Manufacturability of Lab-on-Chip Devices: Dimensional Variation Analysis of Electrode Foils using Visual Technology

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Submitted to the Department of Mechanical Engineering in Partial Fulfilment of the Requirements for the Degree of Master of Engineering in Manufacturing at the Massachusetts Institute of Technology September 2011

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Abstract

Electrodes are necessary components for measuring changes in electrical properties in many microfluidic devices. Daktari CD4 Cell Counter system utilizes an interdigitated electrode foil in order to measure the concentration of the CD4 cells in an assay chamber by measuring the impedance drop. Thus the consistency in the dimensions of the interdigitated fingers in the electrode is critical to the repeatability of impedance measurements. This work involved a thorough variation analysis of the electrode dimensions to characterize the repeatability of the new manufacturing process developed by Daktari.

For this purpose optical imaging was used to obtain high-resolution images of the electrodes and an algorithm was developed in order to estimate the critical dimensions of interdigitated fingers from the images. The results showed that the dimensional variation in the electrodes had insignificant effect on the performance of the electrodes and that the new manufacturing process is capable of producing satisfactory electrodes within the desired target. The relation between the electrode's dimensional variations was found and the effect of critical process parameters was determined in order to maintain the process statistically in control.

Thesis Supervisor: Dr. Brian W. Anthony

Title: Research Scientist
Acknowledgements

The author would like to thank many individuals whose guidance and support were essential in the completion of this work.

Dr. Brian W. Anthony, our thesis advisor, for his professional input, great advice and his involvement since the beginning of the project.

Professor David E. Hardt for his advice on this paper and throughout the academic year and giving the author the opportunity to participate in the program.

It has been an absolute pleasure working with the Daktari team. Their enthusiasm and level of commitment has been extremely encouraging and this project would not have been possible without their guidance.

Jennifer Craig, our writing advisor, for all her valuable and detailed suggestions in writing this thesis. It has been a pleasure getting to know Jennifer and learning from her experiences.

Jacklyn Holmes and Linda Donoghue, you have been great teammates and friends. Your level of commitment has been a key to the success to the projects.

Dean, Matthew and Stephanie for all their supports and assistance.
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Chapter 1: Introduction

1.1 HIV & AIDS

Human immunodeficiency virus (HIV) is a lentivirus that hides in the human body cells for long periods of time and attacks a key part of the immune system – the CD4 cells. CD4 cells are essential for the body in order to fight infections and diseases. HIV can progressively destroy so many of the CD4 cells that the body loses this ability to fight. This condition is called Acquired Immunodeficiency Syndrome (AIDS). The history of HIV and AIDS is a short one. As recently as the 1970s, no one was aware of this deadly illness [1-2].

The HIV epidemic has become a major global public health challenge, with a total of approximately 33.4 million people living with HIV worldwide. Each year around 2.6 million people become newly infected with HIV and around 1.8 million die of AIDS. The worst affected region is sub-Saharan Africa, where more than one in five adults are infected with HIV in some countries [2][3][4].

1.2 Importance of Monitoring

In 2006, the United Nations Member States committed to scaling up services and interventions towards the goal of universal access to HIV prevention and antiretroviral therapy (ART) [4][5], the main treatment for HIV. In order to achieve this objective, it is critical to escalate efforts in identifying eligible patients, effectively managing waiting lists, and closely monitoring any delays in initiation of antiretroviral therapy [3][6].

CD4 counting using flow cytometry is a critical component of the AIDS treatment process. It is used to identify the candidates eligible for ART, as well as on-going monitoring of the immune system and the disease progression [7][8]. Declining CD4-cell counts are considered to be an alarm for the progression of HIV infection. In HIV-positive people, AIDS is officially diagnosed when this count drops below 200-cells/mm³ and antiretroviral therapy should be started [3].
1.3 Monitoring Challenges

The standard laboratory equipment for CD4 testing is often compromised by under resourced facilities, lack of skilled health workers, deficiencies in infrastructure, and high-costs, despite the urgent need for scaling up care services in greatly impacted areas [6][9]. Furthermore, in decentralized hospitals in resource-limited settings, many diagnostic tests simply cannot be performed and are beyond the reach of many HIV-infected people [10]. This issue has resulted in CD4 tests becoming a significant barrier in the efforts to scale up HIV prevention and reaching the planned treatment target in the most affected areas [6].

The World Health Organization and other health organizations have urged the development of simple-to-use, affordable point-of-care CD4 cell counting system to monitor HIV-infected patients in resource-limited settings [9][11]. Microfluidic devices have been advertised as a key candidate to accomplish point-of-care (POC) diagnostics. The technology could permit rapid tests on site, lowering the wait times for results while substantially reducing the cost to the patients [12][13][14].

1.4 Use of Microfluidics

Microfluidics, and specifically lab-on-a-chip (LOC) technology, as a sub-field of MEMS/MST (Micro Electromechanical Systems/ Microsystems Technology) is an emerging technology that enables the manipulation of tiny volumes of fluid (typically in the micro to nano-liter range) in micrometric diameter channels. Since their introduction about 15 years ago, microfluidic devices have been advertised to have potentials for providing a wide range of point-of-care (POC) applications. [12][15] However, microfluidic POC platforms are not yet widely used outside of research laboratories. In order to market such devices and keep pace with increasing interest and demand, further understanding of their fabrication and process optimization is essential. Their performance will also
need to be thoroughly evaluated and validated in the field using clinical trials [13][17][18].

1.5 Point-of-Care Development

Many medical diagnostic organizations such as Inverness, Abbott Point of Care, Daktari Diagnostics, Claros, and Diagnostics 4 All, are taking advantage of these developments and are actively involved in further advancing the development of lab-on-a-chip technology [19-20].

Daktari Diagnostics as a new entrant in the medical device industry has designed a simple and cost-effective point-of-care device for CD4 cell testing. The device, DAKTARI CD4, utilizes microfluidics technology for sample preparation and electrochemical sensing for measuring the CD4 cell concentration that could effectively overcome the barriers of the flow cytometry techniques [9]. DAKTARI CD4 is currently undergoing performance evaluation and optimization, and is en route to clinical trials to obtain patient results and full scale manufacturing for the product is in development.

1.6 Development Challenges

Some of the challenges currently being addressed in the product development and the manufacturing processes are: optimizing the current design; understandings the physical processes of different parts; identifying opportunities for quality control improvement; the ability to predict expected behaviour of manufacturing processes; developing a system for registering the data from patients and identifying manufacturing processes and robust materials for the full scale manufacturing stage.

In this thesis, the quality control of one component of the Daktari system - the 'Electrode foil' - has been investigated. The electrode foil is a PMMA sheet with a conductive layer where the CD4 cell count is performed.
The performance of the foil is affected in many ways by manufacturing variation and the process parameters. Visual techniques were used to understand the variation in the manufacturing process of the foil and investigate the effects of this variation of the performance of the electrode foils.

1.7 The Masters of Engineering Capstone Project

This document is a thesis for the Masters of Engineering in Manufacturing program through MIT's Laboratory for Manufacturing Productivity. The program has a team of students work on research products with a local company. Each of the students focused on a different challenge. The author of this thesis, Kasra Namvari, focused on Variation Analysis of Electrode Foils using Visual Technology. The other team members Jacklyn Holmes and Linda Donoghue focused on testing method and the robustness and repeatability of the electrodes [21], and the impact of design on repeatability of the electrodes and ease of quality assurance [22] respectively. The three theses in combination describe the work done by the students at Daktari Diagnostics in 2011.

1.8 Thesis: Overview

This thesis begins with a description of Daktari Diagnostics Inc., the current product under development and the problem statement in chapter 2. Chapter 3 presents a detailed literature review of the state-of-the-art in MEMS technology, microfluidics and their manufacturing processes, challenges and the visual quality control methods. This is followed by a detailed description of the ‘electrode foil’ in chapter 4 and the methodology used in this work to conduct the dimensional variation analysis for the electrode foils in chapter 5. The results of the analyses along with discussion are presented in Chapter 6. Chapter 7 presents the conclusion and recommendations. The future work is followed in Chapter 8.
Chapter 2: Product and Project Overview

2.1 Company Background

Daktari Diagnostics is a medical diagnostic device company located in Cambridge, Massachusetts, focusing on Medical diagnostic devices. The company is currently in the process of developing a CD4 cell counter for patients with HIV. The CD4 cell counter will be used in the developing world. The device is designed to be portable, robust, cost effective, and deliver results quickly as a point-of-care method. Daktari's CD4 counter will allow doctors to better identify the candidates eligible for ART, as well as on-going monitoring of the immune system and the disease progression of the patients.

2.2 Product Description

The product currently in development at Daktari Diagnostics is a CD4 cell counter that is needed for patients with HIV. The CD4 counter provides information to the caregivers about the concentration of CD4 cells in the patient’s blood. This level shows how strong the patient's immune system is and can guide the caregivers as to when and how much anti-retroviral drugs (ARV) to prescribe. Measuring the CD4 cell count overtime shows how fast the disease is progressing or responding to treatment. Figure 1 shows how Daktari's system is used to measure a CD4 cells. The assay process has three main stages. The stages are: (A) Blood Sample flows through the assay chamber and CD4 cells stick to the antibody. (B) Red blood cells are washed out of the chamber. (C) CD4+ cells are lysed and the difference in impedance is measured [19][23]. Figure 1 shows how the antibodies and cell lysis are used to capture the CD4 cells.
This product would typically be used by a trained operator carrying the portable instrument and a supply of cartridges to patients in remote locations. The device would be used where a flow cytometer is not easily accessible. The operator would prick the patient’s finger with a lancet and allow the blood to flow into the sample entry port of the card. Once a sufficient amount of blood has entered the card, the operator would cap the card, which seals the cartridge, and help the patient with a Band-Aid to reduce the risk of exposure. The capped card would then be put into the instrument and the test would start. Solenoids in the instrument drive out the fluid reagents, stored on the card in blisters, in a very controlled manner. Actuation of valves guides the sample through an assay chamber. Antibodies that were deposited to the electrode foil would capture the CD4 cells. The captured cells’ cell membranes are ruptured, or lysed by a high-impedance solution. The contents of the cells reduce the impedance of the solution. This reduction of impedance is then used to measure the concentration.
of CD4 cells in the blood sample; subsequently the concentration is displayed on the instrument’s LCD display.

2.2.1 The Instrument

The battery-powered instrument was designed for portability. It contains the actuators for the reagents and the valves. The instrument connects to the electrode in the cartridge to read the impedance measurements in the assay chamber. The measurements are used to determine the CD4 cell count in the sample, the rest of the electronics needed to display the results and drive the actuators are contained in the instrument. The instrument also is the user interface while a test is being performed. Figure 2 shows a photograph of the instrument.

![Figure 2: The Daktari Instrument - with parts marked [22]](image-url)
All of the tasks are met by the subassemblies listed below.

1. Frame - the structural element of the instrument. All of the other subassemblies are located using the frame.
2. Door Subassembly - locates the cartridge in place, punctures a vent hole and ensures no bowing in the card.
3. Actuator Subassembly - holds the actuators perpendicular to the frame.
4. Solenoid Subassembly - holds the valve actuators perpendicular to the frame.
5. Outer Casing - protects the internal components from impact and debris and also provides an aesthetic appeal.

2.2.2 The Cartridge

The cartridge is the consumable for the test. The cartridge is a microfluidic device with reagents and the sensing mechanism to measure the amount of CD4 cells in a sample of blood. Figure 3 and figure 4 show a recent iteration of the design. Each cartridge contains the following 7 parts:
1. Backbone – an injection molded PMMA card with microfluidic channels.
2. Lid foil - a transparent PMMA sheet that is laser welded to one side of the backbone to seal the microfluidic channels on the backbone.
3. Functionalized electrode foil – a PMMA foil that covers the 'assay chamber' where the CD4 cell count is performed. This foil has an electrode layer on it. It is then coated with antibody solution, which is used to trap the desired CD4 cells.
4. Blister pack – this part contains the three liquid reagents that perform tasks as they flow through the system.
5. Valve cover- a layer of polypropylene used to create a seal on the valves that are used to direct flow through the system.
6. Housing - an injection molded PMMA element that protects the blister pack and functionalized foil.
7. Cap – a plastic part that seals the blood entry port after the blood is sampled and also closes vents that were necessary to allow capillary flow of blood into the card.
2.3 Problem Statement

At the time of this project, Daktari Diagnostics is in the process of industrializing the manufacturing process for the cartridges for the clinical trials and preparing for product commercialization. This transition requires a great focus on the production processes and capabilities in order to efficiently produce parts and maintain the quality required for the final product.

In the course of this transition, Daktari has encountered several problematic areas where the manufacturing processes were unable to deliver robust parts with acceptable quality as needed. These setbacks were due in combination to current process limitations as well as assay limitations, whereby making the part more easily manufacturable would affect the operation of the final product. In many cases, these issues, such as molding the microfluidic backbone, were solved by minor design alterations or further development and optimization of the existing processes. Others required significant research and development to reach new design features, move to new materials, or develop a new process entirely.

The overlying theme of all challenges in the scale-up process is quality control and mitigation of variation for the final product and assay results. This theme was taken as the main focus the M.Eng project at Daktari, with the goal of identifying sources of variation, determining allowable tolerances to this variability, and offering solutions to monitor and control the manufacturing quality.

In 2010, Linares and Selvakumar [19][23] performed a survey that highlighted the most critical manufacturing challenges facing Daktari at the time. These challenges included potential failure of individual parts as well as part interactions that are critical to operation. The next sections will discuss the progress made in the past year, what challenges are still outstanding, and some additional considerations, both resolved and outstanding, which have influenced
the focus of this project. Based on these observations, the main operation of focus for this project is the production of electrode foils.

2.3.1 Manufacturing Challenges

In this section a range of manufacturing process challenges for the components of the cartridge as well as the interactions of these components with the instrument are described.

2.3.2 Blister Pack Production and Instrument Interaction

Previous research by Linares and Selvakumar [19][23] focused on the formation of the reagent blister packs and modeling the flow behavior to determine the effects of formed geometries and instrument alignment on the flow characteristics and assay performance. Additionally, instrument and cartridge interactions at the valves and electrode pads were also analyzed. This work provided extensive information on component behavior during product operation. Conclusions from this work have led to the optimization of blister geometry, as well as continued work on valve design and flow analysis [19][23].

Recent challenges in regards to blisters and fluid flow are valve leakage and an occurrence of post flow. Valve leakage is one potential cause for unexplained fluid behavior, and led to a redesign of the valve seat geometry, a change in material from blister foil to polypropylene, and experimentation on the instrument actuator tips. These design changes have resulted in improvements, which reduce the risk in these parts. Focus will continue if the problem resurfaces again.

Post flow is a phenomenon where fluid flow continues, sometimes for minutes, after stopping actuation of the blisters. This observation indicated an unexplained response during blister operation, which caused stored energy to
continue pushing fluid after forced actuation. End result, a change to the blister
system, developed by Daktari, mitigated the problem.

2.3.3 Electrode Foil Production

As described in Section 2.2, the electrode foil consists of an interdigitated
electrode pattern on a PMMA substrate. The electrode is critical to the operation
of the Daktari CD4 system, which relies on the electrical readings from the
electrode to determine the cell count. The nature of the impedance reading
makes it sensitive to minor variations in the electrode, which has previously
been a fragile part. Daktari is currently developing and validating a new
manufacturing process to produce more robust parts. To assure accurate assay
results, it is critical to understand the production variability and to ensure
repeatability in electrode manufacturing.

The previous method of electrode production employed Chemical Vapor
Deposition (CVD) to sputter gold over the entire surface of the PMMA substrate,
followed by laser ablation to strip away unnecessary gold, leaving the electrode
pattern behind. The gold electrodes are fragile, require delicate handling, and
create risk for an assay that relies on the exact finger configuration and
continuity to produce repeatable results. In addition to the risk of damaging the
electrodes post-production, the ablation process itself introduced variability.
During ablation, gold particulates would redeposit onto the surface, texturing the
surface and making the foil more difficult to weld to the backbone.

Daktari Diagnostics has recently developed a new process for the production of
the electrode foils. The new process is currently being patented and due to the
patent process, the author is not at liberty to disclose the details of the process or
the parameters through out the course of this paper. The electrodes
manufactured by the new process do not require any additional ablation and the
parts are far more robust as well as faster and less expensive to produce.
While initial observations indicate the viability of these new parts in the product, validation is required before the gold electrodes can be abandoned. To get to this point, Daktari must eliminate or understand how to control any risks associated with the new product, with the large majority of these relating to the quality of the parts produced and the variability that may affect the performance of the CD4 assay.

2.3.4 Selection of subset of Problems

Considering the importance of the functionality of electrode foils and the benefits associated with the newly developed process, it was proposed to conduct a detailed variation analysis on the electrodes, and to compare the results to the gold electrodes in order to validate and understand the repeatability of the new processes. Optical techniques were used to obtain high-resolution images of the electrodes and an algorithm was developed in order to measure the dimensional variations of the electrodes' fingers from the images. In addition the effect of the dimensional variations on the sensitivity of the electrodes was studied in order to understand the impact of these variations on the electrode's performance.
Chapter 3: Background Research

3.1 MEMS

A major drive in modern technology over the past several decades has been miniaturization. The field of microelecto-mechanical systems (MEMS) was founded three decades ago when scientists began making leaps forward in miniaturization [24]. MEMS technologies include many variations of electromechanical devices in the hundreds of micrometer to sub-micrometer scale. Devices range from gears and components to full electrostatic motors and micro-engines. These small-scale systems are much like integrated circuits, with their ability to offer integrated operations and functionalities on a single chip. Commonly used as sensors, MEMS can be produced and incorporated in product designs to handle detection, analysis, and signal processing in a small and repeatable package [25].

As a research tool, MEMS are instrumental in taking measurements and observations that were previously impossible due to difficulty in operating at that scale, such as quantum behavior and sub-molecular phenomena. MEMS are also beneficial to modeling macro behavior for miniaturization purposes as well as resource availability, process control, repeatability of experiments, and degree of observational details. Properly mimicking macro behavior and responses can be a challenge because of the inflation of effects that are negligible or relatively small at the macro level but significant in the micro range, such as adhesion forces.

3.2 Microfluidics

A specific focus spun off from MEMS research is microfluidics, the study of fluid flows through micro-scale structures. These systems are utilized for many purposes including micropumps, microvalves, and micromixers.
Microfluidic devices can function independently or form an integrated system of channels, mixing chambers, nozzles, etcetera, which perform entire processes. This full integration capability, carried over from MEMS development, lends many of the same advantages specified to MEMS. These and other advantages of microfluidics, as highlighted by Land [26], are as follows:

- Efficient use of reagents, minimizing resources and expenses
- Flexible and modular devices which can be combined for scaling
- Faster analysis, with potential for nearly real-time results
- Tighter control of processes through precision with small volumes (especially in use of droplets)
- Low cost of production per unit

Microfluidics are especially attractive for the elimination of moving parts; this elimination greatly simplifies the production process and the integration of the different components. As also stated for the general realm of MEMS technology, one of the greater challenges is accounting for the different dynamics of the small scale. Even moving a fluid through a simple channel must be reconsidered at this scale. Adhesion forces, fluid particle size, boundary conditions, and other phenomenon must be examined to determine the importance of each [25][27].

### 3.2.1 Components of Microfluidics

As previously stated, microfluidic devices perform a range of functions, often in combination with each other. The main components which form these building blocks can be generalized into three main categories, as described by Tabeling [25]: fluidic interconnects, control elements, and fluid injection.

**Fluidic Interconnects**

Interconnects serve as connectors to microfluidic channels from other microfluidic channels, external input, or fluid injection components.
Control Elements
These components, such as pumps and valves, allow the flow of fluid to be controlled and regulated as desired.

Fluid Injection
Injection components, such as microneedles and capillary channels, facilitate the sample preparation and introduction into the microfluidic system.

The components described above pertain to the construction of basic fluid flow within microfluidics. Many more features and tools can be employed for operation, fluid manipulation, and specific fluid processes. Some of these are microvalves, micropumps, microflow sensors, microneedles, micromixers, microfilters and microseparators to give a general sense of the wide array of microfluidics [24][25].

Each of these can be described as multi-purpose tools, bridging the general categories above, and are available for a number of applications through integration in microfluidic platforms.

3.2.2 Microfluidic Device Structure

Microfluidic devices are composed of different layers, each performing a specific functionality. A typical arrangement of a microfluidic device is shown in Figure 5; it comprises a central layer or a “backbone”, external layers, and additional components for flow control and sensing applications.
(a) **External Layer**

The external layer acts as a cover for the central layer and seals the microfluidic channels. At Daktari, this layer is a transparent PMMA film laser welded to the central layer. This film seals the channels with no additional functionality. The electrode film performs the same function over the assay chamber on the reverse side of the cartridge. The PMMA substrate is welded around the channel, serving as the external layer and providing the seal, while also performing the critical electric sensing function of the assay.

(b) **Central Layer**

The central layer is an essential part of microfluidic devices. It contains all the microfluidic channels, valves, vents and waste channels. The fluid is directed through this layer and any additional components connect to this layer. The central layer can contain a very simple channel to a very complex channel system depending on the complexity of the application.
The Backbone is the central layer developed at Daktari and contains all of the features mentioned above and is manufactured as a plastic injection molded component using PMMA (polymethyl methacrylate). Other components of the Daktari CD4 cartridge, including the electrode foil and the blister packs, are mounted to the backbone.

(c) Additional components

Depending on the application, additional components are added to the external and central layers. These components typically perform either fluid flow control or sensing [19][23].

Fluid flow control mechanisms are features on the central layer, such as valves, or external components for directing or implementing fluid flow within the central layer. At Daktari, the blister pack containing the three reagents is an additional component that allows for the delivery of reagents to the central layer and drives the reagents through the system.

Sensing components are typically used for measuring changes in different properties such as temperature, pressure and electrical properties. Daktari's electrode foil is utilized to measure the impedance change in the assay chamber.

3.3 Lab-on-a-Chip Technology

A study by Korb sought to identify potential applications for microfluidics; many of these applications fell into the biochemistry and other related fields. The ability to combine microfluidic processes with other MEMS technology led to the development of lab-on-a-chip devices, which complete portions of or full chemical and biochemical processes. These processes include drug delivery systems, assays, genomics, cytology, and surface patterning, among many others [18].
Typical laboratory operations include individual stages for sample preparation, pre-treatment, separation, and reactions, in addition to measurements, observations, and the interpretation of results. With careful design, complete lab-on-a-chip devices can incorporate all of these procedures and produce results from the input of a small raw sample [18][26]. In addition to the simplicity of the test, the rate can be much faster due in combination to the small samples requiring reactions and the elimination of preparation and material handling steps. Rates can reduce from hours or days of processing to minutes or hours respectively [26].

The Daktari CD4+ system utilizes microfluidic channels in the cartridge to flow blood and reagents that are controlled by actuators and solenoids in the instrument. The design of the product takes a fixed volume of blood, collects the desired cells, and processes the sample to determine and report on the concentration of CD4+ cells. This use of microfluidics takes advantage of a small sample size, minimal sample preparation, and the processing and delivery of results by a single device. This was achievable by the integrated system of fluid introduction, channels and valves, and processing of the sample and reagents. This lab-on-a-chip process supplies a result in less than 10 minutes instead of days and does so with a portable instrument, eliminating the need for elaborate laboratory equipment and training.

### 3.4 CD4 Testing

CD4+ concentrations allow doctors to assess the relative health of a person with Human Immunodeficiency Virus (HIV) or Autoimmune Deficiency Syndrome (AIDS). This result relates to the white blood cell count in a patient’s blood sample and is used to determine when the patient should begin a treatment regime of antiretroviral therapy (ART). Treatment is initiated when the cell count drops below a certain level, which varies depending on available resources. WHO standards call for treatment when CD4 levels fall below 350 cells/μL, although this is often reduced to 200 cells/μL (the official level at which
a patient is declared to have AIDS in resource limited regions such as those of Daktari’s focus [28]. During treatment, additional CD4+ tests are conducted to monitor the effectiveness of treatment and the overall health of the patient in terms of their immune system.

Traditional testing for CD4+ cell concentrations is performed through flow cytometry. This process involves marking CD4 cells from a blood sample with a fluorescent marker, flowing cells past an excited light source, and utilizing a photomultiplier to detect the changes in wavelength as each cell passes. This allows the absolute CD4 cell count to be determined for the given sample [28]. The equipment involved is very large, complex, and the test is time consuming, and must be performed by trained personnel at stationary laboratories. Additionally, they require larger samples and sample preparation prior to CD4 counting. This preparation and testing process can take 18-24 hours to complete, not including other delays. Flow cytometry processes are often in high demand, resulting in long lead times before receiving results [28].

3.4.1 Cell Lysate Impedance Spectroscopy

The Daktari CD4 system takes a different approach to CD4 testing, using cell lysate impedance spectroscopy. In this process, antibodies in the assay channel retain the CD4 cells as blood is flowed through the microfluidic channels. Reagents wash out other cells and ions in the blood and lyse the remaining white blood cells, causing a drop in the measured electrical impedance in the channel. The assay, which determines the concentration of CD4 cells, is completed with an electrochemical sensor, which takes the change in electrical impedance after lysing to determine the concentration in a small sample of blood. The magnitude of the impedance drop has a linear relationship to the number of cells lysed, allowing the number of cells to be determined by converting the change in conductance to number of cells based on the relationship. In testing, these results compare very closely to the traditional flow cytometry methods [19][23].
3.5 Manufacturing

There are a multitude of processes to manufacture microfluidic devices and even more new techniques being developed with a wide array of materials and properties. The manufacturing technique chosen by any microfluidic designer greatly depends on the material and tolerances required in the design.

3.5.1 Backbone Manufacturing Options

Daktari Diagnostics chose to use polymethyl methacrylate (PMMA) for the microfluidic backbone because it interacts well with the fluids and chemical components in the assay. This material choice was carried through with other plastic components in the card in order to maintain compatibility and consistency in material properties.

Polymers, such as PMMA, can be processed in either serial or parallel processes. Serial processes are less desirable due to the response of the polymer to intense localized energy, such as those that occur in milling processes. The long chains that make the polymer can reorient when energized and crystallize in an undesirable form. Therefore, most processing of polymers for microfluidic applications is a parallel process where the entire surface is patterned at once with the use of a mold. Molding applications available include injection molding, micro-casting, and micro-forging [18].

The polymer is formed around the mold to get the desired shape. This process is generally a standard one; however the processes used to make the mold are diverse. Many techniques have been adapted from other industries such as semiconductor manufacturing [18].

One such technique is to apply a photoresist to a substrate and cure the negative of the microfluidic pattern. This mold is relatively quick to manufacture, but cannot produce a large amount of parts. Similarly, using a photoresist and
etching process to make the mold out of silicon can create a more robust mold but requires more processing steps [18].

Electroplating and Electro-Discharge Machining (EDM) are additional options. Electroplating is commonly used in conjunction with physical vapor deposition (PVD). Physical vapor deposition is used to create the initial layer and the electroplating grows on top to create the mold. Electro-Discharge Machining (EDM) electrically erodes away unwanted material from the mold [18].

Daktari uses traditional machining methods to create molds for the plastic injection molding of the backbone, cap, and housing. The mold is expensive and not infinitely flexible to design changes, but each mold can create many parts and does not vary much from part-to-part. This process was also chosen for the speed that injection molding could produce parts in production. Daktari’s partner, who produces the injection-molded parts, can meet the necessary dimensions and tolerances with this process.

### 3.5.2 Electrode Foil Manufacturing Options

The electrode foil performs multiple duties in the Daktari cartridge and requires several steps to produce a complete part. A complete electrode consists of a PMMA substrate, electrode-sensing layer, and antibody solution with a protective sucrose layer. The process to produce complete and functionalized electrodes starts with a sheet of extruded PMMA. This sheet will first have the electrode layer put on the surface before the antibody and sucrose layers are applied by spotting, onto the surface.

There are two methods that Daktari is using to apply the electrode to the substrate. The first method is to use PVD to sputter coat the PMMA entirely with gold, followed by a laser ablation of the excess gold from the surface. Gold was chosen due to its conductivity properties and resistance to corrosion. The ablation process is done by raster (a serial process) or excimer (a parallel process) laser methods, each of which presents unique challenges. The rastering
laser textures the surface while the excimer process causes some amount of gold to redeposit on the surface. Both side effects complicate the later welding process and affect the properties of the electrode. An additional challenge with the gold electrodes is the poor adhesion between the gold and PMMA substrate. Poor adhesion causes the electrodes to be fragile and susceptible to damage, resulting in broken electrical connections and variability in the performance of the assay.

The second manufacturing process was developed at Daktari Diagnostics and the electrodes produced are the focus of the 2011 thesis projects. The electrodes manufactured by this process are much preferred from a manufacturing and durability standpoint. They are faster to produce, configurable for flexible design changes, and are more resistant to physical damage than the gold electrodes.

Once the conductive electrode layer is complete, the antibody and protective layer are applied to the PMMA with the electrode by spotting. The spotter deposits small drops of antibody solution to the surface of the electrode in the location shown in figure 6. The spotter then deposits a protective layer over the antibody. There is the spotting machine located at Daktari’s facility in Cambridge Massachusetts along with one in Germany at Daktari’s partner. The exact pattern of the antibody will depend on the characteristics of the final electrode process and pattern.

Figure 6: Antibody solution location on the electrode
Chapter 4: Background Context: Working of the Electrode

In order to understand the critical dimensions of the electrode, it is helpful to review how the electrode functions and how measurements are taken in the assay chamber. This chapter focuses on the functionality and the operation of the electrode.

The electrode foil (figure 7 and 8) is part of the cartridge that is used to measure the number of captured CD4 cells. The electrode foil also performs two structural tasks when it is welded to the backbone. The electrode foil forms the ceiling of the ‘assay chamber’ where the CD4 cell count is performed and is spotted with antibody solution, which is used to capture the CD4 cells.

![Figure 7: Daktari's interdigitated electrode foil](image)

![Figure 8: The electrode foil welded to the backbone](image)
During operation, the electrode foil measures the electrical impedance within the assay chamber, both before and after lysis, or bursting, of the captured CD4 cells. The measured drop in electrical impedance occurs due to the release of ions from the burst cells and is directly proportional to the number of CD4 cells from the sample. Pads at the end of the electrode pattern establish contacts with the electric connector pins in the instrument. The instrument takes the measured impedance change and converts it to a cell count to display to the user based on a known linear relationship between the impedance drop and cell count.

The quality of the electrodes and the constancy in the dimensions of the fingers and the side rails are vital in the performance of the electrode foils. Impedance measurements (1/resistance) are very sensitive to the sensing area of the electrode. This sensing region is the space between opposing electrode rails. An interdigitated electrode, such as Daktari's, increases the sensing region for a given area by utilizing interlocking fingers. These fingers create a sensing region that winds through the electrode fingers, as indicated by the dotted line below in Figure 9.

![Figure 9: sensing region of the electrode](image)

With a constant gap width throughout the electrode, the sensing region can be modelled based on the characteristic length (the total length of the dotted line) and gap along the sensing area. The proportion of length over gap (L/g) is
inversely proportional to the impedance. Reducing the length or increasing the gap between fingers will increase the impedance. This relationship makes the repeatability of finger width and spacing critical to the repeatability of the impedance drop measurements. Even small variations to finger dimensions will greatly affect the proportion of length and gap, and therefore the resulting impedance drop measurements.
Chapter 5: Methodology

As mentioned in section 3.5.2 Daktari has developed a new manufacturing process for the production of the electrode foils. Although this process seems to be producing viable parts, further validation is required to ensure the quality and the consistency in produced parts. The rest of this chapter describes the methodology carried out in order to understand and characterize the variation of the critical dimensions in the electrodes produced by the new manufacturing process and to understand the impact of these variations on the performance of the electrodes.

5.1 Variation analysis of the electrode

As mentioned in the previous section, the repeatability of the finger width and the finger spacing in the electrodes are critical to the repeatability of the impedance drop measurements or the performance of the electrodes.

In order to characterize the dimensional variation in the electrodes and to validate the repeatability of the newly developed process, a total of 1000 electrodes were manufactured (a quarter of the electrodes needed for the clinical trials). With the aid of different optical techniques, high-resolution images of the electrodes were obtained and the dimensional variations of the finger width and finger spacing were analysed using a custom developed algorithm.

5.1.2 Numerical analysis of the electrodes

An algorithm was developed using Matlab in order to analyse the dimensional variations of the finger width and the finger spacing in the electrodes. This section describes the steps in the algorithm.
**Step 1:** The algorithm reads the scanned image file from a directory.

**Step 2:** With the image-processing feature in Matlab, the algorithm first converts the image into a binary image.

**Step 3:** The algorithm sets the threshold level to 155 (the threshold level was selected by comparing an original image to its binary image with different threshold levels and subjectively selecting a level that best matches the variations in the original image). Figure 10 shows a binary image of an electrode with the set threshold level.

**Step 4:** The algorithm then finds the perimeter pixels of the fingers by converting all non-perimeter pixels into black pixels and leaving the perimeter pixels as white (figure 11).

**Step 5:** The algorithm then takes 650 single-pixel columns across the perimeter pixels (figure 12 and 13).

**Step 6:** Distances of white pixels are obtained in terms of pixels.

**Step 7:** Distances less than 70 pixels refer to finger width and distances higher than 70 pixels refer to finger spacing. (This reflects the design of the current electrodes in which finger width is 130 microns and finger spacing is 250 microns, since 1 pixel is 2.65 microns at 9600 dpi). An alternative method to this step was also used for different electrode designs. The algorithm separates the odd and even distances. Odd distances refer to finger width and even distances refer to finger spacing.

**Step 8:** Pixel distances are converted into microns by multiplying by 2.65 (since the images were scanned at 9600 dpi).

**Step 9:** The *locus finger width* and *locus finger spacing* is obtained at 650 locations along the fingers for all 126 fingers in an image.
Step 10: The values in step 9 are averaged to obtain *single finger width* and *single finger spacing*.

Step 11: the values in step 10 are averaged to obtain *overall finger width* and *overall finger spacing*.

The algorithm is shown in the appendix.
Figure 12: The vertical columns taken down along the interdigitated fingers (step 5)

Figure 13: Close up of vertical columns (step 5)
5.1.3 **High Resolution Scanning**

A Canon CanoScan LiDE 700 scanner was used at 9600 dpi (1 pixel equal to 2.65 microns) in order to obtain images of the electrodes to be analysed in Matlab.

5.1.4 **Minimizing the scanner variability**

To minimize the variability due to the scanner, the electrodes were all placed at the same location on the scanner bed along the scanner's centre shaft guiding the scanner beam, where the beam vibration is expected to be at its minimum. Figure 14 depicts the positioning of the electrode on the scanner bed.

![Figure 14: position of the electrode on the scanner](image-url)
5.1.5 Characterizing the scanner error

The first step in using the scanner was to characterize the scanner error and understand the repeatability and reproducibility of the scanner. A photolithography mask (figure 15) with finger width of 100 ±3 microns was used to characterize the scanner error.

Figure 15: Scanned image of Photolithography mask (finger width 100 ±3 microns)
Repeatability

The repeatability of the scanner was obtained by scanning the photolithography mask a number of times along the scanner's centre shaft without moving the mask or opening the scanner lid and then using the algorithm to measure the finger width. The finger dimensions from each scan were then compared in order to find the repeatability error.

In each scanned image, the differences in the finger width of the 10 fingers of the image were compared at 15 different single pixel columns. The maximum error was found to be 2.65 microns or 1 pixel at 9600 dpi. Table 1 shows the comparison of the 10 fingers between two scanned images for 2 out of 15 columns for each image.

<table>
<thead>
<tr>
<th>Finger number</th>
<th>Scan 1 finger width (microns)</th>
<th>Scan 2 finger width (microns)</th>
<th>Difference scan 1 and scan 2 (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>line 1</td>
<td>line 2</td>
<td>line 1</td>
</tr>
<tr>
<td>1</td>
<td>111.3</td>
<td>111.3</td>
<td>111.3</td>
</tr>
<tr>
<td>2</td>
<td>111.3</td>
<td>111.3</td>
<td>111.3</td>
</tr>
<tr>
<td>3</td>
<td>113.95</td>
<td>111.3</td>
<td>113.95</td>
</tr>
<tr>
<td>4</td>
<td>116.6</td>
<td>116.6</td>
<td>116.6</td>
</tr>
<tr>
<td>5</td>
<td>111.3</td>
<td>108.65</td>
<td>108.65</td>
</tr>
<tr>
<td>6</td>
<td>111.3</td>
<td>111.3</td>
<td>111.3</td>
</tr>
<tr>
<td>7</td>
<td>113.95</td>
<td>113.95</td>
<td>116.6</td>
</tr>
<tr>
<td>8</td>
<td>116.6</td>
<td>116.6</td>
<td>116.6</td>
</tr>
<tr>
<td>9</td>
<td>111.3</td>
<td>116.6</td>
<td>113.95</td>
</tr>
<tr>
<td>10</td>
<td>116.6</td>
<td>113.95</td>
<td>113.95</td>
</tr>
</tbody>
</table>
Reproducibility

The reproducibility error of the scanner was determined by removing the photolithography mask and then replacing it as close to the original position as possible after each consecutive scan.

In each scanned image, the differences in the finger width of the 10 fingers of the image were compared at 15 different single pixel columns.

Table 2 shows the comparison of the 10 fingers between two scanned images for only 2 out of 15 columns for each image. The maximum error was found to be 7.95 microns or 3 pixels at 9600 dpi.

Table 2. Scanner reproducibility error. Comparison between two scanned images. (showing 2 out of 15 vertical lines)

<table>
<thead>
<tr>
<th>Finger number</th>
<th>Scan 1 finger width (microns)</th>
<th>Scan 2 finger width (microns)</th>
<th>Difference scan 1 and scan 2 (microns)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>line 1</td>
<td>line 2</td>
<td>line 1</td>
</tr>
<tr>
<td>1</td>
<td>111.3</td>
<td>111.3</td>
<td>108.65</td>
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<tr>
<td>2</td>
<td>111.3</td>
<td>111.3</td>
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<tr>
<td>3</td>
<td>113.95</td>
<td>111.3</td>
<td>113.95</td>
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<tr>
<td>4</td>
<td>116.6</td>
<td>116.6</td>
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<tr>
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<td>111.3</td>
<td>108.65</td>
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<td>7</td>
<td>113.95</td>
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<td>8</td>
<td>116.6</td>
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<td>9</td>
<td>111.3</td>
<td>116.6</td>
<td>116.6</td>
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<tr>
<td>10</td>
<td>116.6</td>
<td>113.95</td>
<td>119.25</td>
</tr>
</tbody>
</table>
5.1.6 Validating the scanner capability

In order to ensure that the scanned images do in fact depict the dimensional variation of the fingers, a monochromatic camera with a 20X zoom lens (1 pixel equal to 0.521 microns) was used. The monochromatic image was then compared to the scanned image to ensure that the scanner is capable of capturing the finger variation. Figure 16 shows the comparison between the two images for the same fingers in the same electrode.

![Monochromatic and scanned image comparison](image)

**Figure 16: Monochromatic and scanned image of an electrode**

While more detail is visible in the monochromatic image, the scanned image depicts sufficient detail in the finger dimensional variation for the purpose of this research. This comparison validates the use of a 9600 dpi scanner.
5.1.7 Characterizing the dimensional variations

In order to understand and characterize the variation of the newly developed process of the electrodes, 1000 electrodes were produced with constant process input of which a total of 100 electrodes were scanned at 9600 dpi. The finger width and finger spacing together with their corresponding standard deviations were obtained for each electrode by applying the algorithm to their images. This analysis was conducted in order to understand and characterise the variation of the critical dimensions in the electrode based on when it was produced in a run and to determine if the process is drifting over time.

5.2 Impact of the dimensional variations on the electrodes performance

Once the dimensional variations in electrodes were analyzed, it was critical to understand the impact of these variations on the performance and functionality of the electrodes.

As mentioned earlier in chapter 4 the electrode measures the electrical impedance within the assay chamber, both before and after lysis of the captured CD4 cells in order to determine the CD4 cell concentration. Dip testing the electrode is a method being used by Daktari in order to characterize the performance of the electrodes in terms of their sensitivity to impedance measurement.
5.2.1 Dip test

The Dip Test is performed by taking an electrode rinsed with deionized (DI) water and submerging it in solutions of known conductivity to the level where the electrode would come in contact with the fluid in the assay chamber. The electrode is left in the solution for 10 seconds before the impedance reading is recorded from the meter attached to the pads on the electrode. The electrode is dipped in DI water between each solution. Figures 17 and figure 18 show an electrode being dip tested and the level where the fluid enters the assay chamber on one design [27].
Once the impedance is recorded a linear relationship can be found between 1/impedance and the solution conductivity. The slope of 1/impedance Vs solution conductivity is used to compare the variability between electrodes.

5.2.2 Variations measured from the dip test

Once the dip test was carried out for the electrodes, the variation in the performance of the electrodes was measured as percent cell equivalent error. This metric is the easiest way to compare two electrodes with different slopes and variations.

The way the instrument uses the slopes is as follows:

An unknown input number of cells proportionally relates to an input conductivity.

1. The actual electrode doing the measurement reads the input conductivity.
2. 1/Impedance is measured with the actual electrode.
3. The measured $1/\text{impedance}$ is used with the average value line.
4. The output conductivity is then measured.

Figure 19: Procedure for Determining % Cell Equivalent Error [27]

Using the equation of a line $y = mx + b$ and both the actual and expected lines go through zero so $b=0$.

$$\frac{1}{\text{impedance}} = \text{Input}_{\text{conductivity}} \times M_{\text{actual}} = \text{Output}_{\text{conductivity}} \times M_{\text{average}} \tag{1}$$

$$\text{Output}_{\text{conductivity}} = \text{Input}_{\text{conductivity}} \times \frac{M_{\text{actual}}}{M_{\text{average}}} \tag{2}$$

$$\%\text{CellError} = 1 - \frac{M_{\text{actual}}}{M_{\text{average}}} \tag{3}$$
The actual slope can be steeper or shallower than the average slope. Ideally if all the electrodes have the same slope the percent cell error would be zero. However, some of the actual slopes will give positive or negative percent cell error [27].

5.2.3 Dimensional variations and the measured slope from the dip test

As discussed in chapter 4 the repeatability of finger width and spacing are critical to the repeatability of the impedance drop measurements or in other words, the sensitivity of the electrodes. In order to determine the relation between the dimensional variation and the electrode's sensitivity, a total of 20 electrodes were dip tested and the % cell errors were compared to the electrodes dimensional variations.
Chapter 6: Results and Discussions

Using the algorithm described in section 5.1.2 and applying it to the scanned images of the electrodes, a total of 100 electrodes were analyzed from the production batch of 1000 (a quarter of the electrodes needed for the clinical trials) at constant input parameters. The critical dimensions to be analyzed were the finger width and the finger spacing of the electrodes. The desired dimensions were 130 microns for the finger width and 250 microns for the finger spacing. The analysis was performed in order to understand and characterize the variation in the critical dimensions of the electrodes throughout the production run of the new manufacturing process developed by Daktari. Subsequently the effect of the dimensional variation on the sensitivity of the electrodes was studied.

As described in section 5.1.2, the algorithm takes $i=650$ single-pixel column across the interdigitated fingers in order to measure the finger width and the finger spacing in an electrode. In this section, 3 specific terminologies (see figure 20) are used for the finger width:

*Locus finger width* refers to the width at a specific location, $i=n$, for an individual finger.

*Single finger width* refers to the average of all $i=650$ locus finger widths in an individual finger.

*Overall finger width* refers to the average of all the 126 single fingers widths in an electrode.

The same approach is used for finger spacing.
In this section, figures 21 to figure 34 refer to *locus finger width*, figures 35 to 39 refer to *single finger width* and figure 40 refers to *overall finger width*. 
6.1 Locus Finger Width

6.1.1 Locus Finger Width variations in a given finger

To begin with, the variation in locus finger width for any given finger was analyzed. For this analysis a random electrode was selected and the finger width variation was analyzed for 650 different locations (i) for each 126 fingers separately. Figure 21 shows the approach for random finger with 3 different locations. This analysis was performed in order to characterize the locus finger width variations in a given finger.

![Figure 21: Locus finger width variation in a given finger](image)

Figures 22 to 25 show the variation in the locus finger width at 650 different locations, for 4 randomly selected fingers in a randomly selected sample. The reproducibility of the scanner, as stated in section 5.1.5, is 7.95 microns is shown on the figures.

The highest standard deviation in locus finger width in the sampled electrode was 5.7 microns, for finger number 5 (shown in figure 22), with a maximum and minimum values of 145.75 and 119.25 microns respectively. The mean value of locus finger width was 134 microns resulting a tolerance range of 10% (% difference between maximum value of 145.75 and the mean value of 134). Table 3 summarizes the results.
While Daktari has not yet set forth an acceptable tolerance range for the dimensional variation of the electrodes, a tolerance range of ±10% suggests that the new manufacturing process is capable of producing single fingers with a consistent finger width. Table 3 summarizes the results of this study.

Table 3

<table>
<thead>
<tr>
<th>Finger no.5</th>
<th>Locus Finger Width</th>
</tr>
</thead>
<tbody>
<tr>
<td>-locus finger width</td>
<td>-reproducibility error</td>
</tr>
<tr>
<td>180</td>
<td>170</td>
</tr>
<tr>
<td>160</td>
<td>150</td>
</tr>
<tr>
<td>140</td>
<td>130</td>
</tr>
<tr>
<td>120</td>
<td>110</td>
</tr>
<tr>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

Figure 22: Locus finger width variation for finger no.5

Finger no.25

Figure 23: Locus finger width variation for finger no.25
Figure 24: Locus finger width variation for finger no.60

Figure 25: Locus finger width variation for finger no.90
Table 3: Locus finger width variation results for finger 5 in a random sample

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum locus finger width</td>
<td>119.25 microns</td>
</tr>
<tr>
<td>Maximum locus finger width</td>
<td>145.75 microns</td>
</tr>
<tr>
<td>Mean locus finger width</td>
<td>134 microns</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5.7 microns</td>
</tr>
<tr>
<td>Range (difference of extreme values)</td>
<td>26.15 microns</td>
</tr>
<tr>
<td>Tolerance range (percentage difference between maximum and mean values)</td>
<td>±10%</td>
</tr>
</tbody>
</table>

6.1.2 Locus Finger Width variations between all fingers in a given electrode

The next step was the dimensional variation analysis of different fingers in an electrode for a given location. For this analysis a random electrode was selected and the variation between the 126 fingers was compared separately for each location (i) at 650 different locations (i). This approach is shown in figure 26.

![Figure 26: variation between fingers for a given location in an electrode](image-url)
Figures 27 to 30 show the variations for 4 randomly selected locations (i=100, i=250, i=400 and i=550). The error bars are set to 7.95 microns, the reproducibility error of the scanner.

The maximum and minimum *locus finger width* values were 148.2 ± 7.95 microns and 121.9 ± 7.95 microns respectively with a mean value of 136.3 for the sampled electrode. Thus the tolerance range in the sampled electrode was found to be ±4% of the *mean locus finger width* (% difference between maximum value of 148.2 and the mean value of 136.3) with a maximum range of 26.3 microns between the two extreme values.

While Daktari has not yet set forth an acceptable tolerance range for the dimensional variation of the electrodes, a tolerance range as tight as ±4% suggests that the new manufacturing process is capable of producing consistent fingers in an electrode. Table 4 summarizes the results of this study.
Figure 28: Locus Finger width variation between different fingers i=250

Figure 29: Locus Finger width variation between different fingers i=400
Locus Finger Width variation between fingers for location i=550
(electrode no. 400)

Figure 30: Locus Finger width variation between different fingers i=550

Table 4 Locus finger width variation results for fingers in electrode no. 400

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum locus finger width</td>
<td>121.9 microns</td>
</tr>
<tr>
<td>Maximum locus finger width</td>
<td>148.2 microns</td>
</tr>
<tr>
<td>Mean locus finger width</td>
<td>136.3 microns</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.7 microns</td>
</tr>
<tr>
<td>Range (difference of extreme values)</td>
<td>26.3 microns</td>
</tr>
<tr>
<td>Tolerance range (percentage difference between maximum and mean values)</td>
<td>±4%</td>
</tr>
</tbody>
</table>

6.1.3 Locus Finger Width variations for a given finger between different electrodes

The dimensional variation of the same finger in 100 sampled electrodes was analyzed for a given location. This analysis was performed in order to understand how the finger width of a specific location in a specific finger varies in the production run of 1000 electrodes. For this analysis a random finger was selected and a given locus finger width was compared separately for each electrode. This approach is shown in figure 31.

![Figure 31: Locus Finger Width variations for a given finger between different electrodes](image-url)
Figures 32 to 34 show the variations of the *locus finger width* for a randomly selected finger at location (i=100, i=300 and i=600) along the same finger number between different electrodes. The error bars are set to 7.95 microns, the reproducibility error of the scanner.

The highest standard deviation in *locus finger width* was 6.75 microns, for location i=600 (34), with a maximum and minimum values of 148.4 and 119.25 microns respectively. The mean value of *locus finger width* for this location was 134 microns resulting a tolerance range of 12% (% difference between maximum value of 148.2 and the mean value of 134).

Table 5 summarizes the results.
Figure 33: Locus Finger width variation between different electrodes for $i=300$

Figure 34: Locus Finger width variation between different electrodes for $i=600$
Table 5: Locus Finger Width variations for a given finger for 
\(i=600\)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum locus finger width</td>
<td>121.9</td>
</tr>
<tr>
<td></td>
<td>microns</td>
</tr>
<tr>
<td>Maximum locus finger width</td>
<td>148.2</td>
</tr>
<tr>
<td></td>
<td>microns</td>
</tr>
<tr>
<td>Mean locus finger width</td>
<td>136.3</td>
</tr>
<tr>
<td></td>
<td>microns</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>6.75</td>
</tr>
<tr>
<td></td>
<td>microns</td>
</tr>
<tr>
<td>Range</td>
<td>29.15</td>
</tr>
<tr>
<td>(difference of extreme values)</td>
<td>microns</td>
</tr>
<tr>
<td>Tolerance range</td>
<td>±12%</td>
</tr>
<tr>
<td>(percentage difference between maximum and mean values)</td>
<td></td>
</tr>
</tbody>
</table>

It must be noted that this particular analysis is not completely accurate, since each constant location \((i)\) may not necessarily refer to the same exact point on the same finger from one electrode to another. For this reasons, in order to better characterize the variations of the same fingers between electrodes, single finger width variations (average of locus finger width for a finger) have been analyzed between electrodes (see figure 20 for definition of single finger width).
6.2 Single Finger Width

6.2.1 Single finger width variations for a given finger between different electrodes

The dimensional variation of single finger width (average of all locus finger widths) for any given finger was also analyzed throughout the production run of 1000 electrodes.

Figures 35 to 38 show the variation in the single finger width, from one electrode to another, for 4 randomly selected fingers. The maximum standard deviation of single finger width, for the same finger, from one sample to another was found to be 5.9 microns with a maximum value of 150.8 and minimum value of 118.8 microns. A standard deviation of 5.9 microns is only a ±4.5% variation from the mean single finger width value of 132 microns. Moreover, it is reasonable to assume that any variation of one finger could be compensated by an inverse change in another finger within the same electrode. Hence not affecting the overall finger width of an electrode. Table 6 summarizes the results of this analysis.

As highlighted by the straight lines on figures 35 to 38, no significant mean shift was observed for the single finger widths from one electrode to another.
Figure 35: Variation in single finger width across samples for finger no.7

Figure 36: Variation in single finger width across samples for finger no.10
Figure 37: Variation in single finger width across samples for finger no. 85

Figure 38: Variation in single finger width across samples for finger no. 119
Table 6. Variation of a given finger between samples

<table>
<thead>
<tr>
<th>Maximum standard deviation (for a given finger between samples)</th>
<th>5.9 microns or 4.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum single finger width (finger 41)</td>
<td>150.8 microns</td>
</tr>
<tr>
<td>Mean single finger width</td>
<td>132 microns</td>
</tr>
<tr>
<td>Minimum single finger width (finger 41)</td>
<td>118.8 microns</td>
</tr>
<tr>
<td>Range (difference of extreme values, finger 41)</td>
<td>32 microns</td>
</tr>
</tbody>
</table>

6.2.2 Single finger width standard deviation in an electrode

The standard deviation of the single finger width within each sampled electrode is plotted on figure 39. The figure shows that the maximum standard deviation is 7.1 microns. This value is within the limits of the scanner reproducibility error of 7.95 microns and shows that we are at the limit of the scanner capability.

As shown by the straight line on figure 39, the standard deviation of the single finger width for the 126 fingers within each electrode, in a production run of 1000 electrodes, has a consistent average value of 4.6 microns. This consistency suggests that the manufacturing process is capable of producing accurate electrodes and that the dimensional variation within each electrode is insensitive to when it was produced in the run.
6.3 Overall Finger Width

The overall finger width of each electrode was also obtained for the sample of 100 electrodes. The overall finger width of an electrode is the average value of all single finger widths in an electrode (see figure 20). Figure 40 shows a scatter plot of the overall finger width for the 100 sampled electrodes. The error bars are set to 7.95 microns, the reproducibility error of the scanner; see section 5.1.5 for the reproducibility error of the scanner.
The mean value of the *overall finger width* of the samples was found to be 132.2 microns. The maximum and minimum *overall finger width* values (marked in red on figure 40) were 142.2 ± 7.95 microns and 125.2 ± 7.95 microns respectively. Thus the tolerance range in a production run of 1000 electrodes was found to be ±8% of the *mean overall finger width* (% difference between maximum value of 142.2 and the mean value of 132.2) with a maximum range of 17 microns between the two extreme values.

A tolerance range as tight as ±8% suggests that the new manufacturing process is capable of producing electrodes with consistent *overall finger width* in long runs.

Table 7 summarizes the results of the *overall finger width* for the 100 sampled electrodes from a production batch of 1000 electrodes.
6.3 Finger spacing

Apart from the consistency in the finger width, another critical parameter in the performance of the electrode is consistency in the finger spacing. Although the two are directly related, it has been observed that at times the centre line of a finger may slightly displace in the production resulting in constant finger width but a change in finger spacing. For this reason, the single finger spacing and overall finger spacing has also been studied independently.

6.3.1 Single finger spacing variations in the electrodes

The standard deviation of single finger spacing within each sampled electrode is plotted on figure 41. The figure shows that the maximum standard deviation of
the single finger spacing within each electrode is around 11.9 microns or 4.8% of the mean value of single finger spacing.

As shown by the straight line on figure 41, the standard deviation of the single finger spacing for the 126 fingers within each electrode is consistent, in a production run of 1000 electrodes, with an average value of 9.7 microns. This consistency supports the earlier finding that the manufacturing process is capable of producing accurate electrodes and that the dimensional variation within each electrode is independent to when it was produced in the run.

Figure 41: Scatter plot of average standard deviation of finger spacing
6.3.2 Overall finger spacing

Figure 42 shows a scatter plot of the overall finger spacing for the 100 sampled electrodes. The error bars are set at 7.95 microns, the reproducibility of the scanner.

![Image: Scatter plot of overall finger spacing](image)

**Figure 42: scatter plot of overall finger spacing**

The mean value of the overall finger spacing of the samples was found to be 249.5 microns. The maximum and minimum overall finger spacing values (marked in red on figure 42) were 256.7 ± 7.95 microns and 239.7 ± 7.95 microns respectively. Thus the tolerance range in a production run of 1000 electrodes was found to be ±4% of the mean overall finger spacing (% difference between minimum value of 239.7 and the mean value of 249.7) with a maximum range of 17 microns between the two extreme values. This tolerance range is even tighter than the ±8% tolerance range for the overall finger width.

Table 8 summarizes the results of the overall finger spacing for the 100 sampled electrodes from a production batch of 1000 electrodes.
Table 8: Overall finger spacing results

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum average spacing</td>
<td>239.7 μm</td>
</tr>
<tr>
<td>Maximum average spacing</td>
<td>256.7 μm</td>
</tr>
<tr>
<td>Mean average spacing</td>
<td>249.5 μm</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.1 μm</td>
</tr>
<tr>
<td>Range (difference of extreme values)</td>
<td>17 μm</td>
</tr>
<tr>
<td>Tolerance range</td>
<td>±4%</td>
</tr>
</tbody>
</table>
6.3 Process capability analysis

6.3.1 Frequency distribution histograms and probability plots

Figure 43 and figure 44 show the histogram of the overall finger width and the overall finger spacing for the 100 sampled electrodes. Both histograms suggest a normal distribution. The normal probability plot of this data agrees with this hypothesis, see figure 45 and figure 46.

![Histogram of the overall finger width](image1)

![Histogram of overall finger spacing](image2)
Figure 45: Normal probability plot of the overall finger width

Figure 46: Normal probability plot of overall finger spacing
6.3.2 Individual and moving range control charts for individual measurements

In order to verify that the process was in fact statistically in control during the production run of 1000 electrodes, the Shewhart control chart for individual measurements and a moving range control charts were used. This method was employed since the sample size group used is n=1 for the 100 samples in a batch of 1000.

For the control chart for individual measurements, the parameters are

Center line

\[
\bar{x} = \frac{\sum_{i=1}^{m} x_i}{m}
\]  

(4)

Control limits

\[
\bar{x} \pm 3 \frac{MR}{d_2}
\]  

(5)

Where

\[
MR_i = \frac{\sum_{i=2}^{m} |x_i - x_{i-1}|}{m - 1}
\]  

(6)

Figure 47 and 48 show the control chart for individual measurements of the overall finger width and the corresponding moving range control chart. It can be seen that there are no points outside the control limits and the points are almost equally divided above and below the centre line which means that the process was statistically in control during the production of 1000 electrodes.
Figure 47: Control chart for the overall finger width

Figure 48: Moving range control chart for the overall finger width
The corresponding control charts for the *overall finger spacing*, figure 49 and figure 50 confirm that the process was in statistical control.

![I control chart](image1)

*Figure 49: Control chart for the overall finger spacing*

![MR control chart](image2)

*Figure 50: Moving range control chart of the overall finger spacing*
6.4 Dimensional variations and the sensitivity of the electrodes

6.4.1 Dip test of the electrodes with extremes dimension

In order to understand the significance of the dimensional variation of the electrodes on the electrode's sensitivity, the two electrodes, with extreme values of overall finger width and overall finger spacing from the sample, where dip tested according to the procedure stated in section 5.2.1.

The resulting slopes (slopes of 1/impedance Vs conductivity) from the dip test showed only a 1% difference between the two electrodes.

The small difference in the slope suggests that in a production run of 1000 electrodes, the dimensional variation does not significantly impact the sensitivity of the electrodes and the manufacturing process is capable of producing satisfactory electrodes while it is statistically in control.

A number of electrodes with increased finger width (70 microns or 35% increase), with the same interdigitated design, were also manufactured and dip tested and compared to the extremes of the original sample.

Table 9 summarizes the data and figure 51 shows the results of the dip test.

It should be noted that there are in fact 3 lines plotted on figure 51. Since there is only a 1% difference between the slopes for the electrodes with extreme values of overall finger width from the sample, the lines (green and purple) are almost overlapping in the figure.

<table>
<thead>
<tr>
<th>Electrode 600 (maximum overall finger width)</th>
<th>Overall finger width</th>
<th>Overall finger spacing</th>
<th>slope</th>
<th>% slope difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>240</td>
<td>1.21E-05</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Electrode 197 (minimum overall finger width)</td>
<td>125</td>
<td>256</td>
<td>1.22E-05</td>
<td>101%</td>
</tr>
<tr>
<td>Increased finger width</td>
<td>203</td>
<td>178</td>
<td>1.53E-05</td>
<td>126%</td>
</tr>
</tbody>
</table>
6.4.2 Correlation between overall finger spacing and performance

In order to generally understand the relationship between the electrode's dimensional variation and its sensitivity, a total of 21 electrodes have been dip tested and the results of their slope of 1/impedance Vs conductivity has been plotted against their overall finger spacing, see figure 52.

All the data points in figure 52 fall within the region of the mean value of overall finger spacing ±7.95 microns in the y-axis (the reproducibility error of scanner) and the mean value of the slope ±1.8% in the x-axis (the repeatability error of the dip test).

This fact leads to conclude that

a) The finger dimensional variations are smaller than the reproducibility error of the scanner.
b) The finger dimensional variations in a production run have no significant impact on the electrode's sensitivity.

c) We are at the limits of the capabilities of the dip test and the scanning technique and that the variations seen in figure 52 are due to noise or the reproducibility error of the technology used.

d) A relationship between the Overall finger spacing and the electrode's sensitivity cannot be established by comparing only electrodes with the same design dimensions. In order to possibly establish a relationship between the two, electrodes with significantly different overall finger spacing should be compared.

Figure 52: Overall finger spacing Vs slope from the dip test
6.5 Impact of process parameters

The manufacturing process developed by Daktari has four main input process parameters. In order to understand the affect of manipulating these parameters on the quality of the electrodes, a number of electrodes were produced at different setting for each process parameter. Because of the proprietary nature of this research, key parameters cannot be disclosed in this paper. However, in testing at the facility, changing the two parameters A and B were found to significantly affect the quality of the electrodes.

It was observed that by increasing parameter A by only 3 units the single finger width increased by almost 23% or 30 microns in the electrodes. While decreasing Parameter A resulted in a 20% decrease in the single finger width in the electrodes.

Increasing parameter B by only 1 unit resulted in 35% or 45 microns decrease in the single finger width and decreasing the parameter by 1 unit resulted in an increase of 8% in the single finger width.

As well as the changes in the finger width, discontinuities were also observed in the electrodes side rails where all the fingers are connected.

The significant changes observed in the single finger width by manipulating the two parameters imply that process parameters A and B should be thoroughly controlled in a long production run.

Table 10 shows the impact of manipulating the two parameters A and B on the electrode dimensions.
Table 10: Effect of two main process parameters

<table>
<thead>
<tr>
<th></th>
<th>Single Finger Width</th>
<th>Side Rails</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Decreasing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parameter A by 3</td>
<td>Decreases almost 20% or 25 microns</td>
<td>Discontinuity visible</td>
</tr>
<tr>
<td>units</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Increasing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parameter A by 3</td>
<td>Increases almost 23% or 30 microns</td>
<td></td>
</tr>
<tr>
<td>units</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Increasing B</strong></td>
<td>Decreases by almost 35% or 45 microns</td>
<td>Discontinuity visible</td>
</tr>
<tr>
<td><strong>Decreasing B</strong></td>
<td>Increases by 8% or 10 microns</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 7: Conclusion

To conclude based on the current findings, the manufacturing process developed by Daktari has been found to be capable of producing satisfactory parts and to be robust to manufacturing variation.

The *locus finger width* variation within an individual finger had a maximum standard deviation of 5.7 microns and a tolerance range of 10%, suggesting that a single finger in an electrode has a consistent width throughout its length.

The *locus finger width* variation between all the fingers in an electrode had a standard deviation of 4.7 microns with a tolerance range of ±4% and a maximum range of 26.3 microns between the two extreme values. This tight tolerance suggests that all the fingers in an electrode have consistent *locus finger width* when compared to one another.

In a production run of 1000 electrodes (a quarter of the quantity needed for the clinical trials), the tolerance range for the *overall finger width* for the electrodes was found to be ±8% with a maximum range of 17 microns. The tolerance range for the *overall finger spacing* was found to be ±4%, with a maximum range of 17 microns. When the same finger was compared between different samples, the maximum standard deviation of *single finger width* was 5.9 microns.

While Daktari has not yet set forth an acceptable tolerance range for the dimensional variation of the electrodes, the results from this study suggest that the new manufacturing process is capable of producing consistent electrodes in long runs.

The effect of the extreme variations in the finger dimensions from the samples showed minor change in the electrodes performance and sensitivity, resulting in only 1% change in the slope of 1/impedance Vs conductivity. This minor change proves that the dimensional variations from the manufacturing process have no significant impact on the electrode’s performance.
The relationship between the electrode dimensional variation and the electrode performance was analyzed. The data confirmed that the variations are within the boundaries of the reproducibility errors and that we are at the limit of the capabilities of the technology used.

The process was found to be statistically in control during the production run of 1000 parts with no data drifting away from the desired target.
Chapter 8: Future Work

This section summarizes the future work in order to optimize the design and the manufacturing of the electrodes. The future work is divided in the following four main categories:

- Variation analysis of the electrodes
- Quality control of the electrodes
- Electrode design and robustness
- The ability to capture the CD4 cells and filling the assay chamber

8.1 Variation analysis of the electrodes

Increasing the Number of Samples and the Production Run
To validate the capability of the process for clinical trials, a total of 100 samples were investigated from a production run of 1000. Once the production is ramped up the production run is in the order of millions. Hence a larger number of samples should be investigated from a larger production run.

More Accurate optical Technique
Although the scanning technique at 9600 dpi has proved to be a valid technique in picking up the dimensional variation of fingers, it would be beneficial to use more accurate technique in the future to compensate for the reproducibility error of the scanner.

Acceptable Tolerance Range
An acceptable tolerance range should be established for the sensitivity and the performance of the electrodes in terms of the desired slope of 1/impedance Vs conductivity. This essentially would lead to establishing an acceptable tolerance range for the dimensional variation of the electrodes.
8.2 Quality control of the electrodes

Process Parameter Optimization
Work was done by Donoghue and Holmes [29] in early 2011 to optimize the process parameters. This work should be expanded and better understood prior to ramping up the full-scale production.

In-Line Quality Control
An in-line quality control testing method should be developed to monitor the electrodes once the electrode production is ramped up. This method will depend greatly on the electrode production method, electrode pattern, and type of test it will perform.

Automated Process Control
The process has been statistically in control for the run of 1000 electrodes. However once the production is ramped up, it could drift away from the desired target. An automated process control (APC) should be designed to facilitate keeping the process in control.

8.3 Electrode Design and robustness

Optimal Electrode Design
The new manufacturing process is flexible and new designs can easily be explored. Different designs have been explored by Donoghue [22]. The robustness of different designs to defects should be characterized and an optimum electrode design should be established prior to developing a quality control device.

Ageing Study
Before the new electrodes can replace the gold electrodes, an ageing study must be done to ensure that the component remains viable throughout the duration of
its shelf life. The aging study should be done by accelerated ageing in an oven designed for that purpose with samples being tested on a regular basis.

8.4 Ability to capture CD4 cells and filling the assay chamber

Functionalization
The ability to adhere the antibody to the electrode and achieve acceptable cell capture is vital for the performance of the device. The functionalization process must be verified that it is in fact attaching the antibody to the electrode foil without compromising its ability to capture the CD4 cells.

Flow Characteristics
Once the functionalization performance is at an acceptable level, the flow characteristics of the blood and reagents must be observed through the assay chamber. Proper filling of the chamber is necessary for cell capture and the elimination of air bubbles. Design changes may need to be made to the shape of the assay chamber or the location of the antibody on the electrode foil in order to achieve proper filling.
References


[16] Yager Laboratory, Department of Bioengineering, University of Washington, “Microfluidics for Point-of-Care Diagnostics”.


Appendix

Matlab Algorithm

function [locusfingerwidth locusfingerspacing] = main (filename)
%Find the finger width and finger spacing for all fingers
% for each finger for ind=n

% Read image file from directory
m=imread(filename);

% adjust threshold level - and mt is a binary image
mt=(m>155);

% find perimeter pixel in binary image and assign to new image PE
PE=bwperim(mt);

% take single pixel columns along the interdigitated fingers
ind{1}=find(PE(:,400)>0);
.
.
ind{650}= find(PE(:,400)>0);

% Calculate differences between points in pixels
    for i=1:650
        dist=diff(ind{i}(2:end));
    end
% ignore small differences (they are due to dust etc)
    dist=dist(dist>20);

% odddist is less than 70 pixels and Evendist bigger than 70 pixels
%(for the current design where finger width
% is smaller than finger spacing)
    odddist=dist(dist<70);
    evendist=dist(dist>70);

% multiply by 2.65 to convert pixel to micron at 9600dpi res
% 126 is the last finger
    if length(odddist) == 1:126
locusfingerwidth(:,i)=2.65*odddist;
locusfingerspacing(:,i)=2.65*evendist;
else
locusfingerwidth(:,i)=2.65*odddist(70:70);
locusfingerspacing(:,i)=2.65*evendist(70:70);
end
end

% apply to all scanned images in directory

clear all
clc

Files=dir('*.tif')

for i=1:length(Files)
    (Files(i).name)
    % show all locus finger width and locus finger spacing for all images, all fingers,
    % at all ind=n points
    [locusfingerwidth locusfingerspacing]=main (Files(i).name)
    % For each image show average single finger width (126 values per image)
    singlefingerwidth=mean(locusfingerwidth,2);
    % Show the overall finger width of the electrode (1 value per image)
    Overallfingerwidth(i)=mean(singlefingerwidth);
    % Show the standard deviation of the single finger widths in an image
    STDFingerWidth(i)=std(singlefingerwidth);
    % For each finger show single finger spacing (120 values per image)
    singlefingerspacing=mean (locusfingerspacing,2);
    % Show the overall finger spacing of the electrode (1 value per image)
    overallfingerspacing(i)=mean(singlefingerspacing);
    % Show the std of the single finger spacing in an image
    STDFingerSpacing(i)=std (singlefingerspacing);
end