adjustment of the scaling constant  $k_1$ . In either case, no positive derivative should be allowed initially for the viral load. An intuitive way to see this is to note that therapy results in a decline of virions, thus, an increasing virion count could only be the result of fluctuations before therapy, which is not possible since the patient was in steady state at the start of therapy. The second refinement term corresponds to the statement that the average infected CD4<sup>+</sup> T cell lives longer than free virions.

### 3.2.3. Other refinements and performance enhancements

To increase the performance of the method, there are additional refinements possible. For instance, one can limit the range of set points before the computationally intensive ODE solver to decrease the time needed to compute the cost for some of the iterations. Thus it can be profitable to check that the set point  $\leq 0$  at step (ii) of the cost function for experiments where one assumes 100% effective HAART.

At times, it may be advantageous to dictate the search order of parameters. Parameters with low variation between patients (as the results for  $c$  and  $\delta$  in [4] indicate) are then introduced into the search at a later stage. For instance,  $c$  and  $\delta$  can be fixed at

initial values during the first stage of the search until a preset tolerance is met. The search is then continued, this time allowing all parameters to vary.

### **Alternatives to the standard LSQ cost basis**

As noted earlier, the primary computational burden of the parameter search is in solving the model in a numerical ODE solver. Since the generated model curves for a parameter set are available at each step of the search it can be useful to define alternative cost functions that are based on a diverse set of norms. These cost values may then be calculated with minimal overhead at each iteration to allow the researcher some insight into the changes that an alternative cost definition would bring about in the cost landscape.

For instance, one can define a cost that takes into account not only the squared distance between sample level and predicted level at a fixed point in time. Rather, the expanded function computes the distance to the nearest point in two dimensions (i.e. CD4<sup>+</sup> T cell level and time for  $T$ , and viral count and time for  $v$ ). Obviously such a norm would call for calibration experiments and calculations to determine the ideal influence of the time-difference on the cost. In addition to this a tie breaker definition would be needed for points that lie between peaks in the time domain. To quantify the influence of time on the cost function, one can define a time-only cost that searches for the nearest point in time on the generated ODE curve and allow this cost function to run in parallel with the standard LSQ method.

Another option is the creation of zero-range cost functions for use with global solvers. Such cost functions define a range below which the difference between a data point and the computed curve is set to zero. Once the pre-defined tolerance is met for all points, the total cost is zero. This scheme allows global box bounded solvers to search for parameter sets only until a zero is found and then continue along the search tree to find additional parameter sets that meet the zero-range requirements. In this way the global solver can exit with a list of parameter sets that meet the zero-range requirements, which in turn can be used as starting points for local solvers.

It is clear that the parameter estimation capabilities of the basis procedure can be extended with minimal computational overhead. The definition of alternative cost functions and their implementation is an excellent topic for further research.

### 3.2.4. Implementation

Except for the initial data entry stage in *Microsoft Excel*, *Matlab* is used as basis of implementation for analysis, together with the *Optimization Toolbox*. In the user guide [40], details about the *Optimization Toolbox* and its functions are described. For control purposes, searches are also implemented in the *Tomlab* optimization toolbox. The initial search method used in this research is the Nelder-Mead based algorithm [41–43] as implemented in *Matlab*'s function, `fminsearch`.

The code for core functions used throughout the research can be found in Appendix C and the accompanying compact disc.

## 3.3. Alternative search methods

The Nelder-Mead search is not the only possible method that can be used. It is often the case that the most convenient method is not the most efficient. From [43], it is clear that one has to look beyond the Nelder-Mead method if the parameter search must be applied to large volumes of data, and results are expected within reasonable time. Enquiries led to the *Tomlab*<sup>3</sup> optimization package. Since this is a commercial package with consistently updated routines that are rooted in research, it is possible to use global box-bounded searches and other methods to check results from the initial searches without delving into implementation details.

With such commercial packages it is possible to increase the convergence rate and lower the computational cost for the parameter estimates. Also, global solvers allow some degree of backup to check for multiple minima in the parameter space.

One of the aims of future research is to set up different search methods in such a manner that switching between them is automated, and the modifications to the cost are introduced at appropriate stages. Currently such functionality is controlled by the user.

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<sup>3</sup><http://tomlab.biz/>



### 3.4. Summary of chapter

Chapter 3 presents different parameter estimation procedures, with specific attention to the methods that are implemented for this research.

The chapter starts out by discussing a standard least square estimate from literature for the parameters of the basis model in section 3.1.

The drawbacks and limitations of the standard method are considered, and in section 3.2 an estimation procedure is presented that is based on least square distance but includes additional customizable penalty terms.

Modifications and variants of this method are discussed in the context of the basis model and additional refinements of the procedure are presented.

Finally, in section 3.3 the search method used in this research is discussed together with alternatives.

## **Part II.**

# **Results and Conclusions**

## 4. General Results

**G**ENERAL RESULTS from experiments that were performed throughout the research are gathered in this chapter. The chapter contains simulation results that depict the influence of proliferation on the three-dimensional model. The results from validating the estimation routine against generated data are shown and the influence of additional penalty terms in the cost function can be seen. Together with these results an investigation is presented to determine the applicability of data-collection tables in the context of practical data collection at hospitals.

A published experiment [4, 8] is reproduced to validate the estimation procedure and evaluate the influence of assumptions and outside information on the estimation cycle.

The chapter is concluded with the results of an investigation into the variation of parameters of the basis model throughout the pathogenesis of HIV/AIDS. The variations of basis parameters are used to explore dual compartmental dynamics and parameter estimates are linked back to an established model to explain the disease progression from HIV to AIDS.

### 4.1. The influence of proliferation: simulation comparisons

In section 2.5.2 the influence of a proliferation term (2.12) on the basis model (2.1) was discussed from a theoretical perspective, with calculations to determine upper bounds of influence. In this section, the models are compared through simulation, allowing for a visual comparison. Also the rather more difficult question of the proliferation term's influence over varied time scales is investigated through simulation.

Fig. 4.1 shows an example simulation that compares the basic three-dimensional model of (2.1), with and without the proliferation term (2.12) included in the CD4<sup>+</sup> T cell dynamics.

Now the data from the proliferation based model is used to estimate all parameters for the basic model, to get an indication of the agreement between models. The result

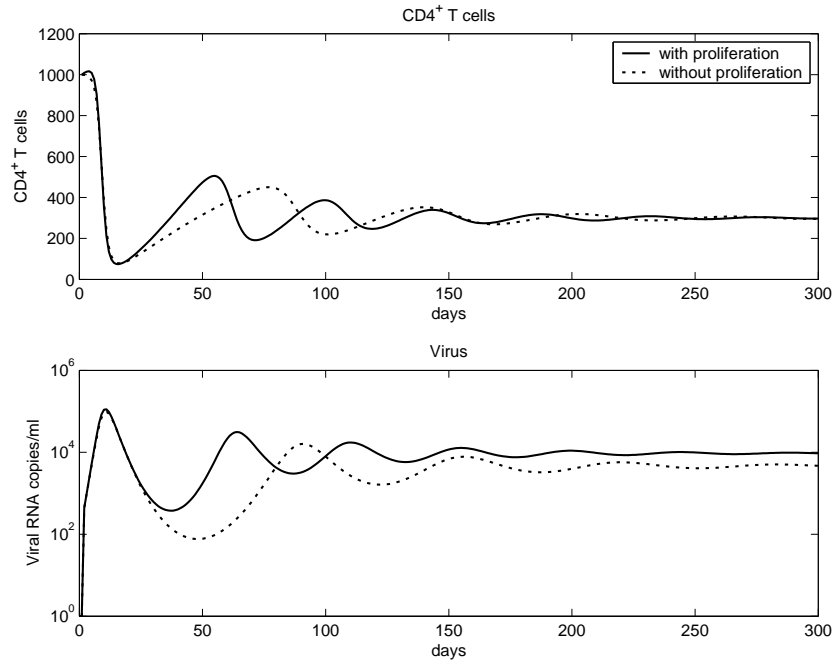


Figure 4.1.: The test basis to compare the three-dimensional model with and without proliferation term for the CD4<sup>+</sup> T cells.

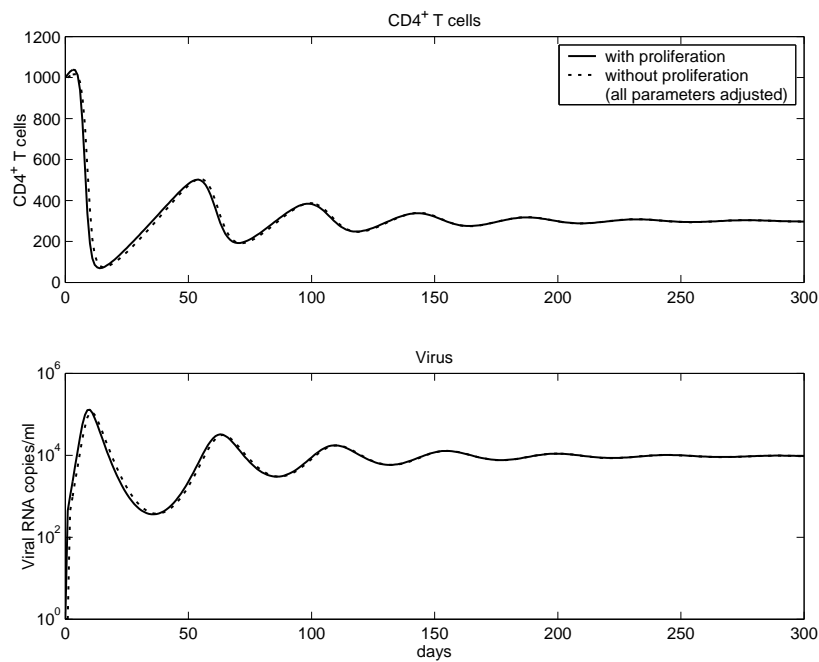


Figure 4.2.: The data from a proliferation based model is used to estimate all parameters for the basic three-dimensional model. The resulting curves agree very well.

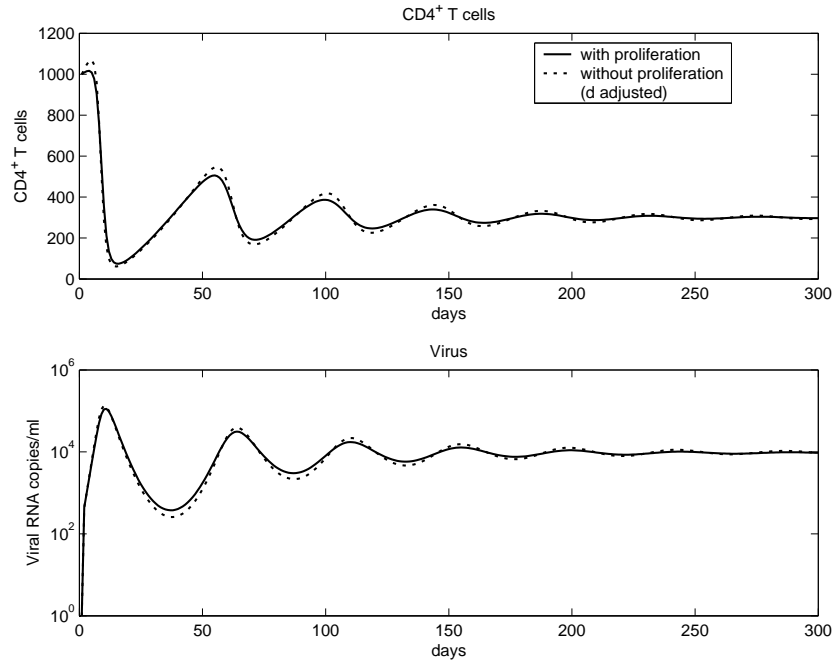


Figure 4.3.: The data from a proliferation based model is used to estimate  $d$  only for the basic three-dimensional model, keeping all other parameters as in the test basis. The difference is more pronounced than in Fig. 4.2 and Fig. 4.4.

in Fig. 4.2 shows that there is a minor difference throughout the modeled time frame. This difference becomes even less as the steady state is approached.

To see if the proliferation could be lumped into the parameter  $d$ , an estimation was performed with all parameters of the basic model fixed at the values of the corresponding parameters of the test set. Fig. 4.3 shows the result for this estimation. Here one can see that the data is quite agreeable, but that there is a distinct deviation at the peaks of the curves. As with the previous estimate, the difference between the curves diminishes with time.

When the previous estimation is repeated, but both  $s$  and  $d$  are allowed to vary, a more agreeable solution is found. In Fig. 4.4 shows the first hundred days of the data. There is no large difference between the two versions of the model and the difference diminishes after about sixty days.

A final comparison allows for the effect of the proliferation term if only a small data-window is available. The results in Fig. 4.5 show how the estimate is near perfect at the start, but the predictions diverge after thirty days. From Fig. 4.5 one can see that the estimates for the  $CD4^+$  T cell level and viral load set points are robust against changes

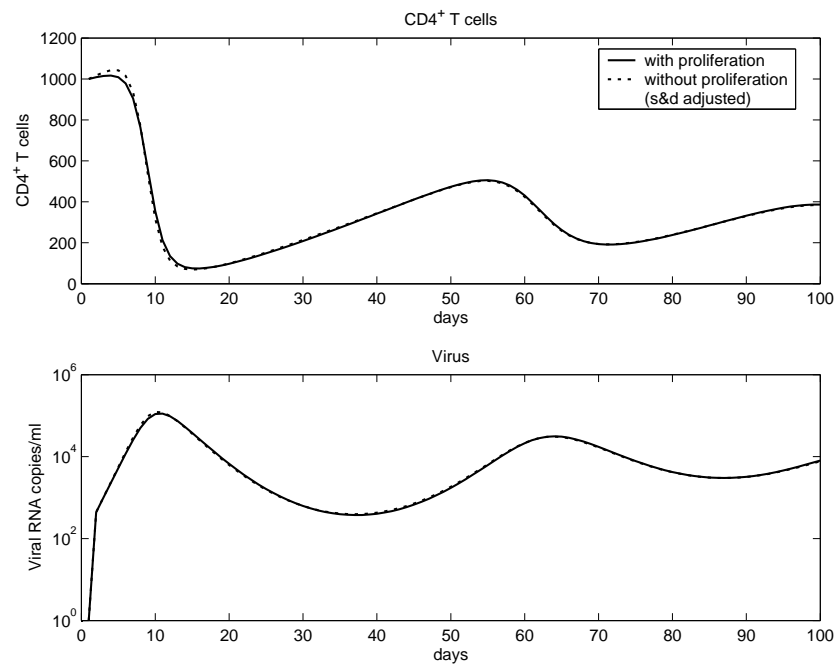


Figure 4.4.: The data from a proliferation based model is used to estimate  $s$  and  $d$  only for the basic three-dimensional model, keeping all other parameters as in the test basis. The difference is more pronounced than in Fig. 4.2 but less than in Fig. 4.3.

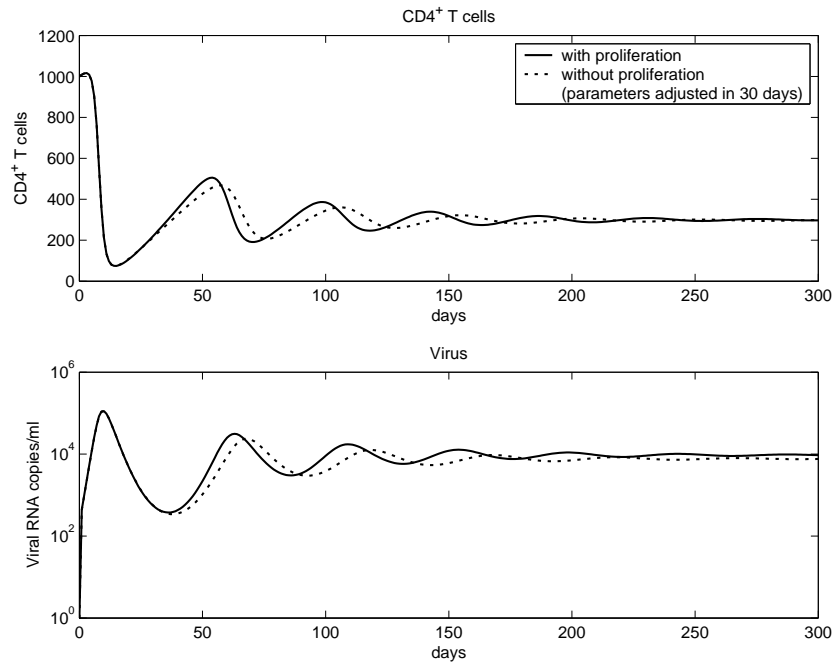


Figure 4.5.: The data from a proliferation based model is used to estimate all parameters for the basic three-dimensional model, but here only the first thirty days are used for the estimate. At first there is no notable difference between the two curves, but after thirty days a deviation develops that becomes more pronounced as time progresses, whereas Fig. 4.2 had an initial difference that stayed consistent throughout the comparison.

in the proliferation term, but that changes in the estimation of the time to set point can be expected.

From the simulations of the two versions of the three-dimensional model, it can be seen that the proliferation term does have a marked influence on the estimation of both  $s$  and  $d$ , rather than on  $d$  only. Once the proliferation has been absorbed into these two parameters, modeled differences are small, and can be ignored in the context of clinical measurements. It is evident that the model parameters for the two versions of the three-dimensional model cannot be compared before the influence of the proliferation term has been accounted for. In the following sections, the three-dimensional model without proliferation term is used as the basis for estimation. From this basis, as an increased number of measurements become available, it is possible to add additional detail to the model and estimate the proliferation parameters.

## 4.2. Method verification

### 4.2.1. Generated data

First, the described procedure is checked against generated, well-posed data, exceeding all requirements of [12]. The underlying test-set is generated with the aim to accommodate the range of parameters described in literature [4, 11, 15, 22, 25, 44–46]. Some parameters vary considerably among authors—here the choice is biased toward the values given in [11] and [4]. For a discussion of parameter and set point variations the reader is referred to [45, 46].

#### Generated data with a random component

As a starting point, a dataset is generated for  $\chi = [10 \ 0.01 \ 5 \times 10^{-6} \ 0.5 \ 3.0 \ 1000]^T$ ,  $\vec{x}_0 = [1000 \ 1 \ 100]^T$  and  $t = 0, 1, 2, \dots, 99, 100$ . Without any random component added to the data, the estimation routine achieves a near-perfect estimation of parameters (results not shown).

A random component with a  $\log_{10}$ -normal variance of 0.6 in the virus and a normal variance of 50 in the  $CD4^+$  T cell count, is added to the data to simulate inaccuracies in measurements. The log-variance in the virus data is based on measurement variances described in [39].

The first estimate of parameters is made without any assumptions added to the cost function. The result is shown in Fig. 4.6, which corresponds to  $\hat{\chi} = [10.2 \ 0.011 \ 7.5 \times 10^{-6}$



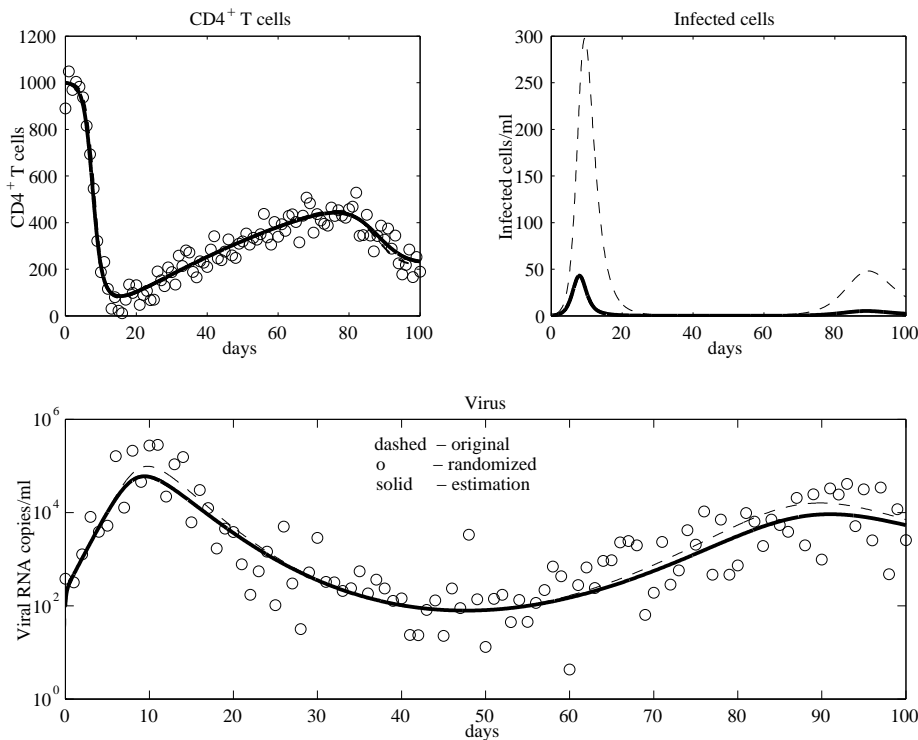


Figure 4.6.: Estimate of parameters on noisy data, without penalty term assumptions added to the cost function. Note the difference of infected cell levels between the original parameter simulation (dashed) and the estimated parameter simulation (solid). Compare Fig. 4.7.

$4.22 \ 0.45 \ 831.23]^T$ . Note that  $\hat{\delta} = 4.22$  and  $\hat{c} = 0.45$  are not correctly estimated. Their values should be exchanged, since the original data has as basis  $\delta = 0.5$  and  $c = 3$ . This difference can be seen in the number of infected cells in Fig. 4.6, but not in the  $CD4^+$  T cell or virus counts. The exchange of  $\delta$  and  $c$  reflects the fact that the  $CD4^+$  T cell and virus counts of (2.1) are symmetric with respect to  $\delta$  and  $c$  [11]. Since the infected cell count is not available to the estimation routine when determining the parameters, the cost function has to be augmented with the knowledge that  $\delta < c$  to correct this exchange. This addition to the cost function was described in section 3.2.2.

After adding the relevant penalty terms to the cost function, a second estimation is performed as shown in Fig. 4.7. Even though the  $CD4^+$  T cell count and the viral levels are nearly identical in Fig. 4.6 and Fig. 4.7, there is a distinct difference in the predicted levels for infected  $CD4^+$  T cells. The infected cell levels are not available as a measurement in practice, but since the data for this test-case is generated from the model, a comparison is possible. In both Fig. 4.6 and Fig. 4.7 the expected level of infected  $CD4^+$  T cells is given in the dashed line. Only in Fig. 4.7 does the predicted

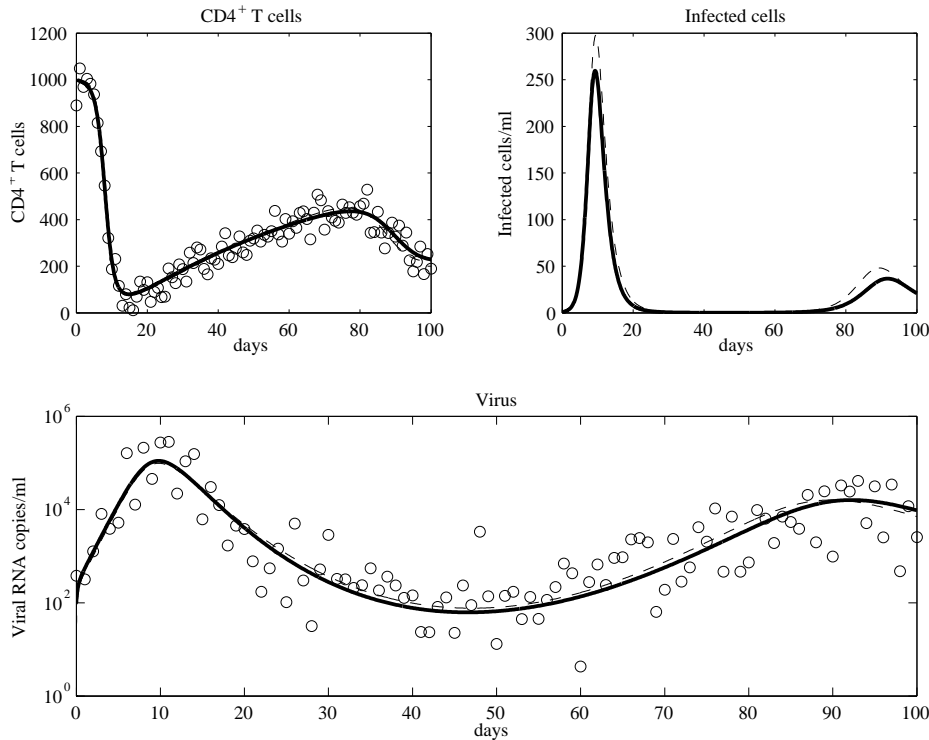


Figure 4.7.: Estimate of parameters on noisy data with a penalty term, assuming that  $\hat{\delta} < c$ , added to the cost function. Note the similarity of infected cell levels for the original parameter simulation (dashed) and the estimated parameter simulation (solid). Compare Fig. 4.6.

level (solid line) match the expected level.

The second parameter estimate is unchanged from the first estimation, except that  $\hat{\delta}$  and  $\hat{c}$  are exchanged, i.e.  $\hat{\chi} = [10.7 \ 0.015 \ 4.5 \times 10^{-6} \ 0.58 \ 2.05 \ 896.49]^T$ . Note that the order of  $\hat{\delta}$  and  $\hat{c}$  is correct. The correct estimation is a direct result of the addition of the penalty term,  $k_2 \max(\hat{\delta} - \hat{c}, 0)$ , to the cost function which implies that  $\delta < c$ . It is clear that the estimation procedure responds correctly when outside knowledge is added. The next section investigates if the estimation is still reasonable with less data-points.

#### 4.2.2. Validating a dataset with a reduced number of data points

The theoretical limit for the minimum number of data points for an estimation of parameters, under conditions of persistent excitation, was derived in [12]. One of the primary applications of the basis model is during the period immediately following the initiation of HAART, where the condition of persistent excitation is met [12]. Also experiments to determine  $c$  and  $\delta$  as in [4, 7, 8] are all performed in the immediate period following the

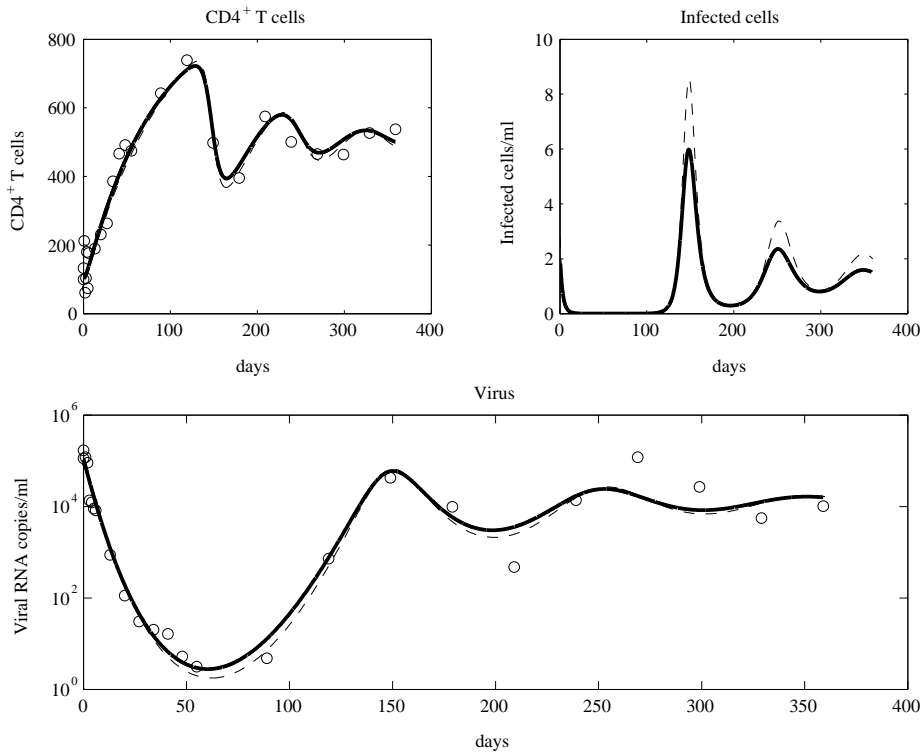


Figure 4.8.: Estimate for dataset with reduced number of points. Original parameter simulation – dashed; Estimated parameter simulation – solid.

initiation of treatment. Yet, it becomes increasingly hard to meet all conditions of identifiability in practical situations. To ensure that sample data complies with identifiability requirements, a time schedule can be set up that allows flexibility in sampling intervals without jeopardizing parameter identifiability after the initiation of HAART [22, 44]. Before such a time schedule for measurements can be finalized, its viability for model identification has to be determined. As an example, to show the applicability of the proposed procedures, a dataset is created with  $\chi = [10 \ 0.01 \ 8 \times 10^{-7} \ 0.5 \ 3.2 \ 4000]^T$ ,  $\vec{x}_0 = [100 \ 2.8 \ 112500]^T$  and the time points taken from the schedule as in Fig. 4.8.

The proposed schedule is intended for use after initiation of HAART, thus the initial  $CD4^+$  T cell level is chosen to be  $T_0 = 100$  and  $v_0$  is chosen near the set point value of  $\chi$  with  $\beta = 8 \times 10^{-6}$  and  $k = 18200$  according to (2.5): After determining the viral set point,  $\beta$  is lowered to  $\beta = 8 \times 10^{-7}$ , and  $k$  is lowered to  $k = 4000$  corresponding to the assumption that HAART in this case is 90% effective in reducing  $\beta$  and about 80% effective in reducing  $k$ . A random component is added to both the  $CD4^+$  T cell and virus counts, as with the previous dataset. The estimate corresponding to Fig. 4.8 gives  $\hat{\chi} = [11.2 \ 0.012 \ 7.4 \times 10^{-7} \ 0.49951 \ 3.89 \ 5156]^T$ .

Table 4.1.: Comparison of results with a published experiment. The half-life for  $c$  is computed as  $t_{c\frac{1}{2}} = \frac{\ln 2}{c}$  and similarly for  $\delta$ .

Patient number	Method	Virus clearance		Infected cell loss	
		$\hat{c}$ (day <sup>-1</sup> )	$t_{c\frac{1}{2}}$ (days)	$\hat{\delta}$ (day <sup>-1</sup> )	$t_{\delta\frac{1}{2}}$ (days)
104	published	3.7	0.2	0.5	1.4
	penalty function	2.03	0.34	0.51	1.36
	$\vec{x}_0$ modified	3.40	0.20	0.50	1.38
105	published	2.1	0.3	0.5	1.3
	penalty function	0.66	1.05	0.66	1.05
	$\vec{x}_0$ modified	2.22	0.31	0.45	1.54
107	published	3.1	0.2	0.5	1.4
	penalty function	2.07	0.33	0.50	1.39
	$\vec{x}_0$ modified	3.07	0.23	0.49	1.41

The assumption that the patient is in steady state was not added to the estimation procedure, since this assumption is only made to approximate a value for  $v_0$ . If such information is available, the accuracy of estimation can be increased.

### 4.3. Reproducing a published experiment

In this section, the parameter estimation of  $c$  and  $\delta$  for three patients in [4, pp. 16–19] is repeated using the custom penalty method. The patients under consideration were treated with protease inhibitors, allowing for additional assumptions to be made about the system. By fitting a reduced system to sample data it was possible to estimate both  $c$  and  $\delta$ . Here the estimation is repeated, but instead of reducing the system equations, the assumptions are added to the cost function as described in section 3.2.2. The method described in this paper is used to extract the same two parameters as in the experiment. Note that *all* the assumptions described in the experiment are included in this estimate. This is done to verify that the procedure described here correctly handles the addition of custom penalty terms. After estimation, results are compared.

There is a distinct, and consistent difference in  $\hat{c}$  between the published results and the estimation by custom penalty function. Since the estimation of  $c$  is dependent on the shoulder region of the virus count [11], a dependence on  $\vec{x}_0$  is to be expected. It is possible to find values for  $\vec{x}_0$ , for which the estimates,  $\hat{\delta}$  and  $\hat{c}$ , are closer to the published

estimates. These nominal values for  $\vec{x}_0$  are found by keeping all parameters at the values published in [4] and instructing the optimization routine to search for the initial values  $\vec{x}_0$  that minimize the cost function. With  $\vec{x}_0$  fixed, one can estimate the parameters again with the same cost function, but without searching for  $\vec{x}_0$ , to find the values given in table 4.1.

The small data window for this experiment does not allow clear information to be found about the initial conditions. From the results it is clear that the estimation of  $c$ , and, to a lesser degree of  $\delta$ , is dependent on outside information about the initial level of the virus concentration. When this information is added to the estimation procedure the results compare very well with those published in [4].

## 4.4. Parameter variation in the basis model

Parameter variation in dynamical models from HIV infection to the onset of AIDS is still in open discussion [15, 18, 45]. The variation in parameter estimates across different studies is addressed in [45]. In [15], the question is raised if the three-dimensional model can be considered as a valid model for longer periods of time, since it does not account for the consistent decline of CD4<sup>+</sup> T cells and the final explosion of virus particles as patients progress from the asymptomatic stage to AIDS.

Since the three-dimensional model cannot describe the complete pathogenesis of the disease with a single set of parameters, the question follows whether a variation of parameters over time could account for the variations in CD4<sup>+</sup> T cell and viral load from infection through the final stages of AIDS. When this question is answered, the variations that are found can be assessed and may form the basis for new discoveries of underlying interactions.

As an example, variations in the parameters  $s$  and  $d$ , which are the source and death rate terms for CD4<sup>+</sup> T cells, could point to plausible source functions that depend on other parameters.

Finding the variation of parameters over disease progression necessarily implies a phenomenological approach, which deviates from the deterministic nature of the model. Thus any results with respect to such variations should serve as starting point for further research, rather than as end result.

This section is divided into two parts. First the variation of parameters in the three-dimensional model is found over the course of HIV/AIDS. These results are combined with additional research and model extension to find a plausible explanation for the emergence of AIDS in asymptomatic patients.

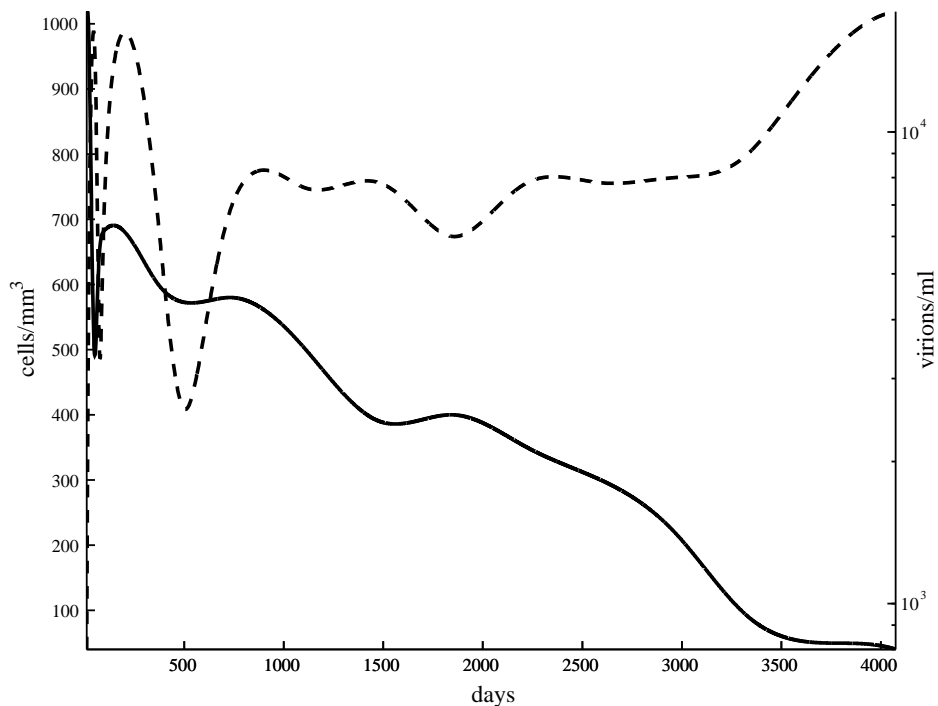


Figure 4.9.: An interpolated version of the dataset from [10], showing the progression of HIV from early infection to AIDS. The solid curve represents the  $CD4^+$  T cell levels and the dashed curve the viral load.

#### 4.4.1. Phenomenological approach to parameter variation in the three-dimensional model

To get an indication of the variation of parameters over the course of the disease, a dataset from [10] is considered in this section. Fig. 4.9 shows the interpolated dataset for virus and  $CD4^+$  T cell levels over the course of the disease. In the early stages after infection, the variation in both  $CD4^+$  T cell and virus levels is clear. This is followed by a period of relative stability and finally, at about 3000 days, the levels indicate progression from steady state to AIDS. This dataset is used in [15] as a benchmark to compare compliance of different HIV/AIDS models to real data.

One concern in [15] is that the model in (2.1) does not track the data from HIV infection until the onset of AIDS with a single set of parameters. It has been noted that patients' parameters change during the course of the disease [18]. By estimating parameters from infection to the onset of AIDS this study offers a simulated confirmation that the parameters of (2.1) must vary to account for the changes in data.

A study of the variation in parameters assists researchers in finding the underlying

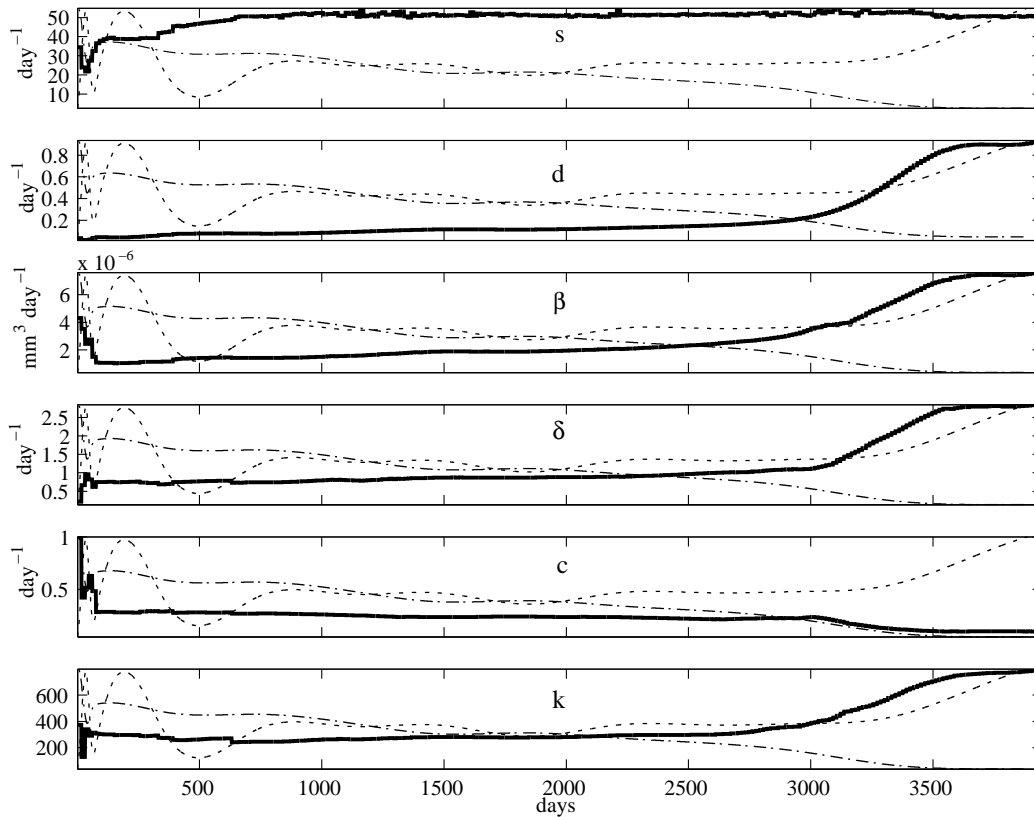


Figure 4.10.: The progressive estimate of parameters over the course of HIV/AIDS. The estimate for each parameter is represented by a solid line.  $s$  is limited to 50. The dash-dot line shows a normalized  $CD4^+$  T cell count and the dotted line shows the normalized virus level. The normalized levels are shown as a visual reference of disease progression and are not part of the parameter estimate.

mechanisms that account for these variations and helps to point out weaknesses in the model where the structure might be changed to facilitate more accurate modeling.

It should be observed that the original dataset is interpolated before it is used to estimate parameters. The parameters are estimated for a fixed data window, which is moved over the data at fixed increments.

Large time difference between measurements, combined with interpolation, result in erratic estimates if a perfect fit of the curve is searched for at each point. To prevent this, the estimation procedure exits with a higher tolerance for error. Fig. 4.10 shows the result for a window size of 60 days, with estimates made 15 days apart. On each graph, the normalized  $CD4^+$  T cell and virus loads are plotted to give a visual reference of the disease progression.

Instead of averaging the estimation over a few windows, the search tolerance is in-

creased and each subsequent search is initialized with  $\hat{\chi}$  from the previous window. This allows for continuity of parameters. Intuitively this scheme corresponds to the assumption that parameters vary smoothly over time as the disease progresses. Thus the estimation procedure factors the results from the previous window into the next estimation, resulting in an estimate,  $\hat{\chi}$ , that is a modification of the previous window's  $\hat{\chi}$ . Each modification is just enough to ensure that the cost value changes by less than the specified tolerance. In conclusion one can see that, at each point, the estimated line is fitted to the interpolated data, and estimation stops as soon as a *possible* (as opposed to a locally *near optimal*) solution is found. An intuitive description of this procedure would be that the parameter estimate for each window is the “nearest plausible neighbor” to the estimate of the bordering windows.

In Figure 4.10, the increase in  $\hat{s}$  is halted by the assumption that  $s \leq 50$ . Most estimations tend toward unreasonable asymptomatic values with the onset of AIDS at about 3000 days. The estimations  $\hat{\delta}$ ,  $\hat{c}$  and  $\hat{k}$  stay fairly constant until the onset of AIDS, whereas the estimations for  $\hat{s}$ ,  $\hat{d}$  and  $\hat{\beta}$  show a steady increase, even before the onset of AIDS.

#### 4.4.2. Exploring dual compartment dynamics as alternative to parameter variation

In the previous subsection, a phenomenological analysis of parameter variation is presented. As indicated before, the variations that are presented should ideally be used as a starting point for further research. The previous results, together with the experience gained through estimating parameters for different patients, allows for the synthesis of a novel interpretation of deterministic models for the onset of AIDS.

##### Basis of analysis

In Fig. 4.10 the parameters seem fairly constant throughout the disease progression, with a definite change at the onset of AIDS. This change of parameters together with the unexplained explosion of the viral level indicates a second compartment that influences the viral levels.

A model that incorporates two compartments is given in (2.17). One of the major drawbacks of this model, though, is that the estimation of parameters is complex and subject to specific measurements from each compartment [13]. Added to this is the distinct lack of data for complete, untreated disease progression, with the data from



[10] being the only source used for comparison in [15]. Thus the deterministic and phenomenological approaches are combined in an effort to explain the progression to AIDS. Moreover, when the problem is explored from this angle, the questions that are raised are more specific, narrowing the field to allow more pointed research.

Recall the extended six-dimensional model (2.17). One of the prominent characteristics of (2.17) is that it is two-compartmental, with each compartment approximately conforming to the lower-dimensional models (2.15) and (2.1) respectively, with a shared pool of virus particles.

$$\begin{aligned}
 \text{CD4}^+ \text{ T cell compartment (2.15)} & \quad \begin{cases} \dot{T} &= s_T - d_T T - \beta_T T v, \\ \dot{T}_1 &= q_1 \beta_T T v - k T_1 - \delta_1 T_1, \\ \dot{T}_2 &= q_2 \beta_T T v + k T_1 - \delta_2 T_2, \end{cases} \\
 \text{Macrophage compartment (2.1)} & \quad \begin{cases} \dot{M} &= s_M - d_M M - \beta_M v M, \\ \dot{M}^* &= \beta_M v M - \delta_M M^*, \end{cases} \\
 \text{Shared viral pool (2.15) \& (2.1)} & \quad \begin{cases} \dot{v} &= \pi_T T_2 + \pi_M M^* - c v. \end{cases}
 \end{aligned} \tag{4.1}$$

The key observation at this stage is the fact that the two compartments in the extended six-dimensional model share a single pool of virus particles. When (2.17) is viewed in the light of (4.1), together with the identification methods implemented in this research, it is reasonable to recast the model into a framework of two three-dimensional models with interaction parameters,

$$\begin{aligned}
 \text{CD4}^+ \text{ T cell compartment} & \quad \begin{cases} \dot{T} &= s_T - d_T T - \beta_T T (v_T + q_{v_M} v_M), \\ \dot{T}^* &= \beta_T T (v_T + q_{v_M} v_M) - \delta_T T^*, \\ \dot{v}_T &= k_T T^* - c_T v_T, \end{cases} \\
 \text{Secondary compartment} & \quad \begin{cases} \dot{M} &= s_M - d_M M - \beta_M M (v_M + q_{v_T} v_T), \\ \dot{M}^* &= \beta_M (v_M + q_{v_T} v_T) M - \delta_M M^*, \\ \dot{v}_M &= k_M M^* - c_M v_M. \end{cases}
 \end{aligned} \tag{4.2}$$

In (4.2), the dynamics of the CD4<sup>+</sup> T cell compartment have been simplified by removing the latently infected cells from the model. Also, the viral load is not shared directly, rather the option is left open to regulate the influence of the viral burdens between

compartments. For instance, this extension allows for the secondary compartment to be located in the bone-marrow and thus have an influence of less than one on the CD4<sup>+</sup> T cell compartment. Constraints  $q_{v_T} \leq 1$  and  $q_{v_M} \leq 1$  are placed on the coupling constants, where  $q_{v_T}$  is the influence of  $v_T$  on the virus pool  $v_M$  and  $q_{v_M}$  is the influence of  $v_M$  on the virus pool  $v_T$ . In order to find the total viral load in the CD4<sup>+</sup> T cell compartment (ie. blood), as measured in viral load tests, one can calculate  $v = v_T + q_{v_M} v_M$ , where  $v_T$  is the virus produced by CD4<sup>+</sup> T cells and  $q_{v_M} v_M$  is the fraction  $q_{v_M}$  of virus particles originating from the virus pool  $v_M$ . Thus, when the viral pool is shared as in (2.17), the coupling parameters are both equal to one.

Even though the recast system (4.2) has more parameters, they conform to the form of (2.1). In this case

$$\begin{aligned} \chi_r &= \left[ s_T \ d_T \ \beta_T \ \delta_T \ c_T \ k_T \mid s_M \ d_M \ \beta_M \ \delta_M \ c_M \ k_M \mid q_{v_T} \ q_{v_M} \right]^T \\ &= \left[ \chi_T^T \mid \chi_M^T \mid q_{v_T} \ q_{v_M} \right]^T, \end{aligned}$$

where  $\chi_r$  is the parameter set of the complete recast system (4.2). The parameter sets  $\chi_T$  and  $\chi_M$  are the parameters of (2.1) for the respective compartments. The parameters  $q_{v_T}$  and  $q_{v_M}$  are the coupling constants between compartments. By recasting (2.17) as in (4.2), the foundation is laid to exploit the identification procedures of the three-dimensional model within a higher-dimensional framework. The concept is illustrated in Fig. 4.11.

## Two stage analysis

As in the previous section, practical analysis is based on the data from [10] as depicted in Fig. 4.9. The shared pool of virus particles in (2.17) and the lack of explanation for CD4<sup>+</sup> T cell decline by the three-dimensional model, together with the review in [15]—where this shortcoming of (2.14), and per implication (2.1), is emphasized—all contribute to the analysis that follows.

In chapter 2, recent research is described that indicates the existence of long lived infected cells with a half-life of weeks, and in some cases even months. Thus it is hypothesized that the final onslaught of virus that can be seen in Fig. 4.9 could be the result of the interaction of these long-lived cells with the virus. Fig. 4.11 illustrates the interaction of the compartments. Also, since the long lived cells share the plasma viral load with CD4<sup>+</sup> T cells, the viral set-point of the CD4<sup>+</sup> T cells compartment can be

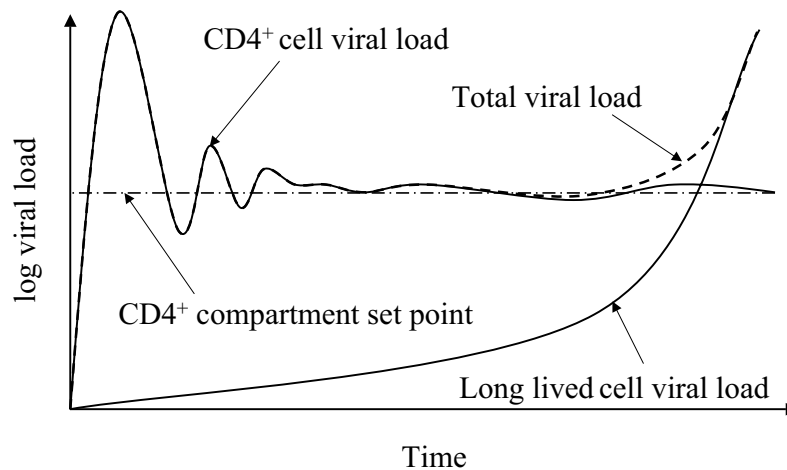


Figure 4.11.: An illustration of the concept of breaking down a higher-dimensional model into compartments to allow identification of parameters for each compartment.

used as a constant in the first stage of analysis. The relatively fast dynamics observed in the early stages of Fig. 4.9 combined with the results from the vaccine readiness study described in chapter 5, indicate that a set-point is likely to be reached within 500 days. Thus the decision is made to analyze the secondary compartment, with the influence of the  $CD4^+$  T cell compartment fixed at its set-point value.

From [47], updated estimations for  $c$  and  $\delta$  are used as starting point for the clearance rate constant of the virus and, in the second stage of analysis, for deathrate constant of infected  $CD4^+$  T cells.

For the first stage of analysis, the basis model of (2.1) is adjusted to reflect the addition of a fixed viral load from the  $CD4^+$  T cell compartment. With this adjustment implemented, the objective function is set to find a set of parameters that mimics the final explosion of viral load, and during the rest of the disease stays comparably insignificant. Since there is no data available for the cells of this compartment, only the viral levels are considered. Thus, in effect, a phenomenological approach is incorporated in this analysis together with links to the mechanistic description allowed by outside knowledge from other research. Fig. 4.12 shows the result of the first stage parameter search. Here the set point influence of the  $CD4^+$  T cell compartment is set to 1000 and  $\hat{\chi}_M = [1, 0.001, 5 \times 10^{-10}, 0.001, 13, 80000]^T$  and  $\vec{x}_0 = [560, 0.001, 100]$ . The curve of the data is steeper than the modeled curve. In this case middle ground is chosen between closely mimicking the lower values at the end of the dataset, and reaching the final value of the

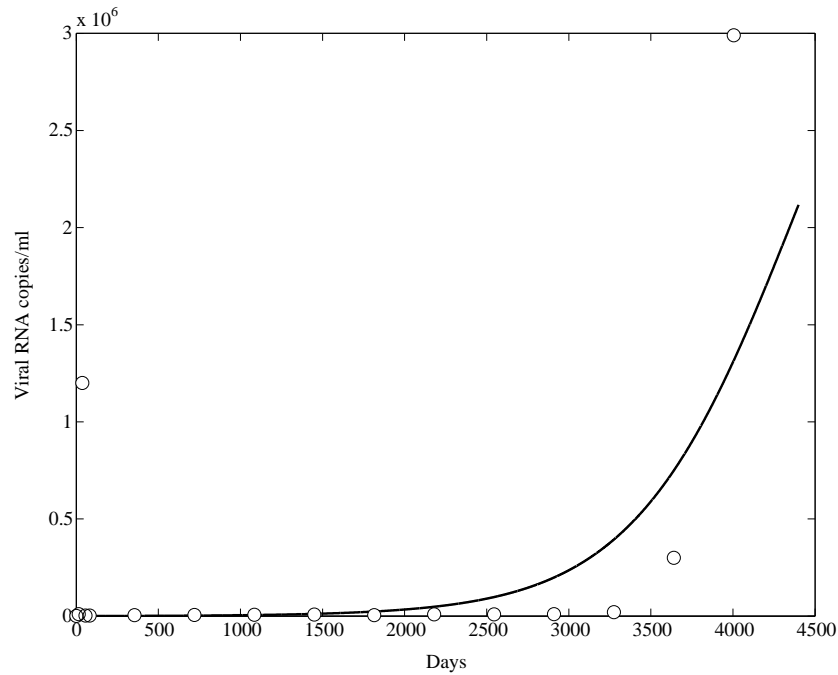


Figure 4.12.: This plot shows the population size of virus, due to long lived cells, with a constant virus set-point from the  $CD4^+$  T cell compartment.

viral load.

Fig. 4.12 might seem to indicate that virus levels produced by long-lived cells follow an unbound exponential curve. It is clear though, that (2.1) settles to a set-point value even in the presence of virus particles from a secondary pool of virus. It is assumed for this analysis that the patient dies due to secondary causes accompanying AIDS before such a set-point is reached. This assumption might also explain why some patients never progress to AIDS. If the dynamics of the long-lived cells in such patients settle at a lower set point, it might be possible that the asymptomatic stage continues, without medical intervention.

After modeling the explosion of virus with constant set-point at 1000 virus particles ( $q_{v_T} \approx 0.15$ ), the curve is saved for stage two of the analysis.

In the second stage, the generated curve from stage one is used as a reference point for the influence of the long-lived cell compartment on the  $CD4^+$  T cell compartment. At each time-point this curve is consulted to find the viral level from the long-lived cell compartment.

As a starting point for the parameter search, the parameter set from chapter 5 is used. In Fig. 4.14 one can see that even an initial guess from the vaccine readiness cohort in

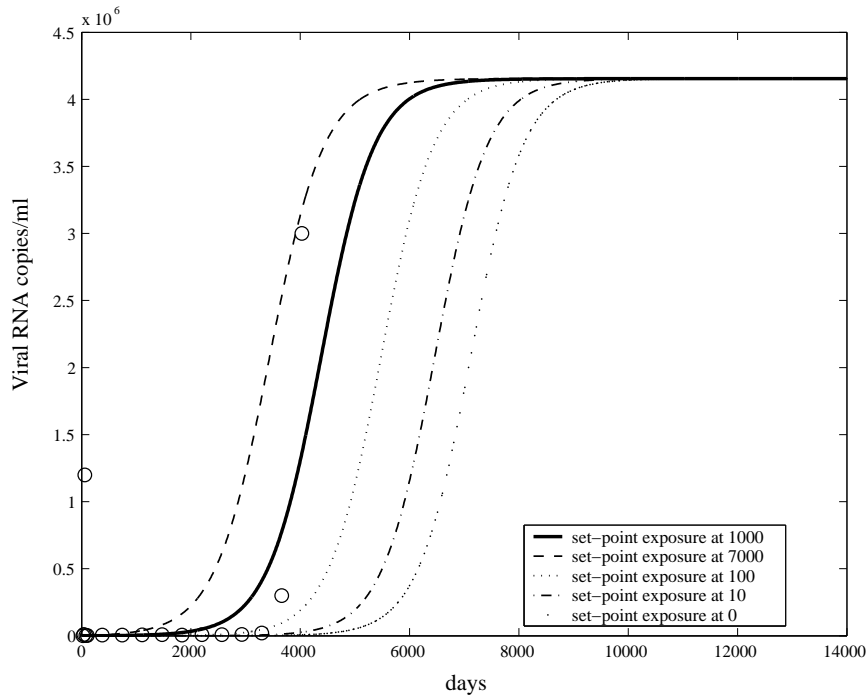


Figure 4.13.: An extended timescale of Fig. 4.12 and variation of set-point exposure levels shows the influence of set-point exposure from the  $CD4^+$  T cell compartment on the progression of viral load for the long lived cell compartment

chapter 5 results in a good approximation of disease progression and the decline of  $CD4^+$  T cells. From this starting point the value of  $c$  and  $\delta$  are adjusted to the updated values described in [47] and the parameter search is initiated. In this case, the model is fitted to the first seven data-points, since the  $CD4^+$  T cell compartment is responsible for the initial variation. The estimation procedure finds  $\hat{\chi}_T = [5, 0.003, 2.7 \times 10^{-7}, 0.62, 20, 70000]^T$  and  $\vec{x}_0 = [990, 0.001, 80]$ . The plot of this parameter set against the original data is given in Fig. 4.15.

### Combining results into a single model

A question that arises from the two stage analysis is, if the parameters that were estimated for each stage of the analysis perform similarly when the compartments are combined into one model, as in (4.2).

With the parameter set  $\hat{\chi}_r = [\hat{\chi}_T^T \hat{\chi}_M^T 0.08 \ 1]^T$ , the recast model (4.2) is simulated and the result can be seen in Fig. 4.16. It is clear, when this figure is compared to Fig. 4.15, that the assumptions hold and only a small difference in modeled results is present.

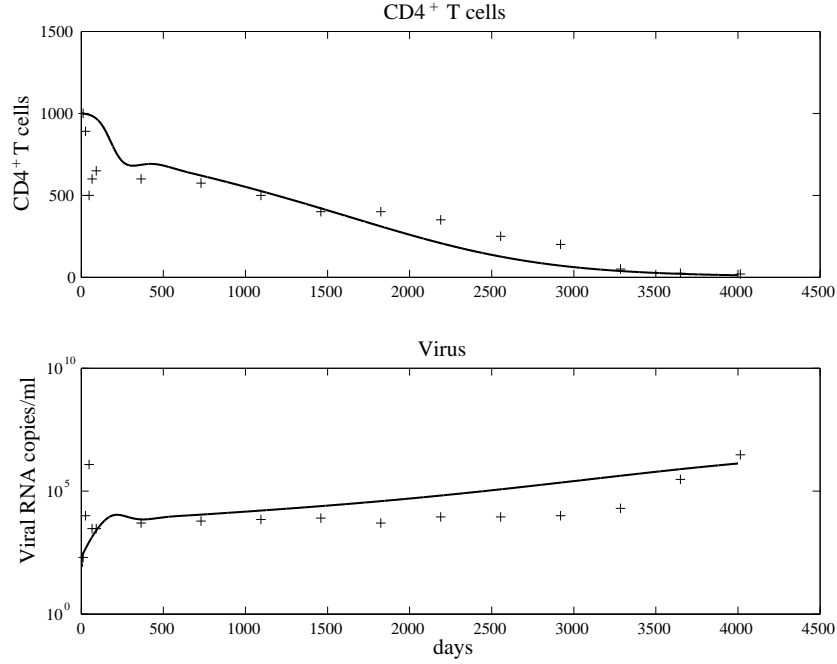


Figure 4.14.: The model curve for the initial parameter set that is used as starting point for the second stage of analysis.

### 4.4.3. Link to existing models

Recall the extended compartmental HIV/AIDS model (2.17) in chapter 2:

$$\begin{aligned}
 \dot{T} &= s_T - d_T T - \beta_T T v, \\
 \dot{T}_1 &= q_1 \beta_T T v - k T_1 - \delta_1 T_1, \\
 \dot{T}_2 &= q_2 \beta_T T v + k T_1 - \delta_2 T_2, \\
 \dot{M} &= s_M - d_M M - \beta_M v M, \\
 \dot{M}^* &= \beta_M v M - \delta_M M^*, \\
 \dot{v} &= \pi_T T_2 + \pi_M M^* - c v.
 \end{aligned}$$

It is important to keep new findings in perspective with earlier research results to allow comparison and clarification of parameters. Modifying a model to suit new ideas can be helpful in initial phases, but is not always desirable, since these modifications remove the direct link to previous research. In order to allow some perspective on the matter, the results from the two stage analysis are linked back to the six-dimensional model (2.17). Since this model has a shared pool of virus by default and the addition of latent cells is present, it presents some obstacles to be overcome before one can run simulations.

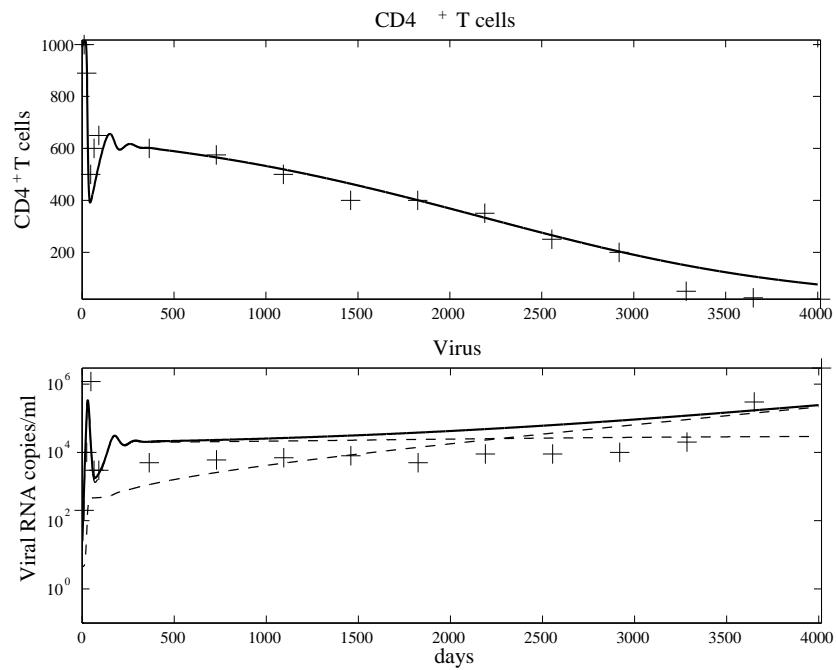


Figure 4.15.: The second stage of analysis, showing how the steady decline of CD4<sup>+</sup> T cells is tracked by the model. The virus level shows the levels due to the two compartments (dashed lines) and the total virus in the blood (solid line).

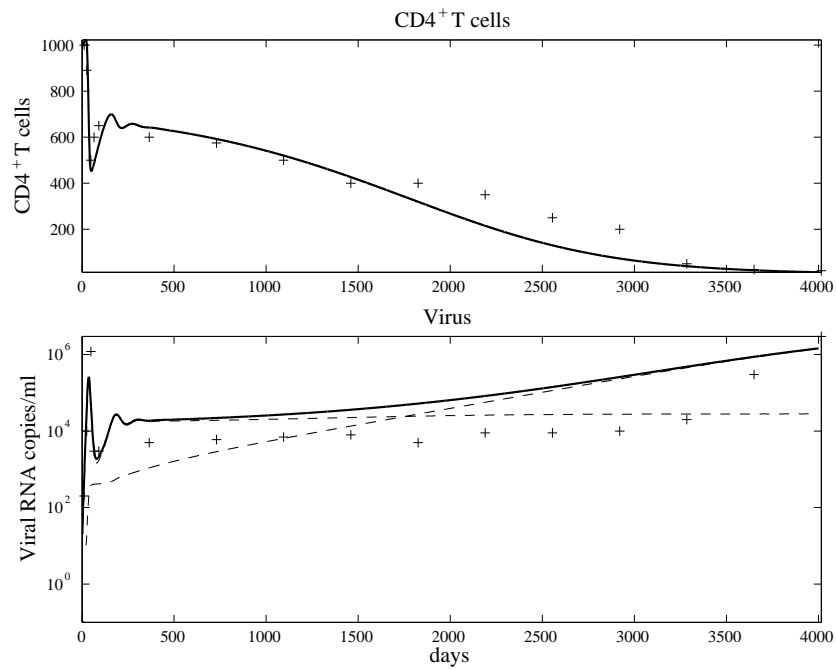


Figure 4.16.: The recast model (4.2) simulated with parameters from the two stage analysis. The virus level shows the levels due to the two compartments (dashed lines) and the total virus in the blood (solid line).



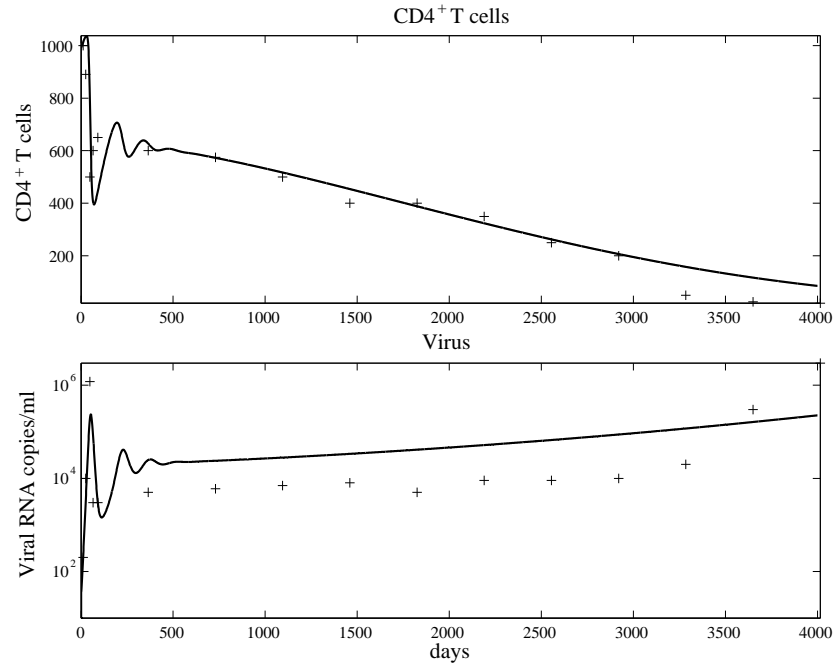


Figure 4.17.: The extended compartmental model of (2.17), fitted to the data of Fig. 4.9

In Fig. 4.17 a plot is shown with a modified parameter set. The parameters are based on  $\hat{\chi}_r$ , with some manual modifications. These modifications are based on personal experience and published parameters [30].

The results are obtained with  $\vec{x}_0 = [990 \ 0 \ .01 \ 10 \ 0 \ 10]$  and  $\hat{\chi} = [s_T \ d_T \ \beta_T \ \delta_2 \ k \ \pi_T \ | \ s_M \ d_M \ \beta_M \ \delta_M \ c \ \pi_M \ | \ \delta_1 \ q_1 \ q_2] = [5 \ 0.003 \ 2.5 \times 10^{-7} \ 0.62 \ 0.6 \ 70000 \ | \ 0.1 \ 0 \ 5 \times 10^{-10} \ 0.0005 \ 16 \ 120000 \ | \ 0.1 \ 0.8 \ 0.2]$ .

## 4.5. Summary of chapter

Chapter 4 presents the general results of this research and is the first of three chapters discussing research results.

The first section contains simulation results that depict the influence of the proliferation term (2.12) on the three-dimensional model. These simulations support the choice of basis model without a proliferation term and give insight into the influence that this term has on model dynamics.

This is followed by the results in section 4.2 where the estimation routine is validated against generated data that shows the influence of additional penalty terms in the cost function. Also, the applicability of data-collection tables with a reduced number of data-points is investigated in the context of practical data collection at hospitals.

Next, in section 4.3, a published experiment [4,8] is reproduced to validate the estimation procedure in the context of accepted results from literature. In this experiment the influence of assumptions and the inclusion of outside information in the cost function are exemplified.

Section 4.4 concludes the chapter with an investigation into the variation of parameters of the basis model throughout the pathogenesis of HIV/AIDS. First a phenomenological approach is followed to find possible variations in parameters. These results are then interpreted to explore dual compartmental dynamics as an alternative to blind parameter variation. This exploration exemplifies the adaption of the estimation procedure to investigate complex systems from the foundation of the basis model. Finally, the results of the dual compartmental dynamics are linked back to an established model to allow additional perspective on the estimated parameters.

## 5. Results from a Vaccine Readiness Study in Southern Africa

**P**ARAMETER estimation for patients who took part in the HIVNET 28 vaccine readiness study is described in this chapter. As noted in [6], HIV viral load may be a critical endpoint in vaccine trials by which to judge efficacy. It is important to define viral dynamics in unvaccinated infected individuals, especially in non-B subtype infections where little information is available. The main aim in this chapter is to determine the viral set point for these patients. The time from seroconversion to reach this set point is also of interest for the trial and analysis of the data allows for initial estimates of parameters for non-B subtype infections.

### 5.1. Description of analysis

#### 5.1.1. Study population

Fifty-one individuals with recent HIV-1 infection were recruited within eighteen months of acquiring HIV-1 infection from four countries in southern Africa (Zimbabwe, Malawi, Zambia and South Africa). Participants were followed at two, four, seven and nine months after enrolment. At each visit, blood samples were obtained for plasma RNA levels, lymphocyte subset analysis and DNA isolation. Participants were not on antiretroviral treatment.

Of the fifty-one participants recruited, ten were from Zimbabwe, six from Malawi, sixteen from Zambia and nineteen from South Africa. The majority of participants (42/51) were female. The median age was twenty-eight and the median interval from seroconversion to first viral load measurement was 8.9 months (interquartile range of 5.5-14.1). Comparison of  $\log_{10}$  RNA copies/ml in participants at enrolment showed no significant differences between countries and, based on this, were grouped as one cohort [6]. The assay used to measure free plasma virus was the branched DNA assay

and the readout was in RNA copies/ml. This is free virus and is not cell associated. It is a measure of budded virus from the cells found in the lymphoid tissue and the blood circulation. The branched DNA assay is a signal amplification assay and is a direct measure of RNA copies. It is assumed that the measured viral loads directly correlate with  $v$  in the basis model.

Only thirty-four patients met the identification requirements in [12], of the seventeen remaining patients ten had enough data points to allow analysis within the context of the cohort and the data of the remaining seven patients was not suitable for dynamic parameter estimation. The sample data for four patients from the cohort is shown in Table 5.1. Since the patients were recruited in the early stage of infection, variation in the viral load and CD4<sup>+</sup> T cell levels are expected. The variation that is evident in Table 5.1 is needed to allow parameter estimation from the data. This follows from the necessary condition of persistent excitation as described in [12].

### 5.1.2. Outline of procedure

The minimum requirement for estimation of parameters is five viral load and four CD4<sup>+</sup> T cell measurements as described in [12]. Thirty-four of the fifty-one participants had enough samples (5 + 4) to meet the minimum requirements. For these patients, the estimation routine was initiated with the set of parameters taken from literature as in section 4.2.1.

After the first iteration of parameter estimates, the model curves were plotted and examined for each patient. For patients where the initial fit was not deemed adequate, the parameters were adjusted manually until no progress was made by manual search. The parameters found in this manner were then averaged out for all patients and used as the new starting point for the final computer based parameter search.

Once the parameters for each patient had been estimated, the set point was calculated using (2.5). The fluctuations in the modeled viral load were analyzed in each patient to find the elapsed time from seroconversion where the set point of (2.5) is reached. The time from seroconversion to the point where the fluctuations fall within 0.5 of the log<sub>e</sub> values of the set point is taken as an estimate for the time to reach set point.

### 5.1.3. Patients with insufficient data points

Of the seventeen remaining patients, seven did not have enough data to be useful for this study. The other ten patients had insufficient data points for a complete evaluation of

Table 5.1.: Data points for four patients of the HIVNET 28 study.

<b>Patient ID</b>	days since seroconversion	viral load copies/ml	cd4 cells copies/ $\mu$ l
<b>P536000015</b>			
<i>(5+4 samples)</i>	391	46 699	
	426	24 463	187
	503	62 364	136
	573	25 079	193
	636	29 821	143
<b>P536000080</b>			
<i>(5+4 samples)</i>	170	3 239	
	247	5 810	451
	317	6 002	437
	366	20 439	455
	457	9 638	524
<b>P541000228</b>			
<i>(5+4 samples)</i>	422	27 941	
	506	50 295	409
	597	34 909	312
	723	33 575	308
	786	18 748	250
<b>P541000242</b>			
<i>(4+3 samples)</i>	411	67 813	
	474	11 569	
	586	39 887	186
	685		178
	775	19 359	272

parameters on their own, but allowed first order estimates in the context of the cohort. For these patients, the assumption was made that  $c$  does not differ significantly from the other patients in the cohort [4]. For a discussion of identifiability in situations where key parameters are fixed, see [12]. To solve the set point estimate for patients with less measurements (say, 4+3 as for P541000242), the clearance rate constant of the virus,  $c$ , was fixed at the average value of the participants with five viral load and four CD4<sup>+</sup> T cell (5+4) measurements. After one initial estimation run, these estimated parameters were used as the initial point to do a second iteration where  $c$  was allowed to vary. Thus, by adding these ten patients to the initial group of thirty-four, parameters were estimated for forty-four of the fifty-one participants.

#### 5.1.4. Parameter ranges and assumptions

Even though the minimum requirements for parameter estimation were met, estimation is still subject to some assumptions. The patients in the HIVNET 28 study were recruited according to strict standards [6], and thus a structured set of assumptions can be laid out:

1. Patients are in the early stages of infection, based on the enrolment data. The midpoint between the last HIV-negative sample and first positive sample is taken as an estimate to the time of seroconversion.
2. The initial CD4<sup>+</sup> T cell concentration does not exceed 1200 copies per microliter, based on the data from healthy patients with similar background.
3. Initial values for the viral load coincide with the first viral load measurement.
4. Patients do reach a steady state in viral load.
5. Fluctuations due to factors outside the model are of a lesser degree in viral load than in CD4<sup>+</sup> T cell count.
6. The order of  $c$  and  $d$  is not dictated in this instance of the cost function, but corrected after estimation.
7. The model parameters for patients in this cohort allow a common initial parameter set to be found from where the search procedure is drawn to a global minimum in the cost function of individual patients.
8. The parameters published for subtype-B infection are close enough to such a common point to be used in the first iteration of the parameter search.
9. The bias introduced by human intervention in deciding on the initial point is negligible.
10. The branched DNA assay is a fair indicator of free virus  $v$  in blood.

Table 5.2.: Parameter estimates for three patients meeting identifiability requirements

$\hat{\chi}$	P536000015	P536000080	P541000228	units
s	3.25	7.88	8.45	$T \text{ mm}^{-3} \text{ day}^{-1}$
d	$1.06 \times 10^{-05}$	0.00811	0.00822	$\text{day}^{-1}$
$\beta$	$5.38 \times 10^{-07}$	$7.96 \times 10^{-07}$	$7.58 \times 10^{-07}$	$v^{-1}(10^3 \text{mm}^3) \text{ day}^{-1}$
$\delta$	0.16	0.38	0.67	$\text{day}^{-1}$
c	0.58	0.69	0.85	$\text{day}^{-1}$
k	1016.9	688.5	2548.2	$v (10^3 T^*)^{-1} \text{ day}^{-1}$
$v_s$	36847	10439	26551	$\text{ml}^{-1}$
days to set point	449	335.5	573.5	days

## 5.2. HIVNET 28 study results

The model predictions of three patients with 5+4 data-points in Table 5.2 are shown in Fig. 5.1. In Fig. 5.2 a detailed view is given for P541000242 (4+3 data points). The markers indicate data points and the corresponding model prediction is shown by the solid line. The estimation of the set point is indicated by a dashed line, and the time to set point is taken at the point where the modeled viral load falls between the dotted lines.

Table 5.2 shows the parameter estimates for the three sample patients that meet the minimum requirements for estimation. From the final estimation, we have the following median estimate of parameters:  $\hat{\chi} = [7.48 \ 0.00085 \ 1.4 \times 10^{-6} \ 0.80 \ 1.56 \ 2834]^T$ .

Thus in this estimate  $\hat{s}$  indicates that 7.48 CD4<sup>+</sup> T cells are produced per mm<sup>3</sup> per day,  $\hat{d}$  indicates that CD4<sup>+</sup> T cells live on average for 3.8 years. The reader is reminded that the first two parameters have to account for the influence of the absent proliferation term as discussed in section 2.5 and 4.1, thus in the light of baseline CD4<sup>+</sup> T cell levels these values indicate that proliferation of CD4<sup>+</sup> T cells can be expected in the cohort, which in turn results in a large estimate for the average lifetime of CD4<sup>+</sup> T cells. The variation of  $\hat{d}$  is also discussed in the next section on confidence intervals.  $\hat{\beta}$  indicates that the infectivity of the virus is  $1.4 \times 10^{-6}$  ( $v^{-1}10^3 \text{mm}^3 \text{day}^{-1}$ ) and the average lifetime of infected cells ( $1/\hat{\delta}$ ) is 1.25 days. On average virus particles remain in the system for 0.64 days ( $1/\hat{c}$ ) before they are cleared, and  $\hat{k}$  indicates that infected cells produce on average 2.834 virus particles per cell per day.

The normal probability plot of  $\log_{10}$  set point estimations for all patients is shown in Fig. 5.3. It is visually clear that the estimates follow a log-normal distribution. The Bera-Jarque parametric hypothesis test of composite normality confirms this [48], with

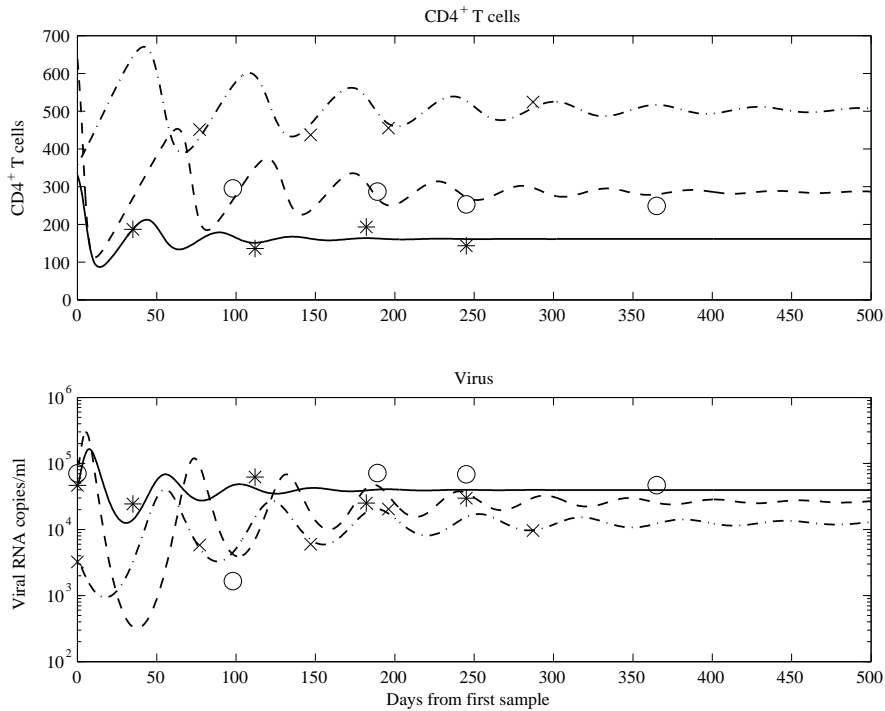


Figure 5.1.: Sample data with model for three patients. P536000015 solid, marked \*; P536000080 dotted, marked o; P525000171 dash-dot, marked x.

a significance level of 0.298.

On the combined plot for model predictions showing the trajectory of viral loads (Fig. 5.4), the calculated median time to set point was 16.57 months (497 days) and the  $\log_{10}$  based median of the calculated set point distribution was 4.08 (12143 RNA copies/ml). In three participants the parameter estimates indicated continuous variation of viral load, indicating that no set point was reached within the modeled time-frame.

### 5.2.1. Confidence intervals

The main aim of the analysis of data from the vaccine readiness trial is to determine a benchmark set point for patients from southern Africa [6]. It turns out that the set point estimation is robust against variations in the parameter estimates for this dataset. The distribution of set points is log-normal with median value of  $10^{4.084}$ . The mean  $\log_{10}$  value is 3.99, and the standard deviation is 0.56, with a 95% confidence interval [20] of [3.82, 4.16] for the mean, and [0.46, 0.71] for the standard deviation.

For the estimation of days to set point, the definition has a direct influence on the result. Since the simulated curve from the ODE solver is used to determine the point



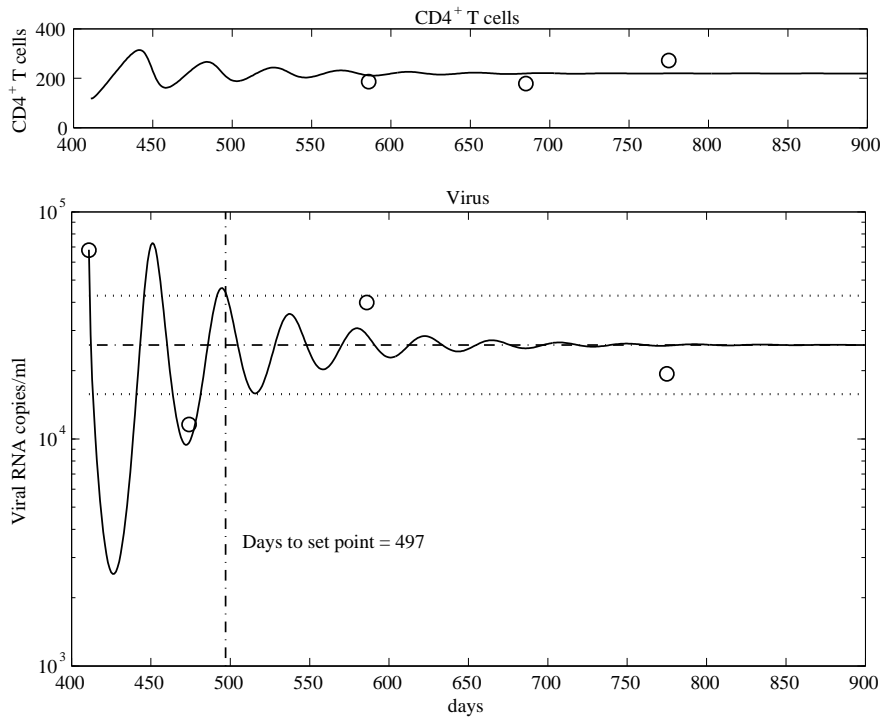


Figure 5.2.: An example, showing the model (solid line) and the original data from P541000242 (markers). The time to set point is also shown.

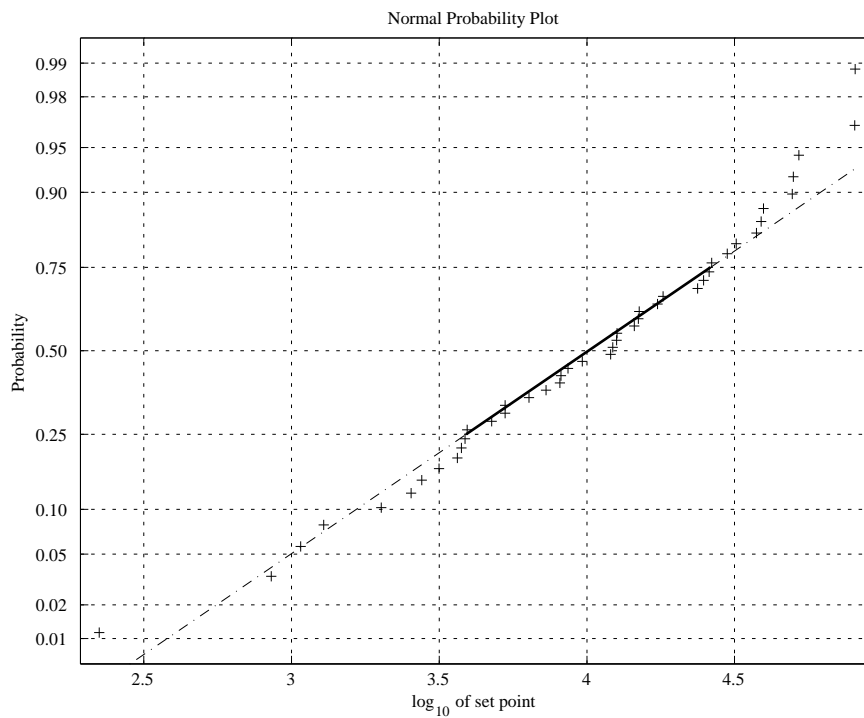


Figure 5.3.: Normal probability plot of set point estimations

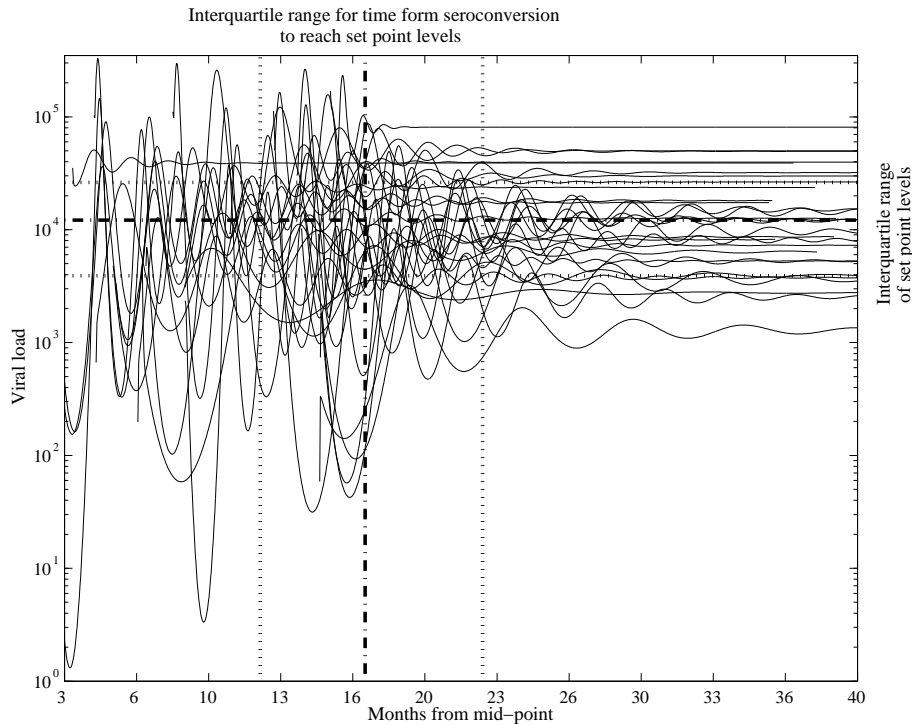


Figure 5.4.: A combined plot of all model estimations, each taken from estimated time of seroconversion.

at which a patient reaches set point, the estimation is bound to be higher as when the samples are used for this estimation. As an illustration of this, one can look at Fig. 5.2. The definition used for this study gives 497 days to reach set point. If the definition is taken as the last data point outside the prescribed range it is 474 days. Both definitions are influenced by the estimate of the time from seroconversion. For the definition in this paper, the mean days to set point is 508 days, with a standard deviation of 187. The 95% confidence interval is  $[449, 567]$  for the mean and  $[153, 239]$  for the standard deviation.

For the parameter estimates it is harder to analyze the results. The confidence intervals for each parameter, assuming a normal distribution, can be easily determined for a group of patients [20], but such closed form equations are not defined for individual patients. Worse still, for non-linear models the parameter estimates are not in general unbiased [20].

The Bera-Jarque test for composite normality ( $\alpha = 0.05, p_s = 0.3$ ) indicates that, of all the parameter estimates for the group, only  $d$  might be considered to come from a normal distribution. Whereas the same test ( $\alpha = 0.05, [p_s p_d p_\beta p_\delta] = [0.86 0.09 0.30 0.18]$ ) indicates that  $s, d, \beta,$  and  $\delta$  might be taken as log-normal. Based on this information,

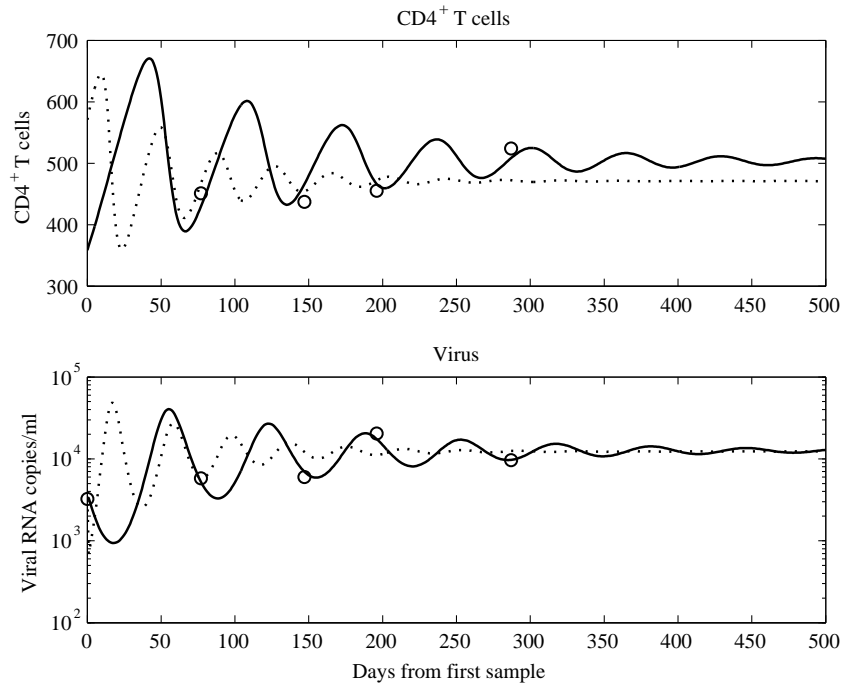


Figure 5.5.: A comparison of model results for two parameter sets for P536000080. The solid line is from the original parameter estimate for the HIVNET 28 study and the dotted line is from the parameter estimate of the box-bounded global search.

it is reasonable to follow the scheme in [20, p. 444], where a logarithmic transformation is applied to parameters in order to stabilize the random variation, without linearizing the model. In this way one can find the mean values for  $\log_{10}(\hat{\chi})$  to be  $[0.8656 \ -4.1501 \ -5.8128 \ -0.13132 \ 0.22551 \ 3.3329]$ , which corresponds to a mean value of  $[7.34 \ 7.08 \times 10^{-5} \ 1.54 \times 10^{-6} \ 0.739 \ 1.681 \ 2153]$  for  $\hat{\chi}$ . The 95% confidence interval for the mean is

$$\begin{bmatrix} 6.28 & 1.41 \times 10^{-5} & 1.22 \times 10^{-6} & 0.629 & 1.435 & 1606 \\ 8.58 & 0.000355 & 1.94 \times 10^{-6} & 0.868 & 1.969 & 2885 \end{bmatrix}$$

and the  $\log_{10}$  based standard deviation for the parameters is  $[0.22301 \ 2.3026 \ 0.33426 \ 0.22966 \ 0.22619 \ 0.41846]$ .

### Box-bounded global solver

As a means to increase the level of confidence in the results, a box-bounded global search is performed using the *Tomlab*<sup>1</sup> constrained global solver. The solver is bounded

<sup>1</sup><http://tomlab.biz/>

by the maximum and minimum estimate for each parameter within the cohort, adjusted approximately by ten percent. Thus the lower limit for  $\hat{\chi}$  is  $[1.7 \ 2.7 \times 10^{-10} \ 3 \times 10^{-7} \ 0.1 \ 0.3 \ 80]$  and the upper bound is  $[26 \ 0.036 \ 1.2 \times 10^{-5} \ 6 \ 10 \ 8500]$ .

For the patients with 5+4 data points, 100 000 function evaluations are performed by the global optimization method. The CD4<sup>+</sup> T cell and viral load trajectories for the box-bounded global parameter estimate are compared to the trajectory of patient P536000080 in Fig. 5.1. On average, the original cost value starting from a fixed initial point is 35% lower than the global search result.

If the third assumption in section 5.1.4 (that the initial viral load for the model and the first viral load data-point coincide), is dropped and the initial value for the viral load is allowed to vary for the box-bounded global solver, then it finds parameter sets with a lower cost for seven of the thirty four patients.

A comparison of the means and variances of the box-bounded global search results to those of the previous section indicate that the estimate for set point is robust against changes in the parameter estimation routine. Visual confirmation of this can be seen in Fig. 5.5, where the set point estimate stays comparatively constant, even though the time to set point is not the same for the two search methods.

A secondary estimation run as performed here can give no formal confidence intervals on individual parameters, all it does is confirm that the original method has indeed found parameter sets that result, on average, in a lower cost. It is thus still necessary to find additional means of analysis.

### Sensitivity analysis

A second approach to find confidence intervals is to perform a sensitivity analysis for each parameter at the initial parameter set and at the final parameter set.

The analysis at the initial set gives an indication as to the available minima in the cost landscape to which the optimization routine might be attracted at the start of the search. If there is more than one possibility, the confidence in the solution is lowered, and one might try distinct estimations from different starting points that are closer to these minima.

The sensitivity analysis at the solution point gives an indication of the goodness of fit and the possible variations at the parameter solution set. Now one can define a benchmark cost value and read the parameter ranges from the sensitivity analysis.

This method is time-consuming and requires human interaction in the decision process.

Once limits are standardized for a cohort, the process can be automated.

In Fig. 5.6 the sensitivity analysis for patient P536000015 is shown at the initial point for the estimation routine and at the final parameter estimation for the HIVNET 28 study. The range of the parameters for this analysis is the same as for the global box bounded search in the previous section. Since the cost landscape at the initial point shows that no parameters fork out, it is considered to be a good starting point. The cost landscape at the final point is shown with a 0.1 “cost-confidence” level drawn in. With this level one would read off the values of the parameters and their ranges in context of the analysis boundaries. Thus, for instance, the line marked with ‘+’ represents  $\delta$  with a minimum at 60% ( $\delta \approx 1.6$ ). The cost curve crosses the 0.1 level at 54% and 64%. This can be converted back to  $\delta \approx 1.3$  and  $\delta \approx 1.9$ . In this way one can determine ranges of applicability for each parameter. Note also the insensitivity of the cost to changes in  $d$ . This indicates that at a 0.1 level practically any value of  $d$  from 0% to 90% could be replaced into the final estimate without notable change in cost. This translates to a large confidence interval for  $d$ . In the context of (2.1) this result makes sense, since, below a certain death rate, the influence of this parameter is swamped by the loss of CD4<sup>+</sup> T cells to infection. This observation is in line with the statistical confidence interval analysis in sections 5.2.1, where the standard deviation for  $d$  is nearly ten times more than the standard deviation of the other parameters.

As with the box-bounded global search, the sensitivity analysis is an ad-hoc method to give an indication of the confidence that one can have in the original parameter estimates (in this case those of the HIVNET 28 study). The sensitivity analysis has the added advantage that it allows for the determination of parameter ranges within a cost-confidence level.

For both methods there is ample scope for further research and some of the proposed starting points in [20] may be well suited to this application.

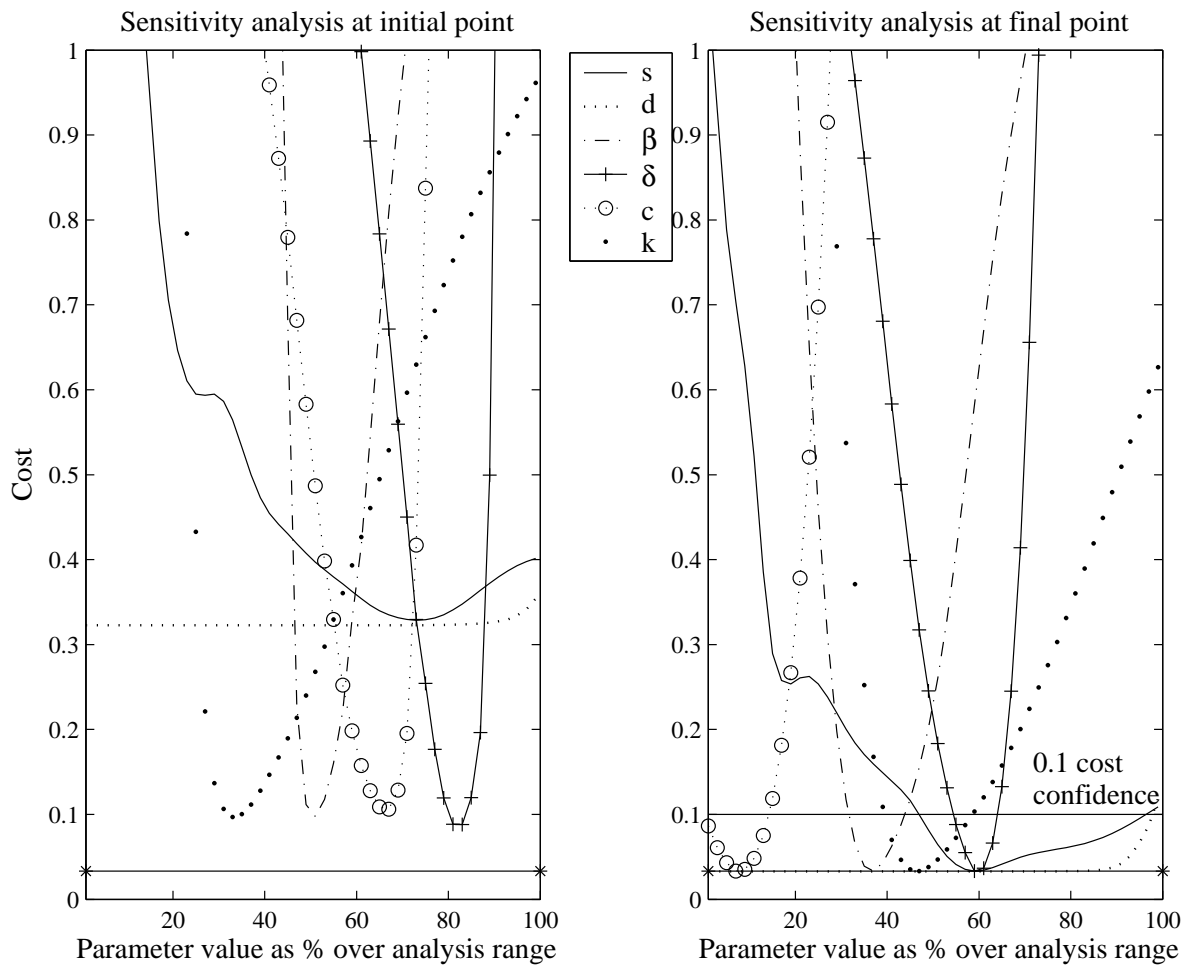


Figure 5.6.: Sensitivity analysis at initial parameter set and final parameter set

### 5.3. Summary of chapter

Chapter 5 presents the results from the HIVNET 28 vaccine readiness study in southern Africa. First the context of the study is described. Since the HIVNET 28 study has a slightly different emphasis than the primary aim of this research, the procedure is tailored to accommodate the given situation, exemplifying the flexibility of the estimation procedure.

The adaption of the procedure for the application to a cohort of patients is presented in section 5.1. This includes a strategy to include patients in the study that would not meet the identifiability requirements under normal circumstances. Also, the necessary assumptions for parameter estimation in the HIVNET 28 context are laid out.

The main aim of this chapter is to determine the set point for patients of the HIVNET 28 study and to find the time from seroconversion, for this set point to be reached. In section 5.1.4, the results show that after approximately 17 months from seroconversion, oscillations in viremia have flattened to a median set point of  $4.08 \log_{10}$ , appearing no different from reported studies in subtype B HIV-1 infected male cohorts [6, 49–51]. Together with these main outcomes, initial estimates for parameters are also presented.

Finally the confidence intervals for parameter estimations of the HIVNET 28 study and a sensitivity analysis for a patient of the cohort are presented.

## 6. Computer Program

### 6.1. Program overview

**B**Y CREATING a computer program, the insights and knowledge gained from research are bundled into a practical tool for people involved with HIV/AIDS. The program has three different access levels that allow users with differing backgrounds to access it. Also there are modules available to conform to varying platform needs. The stand-alone program can be installed on any windows based computer. The web-based applet is intended to allow users to access the program through the internet.

Data entry is performed within the framework of *Microsoft Excel*. Table 6.1 shows the Excel sheet with the minimal data entered. As an absolute minimum, the user must enter a patient id, by which the results can be identified, together with sample data and the days at which they were taken. If the date of the first sample is supplied, the other dates are calculated, otherwise they are shown as “Not supplied” in the date column.

The three access levels to the program are general, advanced and full access. In general user mode, the program allows the access to basis information and procedures. Thus the user can enter information only in standard forms, answer standard questions and receive the standard report with the identified parameters and available information. At the advanced level, users can modify additional search boundaries and access additional information about parameters. Parameters can be fixed at expected levels for a search, and searches with minimal data can be performed by limiting key parameters. In full access mode, the user can modify the cost function directly and customize the search procedure to fit personal needs. The full access mode is intended for users who are familiar with parameter estimation and want to research alternative estimation procedures.

A secondary spin-off from the main program is an educational module that allows students to consider the parameter estimation procedures and become familiar with the modeling of HIV/AIDS. This module is part of the education CD of the University of Pretoria and can also be implemented on web-pages. It has none of the main programs parameter estimation procedures built in, but rather it allows the user to experiment



Table 6.1.: The layout of the Microsoft Excel sheet where the basis information is entered.

## Patient Information Sheet

		<b>Help</b>
<b>Patient ID:</b>	fau96	A string that uniquely identifies this patient
<b>Last Name:</b>	<i>Not Supplied</i>	Optional Last Name
<b>First Name(s):</b>	<i>Not Supplied</i>	Optional First Name(s)
<b>First Positive:</b>	<i>Not Supplied</i>	First Date Where Patient Tested Positive
<b>Last Negative:</b>	<i>Not Supplied</i>	Last Date Where Patient Tested Negative
<b>Infection Date:</b>	<i>Not Supplied</i>	The assumed date of infection.
<b>CD4 Samples</b>		
<i>First CD4 Sample Date</i>	<i>Not Supplied</i>	Date first CD4 Sample is taken
<b>Date</b>	<b>Days</b>	<b>Level (Cells per milliliter)</b>
<i>Not Supplied</i>	10	1000
<i>Not Supplied</i>	25	890
<i>Not Supplied</i>	48	500
<i>Not Supplied</i>	66	600
<i>Not Supplied</i>	91	650
<i>Not Supplied</i>	365	600
⋮	⋮	⋮
<b>Viral Samples</b>		
<i>First viral Sample Date</i>	<i>Not Supplied</i>	Date first viral Sample is taken
<b>Date</b>	<b>Day</b>	<b>Level (RNA copies per micro-liter)</b>
<i>Not Supplied</i>	10	200
<i>Not Supplied</i>	25	10000
<i>Not Supplied</i>	48	1000000
<i>Not Supplied</i>	66	3000
<i>Not Supplied</i>	91	3000
<i>Not Supplied</i>	365	5000
⋮	⋮	⋮

with different parameters by adjusting them manually and visually assessing the effect of these changes in the model. There are pre-evaluated parameter sets available to students for them to compare the manual search for parameters with the computer based search.

## 6.2. From theory to practice

By using the theoretical background as a springboard, a program is created that allows users from diverse backgrounds to increase the effectivity of HIV/AIDS treatment strategies. With the insights obtained from parameter estimation, users can monitor individual patients without the need to understand underlying mathematical details of the model.

Some of the key points for the user are listed below:

- The user interface allows both patients and general practitioners to enter their sample data and receive treatment guidelines. Data can be entered in the familiar environment of Microsoft Excel.
- Information falling outside the data-set can be entered and is incorporated into the estimation procedure to increase the accuracy of estimation.
- The program determines a set of initial conditions from the data and external information for the identification routine without the intervention of an HIV/AIDS modeling expert.
- Inconsistencies in results and data are detected and the user is informed about the discrepancies.
- In situations where a user needs detailed information, or where personal limits must be added that are not currently interpreted by the program, a higher level user can modify the cost function directly and access the raw output from the identification routine.

Some of the underlying functions are listed below:

- The program follows a control-system approach to model identification using a modifiable cost function as described in section 3.2.

This is the key function of the program and in situations where enough data points are available it is adequate for parameter estimation.

- The cost function is LSQ based and combines the LSQ cost with the bounds and prevailing conditions from the user supplied information.

Here the user information from outside the data set comes into play. As an example: When a patient supplies the program with an estimated time since infection that

indicates that the steady state in viral load has been reached, the patient is advised to take medication in conjunction with the load tests, since the basis parameters cannot be determined from the steady state [12]. From the point where medication is taken, the cost function is modified as in section 3.2.2. Thus, the advice to take medication in this case is based on the necessary conditions for parameter estimation.

- The fine-tuning of the cost function is hidden from the user and user input about prevailing circumstances is translated into mathematical terms.

Direct user intervention in the cost function is not necessary for the previous point. Pre-programmed knowledge is incorporated automatically, with feedback to the user. If any assumption needs to be overridden, the user can select to do so without directly editing the cost function.

- A parameter search is done by standard means and the resulting parameters are interpreted for the user.

Once the basis of data and external information is in place, the parameter search commences along standard avenues. It is in the interpretation of results that there is still a great deal of knowledge necessary. Since the parameters are not yet in standard use among practitioners, there is no experience base from which to make recommendations. Currently, the recommendations are made according to general research results as given, for instance, in [22]. The program allows theoretical scenarios to be viewed with different treatment strategies. As more individual experience is gained by practitioners and patients, this information can be used to generate more pointed recommendations.

- In situations where there is not enough data from a single patient, the program can use information from its database to give possible scenarios that allow the user to make initial treatment decisions.

Here the user can benefit from general parameter estimates of other studies like [4]. Since there is no detailed data available for the individual, some parameters can be fixed at expected values to generate a general scenario that contains those initial data points and considers the external information. If, for instance, the patient has been infected for more than fifteen months, it is likely that viral load is in steady state [16], and from a single sample some of the ratios of parameters can be estimated. Even if the finer details are not available, the user still gets an indication of possible scenarios.

As an example, the estimation for a patient is shown in Fig. 6.1, with the correspond-

Estimation graph for PExample:

$s=9.3451$     $d=2.7182e-07$     $\beta=1.473e-6$

$\delta=0.68486$     $c=2.1144$     $k=1953.7$

$T_0=358.24$     $T^*_0=1.6545$     $v_0=3358.7$

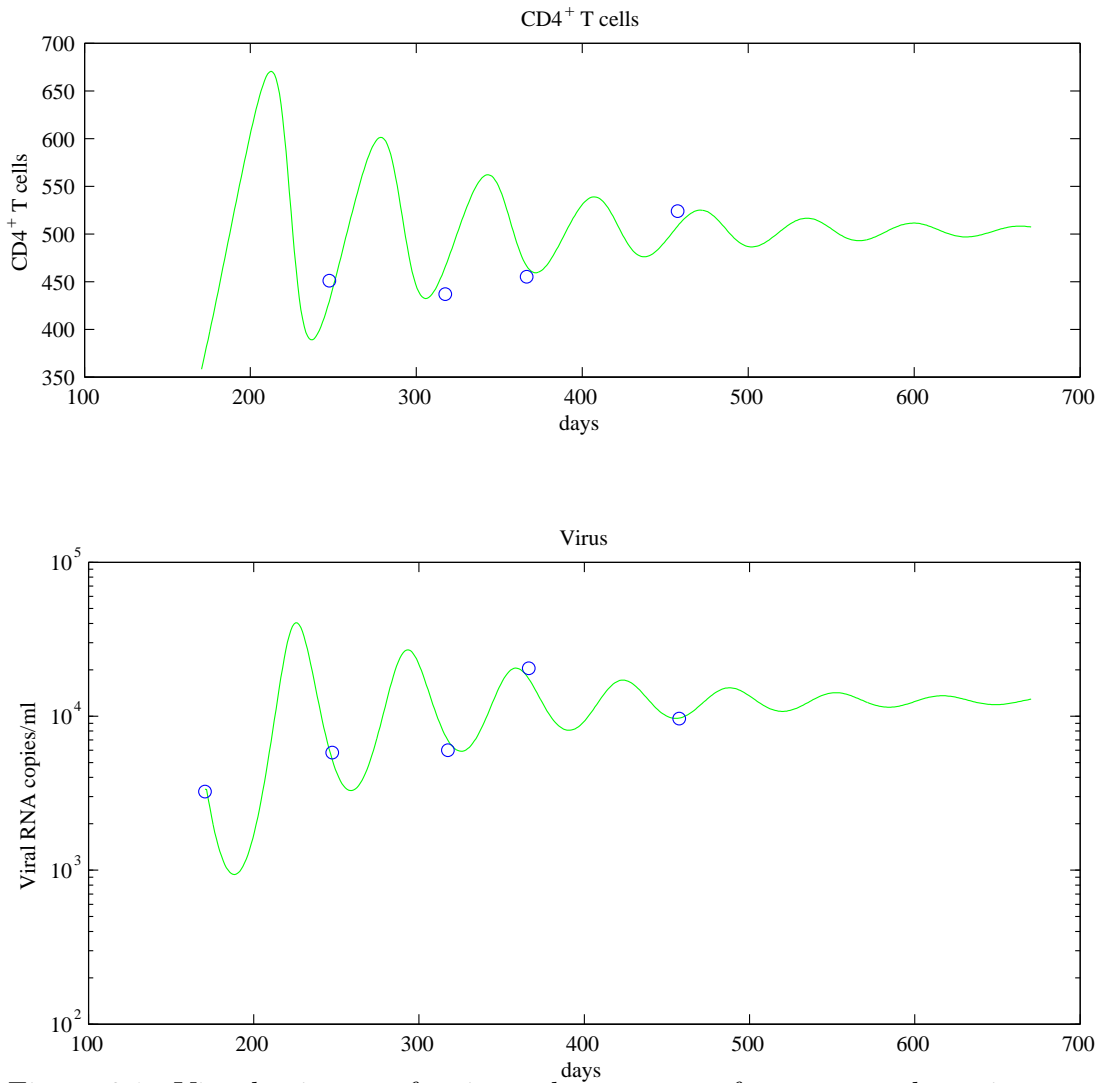


Figure 6.1.: Visual printout of estimated parameters for an example patient

PExample:

Basis Report

-----  
Enough data points were available to determine parameters:

s= 11.409

d= 0.008

beta=  $1.2 \times 10^{-6}$

delta= 1.35

c= 1.31

k= 2889.2

Set-point= 12223

Estimated time to reach set-point = 14 months

Constraints on parameters were set to default.

c<delta

s<50

d>0.008

No additional modifications made cost function

Fit seems adequate: no outliers were detected in data-set.

The spacing between data points is very large, it is important to perform a visual check on the data to ensure that a good fit has been found. It is recommended that samples are taken closer together. For more details see the guidelines for sample spacing.

CD4 cell count seems stable at an acceptable level. The viral set-point falls in the lower range: according to the panel on clinical practices on HIV/AIDS it might be desirable to postpone a rigorous treatment regime.

For visual scenarios of different treatment strengths, please consult the treatment advisor application.

Figure 6.2.: Basis report printout of estimated parameters for an example patient

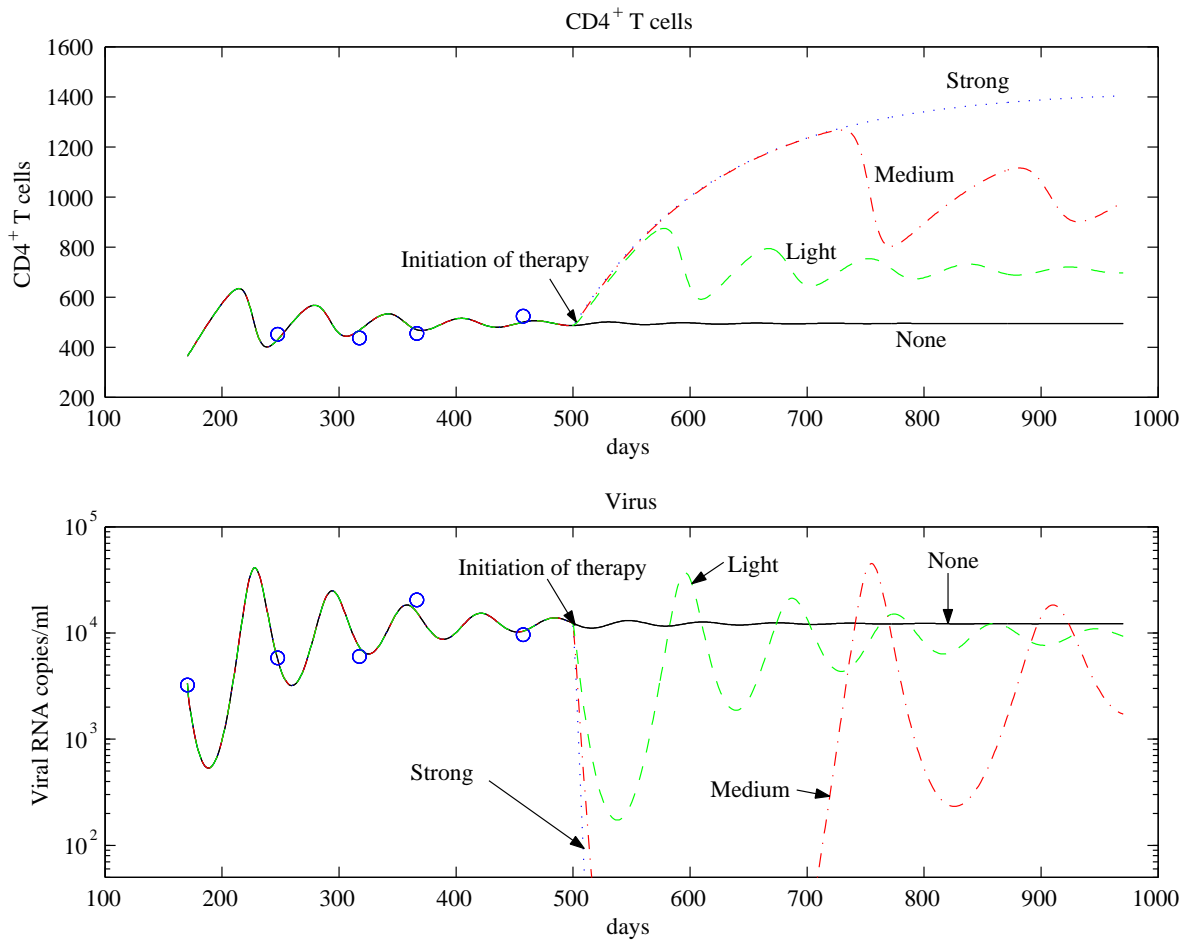


Figure 6.3.: Treatment advisor visual printout for different efficiencies of protease inhibitors

ing report for this patient shown in Fig. 6.2. The report is text based and contains the main information and advice. The user can also see a graphical representation of the parameters in a treatment context and compare different scenarios of treatment as shown in Fig. 6.3. The scenarios represented in this figure are based purely on theoretical values of drug efficacy. Since patients can be monitored after the initiation of therapy, these theoretical values can be augmented by the information gained during treatment. Thus, the trajectory of viral load after initiation of therapy may be used to determine the efficacy of a regime even before viral load is suppressed below the benchmark values described in [5].

The described program is in the early stages of development where only the key functions are implemented. In order to keep the program relevant, the advice generator must be kept up to date in accordance with current guidelines and experience gained from program use. One of the future development goals is to incorporate parameter estimation for higher-dimensional models for patients with enough data points.

It should be stressed that a computer program cannot replace the expert care of a medical practitioner. Program users should always be aware that this program is a tool to help in decision-making and planning of treatment, but that the final decisions lie with the medical practitioner in dialogue with the patient.

### **6.2.1. Code and algorithms**

The program code and description of algorithms can be found in appendices A and C.

## **6.3. Key feature summary**

The developed application uses the theoretical basis of cost function based parameter estimation to allow the medical practitioner to estimate the three-dimensional model parameters for HIV/AIDS patients. The program gives greater insight into the progression of the disease, and helps the practitioner decide on the correct dosage for the patient.

Even though these functions are implemented, the advice of the program currently draws on a small experience base. As the individually tailored parameter estimates become a part of each patient's treatment strategy, the experience of practitioners in the field and insights from research can be incorporated to increase the quality of advice given by the program.

In order to cater for personal experience in treatment strategies, the program can be

used at different levels of complexity. The basis module of the program demands little background knowledge from the user, but for practitioners with experience in mathematical modeling, there is ample opportunity to fine-tune the procedures to conform to the special needs that may arise from their medical situations.

### **Future development**

As of this writing, the modules described in this chapter are in a beta development stage. There is ample opportunity for expansion to all modules, but the initial focus should be to test the basis module for general users with real data. The users guide for the basis model can be found in appendix B.

For the advanced module, integration with existing patient data-base systems should be explored, since much insight could be gained by retrospective analysis of data.

The core functionality of the program is based on parameter estimation procedures. Since optimization procedures and numerical methods are active fields of research, the estimation modules should ideally be linked to specialist package that is continually updated. When the program functions are separated in this way, it is possible to keep up with the advances in optimization, while allowing users to have a consistent and stable interface.

For some patients, data will be available before initiation of treatment and afterwards. If treatment is initiated after an original set of data has been examined, the current program can analyze the data in this context to produce an updated treatment parameter estimate. A useful possibility would be to expand the program so that these differences in estimated parameters can be automatically analyzed in order to quantify the influence of treatment and give an indication of its effectiveness. If this analysis is standardized—as opposed to the general interpretation given at the moment—it would allow for batch analysis of treatment studies to give an indication of effectiveness of different treatment strategies.

One important issue that is not only applicable to the estimation package, is the public awareness about current technology that is available to HIV/AIDS patients. It is important that the public, HIV patients, and especially practitioners, are aware of the importance of early viral and CD4<sup>+</sup> T cell sampling



## 6.4. Summary of chapter

This chapter introduces a computer program as a practical application of this research. A broad overview of the link between theory and practice is given, and the key features of the program are summarized. Some example figures, detailing key aspects of the program are presented, with a discussion of additions and possible expansions to the program.

The users guide for the basis module of the *Dynamic HIV Estimator* can be found in appendix B.

## 7. Conclusion

### 7.1. General results

**T**HE DESIGN and implementation of a dynamic parameter estimation routine allows a diverse base of information from outside the basic dataset to be incorporated in the parameter estimation cycle for the three-dimensional HIV/AIDS model. The results presented in this research show that the developed method can identify parameters in situations where an orthodox LSQ method would fail. Together with the advantage of higher quality estimates in situations where the data is well-posed, the estimation routine can use information from outside the dataset to provide reasonable estimates for data that cannot be used with a pure LSQ method. The presented method is a step forward in the effort to supply patients with individualized parameter estimation. The estimates made in literature were for at most two parameters per dataset, whereas the procedures described herein can estimate all six parameters. It should be noted that the conditions for successful estimation, as described in [12], still apply if to situations where no external information is available to support the basic LSQ cost function.

A standard table that is proposed for data acquisition in hospitals and clinics is analyzed. The results show that the table would contain enough information to extract a good estimation for the parameters of the three-dimensional HIV/AIDS model.

Many experiments in literature have data windows that are too small to make definite conclusions about the parameters from the published data alone. To compare results, external knowledge from articles, or the description of the experiment, can be included in the cost function of the estimation routine. Comparison with a published experiment shows that it becomes increasingly hard to coordinate assumptions and implicit information when analyzing real data.

## 7.2. Parameter variation

Parameter variations during the course of HIV/AIDS are still not well understood. One reason for this poor understanding is the lack of high quality data that can be analyzed. From the results presented in section 4.4.1, one can see that parameters may vary considerably over the course of HIV/AIDS. The data points for the analysis of parameter variation are too sparsely sampled to draw quantitative conclusions, rather the results show that parameters vary over time and that, qualitatively, parameters may vary as presented. For example, since there is a steady decline in  $CD4^+$  T cell levels in the dataset and the results show a corresponding increase in  $s$ , this observation lends support to the use of a density based proliferation term that is commonly used in conjunction with  $s$  [4, 46].

A more densely populated dataset needs to be analyzed before any further conclusions can be made about the variation of parameters in the basis model.

In section 4.4.2, the initial analysis of parameter variation is augmented with a novel two-stage approach of model identification for the six-dimensional model (2.17). It is shown how, by de-coupling the compartments a good initial estimate of parameters can be made. In this context, the higher-dimensional models allow an explanation for the onset of AIDS from HIV without any variation in the model parameters.

## 7.3. Vaccine readiness study

The developed estimation procedure was used successfully to analyze the data from the HIVNET 28 vaccine readiness trial. Despite the sparse data available per patient, the datasets complied with the minimum identifiability requirements, to allow the extraction of the average set point and time from seroconversion until this set point is reached.

Both results are important to form a benchmark for the study of vaccination, since one outcome of the vaccine trial is to achieve a lower set point viral load. The time it takes for patients to reach a steady state gives a good indication of the period for which patients should be monitored to ensure that some samples are taken when the patient has reached a set point.

The results show that after approximately 17 months from seroconversion, oscillations in viremia flattened to a  $\log_{10}$  based median set point of 4.08, appearing no different from reported studies in subtype B HIV-1 infected male cohorts [6, 49–51]. Together with these main outcomes, initial estimates for parameters are also presented.

From the analysis of confidence intervals for set point, days to set point and the individual parameters it is seen that the set point is robustly estimated, thus achieving the main goal of the analysis. The estimate of time until set point is dependent on its definition, and is also more sensitive to changes in parameter estimation.

For a group of patients it is possible to generate statistical confidence intervals for the parameter estimates, but as a result of sparse data, isolated parameter estimates for individual patients of the HIVNET 28 study would result in large confidence intervals on parameter estimates. An ad hoc approach of confidence analysis must be followed for patients in isolation. Possibilities of confidence analysis are presented but the approach needs to be addressed in further research.

Since patients for the study were selected subject to well-defined constraints [6], this allowed for specific conditioning of the estimation routine and a clear layout of assumptions for the group. When estimates for the cohort are combined, the data allows a meaningful first estimate of parameters of the three-dimensional HIV/AIDS model for patients from southern Africa.

## 7.4. Computer program

The developed application uses the theoretical basis of cost function based parameter estimation to allow the medical practitioner to estimate the three-dimensional model parameters for HIV/AIDS patients. The program gives greater insight into the progression of the disease, and helps the practitioner decide on the correct dosage for the patient.

Even though these functions are implemented, the advice of the program is currently based on a small experience base. As the individually tailored parameter estimates become a part of each patient's treatment strategy, the experience of practitioners in the field and insights from research can be incorporated to increase the quality of advice given by the program.

In order to cater for personal experience in treatment strategies, the program can be used at different levels of complexity. The program demands little background knowledge from the user, but for practitioners with experience in mathematical modeling, there is ample opportunity to fine-tune the procedures to conform to the special needs that may arise from their medical situations.

One important issue that is not only applicable to the computer estimation package, is the public awareness about current technology that is available to HIV/AIDS patients. It is important that the public, HIV patients, and especially practitioners, are aware of

the importance of early viral and CD4<sup>+</sup> T cell sampling

## 7.5. Further research

Imagination is more important than knowledge — *Albert Einstein*

... and a good question is worth a thousand answers.

- The core functionality of the program is based on parameter estimation procedures. Since optimization procedures and numerical methods are active fields of research, the estimation modules should ideally be linked to a specialist package that is continually updated. When the program functions are separated in this way, it is possible to keep up with the advances in optimization, while allowing users to have a consistent and stable interface.
- In discussions with Markus M. Edvall and Kenneth Holstrom in connection with *Tomlab* it became evident that there are indeed “better” approaches to parameter estimation than the Nelder-Mead method. Also, in [43] the author states that the Nelder-Mead is a good robust starting point! Thus there is definitely room for expansion in this area of the estimation routine.
- With some of the sparse datasets in the HIVNET 28 study it is possible to find parameter estimates where the cost level is so low that it seems too good to be true. Personal experience indicates that such situations arise from data where the viral load might already be in steady state. Further research is needed to find an estimator that indicates whether data is persistently exciting (which is a necessary condition for successful estimation [12]).
- Since the data is sparse in the previous point, an estimate for persistent excitation might be just as hard to find as an estimate of the parameters (or even impossible with all the variances in the measurement). A good idea might be to add a check for persistent excitation after the numerical ODE solver. This would then constitute an “identifiability cost”. One could then use the argument that, if the identifiability cost indicates that the estimate model is not persistently exciting, then, either the sampled counterpart is not persistently exciting (which indicates that the estimation is useless without further information), or the search method got stuck at a local minimum where the estimate is not persistently exciting (which indicates that the search procedure should be restarted with a new initial point).
- Since the latest estimates of the viral clearance rate,  $c$ , are in the range of 23(!) [47], one must consider the possibility that the parameter estimates for the basis model

(as found from initial points taken from earlier research), are in fact describing the latent and active cell interactions and miss the fast dynamics of  $c$ . To find out if this is the case, one could estimate parameters of four-dimensional model for the same data, but with  $c$  fixed at 23.

- The previous point goes together with the question of pharmaceutical delay: If the viral clearance rate is indeed as high as described in [47], how are the viral dynamics separated from the pharmaceutical delay?
- Since the current definition of treatment effectivity results in sharp changes of viral set point over a small range of treatment efficacy, attempts are made to refine the model to be robust against parameter variation and allow low steady states [45,46]. The question should be considered whether the increased complexity of simple models is warranted, or if it is not better to re-define the definition of treatment effectivity separately from the model. Most of the assumptions that describe treatment efficacy lend themselves to model simplification, rather than the description of the true influence of treatment on the system (see [4,45,47]). It is conceivable that a change in treatment definition would allow earlier research to stay relevant, while at the same time accommodating new insights into treatment strategies.
- The previous point is also important to end-users of dynamic HIV/AIDS models. The current standard of single blood samples for the basis of treatment decisions [5] will stay more effective than the decisions based on models, as long as there is no standard model to use, from where the medical community can build an experience database. It is important that a standard model is used that balances complexity with identifiability and the predictions that can be made from such a model. As long as there are continuous changes in the model this will prevent users from becoming familiar with parameter ranges. Even if some of the parameters (as for instance death rate of healthy cells in the basis model without proliferation) have to accommodate multiple interactions, there are bound to be correlations between the disease and parameter estimates that can be exploited with consistent use of the model.

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# **Part III.**

## **Appendices**

# A. Search Methods

## A.1. The Nelder-Mead search method

The primary source of the Nelder-Mead search method is found in [42] but most implementations are based on any of the *Numerical Recipes* books, of which [43] is a C-based example.

This appendix gives some background information with a description of the method that was found to be a helpful introduction to the method, adapted from a web page. Since internet sources are not guaranteed to stay valid, permission was obtained from the author to reproduce some of the material here.

The Nelder-Mead search method that forms the basis for the parameters search, is a simplex search method where  $f(x)$  has to be minimized for the parameter set  $x$ . The main part of the algorithm, as translated from the *Matlab* source for `fminsearch`, is as follows:

```

repeat
   $\bar{x} \leftarrow \sum_{k=1}^n v_k/n$ 
   $x_\rho \leftarrow (1 + \rho)\bar{x} - \rho v$ 
   $x \leftarrow x_\rho; f_{xr} \leftarrow f(x)$ 
  if  $f_{xr} < \min(fv)$  then
    {calculate the expansion point}
     $xe \leftarrow (1 + \rho\chi)\bar{x} - \rho\chi v_{max}$ 
     $x \leftarrow xe; f_{xe} \leftarrow f(x)$ 
    if  $f_{xe} < f_{xr}$  then
      {expand}
       $v_{max} \leftarrow xe$ 
       $f_{v_{max}} \leftarrow f_{xe}$ 
    else  $\{f_{xe} > f_{xr}\}$ 
      {reflect}
       $v_{max} \leftarrow xr$ 
       $f_{v_{max}} \leftarrow f_{xr}$ 

```

```

end if
else {  $f_{xr} \geq \min(fv)$  }
  if  $f_{xr} < f_{v_n}$  then
    {reflect}
     $v_{max} \leftarrow xr$ 
     $f_{v_{max}} \leftarrow f_{xr}$ 
  else {Perform contraction}
    if  $f_{xr} < f_{v_{max}}$  then
      {Outside contraction}
       $xc \leftarrow (1 + \psi\rho)\bar{x} - \psi\rho v_{max}$ 
       $x \leftarrow xc; f_{xc} \leftarrow f(x)$ 
      if  $f_{xc} < f_{v_{max}}$  then
        {contract outside}
         $v_{max} \leftarrow xc$ 
         $f_{v_{max}} \leftarrow f_{xc}$ 
      else {shrink}
        for  $k = 2$  to  $n$  do
           $v_k \leftarrow v_{min} + \sigma * (v_k - v_{min})$ 
           $x \leftarrow v_k; f_{v_k} \leftarrow f(x)$ 
        end for
      end if
    else
      {Perform an inside contraction}
       $xcc \leftarrow (1 - \psi)\bar{x} + \psi * v_{max}$ 
       $x \leftarrow xcc; f_{xcc} \leftarrow f(x)$ 
      if  $f_{xcc} < f_{v_{max}}$  then
        {contract inside}
         $v_{max} \leftarrow xcc$ 
         $f_{v_{max}} \leftarrow f_{xcc}$ 
      else {shrink}
        for  $k = 2$  to  $n$  do
           $v_k \leftarrow v_{min} + \sigma * (v_k - v_{min})$ 
           $x \leftarrow v_k; f_{v_k} \leftarrow f(x)$ 
        end for
      end if
    end if
  end if

```

```

    end if
  end if
end if
until max(|vk - vmin|) <= tolx && max(|fvmin - fvk|) <= tolf k=1:n

```

The Nelder-Mead method is a popular search method, used in situations where discontinuities may be expected in the cost landscape. An internet search reveals many relevant pages, with descriptions that are rather more understandable than the algorithm presented on its own. The following description of the method is adopted from lecture notes for a course in *Ecological models and data* by Ben Bolker, available on the internet<sup>1</sup>. The section is entitled *Discontinuities etc.: Nelder-Mead (“simplex”, “amoeba”)*

The first problem that derivative-based methods (such as quasi-Newton) have are that they can be messed up when the cost function has sharp changes in it. When the quasi-Newton method gets to a flat part of the graph, it gets faked into thinking it’s found the answer (since the derivative is zero); when there’s a vertical discontinuity in the graph, it gets faked into extrapolating wrong. There are elaborate methods that avoid the use of derivatives (e.g. Powell’s method in Numerical Recipes), but a simple brute-force option is the simplex method invented by Nelder and Mead (called the “amoeba” in Numerical Recipes). Rather than starting with a single point guess at what the parameters are, this method picks a number of points which form a simplex—the simplest shape possible in an n-dimensional space. In two dimensions, this is three points (each of which represents a pair of parameter values) forming a triangle; in three dimensions, it’s 4 points (each of which is a triplet of parameter values) forming a pyramid or tetrahedron; in higher dimensions, it’s n+1 points (we call this shape a “hyper-tetrahedron” or a simplex). We then evaluate the likelihood/sum-of-squares at each point and move the worst point according to the following set of rules:

- start by going in what seems to be the best direction by reflecting the high (worst) point in the simplex through the face opposite it;
- if the cost at the new point is better than the best (lowest) other point in the simplex, double the length of the jump in that direction;

<sup>1</sup><http://www.zoo.ufl.edu/bolker/emd-2000/lect12.html>

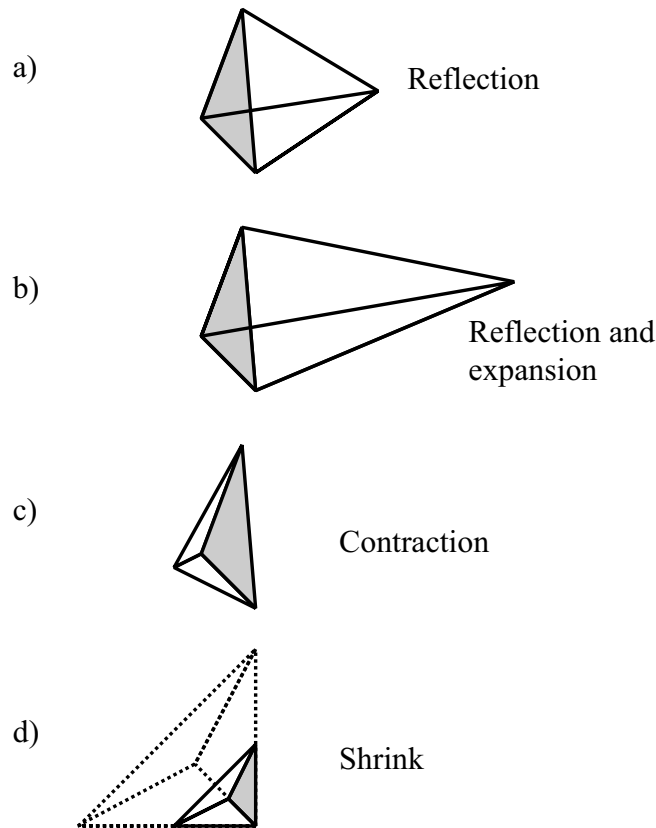


Figure A.1.: The possible rules applied to a trapezoid: a: reflection, b: reflection and expansion, c: Contraction, and d: multiple contraction or shrinkage. Source: [43].

- if this jump was bad—the height at the new point is worse than the second-worst point in the simplex—then try a point that’s only half as far out as the initial try;
- if this second try, closer to the original, is also bad, then we contract the simplex around the current best (lowest) point.

The amoeba works in a wide variety of situations, although it’s not foolproof (nothing is) and it’s not very efficient.

In Fig. A.1 graphical illustration (taken from Numerical Recipes [43, p.409]) of the rules applied to a trapezoid:

The example in Fig. A.2 shows what the simplex does with an exponential model surface in two dimensions.



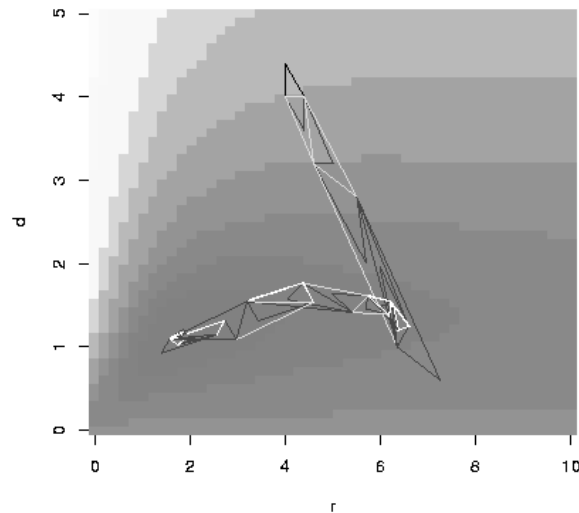


Figure A.2.: An example run from the web lecture notes<sup>1</sup>.

We give the amoeba ( $r = 4$ ,  $d = 4$ ) as starting coordinates and it displaces these coordinates slightly to get its starting simplex (black). Thereafter, it takes steps alternating between simple reflection (dark triangles) and doubled reflection (light triangles), until it finds that it has gone too far and “turns the corner” to start decreasing  $r$ . Occasionally, a simple reflection is too far—usually when the amoeba is trying to turn a corner—and the triangle is white. In any case, the amoeba does eventually get to the right place, although it takes 48 cycles to get there.

## B. Miscellaneous

### B.1. The Dynamic HIV Estimator users guide

This guide is intended to be a concise walk-through for the general user to allow for effective parameter estimation of the three-dimensional model of HIV/AIDS using the *Dynamic HIV Estimator*.

#### B.1.1. Introduction

Thank you for evaluating the basis module of the *Dynamic HIV Estimator* for people living with HIV. This program helps the user to find out more about the current status of the HI-virus in an individual patient.

This program is based on current research that models the behavior of the HI-virus within individual patients. Instead of finding only a level for the CD4<sup>+</sup> T cells and viral particles, this program allows you to determine a range of values that can help with planning and decision making when choosing a treatment strategy.

This users guide will take you through the necessary steps to evaluate the basis module of the *Dynamic HIV Estimator*. Before we begin with the guided tour, there are some important facts that you need to know about programs that are based on mathematical models.

#### **Important**

This program produces a wealth of interesting information, some of which can be applied directly to each patients situation. It is important to note that all predictions are based on the *limited* ability of the computer model to describe the current, active dynamics of the virus within a patient. The program uses this *limited* ability to predict future trends of the disease in the patient, using the same model. Even though the results are based on thorough research, there are still many areas where even the experts disagree on the interpretation of these results. Always keep this in mind when using programs that help

to describe systems and predict future scenarios: even as the weather can be predicted with ever more certainty, there are still some days when rains come unexpectedly.

This program needs to be evaluated by users like yourself to increase its usability and the quality of results. As more feedback is gathered from individual users, the program can be improved to allow better and more precise analysis of HIV in individuals.

The next section describes the information that you will need in order to use this program. Please read it carefully before you start using the program. Any estimate can only be as good as the data that is supplied.

### B.1.2. What you will need before you start

Before you start using the program, there is some basic data that needs to be gathered. This data will be entered in an Excel sheet in *Microsoft Excel* before the *Dynamic HIV Estimator* is used. Remember that the computer program cannot correct mistakes that the user makes when entering data, thus it is important that the data is entered correctly.

#### What you will need

Absolutely Essential	Limit	Recommended
CD4 <sup>+</sup> T cell samples with dates	4 samples	more than 8 samples
viral load samples with dates	5 samples	more than 8 samples
Choose an ID by which you can identify your results.		
<b>Recommended</b>		
Estimated date of infection	within 10 days	exact date
Symptom duration	within 5 days	within 2 days
Age in years		
Gender		
<b>Treatment Information:</b>		
It is essential to list all treatment information if applicable.		
Date of commencement and termination of treatment is important.		

Table B.1.: The information that needs to be gathered before the *Dynamic HIV Estimator* can be used.

The first, and most important information needed to estimate parameters, is the CD4<sup>+</sup> T cell and viral load data. As an absolute minimum, with no additional informa-

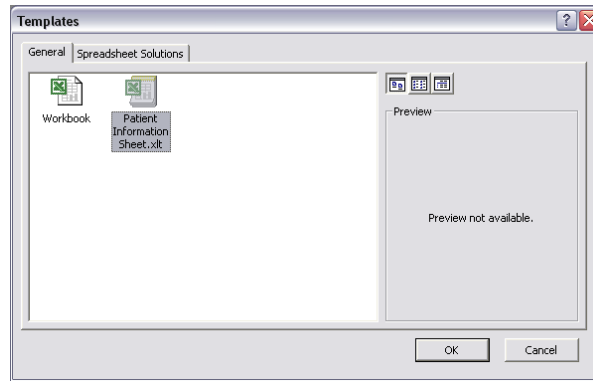


Figure B.1.: The new file dialog where the “Patient Information Sheet” is chosen.

tion, one needs at least four  $CD4^+$  T cell counts, and five viral load levels. Even though this is the minimum, it is far better to have more data points available when estimation commences.

Also note that the samples should be taken as close as possible to the infection date to ensure that the data is from the early stages of infection. Or, if the disease is at a later stage, the samples should be taken directly after potent antiretroviral treatment commences. Currently there are no fixed guidelines to the spacing between samples, but eight weekly samples at the initial stages after infection should allow for a good estimate. Samples after the initiation of therapy should be closer together at first (6 samples spaced two days apart, and then weekly samples).

Once this important information is available, the other information might still help to increase the accuracy of estimation, thus if there is any information available about the infection date, the duration of the symptoms, patient age, and gender these might all help to increase the accuracy of estimation.

### B.1.3. Entering the data

Once you have all the information available, proceed to open a new excel file (click on File→New) and choose the “Patient Information Sheet” template as basis (see Fig. B.1).

This opens a new worksheet with the view shown as in Fig. B.2

Proceed to enter all available information in the appropriate places. The data for this example is adapted from real patient data, and Fig. B.3 shows the Excel sheet with the information added. Once all the data is entered, save the file under an appropriate filename. A good idea is to use “PIS(patient id).xls”, where (patient id) is replaced by

	A	B	C	D
1	<b>Patient Information Sheet</b>			
2		Data	Help	
3	Patient ID:	Please Enter An ID	A string that uniquely identifies this patient	
4	Last Name:	Not Supplied	Optional Last Name	
5	First Name(s):	Not Supplied	Optional First Name(s)	
6	First Positive:	Not Supplied	First Date Where Patient Tested Positive	
7	Last Negative:	Not Supplied	Last Date Where Patient Tested Negative	
8	Infection Date:	Not Supplied	The assumed date of infection. If left blank, the midpoint between First Positive and Last Negative Date is taken.	
9	Gender:	Not Supplied		
10	Age:	Not Supplied		
11				
12	<b>CD4 Samples</b>			
13	Baseline Value	Not Supplied	The CD4 cell level before infection, if available	
14	First CD4 Sample Date	Not Supplied	The date that the first CD4 Sample is taken	
15	Date	Days	Level (Cells per micro-liter)	
16	Not Supplied	Not Supplied		
17	Not Supplied	Not Supplied		
18	Not Supplied	Not Supplied		
19	Not Supplied	Not Supplied		
20	Not Supplied	Not Supplied		
34				
35	<b>Viral Samples</b>			
36	First viral Sample Date	Not Supplied	The date that the first viral Sample is taken	
37	Date	Day	Level (RNA copies per milliliter)	
38	Not Supplied	Not Supplied		
39	Not Supplied	Not Supplied		
40	Not Supplied	Not Supplied		
41	Not Supplied	Not Supplied		
42	Not Supplied	Not Supplied		
60				
61				
62				
63				
64				

Figure B.2.: The empty Patient Information Sheet after creating the new file.

The screenshot shows a Microsoft Excel spreadsheet titled "Patient Information Sheet filled in.xls". The spreadsheet is organized into several sections: Patient Information, CD4 Samples, and Viral Samples. The data is as follows:

	A	B	C
1	<b>Patient Information Sheet</b>		
2		Data	Help
3	Patient ID:	PExample	A string that uniquely identifies this patient
4	Last Name:	Example	Optional Last Name
5	First Name(s):	Example	Optional First Name(s)
6	First Positive:	13-Mar-00	First Date Where Patient Tested Positive
7	Last Negative:	23-Nov-99	Last Date Where Patient Tested Negative
8	Infection Date:	17-Jan-00	The assumed date of infection.
9	Gender:	Male	
10	Age:	30	
11			
12	<b>CD4 Samples</b>		
13	Baseline Value	Not Supplied	The CD4 cell level before infection, if available
14	First CD4 Sample Date	17-Jan-00	The date that the first CD4 Sample is taken.
15	<b>Date</b>	<b>Days</b>	<b>Level (Cells per micro-liter)</b>
17	03-Apr-00	77	451
18	12-Jun-00	147	437
19	31-Jul-00	196	455
20	30-Oct-00	287	524
34			
35	<b>Viral Samples</b>		
36	First viral Sample Date	17-Jan-00	The date that the first viral Sample is taken
37	<b>Date</b>	<b>Day</b>	<b>Level (RNA copies per milliliter)</b>
38	17-Jan-00	0	3239
39	03-Apr-00	77	5810
40	12-Jun-00	147	6002
41	31-Jul-00	196	20439
42	30-Oct-00	287	9638

Figure B.3.: The Patient Information Sheet, after the data is filled in.

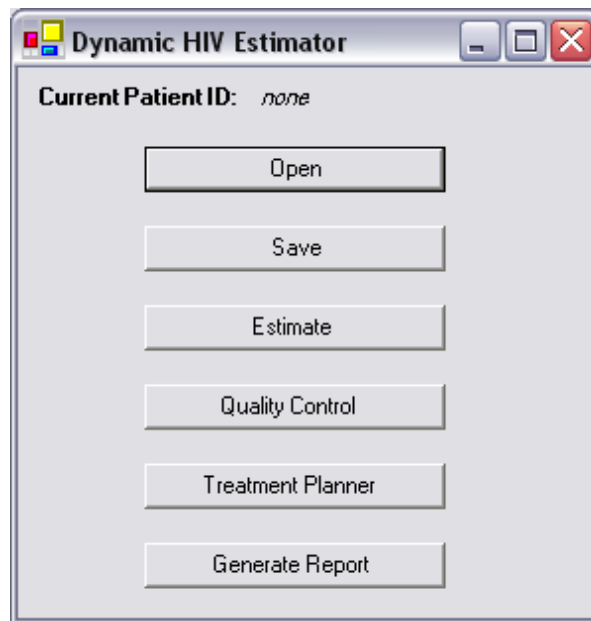


Figure B.4.: The *Dynamic HIV Estimator* when it is run, before a patient file is chosen.

the actual id entered in the file. This allows for easy identification of the file at a later stage.

#### **B.1.4. Performing the estimations**

Now that the data is in place it is time to open the *Dynamic HIV Estimator*. Once the program is running, it looks like Fig. B.4. Simply click on the open file button and choose the file that was saved in the previous step.

When the file is opened, the patient id appears in the program window as in Fig. B.4. Click on the estimate button. This step can take some time, depending on the data, so you might want to make a cup of coffee, do the laundry, or anything that takes some time to do...

Once the estimation is completed, a visual display of the estimated model is given as in Fig. B.6. Check this this display to ensure that the solid line is close to the data points as in Fig. B.6.

It is a good idea to save the results at this point.

Now you can decide to run quality control on the estimate. This is an extremely lengthy procedure, and that is why it is not included in the estimate. All it does is to check if the same model is estimated, using different methods, each of which is possibly a bit slow. In any case, this step is optional, but if you need some additional confidence

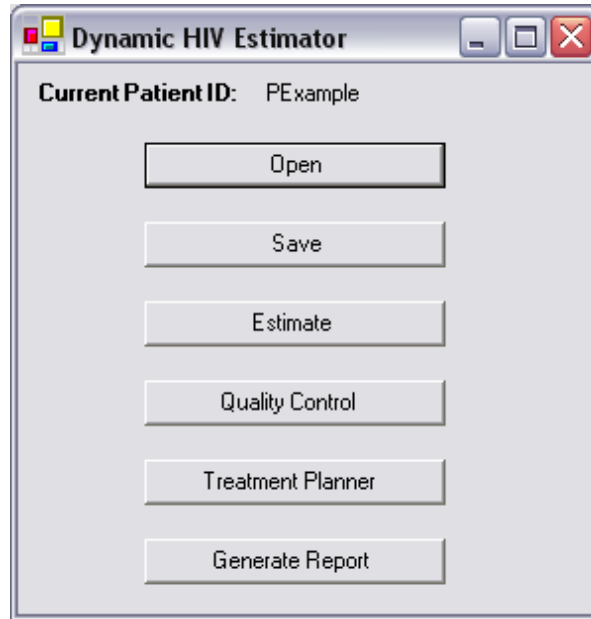


Figure B.5.: The *Dynamic HIV Estimator* once a Patient Information Sheet is opened. Note that the patient ID appears in the window.

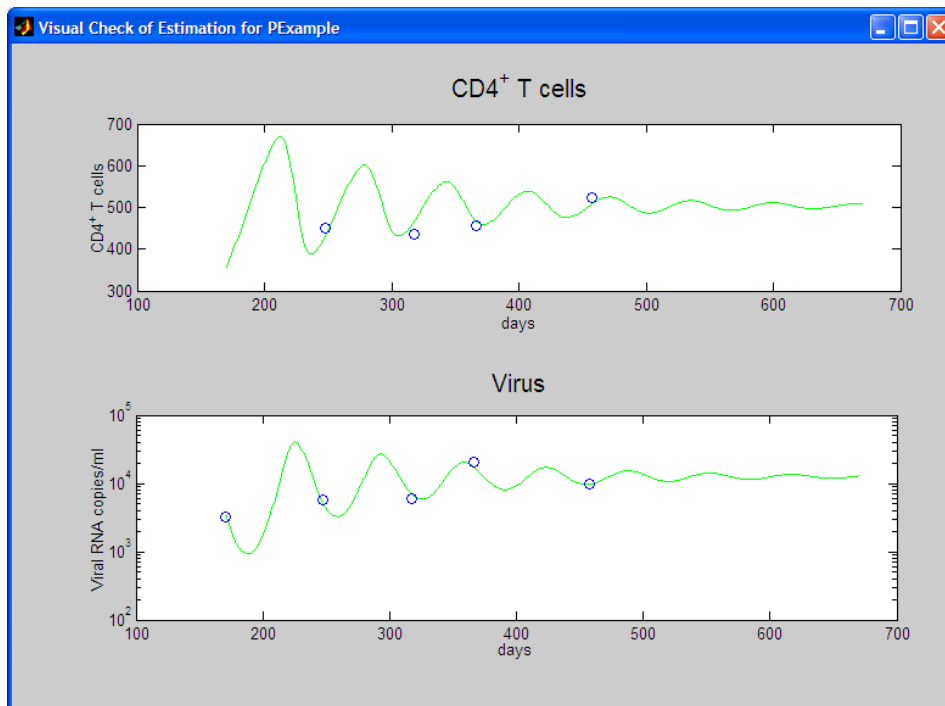


Figure B.6.: The visual display of the estimation. This window must be checked to ensure that the solid line is close to the data points.



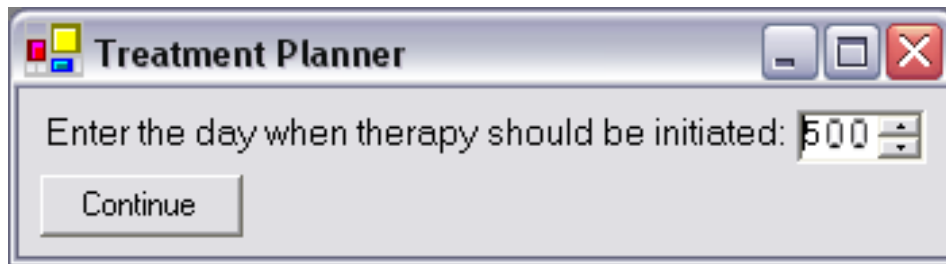


Figure B.7.: It is as simple as setting a choice for the day when the treatment should commence. Here 500 is chosen

in the results, let it run over-night some time.

Again, if you chose to do the quality check, it is a good idea to save the information once the quality control is complete. In any case, whether quality control was done or not, you can proceed to the next section.

### B.1.5. Treatment Planning

For the basis-module, there is one simple option that can be changed for the treatment planner: When to initiate therapy. This is entered in terms of days. For each value entered, the planner calculates the result of three different therapies started at the preferred day.

Thus it is a simple matter of deciding on the time to start with therapy, and the computer does the rest. In Fig. B.7 one can see a choice of 500 days. Click on the continue button, and the result appears as in Fig. B.8

### B.1.6. The report

Once you have completed the estimation cycle, you can click on the *Generate Report* button. This generates a text file with the same name as the patient id, and a “.txt” extension. This file contains all the relevant information about the estimation that you have performed. A copy of the report is also saved in the *Excel Sheet* if you choose the save option.

In Fig. B.9 you can see the report for our example patient.

### B.1.7. Where to go from here

If you need further information, or would like to perform an in-depth analysis of your data, you can always upgrade to the advanced module of the *Dynamic HIV Estimator*.

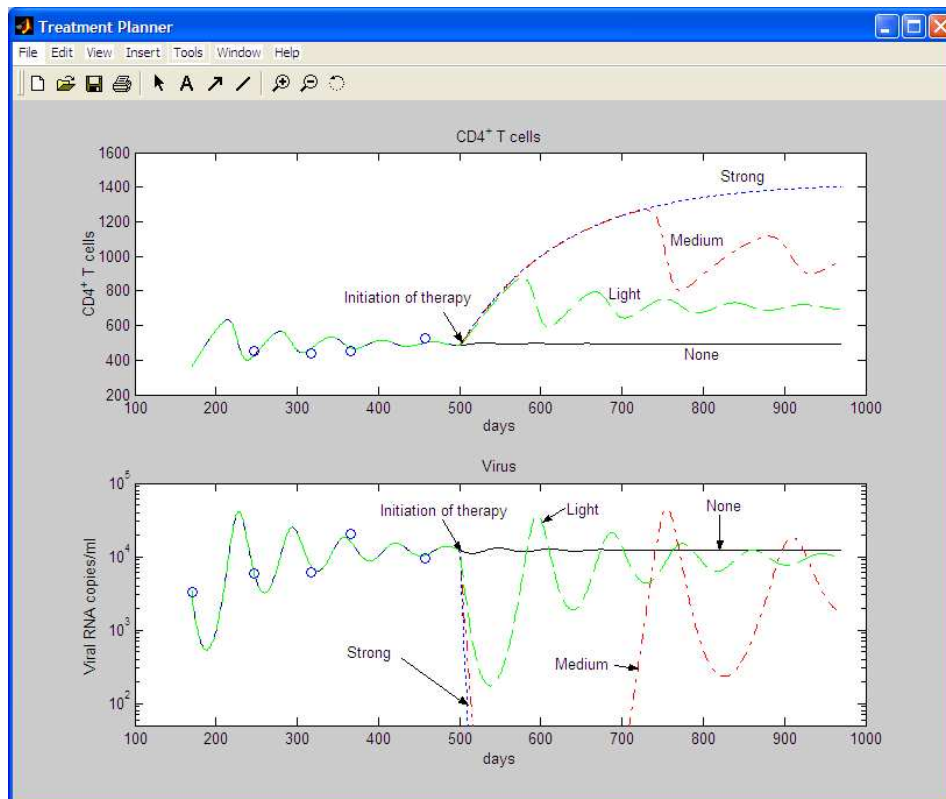


Figure B.8.: The result when choosing to start treatment at 500 days for the example patient.

PEXample:

Basis Report

-----  
Enough data points were available to determine parameters:

s= 11.409

d= 0.008

beta=  $1.2 \times 10^{-6}$

delta= 1.35

c= 1.31

k= 2889.2

Set-point= 12223

Estimated time to reach set-point = 14 months

Constraints on parameters were set to default.

c<delta

s<50

d>0.008

No additional modifications made cost function

Fit seems adequate: no outliers were detected in data-set.

The spacing between data points is very large, it is important to perform a visual check on the data to ensure that a good fit has been found. It is recommended that samples are taken closer together. For more details see the guidelines for sample spacing.

CD4 cell count seems stable at an acceptable level. The viral set-point falls in the lower range: according to the panel on clinical practices on HIV/AIDS it might be desirable to postpone a rigorous treatment regime.

For visual scenarios of different treatment strengths, please consult the treatment advisor application.

Figure B.9.: Basis report printout of estimated parameters for an example patient

The advanced module allows for additional options in both the estimation procedure, and the treatment planning. Instead of working with a pre-defined set of options this module allows for adjustments to be made to each treatment scenario on a separate basis.

Please feel free to mail any comments, questions and ideas to:  
Ruben Filter at [ruben.filter@tuks.co.za](mailto:ruben.filter@tuks.co.za) or [s98013051@student.up.ac.za](mailto:s98013051@student.up.ac.za).

## B.2. Ruminations on the creative process, parameter searches and poetry

The idea behind this section is to add some details about the creative process and how it helps with research<sup>1</sup>. At the same time it allows the interested reader a glimpse into my (Ruben Filter's) thought patterns. It provides a section that has not gone through the lengthy revision process and does not blame everything that was done on the third person<sup>2</sup>.

### Your love is like a global minimum

When people in our community hear (and since we are a pretty small community people are bound to hear) that I write poems, they tend to ask how an engineer comes to do such things (most of them know from somewhere that I studied engineering). This is similar to friends at varsity who want to know what engineering has to do with AIDS. Which in turn is similar to the questions that continue to pop up in my mind.

Now, since I am wondering about such things myself, I have included here some sample *poem searches* (after years of parameter searches, how can I ever *write a poem* again?).

### The simplex method

Start with potentially good-looking gibberish (the initial point—everybody has to start somewhere<sup>3</sup>). Better gibberish does not guarantee a better poem, just as an initial point with low cost, but near a local minimum, is not necessarily preferable to a high point on some well shaped slope:

*A man woke up one morning thinking he was green.*

This line does not go down well as a poem, but it sure sounds like gibberish to me, so I start the search for a poem from here, and find:

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<sup>1</sup>Also an excuse to put some poems in my dissertation;-)

The first paragraph of this section is an example of a thought that I wrote down and came back to, as described later on in the text

<sup>2</sup>Whoever that may be:-)...

<sup>3</sup>see for instance all those lengthy poems by the masters (eg. The love-song of Alfred J. Purfrock – *T.S. Eliot*) with strange languages and quotes from other long dead poets at the start...

*A man woke up one morning, believing he was green  
and since this is an ailment of no small concern  
he called in sick at work and took a stroll in the park,  
taking pains to avoid the paths and keep to the grass  
where nobody would see him in his peculiar state.*

RUX

### Box-bounded global cluster search

Choose a theme (the bounding box, but let the limits be fuzzy for the brainstorm), do a brainstorm (generate a search tree) and pick a few ideas that seem to have the most potential (using some form of poetized cost function will do). For good measure, since this is poetry, include the one piece of gibberish that seems to have the least potential, just for fun. Once you have the starting points proceed as before with the potentially good-looking gibberish.

### Constrained minimization

Allow me to use as example the W.S. favorite, a sonnet:

*Place severe restrictions on form,  
so one can fairly easily see  
that this is intended to be poetry,  
but what, with everyone rattling at the norm,  
it seems that even the forlorn  
old pieces written on the go  
can be a starting point to flow  
into this shape as a sojourn.*

*It is then so, that on the go  
the piece is not complete at all  
and there is much to be required  
before it's good for poets in the know.  
Yet, for each poet since the fall,  
a sonnet's limits never seemed too tall to be attempted.*

This piece was written on the go, keeping with some of the sonnet's limitations<sup>4</sup>—and it shows. Now let's assume, in our parallel universe, that this is how far the *Nelder-Mead* method came before it exited with: **Maximum Number of iterations exceeded**, then we should not give up hope but rather fire up some local search, let's say *Quasi-Newton* (or *Quasi-Shakespear;-*) as the flavor of the day.

<sup>4</sup>Of which the 14 line limit is probably best known.

Back to the little example: even if I change my poem search modes and try to fix up the beat to resemble iambic pentameter<sup>5</sup>, within my current tolerance settings I end up with:

*We place severe restrictions on the form,  
so anyone who looks can easily see  
that such a piece must be true poetry.  
Now, with everybody rattling at the norm,  
it seems that even this, the most forlorn  
old piece, as written in a hurry—on the go—  
can be restricted, as a starting point, and flow  
from there through shapes as temporary sojourn.*

*And so it is, as it is with poems on the go,  
this small piece is not complete at all:  
there's more, much more of it to be required  
before it's good for poets in the know.  
And yet, for every poet since the fall,  
limits never seemed too tall to be attempted.*

Which is clearly a far cry from any classic and safe of ever becoming one. The problem is that for this subject matter I could set up boundaries as much I like, and—even if I could meet all the requirements for a proper Shakespearean sonnet (SS)—with this subject matter, it would never be good beyond its short-lived purpose as example in an appendix of a masters dissertation.

Parameter searches display a similar tendency in situations where one might expect quite extraordinary or even contradictory results: if there are limits imposed on the search, these limits ensure that the results look like valid parameter search results (just as my little example might look like an SS), even when the possibility is present that it is refined gibberish<sup>6</sup>.

When one attempts to search for a parameter set manually (as I did at many points throughout my research), the experience is very similar to writing a poem in form. There are some prescribed limits on the parameters (from literature), but more importantly, the data-points are the fixed markers<sup>7</sup>. Now, if the data is corrupted or obscure in any

<sup>5</sup>Five beats per line. To keep this up for fourteen lines within the rhyme scheme seems very restricted. . .

<sup>6</sup>On the other hand, let us not forget that it is in this precise form that W.S. created some memorable pieces (including all the forgettable ones stemming from his global searches ;-).

<sup>7</sup>This is similar to the line endings in the sonnet that were used as pivot point in the first example, since the sonnet dictates that they should rhyme in a particular way. Then there is half-rhyme and such, which would incur a higher cost, but may be the only way out since English is not a rhyming language like Italian (which could be thought of as the Italians having less noise to contend with in their mother tongue sonnets).

way, we sit with the same problem as in our example above: No matter how close we come to the proposed ideal (lets say for instance the poems cost is defined as the number of SS requirements that are not met), the poem could even meet all the requirements, but without good subject matter we are nowhere. Sad as it may seem, the same holds true with HIV sample data.

### **To sleep or not to sleep: Research and the sleeping pattern of the researcher**

I write this after a good nights rest, and before that, a restless/sleepless week of reporting...

What is the influence of sleep on the research cycle<sup>1</sup>?

I have to meet a deadline for the main part of the dissertation, so I will leave it at that, continuing this excursion with a creative experience I had today.

### **A chair standing in the door**

Take for instance a chair, standing in an open door. One could walk past and forget about it or, in the name of order, move it back to its rightful place amongst the others at the head of the table.

Or, one can wonder about chairs that walk around the house at night and stop by day or the very moment they suspect that humans are on the way. It could make for a great children's book with elaborate stories of how the chairs enjoy being sat upon, and how they attend weddings in their masses just so that happy people can sit on them. . . The possibilities are endless and delightful: What if the chair standing in the opened door just got scared; realized that it is just a chair that had been standing in that room for all its unnatural life

(at which point one could bring in the memories of its natural life in the forest with the birds sitting on its branches [from there the joy of letting living beings sit on it]. Or one could create a propaganda children's book for Greenpeace where the flashback is about the day the loggers came. . .)

And when the chair finally decided to leave, it got as far as the open door before it realized that it was just a dead old chair and could never make it on its own through life, the world and all its forests. So it just stood there and waited for a human to pick it up and put it back on its rightful place, at the head of the table.

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<sup>1</sup>Another one of those *notes to self* that would need to be expanded in a more formal style. . .



On the other hand there is always the cat that cannot open doors, my brother—who can—and the chair that is a simple (albeit temporary) solution to keep a door open for the cat to pass through on a windy day in June<sup>8</sup>.

\* \* \*

There is nothing strange about a chair, neither are we astounded by people who walk through walls—everyone knows how to use a door. But, when a chair stands in the doorway it triggers something in the mind of a tired researcher that cannot be explained by the chair, or the door, on its own.

Thusly with research: When seemingly simple matters are combined in unexpected ways (by chance or by design) there is always potential for growth and exploration. It is the duty (and joy) of a researcher to steer this vast potential along the lines of his/her study, living the balance between getting lost in the infinite possibilities and getting stuck at a local minimum (that forms such a comfortable hammock at times, it is tempting to enjoy life in the comfort zone).

### **When creativity strikes**

We all know the feeling: no-one can (reasonably) strike without a (possibly low paying) job, and creativity has a tendency to surprise its employers.

For me, personally, it was (is!, how else could this section make it into my masters dissertation?) very hard to keep focused on reporting about my work, when every section is a potential doorway to discovery. At each stage of the reporting process I had to look at diagrams, notes and ideas in my notebook and wade through comments in my code, all of them filled with possibilities. In this way, the research turned out to be a dynamic process that has a mind of its own, and seems never-ending.

To keep myself from leaving the reporting task behind to explore new ideas, one attempt that worked fairly well was: I keep my notebook open beside the computer with some sections marked for the purpose of diverting (seemingly) creative ideas so that they do not distract me from my main task. In this manner I wrote down the thoughts that were nagging at the back of my head, and since they were on paper, I could continue without the worry that I might forget the brilliant thoughts. One spin-off is that, by writing down these thoughts, more often than not, one realizes that they weren't that brilliant after all, but rather part of some (subconscious?) master plan to

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<sup>8</sup>I have since heard from my mother that my brother had put the chair in the doorway as a temporary measure to save me from treading in the cat's \*, which had dried up by the time I passed that way:-D.

keep me from finishing my dissertation.

Also, when creativity strikes (interpretation one: you are creative): it is a good idea to work on those parts of the document that need to be created from scratch, and not on those boring jobs of re-hashing old research into your context. Then, when creativity strikes (interpretation two: you are not creative, no matter how hard you try): work on the revision of old work and do the rudimentary tasks that need time, but not creativity. The strange part is (in my case at least) that, as I go through the boring stuff, my creativity gets agitated, because I seem to get along without using it much; then, at the most unexpected times, it strikes again (interpretation one).

I have found that, as random and mystical as some might make out creativity to be, there is a definite link to external circumstances and general quality of life. When my father passed away fifteen months ago, it was as if something in me had tripped a circuit-breaker responsible for my creativity. In the last few months I had the wonderful opportunity to conduct my studies from home (which, at this writing, is on my brother's farm where I grew up). I had the luxury of prepared meals and my family allowed me to focus exclusively on my research. In this environment, where I did not need to worry about everyday tasks, it soon became evident that my creativity returned with such force that I cannot keep up in terms of reporting.

There is a certain standard that is expected in a dissertation, and, since this standard is based on valuable experience of others, it is not given to anyone to disregard the limitations and guidance that the standard provides. Nonetheless, there are some results and ideas that I had to put on paper that do not fit into the research, but could be helpful to anyone wondering about the type of person I am and some of my modes of operation. For these people, who like to get to know the author outside the prescribed norm, I included this excursion. I hope you enjoyed the random thoughts and ideas as much as I enjoyed sharing them.

RuX

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## C. Code description

This appendix contains a short description of the main *Matlab* files that were used throughout this research. Functions are listed alphabetically together with their help text.

### C.1. General Files

#### **cost3d.m**

function cost=cost3d(x,cd4,virus,costType)

COST3D returns a cost value for the three-dimensional model based on parameters and data points. CD4<sup>+</sup> T cell and virus data are column vectors containing the day of each sample and the sample value. costType corresponds to the cost type in COSTCORE. COST3D assumes that all parameters are already checked. If no initial conditions are supplied, it takes the first data-points as guideline for cd4\_0 and virus\_0 and infected cells\_0 is set to log10 of the first virus sample. parameters: s=x(1); d=x(2); beta=x(3); delta=x(4); c=x(5); k=x(6); if specified: T =x(7); T\*\_0=x(8); v\_0 =x(9);

#### **costcore.m**

function cost=costcore(cd4,cd4estimate,virus,virusestimate,option,varargin)

COSTCORE is an internal function used by functions like COST3D. It returns a cost value based on data points and an estimate thereof. option specifies the cost type and additional penalty terms can be passed through varargin.

#### **decodetreat.m**

function eff=decodetreat(treatment,t)

DECODETREAT returns the effectivity ratios for each parameter of the basic model, given a treatment strategy in treatment and time t.

**fit3dmodel.m**

```
function cost = fit3dmodel(x,xo,xsp,tcd4,cd4,tvirus,virus)
```

FIT3DMODEL returns the cost based on the model search parameters,  $x$ , initial point,  $xo$ , and the data points in  $tcd4$ ,  $cd4$ ,  $tvirus$ ,  $virus$ .  $xsp$  is used to select the parameters that are allowed to vary during the search, thus  $xo$  contains the initial conditions and the values in  $x$  are inserted into  $xo$  as described by  $xsp$ .

For example if  $x = [0.3 \ 4]$ ,  $xo = [10 \ 0.01 \ 6e-5 \ 1 \ 2 \ 500 \ 1000 \ 0 \ 1]$  and  $xsp=[4 \ 5]$ , then the cost is computed for the parameter set  $[10 \ 0.01 \ 6e-5 \ 0.3 \ 4 \ 500 \ 1000 \ 0 \ 1]$

This function is used by the wrapper function `optimrfestimate`.

**generate3dsamples.m**

```
function [Ys,Y]=generate3dsamples(x,Ts,variation,nSamples);
```

GENERATE3DSAMPLES generates  $nSamples$  with parameter set  $x$  and the three-dimensional model at time points  $Ts$ . Variation is a vector with normal variation for  $cd4$  as the first part of variation and log10-normal variation the second part of variation. Returns the Sampled data in  $Ys$  ( $cd4=Y(:,1)$ ,  $viral=Y(:,2)$ ) and the original model output in  $Y$ .

**generate\_reducedtable.m**

`generate_reducedtable.m` is a script to generate sample data for the sample set with a reduced number of sample points.

**getmodel.m**

```
function [model,x_0] = getmodel(modeltype)
```

GETMODEL returns the model specified by `modeltype`. `GETMODEL('3d')` returns a handle to the basic 3d model for use with the ODE solver. Model types with If no input argument is supplied, the basic 3d model is returned `'3dfixpro'` returns the 3d model with a fixed proliferation term `'cdpro'` returns the 3d model with a  $CD4^+$  T cell dependant proliferation term `'perelson4d'` returns the 4d model of perelson

If two output arguments are specified, the default parameter set is returned together with the model function.

Higher dimensional models, and models for the two-stage analysis are also available. See the code for details.

**HIV28\_c.m**

HIV28\_c contains the (non-linear) set-point constraint for the *Tomlab* solver for the the HIVNET 28 data.

**HIV28\_dc.m**

HIV28\_dc contains the jacobian of the (non-linear) set-point constraint for the *Tomlab* solver for the the HIVNET 28 data.

**HIV28\_f.m**

function [r] = HIV28\_f(x,data,data2)

HIV28\_f is the cost function for the *Tomlab* run on the HIV28 data set.

x is the parameter set. DATA1 and DATA2 are the data points. If called with one parameter, x is the parameter set and the data values are used from those stored in persistent variables.

Initialize the presistent variables with HIV28\_f(d1data,d2data).

**HIV28\_f\_fixed.m**

function [r] = HIV28\_f\_fixed(x,data,data2)

HIV28\_f\_fixed is the same as HIV28\_f except that a fixed proliferation term is used in the three-dimensional model. See HIV28\_f for usage details.

**interactiveestimation1.m**

INTERACTIVEESTIMATION1 is the GUI for the user. It is created with the Matlab GUIDE.

interactiveestimation1 is the M-file for interactiveestimation1.fig interactiveestimation1, by itself, creates a new interactiveestimation1 or raises the existing singleton.

H = interactiveestimation1 returns the handle to a new interactiveestimation1 or the handle to the existing singleton\*.

interactiveestimation1('CALLBACK',hObject,eventData,handles,...) calls the local function named CALLBACK in interactiveestimation1.M with the given input arguments.

interactiveestimation1('Property','Value',...) creates a new interactiveestimation1 or raises the existing singleton\*. Starting from the left, property value pairs are applied to

the GUI before `interactiveestimation1_OpeningFunction` gets called. An unrecognized property name or invalid value makes property application stop. All inputs are passed to `interactiveestimation1_OpeningFcn` via `varargin`.

### **optimrfestimate.m**

```
function [x, xo]=optimrfestimate(tcd4,cd4,tvirus,virus,xo,xsp,MaxFunEval,tol);
```

OPTIMRFESTIMATE is the wrapper function from where parameter searches are started. The data for the parameter estimation is contained in `tcd4`, `cd4`, `tvirus` and `virus`. `xo` contains the initial point for the parameter search and `xsp` contains the selected parameters for which the search should be performed. `MaxFunEval` sets the maximum number of function evaluations and `tol` sets the tolerance of the search.

Only the data and initial conditions are compulsory parameters.

See also `FIT3DMODEL` for further details of selective parameters.

### **paramestimatelsq.m**

```
function paramest=paramestimate(T,Y)
```

PARAMESTIMATE performs standard LSQ parameter estimation.

`T` is the time points column vector and `Y` a matrix with column 1 = `cd4` cells and column 2 = virus data.

### **patientget.m**

PATIENTGET Get PATIENT OPTIONS parameters.

`VAL = PATIENTGET(OPTIONS,'NAME')` extracts the value of the named property from integrator options structure `OPTIONS`, returning an empty matrix if the property value is not specified in `OPTIONS`. It is sufficient to type only the leading characters that uniquely identify the property. Case is ignored for property names. `[]` is a valid `OPTIONS` argument.

`VAL = PATIENTGET(OPTIONS,'NAME',DEFAULT)` extracts the named property as above, but returns `VAL = DEFAULT` if the named property is not specified in `OPTIONS`. For example

```
val = patientget(opts,'LineStyle','r');
```

returns `val = 'r'` if the `LineStyle` property is not specified in `opts`.

See also `PATIENTSET`.

**patientset.m**

PATIENTSET Create/alter PATIENT OPTIONS structure.

OPTIONS = PATIENTSET('NAME1',VALUE1,'NAME2',VALUE2,...) creates an integrator options structure OPTIONS in which the named properties have the specified values. Any unspecified properties have default values. It is sufficient to type only the leading characters that uniquely identify the property. Case is ignored for property names.

OPTIONS = PATIENTSET(OLDOPTS,'NAME1',VALUE1,...) alters an existing options structure OLDOPTS.

OPTIONS = PATIENTSET(OLDOPTS,NEWOPTS) combines an existing options structure OLDOPTS with a new options structure NEWOPTS. Any new properties overwrite corresponding old properties.

PATIENTSET with no input arguments displays all property names and their possible values.

See also PATIENTGET

**plot3d.m**

PLOT3D plots the three-dimensional HIV model with patient data.

PLOT3D(X) plots the 3d model only, X is the parameter set with initial conditions in X([7 8 9]) and default time limits = [0 100]

PLOT3D(X,OPTIONS) uses the settings as created with PATIENTSET initial conditions specified in options take precedence over those specified in X. If 'RawData' is specified in OPTIONS the time limits are set to [min(RawData(1,:)) max(RawData(1,:))].

See also PATIENTSET, PATIENTGET

**plotparam.m**

plotparam.m is a script file to plot the six parameters of the three-dimensional model on a single figure with the interpolated data in the background.

**plotsens.m**

PLOTSENS(P,iPnr) plots the sensitivity analysis for structure P where P has P.f\_var, a matrix of the cost and P.p\_var the parameter values. P is generated with the function SENSITIVITY\_ANALYSIS

P.x is the stationary point from where the analysis is performed. If x is supplied as a third parameter, this point is printed in the table instead of P.x.

See also SENSITIVITY\_ANALYSIS.

### **plotsens6.m**

PLOTSENS6(P,iPnr) is the same as PLOTSENS(P,iPnr) but does not plot the information for the initial values of the CD4<sup>+</sup> T cell, infected CD4<sup>+</sup> T cell and virus level.

### **savepatient.m**

function success=savepatient(f,p)

SAVEPATIENT saves the basic patient data in p to the filename specified in f in a csv based format.

### **sensitivity\_analysis.m**

SENSITIVITY\_ANALYSIS analyzes the parameter sensitivity for the three-dimensional model.

P=SENSITIVITY\_ANALYSIS(X,DATA\_CD4,DATA\_VIRAL,X.L,X.U,X\_SPACE,NS)  
Returns a structure P that contains the sensitivity analysis for the parameter set as specified in X, the data in DATA\_CD4 and DATA\_VIRAL. The analysis range is determined by X.L, X.U and the space type is specified by a boolean vector in X\_LOGSPACE ([1 1 1 1 1 1 1 1 1] means all logspace and [0 0 0 0 0 0 0 0 0] means all linear). nS sets the number of samples taken over the analysis space for each variable.

See also PLOTSENS.

### **sim3dbasis.m**

SIM3DBASIS simulates the three-dimensional model with the option of adding a basis level from a secondary compartment for printing estimation results and the results for the two stage analysis.

The function plots the CD4<sup>+</sup> T cell and viral levels and if requested, returns a time vector T and the corresponding state matrix Y.

function [T,Y]=sim3dbasis(x,xo,timelimits,patientdata,lineformat,basislevel)

### **sim4d.m**

SIM4D plots the simulation for the 4d model.



[T,Y]=sim4d(x,OPTIONS) where OPTIONS are created with PATIENTSET

For implementation, the proliferation term is left out of the first equation and the parameter sequence is kept to correspond to the 3d model as far as possible.

$x=[s \ d \ \text{beta} \ \text{delta}_2 \ c \ k_2 \ \text{delta}_1 \ q \ k_1]$  and  $x(10:13)=x0=[ \ T \ T_1 \ T_2 \ v ]$

see also PATIENTSET, PATIENTGET

### **tomscript\_estall.m**

tomscript\_estall is a script to re-estimate all parameters for the HIVNET 28 cohort using Tomlab toolbox functions.

### **vsetpt.m**

VSETPT returns the viral set point for the three-dimensional model.

VSETPT(x) returns the set point only

[setpt,daystosetpt]=vsetpt(x,T,Y) where T and Y are simulation results from SIM3D returns the set point for the viral load and the number of days for this set point to be reached.

## **C.2. Additional files and figures**

All files listed in this appendix can be found on the accompanying CD. Additional files and figures are also available. In particular, the figures depicting all estimates for the HIVNET 28 study are available under the HIVNET 28 directory.