# A Comprehensive Study and Validation of High-Throughput Microscale Electrode Production Using Thermal Transfer Printing Techniques

by

Nikhil Jain
B.S. Mechanical Engineering (2011)
University of California, Berkeley

Submitted to the Department of Mechanical Engineering
in Partial Fulfillment of the Requirements for the Degree of

Master of Engineering in Manufacturing

at the

MASSACHUSETTS INSTITUTE OF TECHNOLOGY

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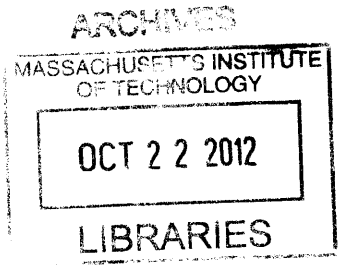




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# Abstract

Adoption of Lab-on-Chip technology, which combines microfluidics with laboratory processes, is steadily increasing within the global health diagnostics field. An important element of this technology is the sensing and measurement capabilities of associated microelectronics. This thesis presents a critical analysis of the use of thermal transfer printing techniques for the manufacture of microscale electrical conductors used for biochemical assays- in this instance, cell lysate spectroscopy. This process affords advantages over traditional techniques such as chemical vapor deposition because of its compatibility with a variety of materials and ability to produce durable electrodes that can perform in the harsh environments that characterize many targeted areas where adequate access to laboratory diagnostic equipment is severely limited.

Commercialization of the process to meet global demand is contingent upon the development of this process at its more rudimentary stages. This study attempts to validate the exponential scaling of this process, including qualification of manufacturing setup, optimization of operational parameters, and detailed analysis of full production runs. The maximization of sensitivity while simultaneously minimizing variation in electrode production presented the primary challenges of this work. It is concluded that a careful balance of process parameters can produce high quality, identical electrodes consistently at the thousands-level production throughput. A variation of only 2.2% in electrode sensitivity revealed that with the determined optimal process settings and in-line quality control, the success even further production scaling to better meet market demand is feasible.

Thesis Supervisor: Dr. Brian W. Anthony
Title: Lecturer, Mechanical Engineering

# Acknowledgements

This success of this work was dependent upon the support of several individuals, and would not have been possible without their considerable assistance.

First, my peers at MIT who also performed their research at Daktari: Tejas Inamdar, Benjamin Judge, and Aabed Saber. They provided academic assistance, emotional relief, as well transportation before and after all of the long days spent in lab.

I would like to thank Dr. Brian Anthony and Professor David Hardt for allowing me to have this unique and exciting experience at MIT, from which I think I have learned more than ever in such a short period of time.

At Daktari, I must thank Bill Rodriguez, Aaron Oppenheimer, Amy Cai, and Josh Anthony amongst others for providing this amazing opportunity. The experience I had while working at Daktari was incredible and I am thoroughly content with my decision to join the team during my studies at MIT. These individuals ensured I had the resources necessary to perform my work, a supportive environment in which to operate, and advice whenever I needed it. I wish them and the company all the best in the future.

One individual at Daktari I have not included in the above list is Rob Etheredge. Rob was my primary mentor and could not have acted any better in this facility. His unparalleled knowledge in this field was crucial to my success. He made himself accessible to my incessant questions and concerns despite the demanding time requirements of his own work. And his humor and charismatic personality made all of the efforts, especially during the most desperate of moments, enjoyable.

Lastly, I would like to thank my parents, Abhi and Karen, and sister, Aarti, for their unwavering support for as long as I have known.

# Table of Contents

# List of Figures

# List of Tables

# Chapter 1 Introduction

This research focuses on development of an electrode production manufacturing processes for Daktari Diagnostics's CD4 counter medical device. Daktari's mission is to create a low-cost system capable of accurately measuring CD4 levels with a portable, and easy to use product which will benefit millions of HIV-infected patients who cannot access expensive diagnostics. Significant innovation in microfluidic manufacturing methods are required for the success of the Daktari CD4, specifically with regard to controlled manipulation and measurement of bio-fluids and assembling microfluidic systems to demanding tolerances at low cost. This paper focuses on optimization and validation of a novel technique for manufacturing electrodes used for measurement for this system, in an attempt to verify production capabilities at high throughput levels. To better understand the project, a brief context and background is necessary.

## 1.1 Company Background

Daktari Diagnostics is a medical diagnostic device company located in Cambridge, Massachusetts. Daktari's mission is to create simple, accurate, and affordable products that address the pressing challenges in global health. The Daktari team of engineers, scientists, physicians, and global health experts is uniquely dedicated to making high-performance products specifically designed for resource-deficient markets. They are committed to delivering critical diagnostic test results to clinicians and patients across the globe. With the slogan, *today there is no place out of reach*, Daktari is developing a point-of-care (POC) CD4 level counter specifically designed for patients with HIV in poorly developed environments (Figure 1-1). [1]

## 1.2 A Global Health Need

### 1.2.1 HIV & AIDS

Since 1981, over 30 million people have died from AIDS. In 2010 alone, it is estimated that 1.8 million died from AIDS and 2.7 million have been infected by HIV. Today, there are more than 35 million people living with HIV and AIDS worldwide, and this value is continually increasing according to the UNAID's *2011 World AIDS Day Report*. [2]

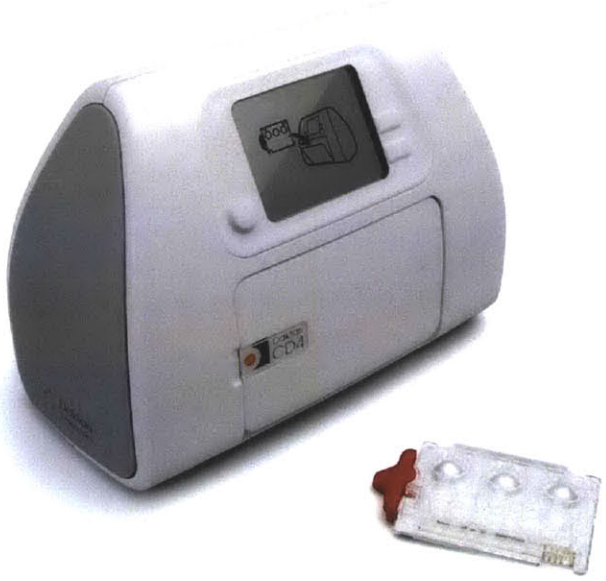

**Figure 1-1: Daktari's first product, the Daktari CD4.**

Human Immunodeficiency Virus (HIV) is a virus that attacks the human body's immune system. Specifically, HIV affects CD4+ cells, (also known as T cells or T-helper cells, a type of white blood cell) which coordinate the immune system to fight diseases by sending signals to activate the body's immune response once foreign bodies like viruses and bacteria have been detected. CD4+ cells are attacked and impaired by HIV, until the body's immune system loses its ability to combat disease, resulting in an increased risk to opportunistic infections, a medical condition known as Acquired Immune Deficiency Syndrome (AIDS). [3]. Healthy, HIV-negative people have CD4+ counts of 600-1200 cells/$mm^3$. [4] A CD4+ count of less than 200 cells/ $mm^3$ signifies as an AIDS diagnosis. [5] The measurement of CD4 is therefore useful to:

- Measure the immune system strength
- Accurately indicate when to start treatment to
    - prevent drug resistance caused by premature medicating
    - reduce the risk of patient drug-related side effects
- Monitor effectiveness of HIV treatment every 3-6 months

Flow cytometers, the modern standard equipment for performing CD4 diagnostic cell counts, are complex machines that cost on average $30,000-$150,000 and take up to 24 hours to provide results. While millions of people now have access to life-saving drugs, 70% of the worldwide HIV infected population does not have access to necessary diagnostics, preventing them from receiving proper treatment. [6]

### 1.2.2 Point-of-Care Development

Across the industry, there exists a tendency to relocate health care services away from centralized hospitals and directly to the patient at the point of care. While this trend provides patients with options in countries with mature infrastructure, point-of-care (POC) technologies may be the most viable way to treat patients in resource-limited settings as well. The quality of healthcare facilities varies widely in developing countries and rural branches commonly have only basic equipment. In fact, access to electricity and running water cannot be guaranteed. In addition to the scarcity of resources, healthcare working may have little training to operate complex equipment, with documented shortages seen in many African countries as poor as one qualified technician per million people. [7] Therefore, there is a need for diagnostics that feature reproducible and accurate results in a short time frame while still being low cost and require little user training. The POC technologies must be rugged, portable, and consume little power in order to be operational in a variable environment for maximum efficacy in the health of users in resource-limited settings. [8]

## 1.3 LOC to POC

Lab-on-a-chip (LOC) technology has been applied to several of the four most common centralized hospital laboratory techniques: blood chemistries, immunoassays, nucleic-acid amplification tests, and, most relevant to CD4 counting, flow cytometry. Promising LOC medical diagnostic systems are being developed to obtain results from complex fluids with efficiency and speed without the need for an

expert operator, attributes that will make POC applications possible for the most resource-limited settings. [9]

### 1.3.1 Microfluidics

Most LOC systems utilize emerging microfluidic technology. The term microfluidics refers to the science and technology of systems that process or manipulate small (< $10^9$L) amounts of fluids and use channels with proportional dimensions of tens to hundreds of micrometers. [10] In microscale fluidic systems, surface tension, energy dissipation, and fluidic resistance significantly contribute to flow dynamics. In particular, the Reynolds number, which compares the effect of the fluid momentum to the effect of its viscosity, can become very low, resulting in a highly laminar flow systems in which fluids do not regularly mix. [11]

### 1.3.2 Advantages

These small scale systems are advantageous for many reasons; the low fluid volumes required mean lower storage volume requirements, lower cost with reduced material usage, and less waste. Also, small channels allow for more rapid testing procedures including heating, mixing, and diffusion as necessary. Smaller size and lower energy requirements allow for high parallel processing for increased throughput, all while permitting the use of disposable systems, a stark contrast to multi-million dollar laboratories in many hospitals. [12] The primary demonstrated advantages of microfluidic devices are outlined as follows:

- Efficient use of reagents helps minimize costs
- Modular design allows flexibility
- Faster results (potential for real time analysis)
- Precise control over small fluid volumes
- Low cost of production

A wide array of microfluidic components such as micro pumps, valves, mixers, and more have been developed. A development that is analogous to the semiconductor industry, has

led to highly integrated LOC systems now applicable to the medically beneficial POC diagnostic devices. The core technology of Daktari is based on recent LOC microfluidic advancements.

### 1.3.3 Industry Note

Despite academic and commercial interest to create microfluidic medical diagnostic devices, few technologies have progressed beyond academic publication to become commercially available products. Micron scale features that permit precise control over solution manipulation conflict with the low cost mass manufacture requirements for resource limited settings. Extremely small assemblies are more susceptible to the physical anomalies that are otherwise ignored in macro application. Tolerance must be scaled accordingly to the micro geometry and practitioners are confronted with uncharacterized phenomena that present new obstacles. Costs much be additionally balanced against by market drivers, the people and governments that are purchasing the POC product. Daktari is at the forefront of developing product in this space, frequently innovating to solve new challenges.

## 1.4 Product Development

Daktari's first product will be Daktari CD4, a CD4 cell counting system designed to be portable and robust enough to be used in settings ranging from a doctor's office to the most remote settings (Figure 1-1). The product's technology overcomes two critical barriers to realization: complex sample preparation and fragile, expensive optical sensors that restrict flow cytometers from POC HIV testing. [1]

### 1.4.1 Cell Chromatography

Daktari's sample preparation technology, known as microfluidic cell chromatography, isolates cells and other particles in a microfluidic sensing chamber. It does not require pipetting, labels, reagents or other complex manual steps typical of other blood testing procedures. For Daktari CD4, this is accomplished by the binding of whole blood, including cells with CD4, to antibody

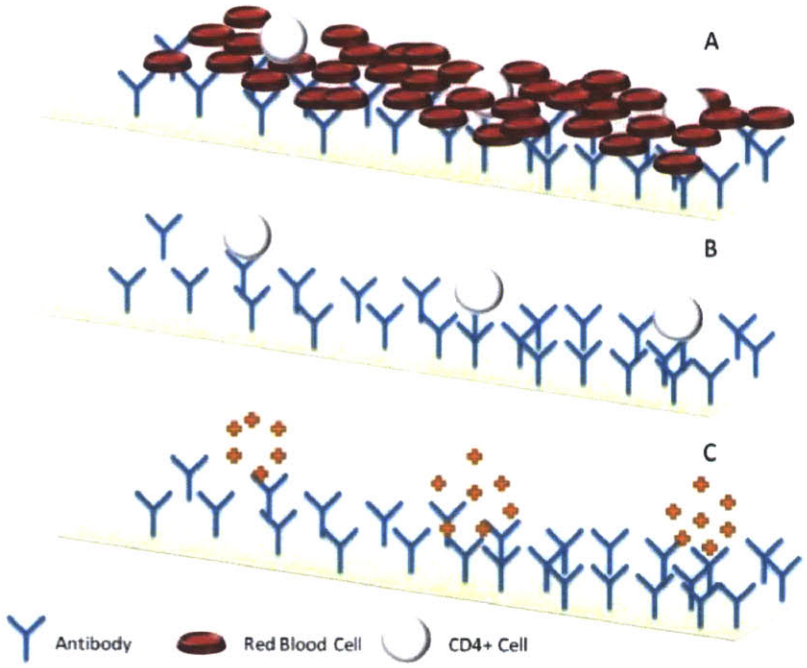


**Figure 1-2: Daktari's patented microfluidic cell chromatogyaphy (A and B) followed by electrochemical sensing (C).**

manufacturing into the channels. Then, a specific shear force is applied to wash loose cells out of the chamber, save for the cells with CD4 protein bound to antibody. This is depicted in Figure 1-2, steps A and B. [13, 14]

### 1.4.2 Electrochemical Sensing

Daktari CD4 also takes advantage of a second innovation, lysate impedance spectroscopy. The system uses simple sensors that count the captured CD4 cells by measuring their internal contents electrically. A handheld instrument interprets the

electrical signal, and reports the CD4 count within minutes [1]. More specifically, the CD4 cells membranes are ruptured, or lysed within a high-impedance solutions (Figure 1-2, step C). The ionic contents of the cell are released into the solution in the channel and the change in impedance is measured. This change in conductivity is proportional to the CD4 count and used to directly determine a simple diagnostic reading. The method proves to be inexpensive and robust, as it is unencumbered by the lenses, cameras, filters or complex options of many diagnostics tests like flow cytometry. [15]

### 1.4.3 Pressing Challenges

Daktari faces a number of challenges in production of their CD4 system. These include:

- Controlling fluid flow at microscale levels
- Fabricating microscale devices on mass producible materials
- Using and storing sensitive solutions in off-the-shelf materials
- Maintaining low costs in the fabrication and integration of electrical components

Creating micro channel pathways and fluidic delivery mechanisms to high geometrical tolerances that allow precise control over the solution is key. Precise parameter control is critical for accurate results for the effective treatment of the end user, however it must be balanced against the costs incurred by the people and governments of developing countries that are purchasing the POC product. Therefore, there is a need for significant manufacturing optimization and/or the development of more capable processes that meet stringent microfluidic product functional requirements, while being amenable to both manufacturing and market needs.

## 1.5 The Masters of Engineering Capstone Project

This document serves as partial fulfillment of the graduation requirements for the Masters of Engineering in Manufacturing program at the Massachusetts Institute of Technology, through the Laboratory for Manufacturing Productivity (LMP), co-advised by Professor David Hardt and Lecturer Brian Anthony. Four students actively worked at Daktari, each focusing on specific challenges in the manufacturing of HIV diagnostic. The author of this thesis, Nikhil Jain, focused on a reducing variability in high-throughput electrode production, with a primary emphasis on system-critical impedance measurements. Benjamin Judge developed a new process for the heat-sealing of microfluidic channels with sensitive geometries [16]. Tejas Inamdar and Aabed Saber focused on characterizing the effect of bonding process parameters on bonding strength and fluid flow response to actuation [17, 18]. Due to similarity in background of work performed at Daktari, the portions of the introductory sections of the four theses were written collaboratively. Together, this work represents an in-depth analysis of a few of the company's most important manufacturing issues at the time of writing.

## 1.6 Problem Statement

In response to Daktari's need to adopt technology developed in laboratory settings to mass-scale production for widespread markets, this thesis focuses on a full-scale validation of the electrode production process. The geometric sensitivity of the electrodes produced using this proprietary metal patterning deposition method is extremely high due to the microscale nature of the analysis. A full characterization of the process from machine installation and material inspection, to production parameter effects for minimizing variability along full-length production runs is detailed and used to refine the process for future quality controlled electrode manufacturing at commercial scale throughputs.

## 1.7 Thesis Overview

This thesis first presents a company profile, product, and describes the problem being addressed. Following is an overview of the specific product at hand and its role and importance in the system. After, an outline of the manufacturing process is presented along with a background of process validation according to Federal Drug Administration (FDA) outlines. A thorough discussion of the steps taken by the author to validate the manufacturing process including testing results of the analysis follows. Lastly, conclusions from the work are detailed and recommendations for specific process improvements as well as production as a whole at Daktari are detailed.

# Chapter 2 Electrode and its Function

## 2.1 Role of Electrodes in CD4 System

Each electrode serves as a critical and sensitive component of Daktari's system. The system is designed to be minimally invasive and mass-producible at cost-effective levels; these constraints have resulted in the product being focused in microfluidic technology and complementary electrical-based measurements. It intricate design makes it delicate to microscopic imperfections and irregular dimensions that can significantly affect its performance. To better understand the importance of the electrode, a summative outline of its role within the overall system follows, along with specifications of its design.

### 2.1.1 The Instrument

The battery-powered instrument is designed for the simplest possible user interaction and portability. The standalone instrument contains the actuators for

driving the reagents and operating 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, and the rest of the electronics needed to display the results and drive the actuators are contained in the instrument. Figure 2-1 shows how the disposable cartridge will go into the unit. Inserting the cartridge is similar to how a cassette goes into a cassette player. All of the tasks are met by the subassemblies listed below.

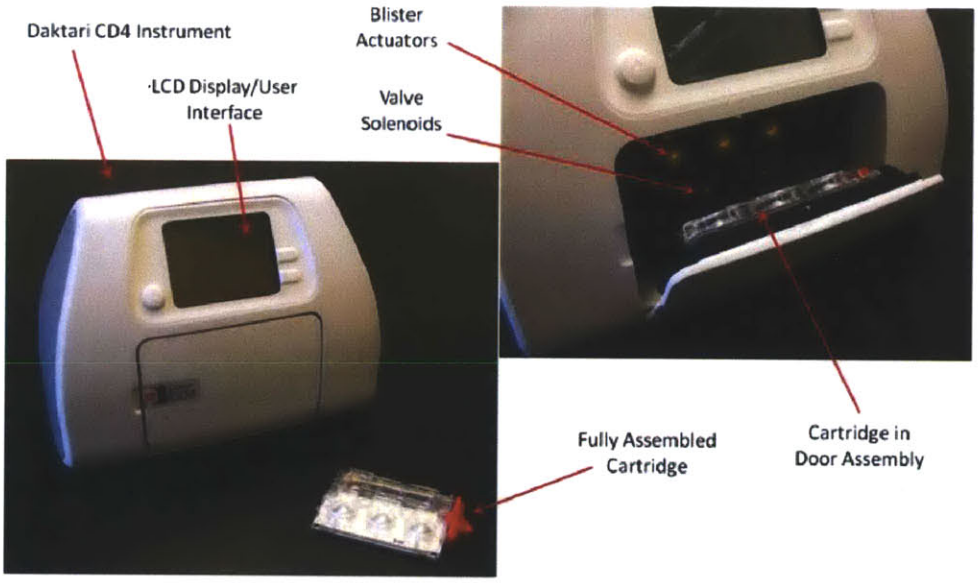


**Figure 2-1: Daktari Instrument [19-21]**

1. Frame - the structural element of the instrument. All of the other subassemblies are located using the frame.
2. Door Subassembly - locates the cartridge, punctures a vent hole and ensures no deformation of 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.

### 2.1.2 The Cartridge

Each test cartridge is consumable for the test, to be immediately disposed of after the assay is performed. 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 2-2 shows a recent iteration of the design. Each cartridge contains the following 7 parts:

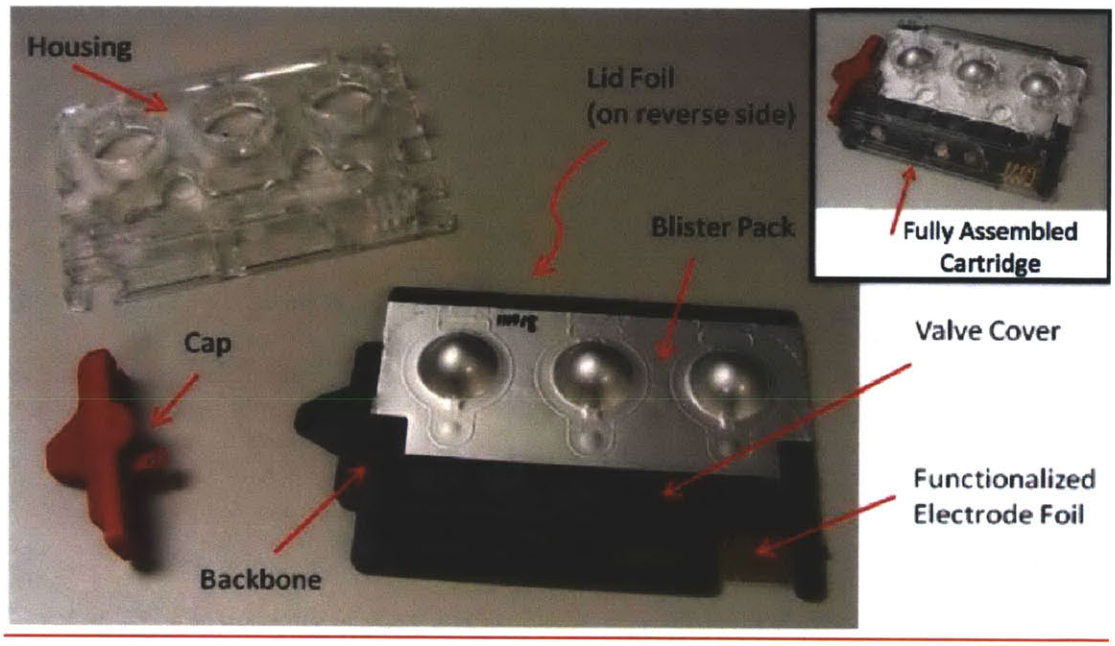


**Figure 2-2: Daktari cartridge with primary components labeled [19-21]**

1. Backbone – an injection molded PMMA (polymethylmethacrylate) 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 – the three semispherical objects in Figure 2-2 contain the three liquid reagents that perform tasks as they flow through the system.
5. Valve cover- a layer of polymer 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 polymer component 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.

## 2.2 Electrode Criticality

In order to make the test more accessible, the Daktari system takes a different approach to CD4+ cell testing by using cell lysate impedance spectroscopy. Flow cytometry is a far more established procedure for HIV diagnostic purposes but access to laboratories with the resources required to perform this operation is extremely limited in the company's market area. Instead, cell lysis involves the placement of antibody along the fluidic channel in which the targeted blood will flow. These antibodies are specifically selected to capture CD4 cells only. After reagents are flowed through the same channel to flush out all other cells and ions, the captured CD4 cells are lysed. More specifically, the environment around the cells is flooded with fluid of low salt concentration which then causes osmosis to occur. The cells ingest water in order to establish equal salt concentration inside and outside until they burst and release their ions. During this process, electrical impedance is measured and the change in impedance after lysing can be linearly correlated to the CD4 cell content of the blood.

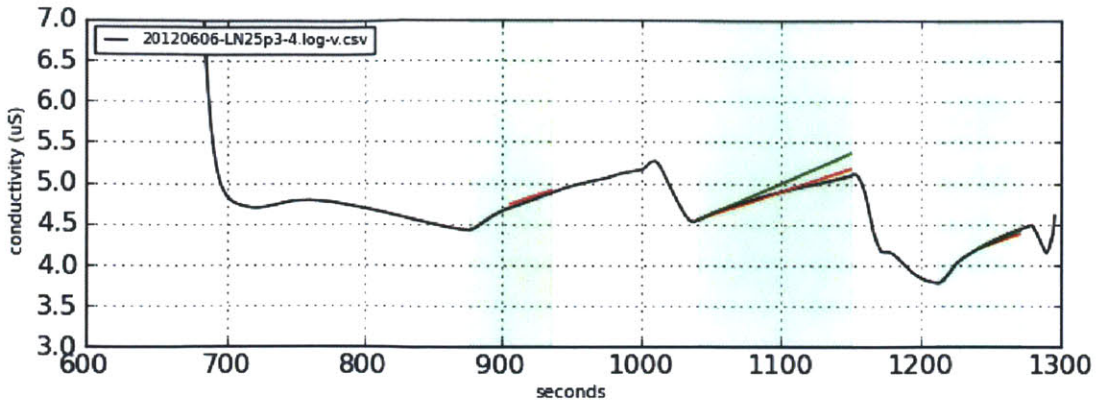


**Figure 2-3: The measured conductivity of a blood sample during a single test. The change in conductivity is directly related to the measured cell count.**

Figure 2-3 depicts the measured conductivity of a card during testing. Conductivity, the inverse of impedance, changes has blood and reagents are flushed through the system. For the purposes of measuring the CD4 count in a known volume of blood, the conductance of the blood is measured at 1200 seconds, before lysing, and then again 1300 seconds, when lysing is complete. This change is proportionally related to the cell count, calculated with a pre-calculated system-static variable.

$$Cell\ Count = Constant * \Delta Conductivy$$

Because this relating constant is fixed the within system, and onboard calibration of individual electrodes is unavailable, it is crucial to minimize the differences between electrodes, or production variation, as well as implement an electrode design that minimizes error reporting.

### 2.2.1 Measuring with Electrodes

Calibration of the electrode involves determining the relationship between known conductive solutions and the corresponding electrical impedance measurements, as sensed by each electrode. This relationship has been shown to be linear in expected conductivity regions from previous unreleased studies at Daktari. Quantification of the slope of this relationship, also referred to as the "sensitivity" of the electrode involves testing. By measuring the impedance seen by the electrode from a set of

solutions of known and controlled conductivity, a line can be "fit" through these points. The slope of this line is then used as the constant in the aforementioned equation, applied in the opposite manner- to relate known impedance measurement to the find the conductivity of the solution.

### 2.2.2 Cell Equivalency and its Relationship to Sensitivity

The slope, or system-wide constant as defined above, is established based on an average of calculated slopes of many production cards. Deviation from this slope will therefore exist in every card, resulting in equivalent cell reading errors. Therefore, minimization of this variation is important.

This error is commonly referred to as cell equivalent error and will henceforth be referred to in this manner. Cell equivalent error is equal to the difference between the calculated cell count based on the unknown particular characteristic slope of the electrode and the corresponding constant, intersystem-wide value. This is represented in the Figure 2-4 below; on a graph relating the 1/impedance, or

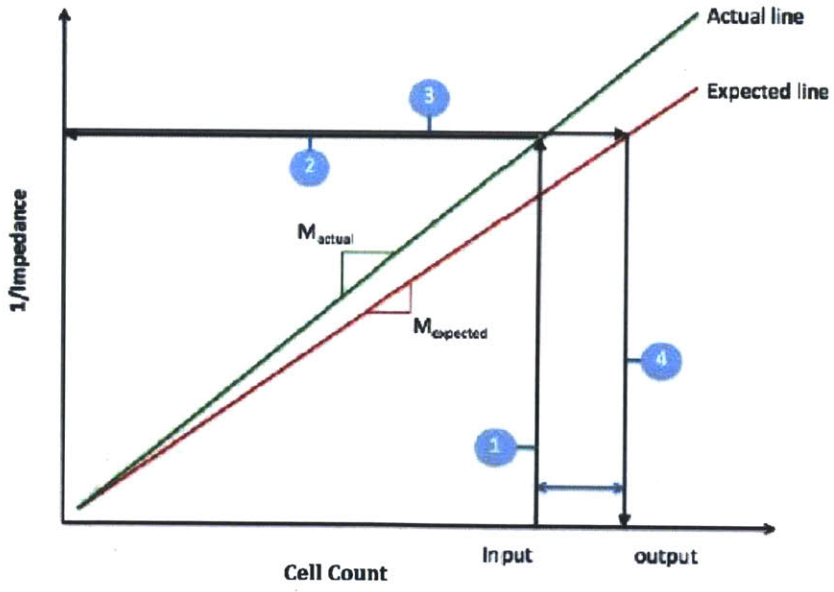


**Figure 2-4: Graphical representation of cell equivalent error. [19-21]**

conductivity, to cell count, the red and green lines represent the actual slope value

of the given electrode and the calculated average, respectively. In sequence, (1) the blood solution closes the electrical circuit, generating (2) a conductivity value recorded by the system. Mathematically, this value is inserted into the preset linear relating equation (3), given by the red line, which then results in a reading (4) of cell count. The error in the measurement in this case is the difference between (1) and (4).

It can be seen that a steeper slope, referred to as an increase in sensitivity, as shown with line set B would reduce the error seen (Figure 2-5). Therefore, the cell equivalent error reported by the system is reduced, and a more accurate CD4 cell concentration reported.

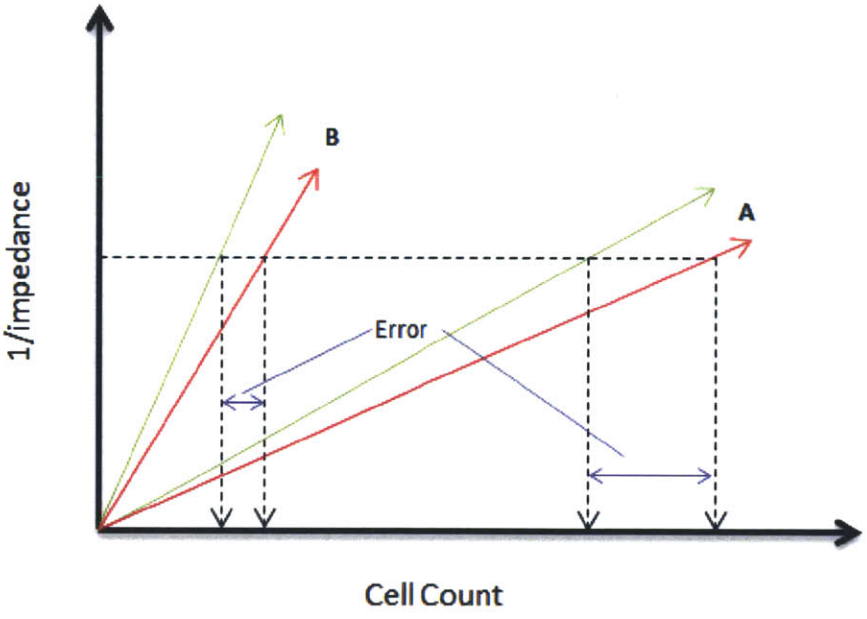


**Figure 2-5: Increasing sensitivity reduces measured cell equivalent error.**

### 2.2.2 Variation in Electrode

While it is important to increase sensitivity to reduce the effect variation has on cell reporting, targeting a reduction in variation between electrodes is another important need. The electrode contains mainly microscale features, designed to specifically to achieve the former- but these same features are difficult to

manufacture, which in turn traditionally causes difficulty in minimizing deviations from the desired outputs. In Figure 2-6, metallic deposit lines, 150 microns in width, on a sample electrode are imaged via interferometer, showing discrepancy in thickness of deposit all along the surface. Therefore, a study of optimizing both these electrode production characteristics is conducted in this study.

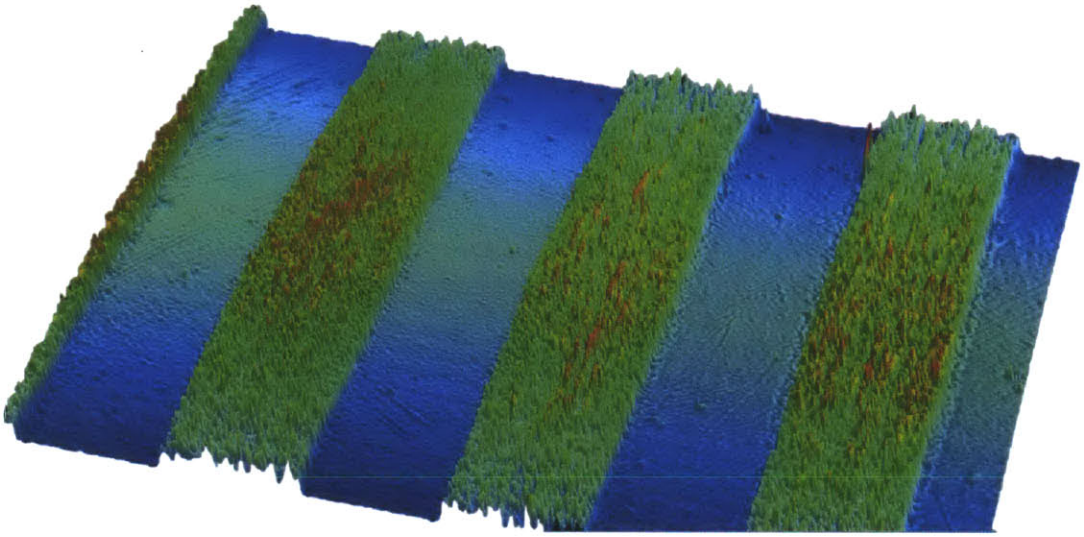

**Figure 2-6: Image of deposit variation in electrode "fingers" using an interferometer.**

## 2.3 Electrode Design

The assay is performed in a microchannel on the card, referred to as the cuvette. A cuvette is typically used to house samples for spectroscopic-related experiments; in Daktari's case, the channel is utilized for cell chromatography and electrochemical sensing. The channel, with a depth of 50 um along its 4mm x 50mm area, is molded into a 2mm thick PMMA card and covered with an electrode.

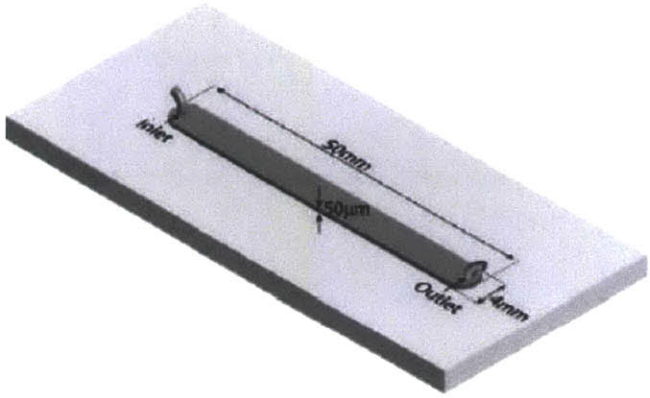


**Figure 2-7: A CAD model of the cuvette on which the electrode sits. [16]**

### 2.3.1 Specifications of the Electrode

While in operation, the electrode measures the impedance between its conductive traces in the assay chamber before and after the cells have been lysed. This measurable drop in electrical resistivity is due to the ions released from burst cells, which assist the movement of electrical current. A measured increase in conductivity is recorded, which can be directly correlated to the number of cells remaining in the chamber.

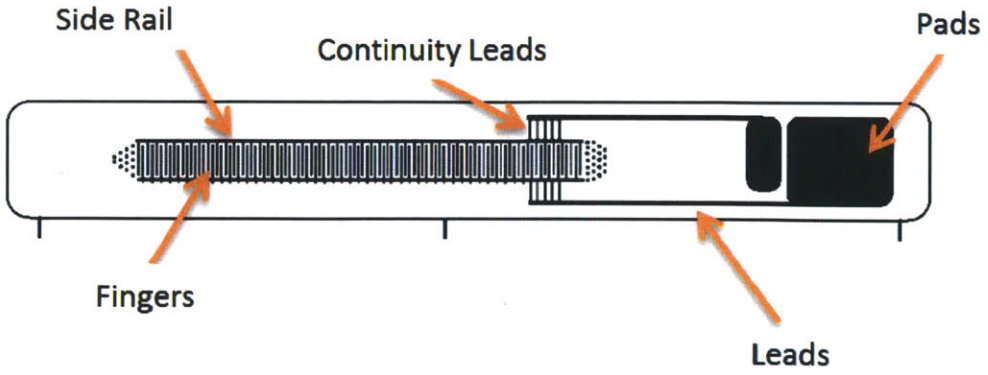


**Figure 2-8: the features of Daktari's interdigitated electrode.**

The electrode itself consists of two relatively large pads, which are touched by contacts built into the instrument. These pads are connected to a series of fingers

which are staggered (see figure 2-8). This design minimizes the separation between the two sides of the electrical circuit, while allowing a large interaction area. Design of this interaction area is the single-most crucial factor in maximizing sensitivity.

### 2.3.2 Key Dimensions of Electrode

The electrode design is based upon two primarily goals: minimize output variation and maximize sensitivity as covered in section 2.2.

This sensitivity corresponds to the characteristic length, marked as the dotted red line in Figure 2-9. This length is the ratio of its length to the gap width; this factor is inversely proportional to the impedance reading.

$$\frac{L}{w_g} \propto \frac{1}{Z} \tag{1}$$

where L represents this characteristic length, $w_g$ the gap width, and Z the measured impedance.

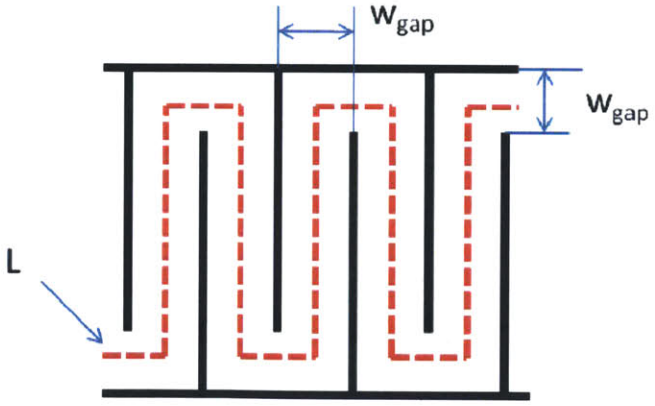


**Figure 2-9: Key characteristic dimensions of the electrode design. [19]**

It is critical to maintain a constant gap width between fingers in order to minimize variability between electrodes; as the distance increases, the measured resistance increases as well and vice versa. Done non-uniformly, error is introduced due to the

instruments universal electrode model. The resistivity of the electrode is determined by:

$$\rho = R * \frac{A}{l} \tag{2}$$

where $\rho$ is the resistivity, R is a characteristic constant of the solution, A represents the cross-sectional area between adjacent fingers, a constant, and l is the distance between them. Therefore, maintaining a constant geometry is vital for uniform resistivity.

It should be noted that while the electrode is *designed* for maximum sensitivity using the aforementioned geometric constraints, sensitivity is also susceptible to manufacturing issues. The quality and consistency of the interdigitated electrodes, both in design and actual production is critical for the Daktari System.

### 2.3.3 Study on Optimizing Sensitivity

A previous study by Donoghue in 2011 [19] focused on identifying design strategies to maximize sensitivity and minimize variability in production runs. In this paper, the aforementioned theoretical relationship between sensitivity and characteristic length and width were verified. Donoghue concluded that increasing the length of the sensing region over the cuvette channel was important for sensitivity purposes.

Longitudinal fingers (along the channel) proved to most effective. However, because of the reduced finger count, the effect of lost connectivity to a single finger would drastically change the characteristics of the electrode. In addition, increased post-production processing reduced cost-effectiveness. [19]

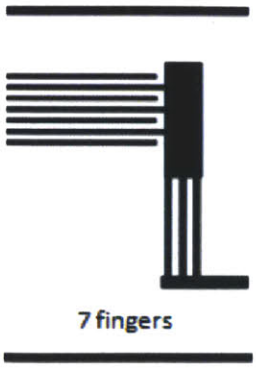


**Figure 2-10: Electrode design with longitudinal fingers. [19]**

### 2.3.4 Studies on Robustness and Variation

During this same period, two simultaneous studies were performed on the robustness of the electrode. [20, 21] This work further proved that electrodes were susceptible to finger loss, as well as susceptible to defects when bent. However, visual scanning and testing of electrodes revealed that the production variation was minimal and not a significant effect on electrical measurements.

## 2.4 Design Iteration

While the previous studies were revealing towards the repeatability of the process, they were conducted on small production levels, on runs of 100s of electrodes. Production verification on a more thorough level is necessary for several reasons.

As Daktari nears clinical trials and beyond, production requirements will increase as well. An exponential increase in throughput, to runs of 1000s of electrodes requires careful study to ensure quality product is manufactured throughout the process.

In addition, after aforementioned-studies, the card was redesigned for better flow performance. This redesign included a shortening of the cuvette. In order to accommodate this while maintaining high sensitivity, the interdigitated electrode

fingers were brought closer together and made thinner. This increased risk of production defects such as breaks and shorts in the conductive trace.

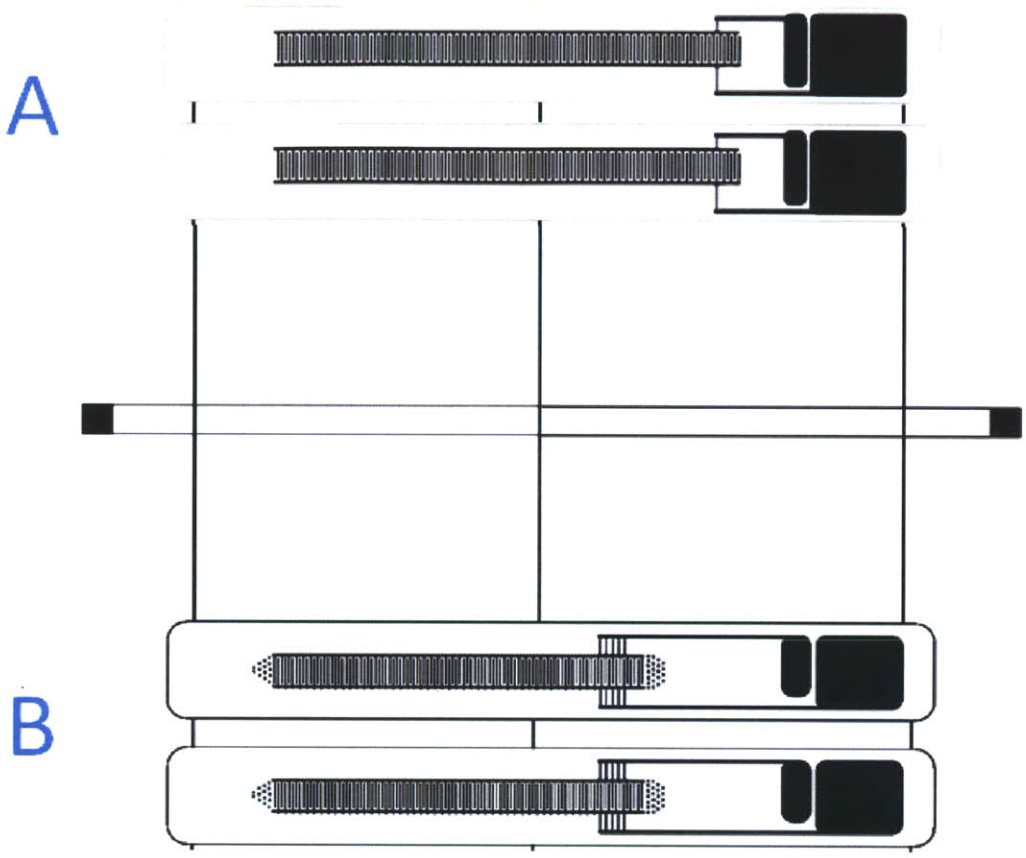


**Figure 2-11: The new "B" electrode design includes a shorter cuvette length, which in turn forced the fingers closer together to maintain equivalent sensitivity.**

Lastly, materials and equipment used in the production process (detailed in Chapter 3) were altered. These actions were made necessary based on material supplier issues. The cumulative effect of all of these process changes warranted the need for a full scale comprehensive validation of the high volume production of electrodes, from supplier to finished product and instrument use.

# Chapter 3 Electrode Manufacturing

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; to ensure accurate assay results, it is critical to understand proponents of variability in production and verify repeatability in electrode manufacturing. In general, many microfluidic devices such as Daktari's are highly sensitive to physical and process anomalies; therefore, the minimization of production variability is critical. Because impedance is directly correlated to CD4 cell count, a thorough statistical analysis of electrode production operating parameters must be performed to minimize errors in diagnostic reports.

Daktari Diagnostics has developed a new production process for constructing the electrodes for this device. This thesis focuses on the validation of this process at

throughput levels more reflective of full-scale production targets. A full scale validation involves three stages: Instrument Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). A brief overview of this specific manufacturing system and process is presented in this chapter. Following this, a detailed validation study, including performed test procedure to fulfill the aforementioned qualification stages, is documented and presented.

## 3.1 Global View of Electrode Production

The electrodes production process begins with sourced stock material and ends only when each individual electrode is secured onto a card. The process is shown in Figure 3-1.

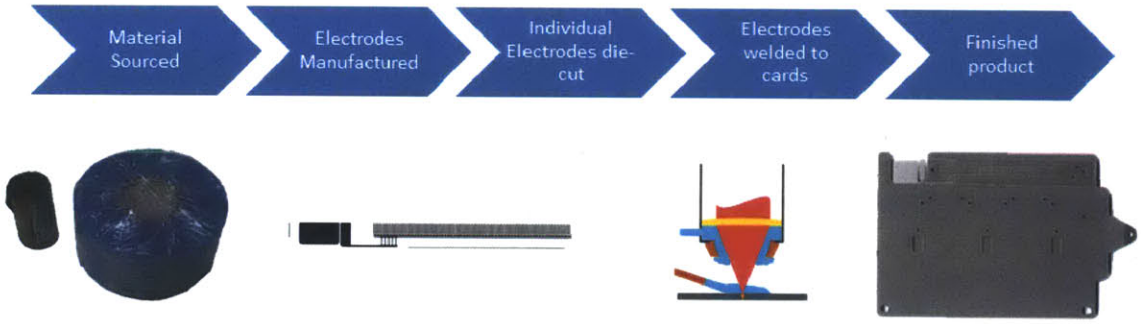


**Figure 3-1: Entire production cycle of an electrode.**

This study primarily focuses on optimization of the 2nd step, or the Daktari controlled part of the process- the production of the electrodes before being attached to cards. However, quantification of the effects of full process is encompassed as well.

## 3.2 1st Generation Manufacturing Process

A 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.

Gold was chosen due to its conductive properties and resistance to corrosion. Poor adhesion between the gold and PMMA caused the electrode to be fragile and susceptible to damage, resulting in broken electrical connections and variability in the performance of the assay. This made the gold electrodes fragile, required delicate handling, and created risk for the assay that relies on the exact finger configuration and continuity to produce repeatable results. [19]

In addition to the risk of damaging the electrodes post-production, the ablation process introduced variability. The ablation process was done by raster (a serial process) or excimer (a parallel process) laser methods. The rastering laser textured the surface of the substrate, while the excimer process caused fragments of ablated gold to redeposit on the surface. Both side effects made the foil difficult to weld to the backbone and affected the properties of the electrode. [19]

The method validated in this work directly generates the electrode pattern onto the PMMA substrate, with no further processing to generate the desired sensor pattern. The process, using developed thermal transfer printing technology, is faster to produce, configurable for flexible design changes, and creates more durable parts than the gold electrodes. Therefore, the electrodes produced by this process are much preferred from a manufacturing and durability standpoint.

## 3.3 Thermal Transfer Printing

Thermal transfer printing involves the coating of a substrate with material from a heated ribbon. Typically, these two materials are spooled under a thermal print head, which heats and deposits the desired material in the desired pattern. First invented in the 1940s by the SATO Corporation, the process is primarily used to make barcode labels, price tags, and tickets. Other processes have been used to accomplish similar tasks but are incapable of meeting the high level specifications of Daktari's product. Photolithography, electron beam lithography and other related methods are well-suited for microelectronics, but face considerable challenges for plastics applications. From a financial perspective, there exist large capital and

operating costs in this complex, and high-refined operation. The process itself can only be used with a limited range of materials, and requires resists, solvents, and other chemicals which can be abrasive on substrates.

Thermal transfer printing provides resolution capabilities that meet Daktari's requirements, while allowing for significant flexibility in material selection. Like ink jet printing used primarily for consumer paper printing, this process is a digital technology allowing each print to be unique; this in turn allows rapid design cycles for printed electronics. It distinguishes itself from this more prevalent technology though in its ability to produce very well-defined sharp lines, with controlled thickness- two important characteristics for the manufacture of electrodes. One of the primary challenges involves the adhesion of the conductive trace to the substrate, though well-developed solutions exist. [22]


**Figure 3-2: Barcodes are typically made using thermal transfer printers due to their high precision and speed.**

### 3.3.1 Thermal Transfer Printer

The main component of the process is a thermal printer (Figure 3-3). It contains built-in spooling components that move media from raw material inserts, under the printing interface and then to a waste or product roll as appropriate. The printer head is a sourced component that is integrated into the printer by the machine designer. It is installed by the nip- near the left edge of the cutaway image (Figure 3-4). The nip is the location where the material is separated after the printing interface into waste and product. [23] Adjustable pressure pods are placed over the printhead which is then translated to the material passing underneath. This process as a whole is controlled via onboard controllers (not shown) which run firmware

which control the pattern to deposit as well as the printing parameters. Settings can be input by the user via a USB-attached computer.

**Figure 3-3: The Zebra R110Xi4 thermal transfer printer.**

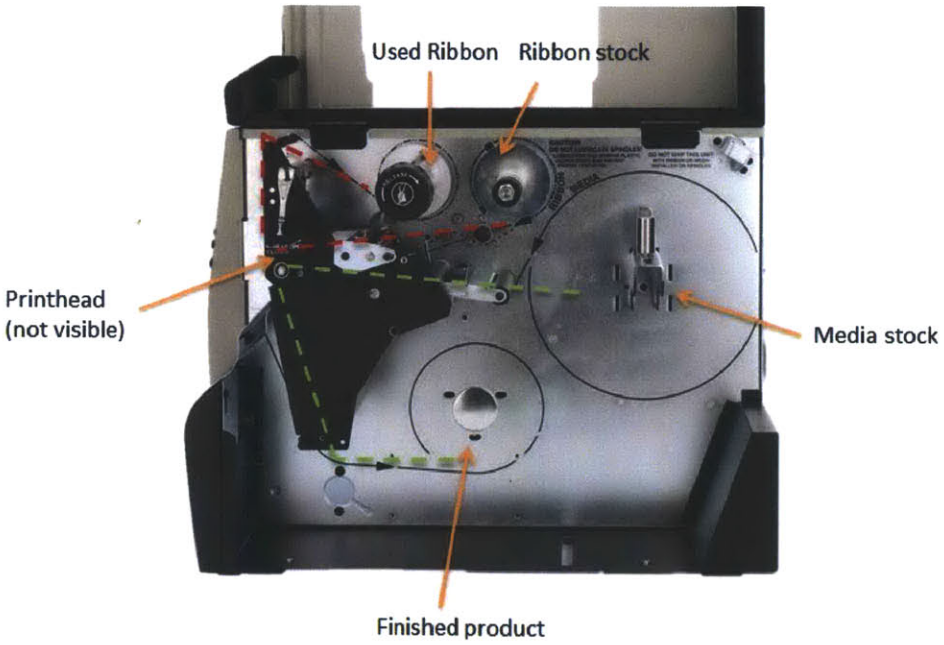


**Figure 3-4: The shell of the printer depicting the spooling interfaces and roll path.**

### 3.3.2 Thermal Printhead

The thermal head consists of an array of heating elements, typically resistors that are arranged as in a matrix of dots. These heatable elements are spaced typically 200 to 600 per inch. Current is passed through the desired elements, quickly heating the surface, which then heats the thermosensitive regions of the ribbons.

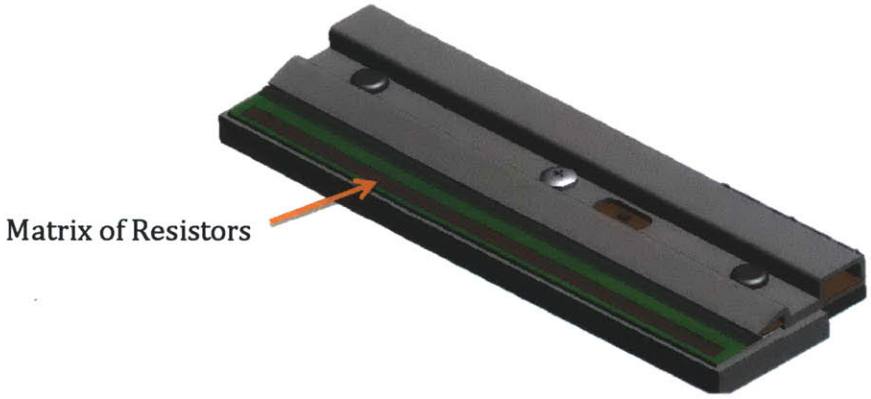


**Figure 3-5: One of many thermal printheads manufactured by Kyocera. [24]**

### 3.3.3 Metallic Ribbon

The thermal transfer ribbon is made with a base substrate of polyester film with a thickness of 3 to 9 microns. One side of the film is coated with a heat resistant low-friction layer for delicate passing under the print head. Opposite this is a series of layers designed to release and attach a consistent deposit on the desired substrate. The ribbon can be engineered to be highly resistive or conductive, and designed for

**Figure 3-6: Sourced Metallograph Conductive Ribbon. [26]**

best adhesion to the desired substrate. [25] The ribbon used in this work, Metallograph Conductive Thermal Transfer Ribbon, is produced by International Imaging Materials, Inc. As depicted in Figure 3-7, the polyester layer has a "release layer", an aluminum layer, and a resin. When heated, the release layer allows the separation for the aluminum and resin. This resin then acts as an adhesive between the aluminum and the substrate. The height of this released material is approximately 0.8 microns, with an aluminum thickness of 2600 Angstroms. [26]

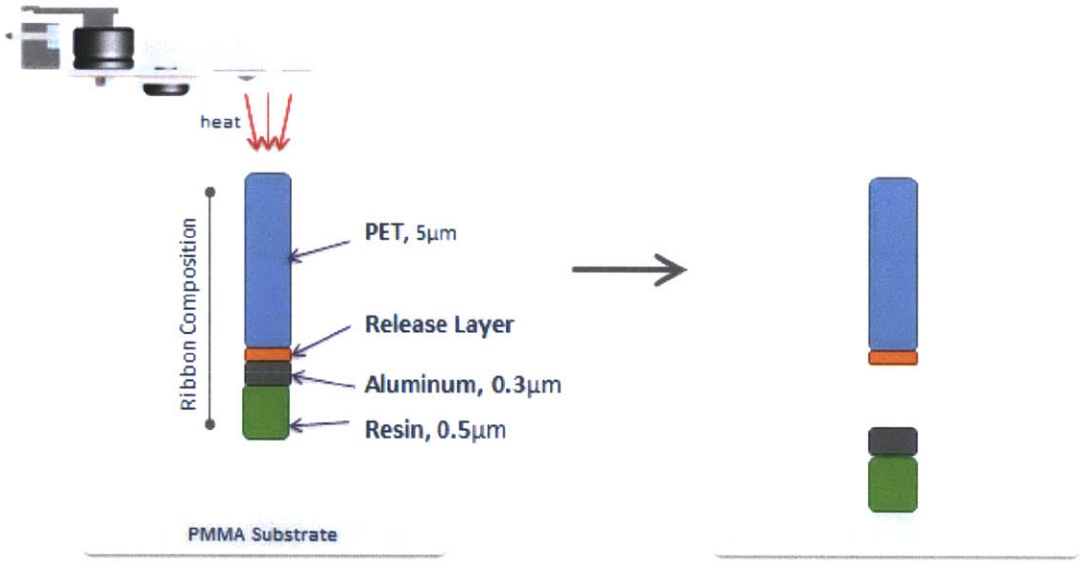


**Figure 3-7: The release process underwent by the ribbon due to heat applied from the thermal head. Note: not to scale.**

### 3.3.4 Printing Process

Spools feed two materials under the print head: one is the aforementioned ribbon, the second is a polymer-based plastic. In this work, a Polymethyl Methacrylate (PMMA) substrate serves as the medium upon which the metal is deposited.

The two sheets pass under the thermal head and then are pulled apart, leaving a used ribbon and the patterned substrate. The transfer happens when the ribbon has been melted enough such that the adhesion to the receiver or substrate is greater

than that to the ribbon carrier film. [25] The makeup of the print is sensitive to the both the geometric parameters involved in the material and roll path, as well as the manner in which the transfer is performed.

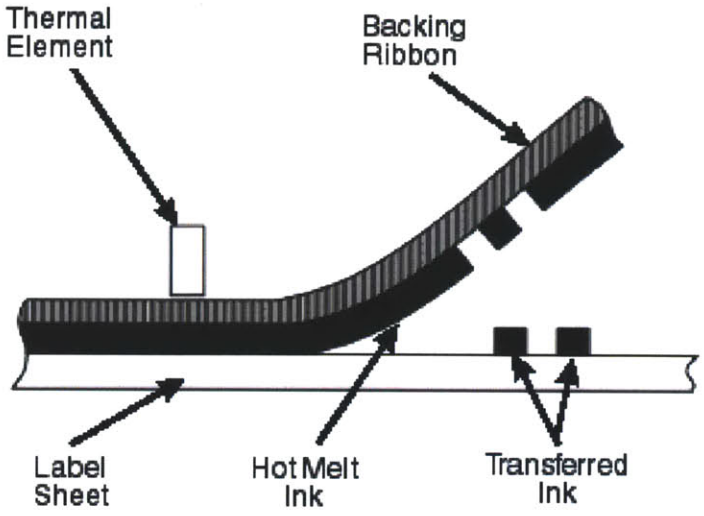


**Figure 3-8: The thermal transfer printing process. [25]**

## 3.4 Previous Validation & Changes

As described in section 2.4, previous work studied the performance of this process in meeting Daktari's specifications. However, after the work was finished, significant changes were made to the process. The thermosensitive ribbon used previously was from an aged production run. However, the vendor attempted to run additional material, the product performed very poorly with an unchanged process. Flash, or excess random metallic deposit, covered the printed electrodes. In response, new ribbon was sourced. Significant changes in the ribbon have been detected between these vendor production runs due to its extreme sensitivity to processing parameters and environment. Therefore, a careful inspection and testing of the new material lot is crucial for qualification.

Several additional changes were made to the process for improved performance that are cannot be detailed in this work due to proprietary reasons. These involved both the setup and use of equipment, accessories, and materials, therefore having significant influence on outgoing product. The cumulative effect of all of these process modifications warranted process revalidation as a whole.

## 3.5 Project Objective

In summary, the microfluidic industry has a need for developing methods of assembly and additional functionality for systems with micron scale features and tolerances. Daktari has a proprietary method for manufacturing electrodes, producing high quality circuitry for electrochemical sensing at a very low cost. As the company nears clinical trials and commercial production, its manufacturing capability and throughput must increase concurrently. Previous validation efforts were performed with minimal sample sizes and prior to significant process modifications. This thesis documents the validation of the electrode production process, on runs of 1000s of electrodes, with the goal of optimizing the process for highest sensitivity and least variability in quality. Each tested electrode undergoes a full cycle of analysis, from incoming inspected raw material, through printing, die cutting at a partner facility, shipment to a welding facility and is then finally returned to Daktari. The electrodes are tested at various points along the process in order to develop a model on the effect of manufacturing production capacity expected when full-scale commercial activity commences.

# Chapter 4 Installation & Operational Qualification Part I

This chapter presents the first part of a validation process, involving the installation and proper operation of equipment, as well operational qualification study. This study analyzes the characteristics of this application of thermal transfer printing technologies for the purposes to detailing the significance and effects of each process parameter. From this, an optimal performance setup can be ascertained and used for future production.

## 4.1 FDA Validation

Process validation involves the collection and evaluation of data, from the early stages of process design until commercial production. Even in commercial production, the continual quality assurance is necessary for acceptable process

control. Validation is performed in order to ensure that all aspects of production including installation, operational parameters, and full length cycles are performed appropriately according to set standards. This is especially relevant to the medical industry, regulated by the Federal Drug Administration, in which it is critical for invasive drugs, diagnostic tests, and operational procedures to perform properly. [27]

To perform such validation, scientific evidence must be collected to ensure the process is consistently delivering quality products. There exist three stages of validation according to the latest guidance protocol published by the FDA:

1. Installation Qualification (IQ): the establishment of evidence that the process equipment is installed and adhering to manufacturer's specification.
2. Operation Qualification (OQ): the establishment of evidence that process control limits result in product that meets established requirements.
3. Performance Qualification (PQ): the establishment of evidence that the process consistently produces product which meets said requirements. [SOP 014 Daktari]

In advance of full-scale commercial production at Daktari Diagnostics, all three of these important stages are covered.

### 4.1.1 The Role of Validation from a Daktari Perspective

Validation is performed for many reasons. Materials supplied for complex manufacturing procedures can contain inconsistencies which will produce inhomogeneous product. Validation offers assurance that the process is reasonably protected against sources of variability that affect production output [28]. In addition, the process itself must be in control throughout operational runs. To adequately and properly understand parameter and variability effects on output, a study must be performed to development process knowledge and quantify relationships, including multivariate interactions between the aforementioned

"inputs". This can help establish ranges of incoming material quality and equipment parameters.

Most importantly, process validation is performed to determine if the process is capable of reproducible commercial manufacturing. This includes the proper design of a manufacturing facility including a qualification of the performance of equipment involved. Subsequently, an OQ and PQ study combines this equipment and utilities qualification with the process itself, and control procedures utilized.

## 4.2 Installation Qualification

### 4.2.1 Overview

In this initial qualification stage, documentation is generated on the installation of the equipment. This includes procedures such as the documentation of equipment in service, calibration of said equipment, protocols for cleaning and cleanliness assessment, and software documentation. The proper installation of the manufacturing setup and safety features are encompassed through this process.

### 4.2.2 Application to Electrode Production Process

The electrode production process is unique due to the manner in which the equipment is set up and materials utilized. The IQ protocol involves documentation of proper installation, operation, and maintenance of the printer itself, accessory rewinders, barcode design software, and modifications necessary in order to produce acceptable conductive traces. [29]

First, while material stock is not in the machine, the maintenance can be performed. This is largely focused on cleaning any deposits, particularly with regards to surfaces that come into contact with the sensitive ribbon. Using isopropanol wipes, the roller bars, print head, and platen must be thoroughly cleaned and dried with wipes. All other areas cleaned using compressed air, focusing on crevices or areas where contaminants typically collect. Debris can be pulled into the printing

interface and quickly ruin batches of outgoing material so it is important to regularly perform this cleaning.

The operation of the machine requires several steps:

- The PMMA substrate must be thread up, and protective liners removed as shown in Figure 4-1. These liners must be fed to an external rewinder which is tensioned to hold material taut.

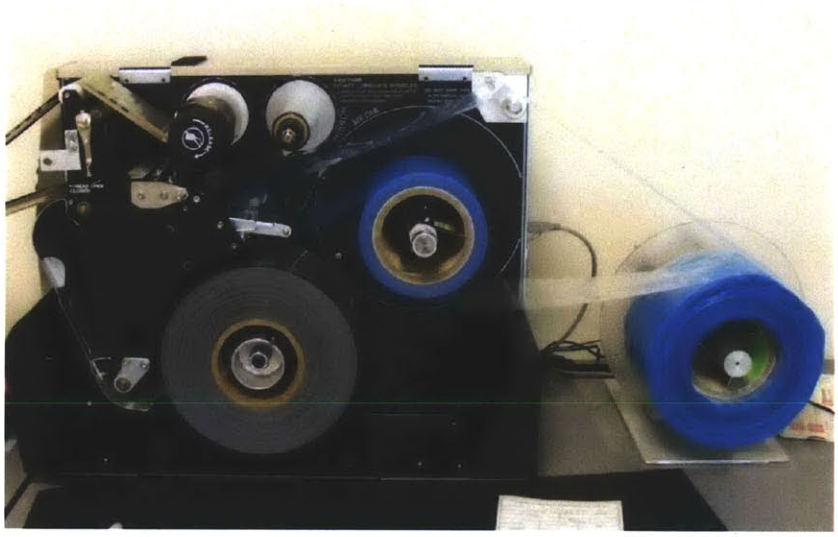

**Figure 4-1: A photo of the machine and accessory equipment set up according to IQ protocol. Note the positioning and flow of equipment and materials, respectively.**

- The ribbon must be thread up without wrinkling. Periodic removal of waste is necessary.
- When the machine is turned out, any error codes displayed on the embedded screen must be cleared.
- Embedded sensors positioned at various points in the equipment (not visible in Figure 4-1) must be overridden using covers
- The finished product must be rewound onto a core installed upon an internal rewinder.

Calibration of the equipment is performed by observing printed standard patterns using a microscope. Careful attention is focused on verifying integrity in critical dimensions.

## 4.3 Operation Qualification- Part I

### 4.3.1 Overview

Operation qualification, also known as process performance qualification, involves the collection of data and objective evidence to establish process parameters and control limits that result in the product meeting specifications. [30] In this stage, the process parameters are challenged to assess the limits and expected performance of the product under various production scenarios. By collecting this data, a model can be developed of the process and continual controlled adjustments can be made while maintaining control. At this stage, the robustness of the process can be assessed and operational ranges established. [31]

### 4.3.2 Application to Process

Because of its novelty, the electrode production process at Daktari has never been fully studied and modeled at high through-puts. Preliminary studies indicated that the process is extremely sensitive to its operating parameters and without proper process specifications, significant variation from run to run has been exhibited. In order to optimize the process for error and variability minimization, a full study is performed in this work to explore the manufacturing design space and verify the robustness of the process as a whole from incoming raw material through production until the final card is secured.

### 4.3.3 Ribbon Material Selection

The first step involves the selection of optimal material, with the goal of maximizing sensitivity and reducing variability. As specified in Section 3.3.3, the ribbon consists

of a number of thin layers, which serve to cleanly and accurately deposit the desired pattern. Procedure and results of this study are excluded from publication though, due to proprietary content.

From this testing, a conclusion was made to utilize ribbon with a $2600^{0}$ aluminum layer for best performance.

### 4.3.4 Production Process Parameters

In order to develop a model of the variability in electrode production, the manufacturing variables need to be identified. Some of the parameters are uncontrollable and grouped into noise factors in the statistical analysis of this study. However, it is important to identify all sources.

**Ribbon & Substrate**

Optimal raw stock was selected for the purposes of this study. The lot of material was unchanged throughout experimentation.

**Print Speed**

The print speed can be mandated through the on-board printer software. This significantly affects the throughput of the manufacturing process as well as the thermal buildup at the print head. The capable range is from 1 inch/second to 6 inches/second.

**Load**

The pressure applied on the printhead is performed by spring-loaded pods. Each spring can be adjusted independently to best match the print requirements. Therefore, production runs can be performed with varying numbers of pressures points, 1 to 3, and pressure values, 35N to 50N.

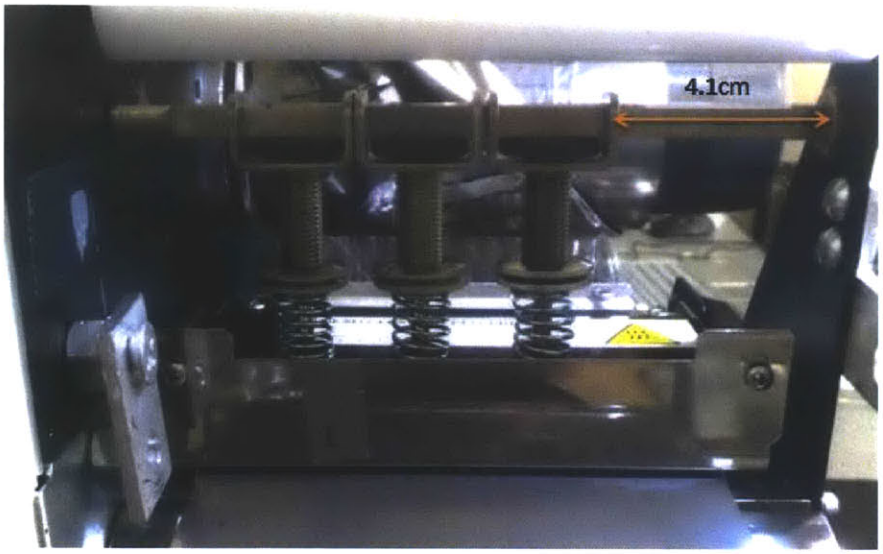


**Figure 4-2: Location of podheads relative to printing surface.**

In this study, all three pressure heads remained the same position focused over the more delicate sections of the electrode. It was seen in preliminary testing that increasing the area on which the load is focused leads to better results. In this study, the 3 pressure heads were positioned over the sensitive electrode fingers as depicted in Figure 4-2.

## Energy Input

The energy input to the print head, indicative of the heat at the head-ribbon interface, can be modified via a non-linear dimensionless parameter from 1 to 30. This is set by the firmware developed by the sourced company. The energy is sent to the resistors that line the critical section of the printhead and interact with the release layer of the ribbon. The head operates around this nominal value while processing the desired pattern.

**Roll Path**

The tensioning in the ribbon and angle of separation between the two materials after printing are crucial as seen through preliminary studies. Tension significantly affects the print quality though no comprehensive study has been performed on the area to date. From a preliminary study, it was hypothesized high tension can lead to early separation between substrate and ribbon and therefore, poor layer deposit on the substrate PMMA. On the contrary, low tension can cause undesirable flash metallic deposits, due to the longer contact time between the two spooled materials, which can short tight tolerance electric circuitry. However, a full study of this has yet to be undertaken.

**Electrode Design**

In order to perform the most relevant qualification for Daktari purposes, the current electrode design at time of writing was used for testing. As mentioned in Chapter 2, numerous requirements direct electrode design. These include channel size, sensitivity including characteristic length and gap width between fingers, as well as robustness against continuity breaks.

### 4.3.5 Design of Experiment

This study focused on three parameters set by the instrument itself: the speed, the energy, and the pressure. For pressure, each of the pod heads were set at the same level for experimental simplification.

The goal of this study is to use these three process parameters to develop a model of electrode production variability in order to establish control limits to be documented in a full scale process validation. This includes a documentation of the

fail rate as well as the impedance variation at each setting. Failure is defined as microscopically visible shorting within the circuits, as well as continuity breaks and categorized as appropriate. It is desired that the results of this study reveal the capabilities of the system as well as the effects of the parameters on output and that of post-production processing.

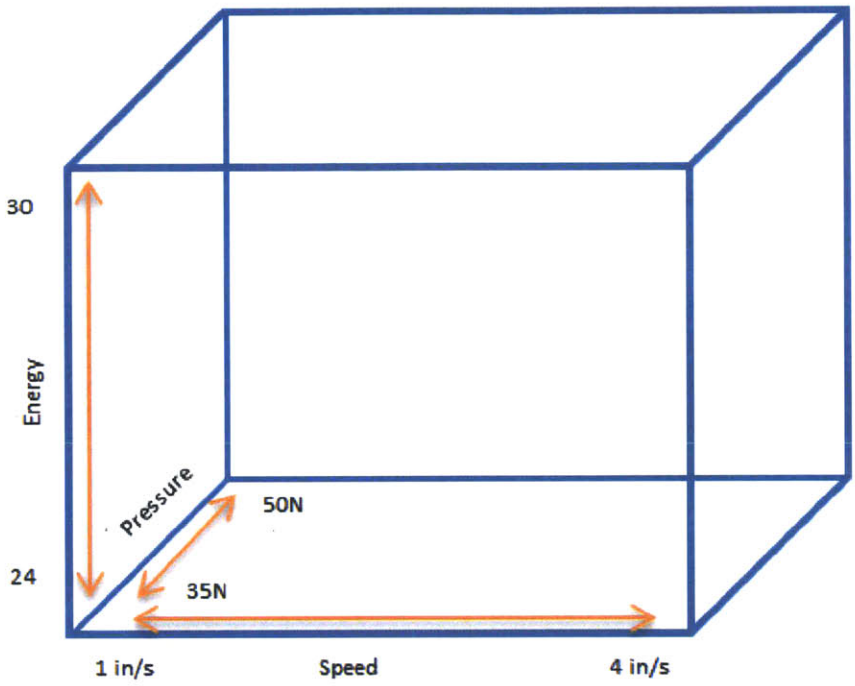


**Figure 4-3: A visual representation design space.**

As detailed in Section 3.1, the electrodes require significant processing outside of the thermal transfer roll-to-roll printing before production is complete. This study will document the effects of each step in the process. The QC positions are shown in the dark shaded regions below in Figure 4-4.

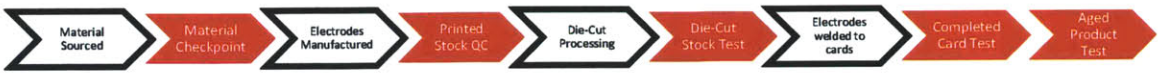


**Figure 4-4: Quality control test points (dark shading) in full-scale electrode production. Processing stages are denoted in blue.**

### 4.3.6 Visual QC

After samples are produced on a large roll and separated according to each set of production parameters, commonly referred to as "pull points", quick visual examination reveals parameter combinations unsuitable for production at the fundamental level. Points at which this was not obvious were approved for the next processing step, die-cutting.

### 4.3.7 Die Cutting

After printing, the roll of electrodes is moved to a die cutter where each electrode is cut from the roll to fit the recessed slot on the card. Typically, the roll is sent to a die cutting vendor where a complex vision system using cameras to detect printed interstitials is used for high speed throughout. Instead, the roll was cut manually on-site to minimize turnover time and because electrodes were selected at intervals for testing, rather than an entire run.

The die cutting process begins with the PMMA with aluminum deposit lined up on top of a thin polyester sheet. This sheet is meant to protect the tool from contacting the base plate, causing denting, chipping, and other detrimental wear. Prior to using, the press is calibrated to finish its throw when just making light impact on the polyester sheet. Therefore, when in operation, the cutting tool penetrates though the PMMA sheet, before coming to a hard stop as it contacts the polyester. This process was performed for 10 electrodes at each point.

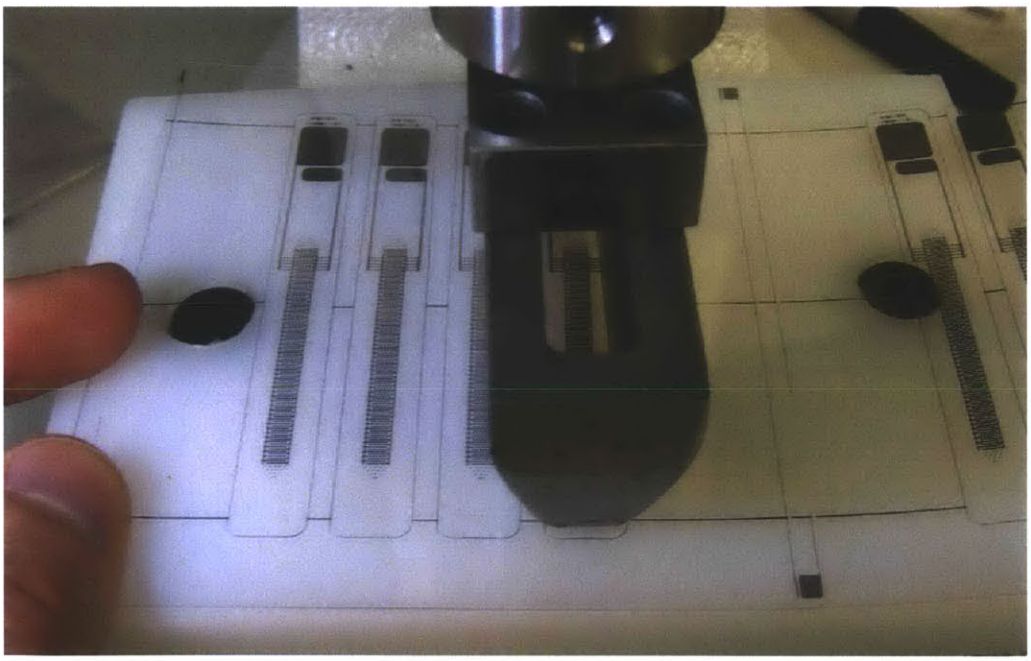

**Figure 4-5: The die-cutting tool being positioned over a series of electrodes.**

**Figure 4-6: Series of electrodes before die cutting on left, single cut electrodes on right.**

### 4.3.8 Dip Testing

#### 4.3.8.1 Cleaning

After the electrodes are cut to size, they are cleaned to remove all residues both from alien objects and from loose ribbon deposit. Antiseptic wipes with 70% isopropyl alcohol are lightly rubbed against the surface of the fingers. This has process has been proven not to damage the desired electrode pattern while removing residue or undesirable foreign objects. Kimtech Kimwipes are then used to dry the electrode, and remove loosened material off the surface.

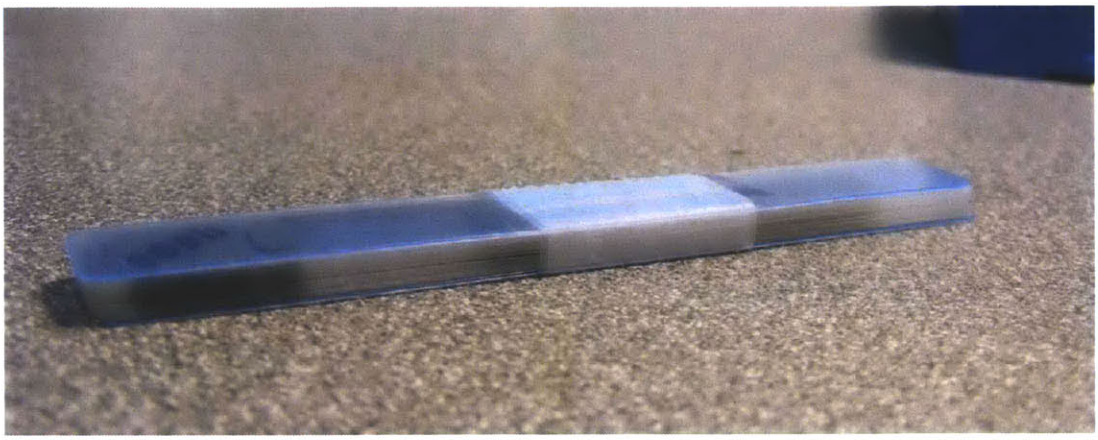

**Figure 4-7: A set of cut and cleaned electrodes.**

### 4.3.8.2 Conductivity Solutions

In order to test the electrodes for electrical continuity and circuit impedance, the electrodes are placed in conductivity standard solutions. These standards, from Omega, are filled with trace amounts of potassium chloride to meet the conductance level desired.


**Figure 4-8: Omega conductivity solution used to prepare solution standards for electrode calibration.**

#### 4.3.8.3 Dip Test Setup

While the shelf life of the conductivity solutions is 1 year, when placed in test tubes that can leach or exposed to air, the conductivity values can drift. To compensate for this, the solution is cycled through a flow cell monitoring system which continuously reads the solution characteristics in real-time. The flow cell is connected to a Pharmacia Biotech Conductivity Monitor with a voltage output measured with a Hewlett Packard 974A multimeter.

The electrode is placed in a plastic test tube with several ports: A slot at the top is cut wide enough for the easy insertion and removal of the electrode. On the sides of the chamber, one entrance port is cut to push the liquid from the standard solution, through the flow cell and into a dipping tube. The other port serves as the exit in order to maintain a consistent fill level. This tube is connected back to the system so that the solution is recycled throughout the dipping process.

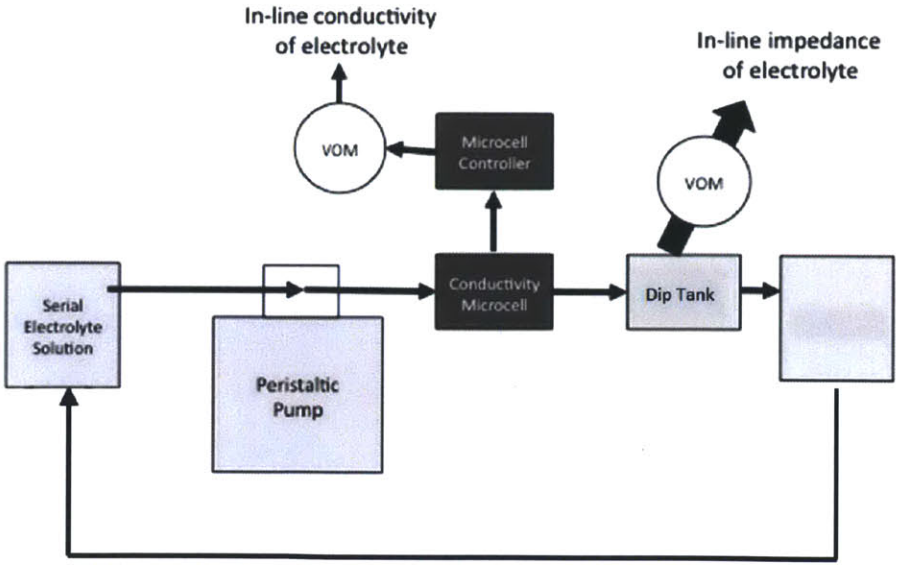


**Figure 4-9: Graphical representation of dip test setup. [32]**

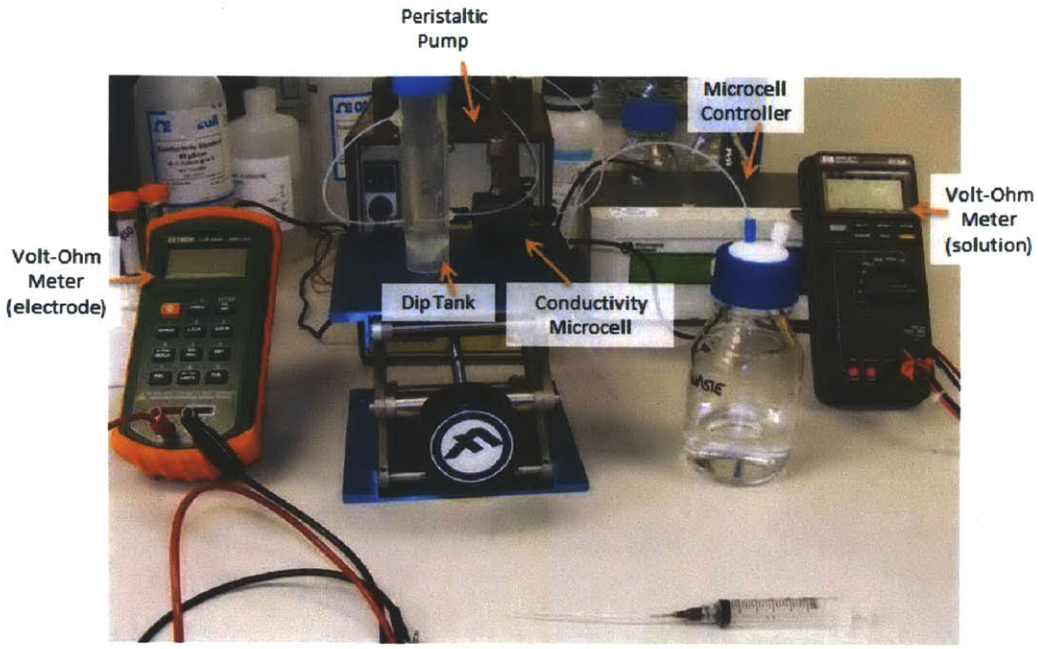


**Figure 4-10: Electrode dip testing setup.**

The fluid is pumped in Polytetrafluoroethylene (PTFE) tubing through the system using a Gilson minipuls 2 peristaltic pump that can be set to dimensionless speeds ranging from 1 to 999 on an internal vernier scale. It was proven in previous studies that flowrate has an insignificant effect on impedance measurements. Therefore, for this test, the speed was set to 650, calibrated to be steady at 585 ul/min volume displacement solely to ensure adequate solution homogeneity.

#### 4.3.8.4 Procedure

Prior to dipping electrodes, calibration of the flow cell occurred. As the ambient temperature and humidity vary, the flow cell can be biased without obvious indication. Therefore, calibration of the system is accomplished by running fresh calibrated standard solution at multiple points to derive the linear relationship

between real-time solution conductivity and microsiemen level. Solutions used to find slope were deionized water, 4.5, 9, and 22.5 µS/cm solutions.

**Table 4-1: Calibration table for flowcell and conductivity monitor**

| Solution | Measured Volts |
|---|---|
| 0 µS (DI Water) | -0,54 mV |
| 4.5 µS | 2.98 mV |
| 9.0 µS | 8.79 mV |
| 22.5 µS | 23.26 mV |
| Relationship: (µS) = 0.925*(mV)+1 | |

Once calibration is accomplished, the dipping tube is filled with 10 µS/cm solution due to its close proximity to expected blood values. The pads of the electrodes are then connected to a separate multimeter, in this case an Extech LCR meter model number 380193. The electrode is then carefully dipped into the solution, making sure to avoid touching the alligator clips from the meter to the solution. Allowing time to reach steady state, a reading is taken and current solution conductivity is noted. This procedure was performed on each of the 10 electrodes at each pull point.

## 4.4 OQ Part I Results

The first part of the Operational Qualification revealed the significance of each parameter during printing. The pull points were tested in random order to in order to reduce the effect of drift over time in the testing procedure. Due to a number of pull point failures, primarily located at parameter extremes, at which visual QC showed obvious print quality issues, a full DOE design analysis was not possible. However, the data still indicated clear parameter influences and interactions.

The results are as follows:

**Table 4-2: Results of Electrode Dip Testing with highlighted rows indicating failure rates above specification limits. Sample size is 10 for each pull point.**

| Pressure | Speed | Energy | Failure (%) | CV (%) of Sensitivity | Sensitivity (Kohms) |
|---|---|---|---|---|---|
| 35 | 2 | 26 | 9.09 | 2.76 | 5.49 |
| 35 | 2 | 28 | 0 | 1.37 | 5.348 |
| 35 | 2 | 30 | 28.57 | 1.75 | 4.799 |
| 40 | 1 | 24 | 37.5 | 2.68 | 6.007 |
| 40 | 1 | 26 | 23.08 | 4.69 | 6.81 |
| 40 | 2 | 24 | 41.18 | 2.66 | 7.26 |
| 40 | 2 | 26 | 41.18 | 1.47 | 4.839 |
| 40 | 2 | 28 | 41.18 | 0.95 | 4.738 |
| 40 | 2 | 30 | 37.5 | 1.83 | 4.633 |
| 40 | 3 | 28 | 16.67 | 3.34 | 5.933 |
| 40 | 3 | 30 | 0 | 1.87 | 5.33 |
| 40 | 4 | 30 | 52.38 | 1.45 | 5.859 |
| 45 | 1 | 24 | 9.09 | 1.88 | 6.785 |
| 45 | 1 | 26 | 9.09 | 2.4 | 6.384 |
| 45 | 1 | 28 | 50 | 1.95 | 5.633 |
| 45 | 2 | 24 | 0 | 2.29 | 6.258 |
| 45 | 2 | 26 | 9.09 | 1.64 | 6.154 |
| 45 | 2 | 28 | 9.09 | 2.42 | 5.83 |
| 45 | 2 | 30 | 23.08 | 3.07 | 5.735 |
| 45 | 3 | 28 | 9.09 | 2.75 | 7.548 |
| 45 | 3 | 30 | 52.38 | 2.21 | 6.739 |
| 45 | 4 | 30 | 9.09 | 2.63 | 7.371 |
| 50 | 1 | 26 | 0 | 2.27 | 6.322 |
| 50 | 1 | 28 | 23.08 | 3.01 | 5.965 |
| 50 | 2 | 24 | 0 | 1.85 | 6.607 |
| 50 | 2 | 26 | 0 | 2.64 | 5.865 |
| 50 | 2 | 28 | 23.08 | 2.95 | 5.427 |
| 50 | 2 | 30 | 33.33 | 2.93 | 5.198 |
| 50 | 3 | 28 | 16.67 | 3.05 | 6.906 |
| 50 | 3 | 30 | 23.08 | 2 | 6.782 |
| 50 | 4 | 30 | 16.67 | 2.88 | 6.935 |

The rows highlighted in dark shading represent pull points at which electrical failure, most commonly shorting between fingers or breaks along the leaders and

rails, in greater than 30% of samples. These parameter settings were noted and immediately removed from further processing due to the obvious reduction in cost effectiveness.

This data collection revealed the effect of pressure on performance. As seen in Figure 4-11, showing a contour plot of the combination of speed and pressure in relationship to performance variability, it can be seen that one or more of these factors has a limited influence due to the homogeneity of the image. With lower relative pressure, values below 42 N, pressure begins to have an effect; in this case extreme speeds, both on the high and low side, can cause unwarranted effects. However, a balance of speed with lower pressure initially appears to improve performance. This analysis overlooks the failure rate indicated in the Table 4-2 above. At a pressure of 40 N, and a middle speed setting of 2 inches per second, the failure rate jumps above 40%. This is unacceptable for logistical purposes. Therefore, the primary conclusion from this chart indicates a need for pressure greater than 42 N in order to avoid making the system more sensitive to other

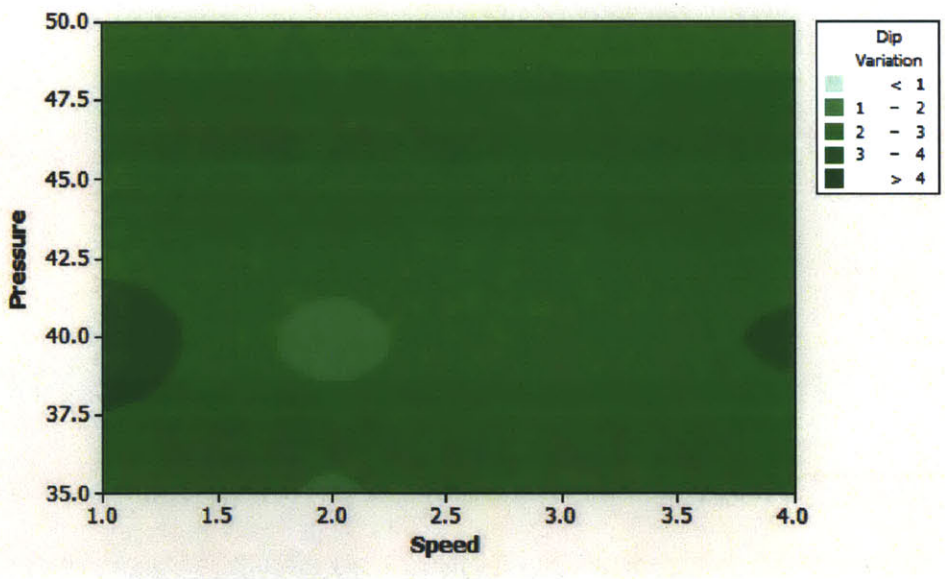


**Figure 4-11: A contour map revealing the effect of pressure and speed on impedance variation. The lighter colors reveal better performing regions, while the darker indicate the opposite.**

parameters.

On the plot of pressure versus energy in Figure 4-12, a similar trend is apparent. Operation with the combination of low pressure and high energy results in poor quality. Again, most of the figure is washed at one level, signifying less of an influence of one or more of the factors. Based on this data, it can be hypothesized that the individual influence of the factors is minor when analyzing variation.

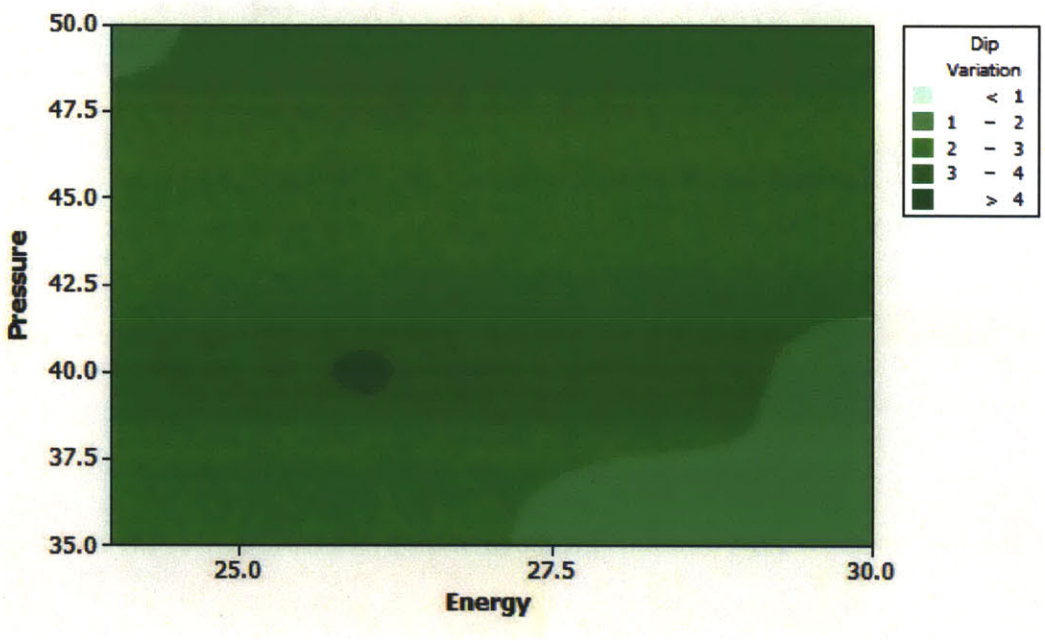


**Figure 4-12: A contour map depicting the effect of energy and pressure on impedance variation. Much like the previous figure, it shows that CV is not heavily influenced by these two factors individually.**

However, the interaction of energy and speed provides a much clearer picture. Figure 4-13 shows a gradual drop in CV with speeds around 2 inches per second, with energy input at levels between 26 and 28. As speed is increased, the print head is unable to produce as accurate product, especially with lower energy.

Counteracting an increase in speed with additional resistive heat at the interface helps but due to equipment limitations, is not possible.

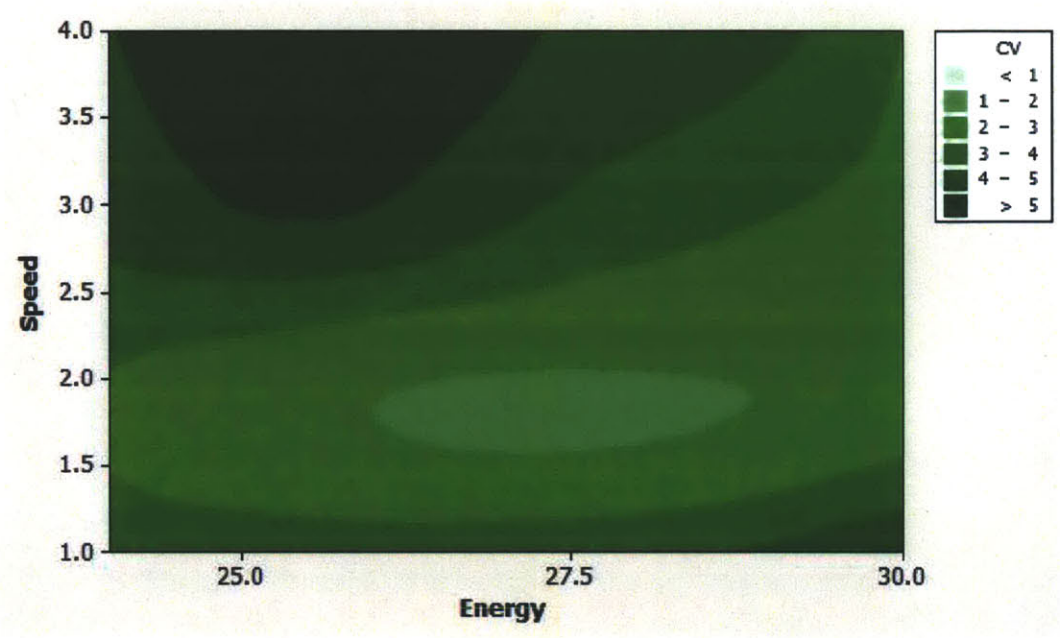


**Figure 4-13: A contour map showing the effect of speed and energy on the response CV. This image shows the optimal area surrounded by continually increasing variation.**

From this information, the highest performing pull points, based failure rate and performance can be ascertained. These have been tabulated in Table 4-3.

**Table 4-3: A direct comparison of the top performing electrode production pull points.**

| Pressure | Speed | Energy | Failure (%) | CV (%) | Sensitivity (Kohms) |
|---|---|---|---|---|---|
| 45 | 2 | 26 | 9.09 | 1.64 | 6.154 |
| 45 | 2 | 28 | 9.09 | 2.42 | 5.830 |
| 50 | 2 | 26 | 0 | 2.64 | 5.865 |
| 50 | 2 | 28 | 23.08 | 2.95 | 5.427 |

This data indicates that the operating parameters of 45N, 2 in/s, and 26 in energy produce product with optimal balance between high electrode sensitivity and low variability, while maintaining a low failure rate. This balance of parameters accommodates adequate thermal adjustment as the ribbon is heating and cooled while preventing overstressing of the materials as they pass under the thermal head. While it is tempting to simply use this analysis to justify all production in the future, it is important to realize that the electrode production process involves significant post-printing processing. A full validation requires full process analysis. The electrodes require welding and testing as full manufactured cards in order to provide a complete assessment.

# Chapter 5 Operational Qualification Part II

As shown previously, the electrodes, after being die-cut, are welded to cards by a Daktari contractor. This process involves transatlantic shipment and handling in addition to the energy-intensive laser welding process. In order to understand the effect of the electrode production process on finished product, it is necessary to subject the samples studied in the previous part of the OQ to the further steps required to more accurately model product as expected after commercial release. Results and conclusions from the first part of the OQ are not meaningless; general trends are indicated and better performing electrodes can be identified. This part of the process is controllable as an in-house production process. However, electrodes that performed well at this mid-point QC stage could be susceptible to damage from further processes. An assessment of this is useful for future production planning. Therefore, the second part of the OQ validation, involving the entire process of card attachment, is performed and documented below. [33]

## 5.1 Welding

After printing, the electrodes are packaged and shipped to a contractor for welding. The bare cards, which are also manufactured at this facility, are fitted with an electrode over the cuvette. The laser weld is placed in two positions; one directly around the cuvette as indicated by the red line in Figure 5-1 and one outside of the printed region. This second one does not come in contact with metallic deposit as is the case with the first one, and therefore an assumption has been made that it has little to no effect of electrode chemical analysis. After this process is complete, the completed cards are returned to Daktari for further testing.

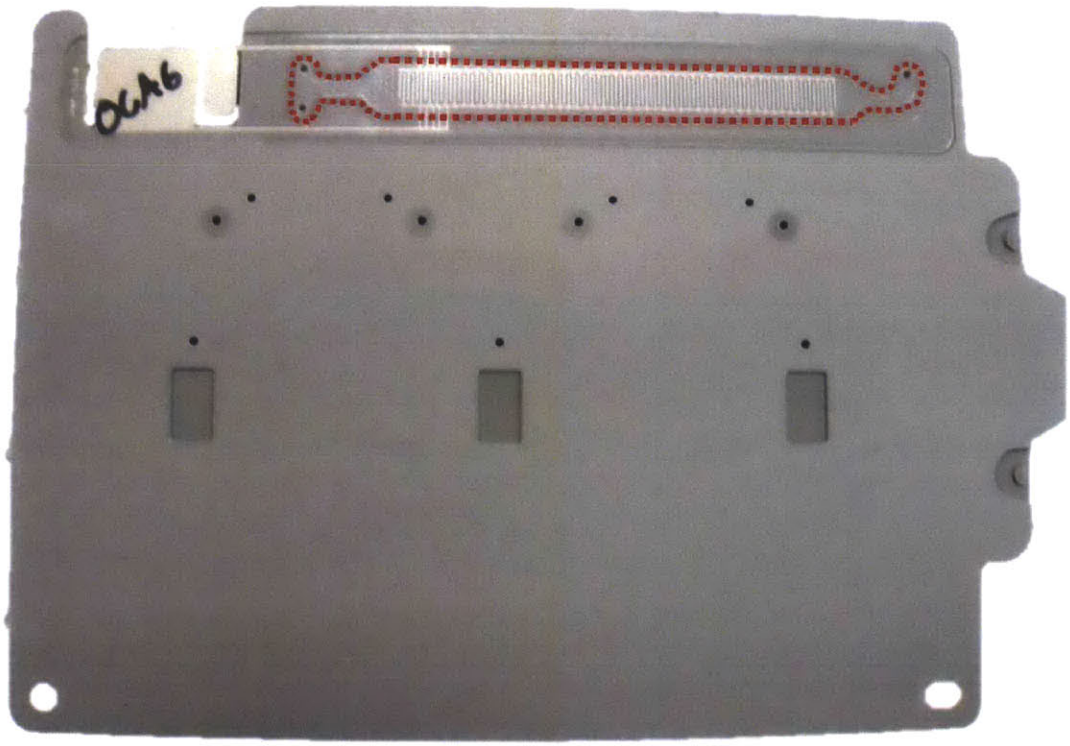

**Figure 5-1: The weld line around the cuvette, attaching the electrode to the blank card.**

## 5.2 Full Card Testing

### 5.2.1 Test Setup

The setup for full card testing is similar to that used during the dip testing except the dip tank is replaced by with a welded card. This card is prepared for testing with the insertion of Eppendorf epTIPS LoRetention 200uL tips, and secured with hot glue which also serves the purpose of blocking fluid flow to other parts of the card and externally.

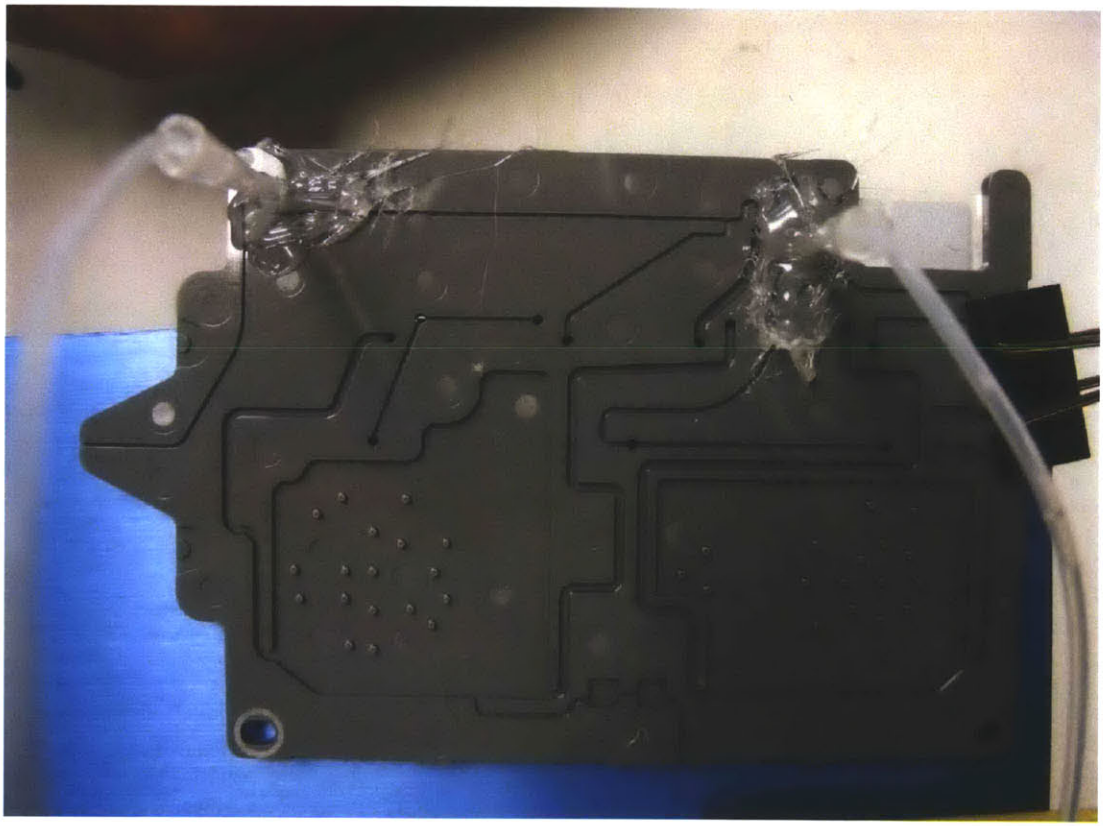

**Figure 5-2: The card is plumbed with pipette tips. Hot glue is used to secure the tips and block the other channels**

In addition, for more thorough analysis, more than one solution is flowed through the card. Previously during dip testing, one solution at 10 us/cm provided a single data point indicating the electrode's electrical characteristics. However, to accurately quantify the electrode's performance relating electrical resistivity to

solution conductivity, multiple points are need. While this relationship is linear, multiple points are necessary to calculate a "best-fit" line.

### 5.2.2 Making Solutions

To use multiple conductivity standards, they must be accurately diluted from a standard. A bottle of 450 μS/cm +/- 1% was used and gravimetrically diluted to 0.9, 4.5, 8, and 22.5 μS/cm using DI water, pipettes, flasks, and a mass balance.

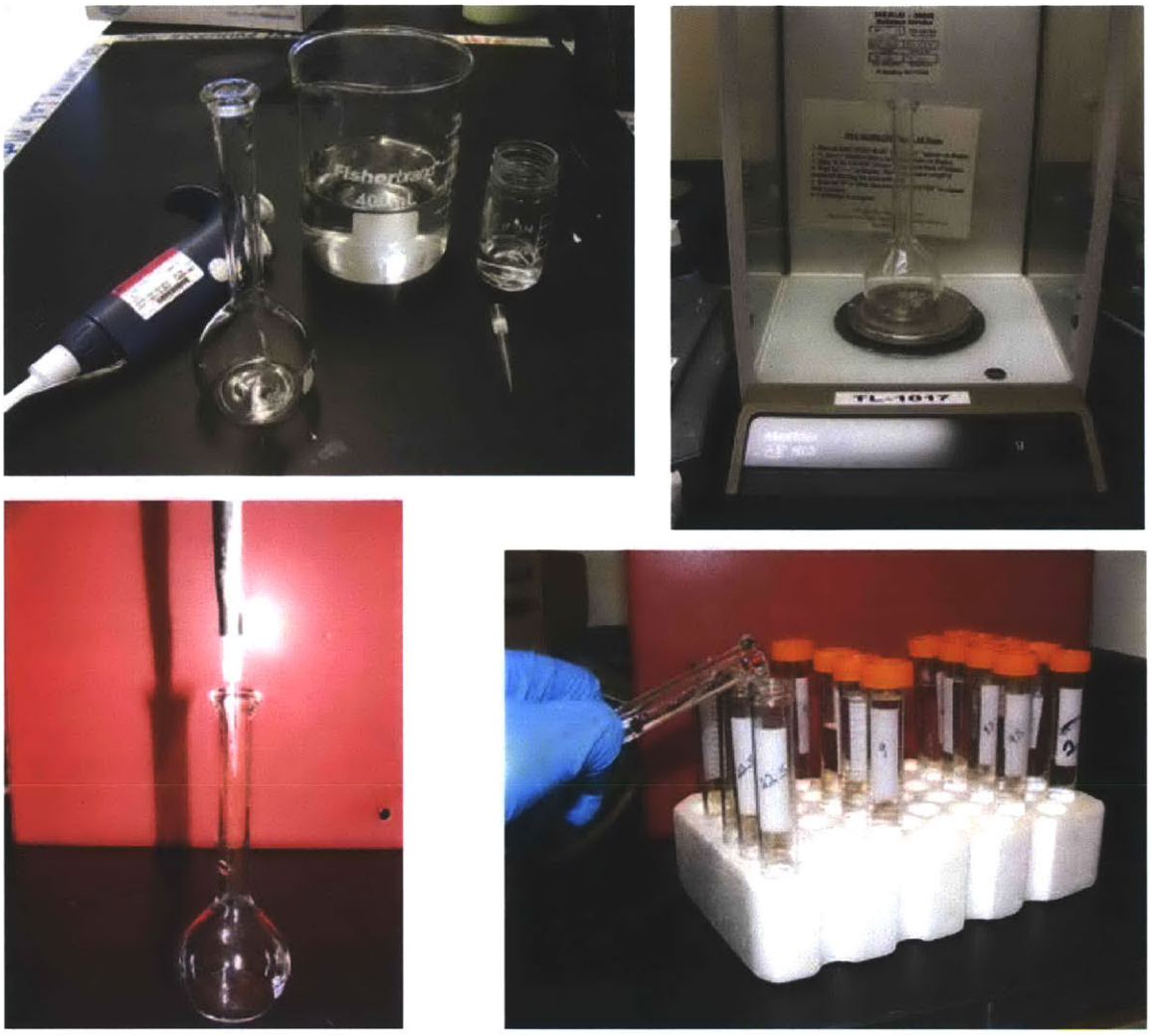


**Figure 5-3: In top left, the pipette, pipette tips, and glassware used to make 50mL of solution. In bottom left, the solution is pipetted into the volumetric flask. In top right, the flask is weighed on a calibrated balance. In bottom right, the solutions are transferred to test tubes.**

### 5.2.3 Procedure

This procedure is again similar to that used during dip testing. However, unlike the dip testing which was primarily testing variability only, the cards are tested with four different conductivity solutions in order to ascertain the *slope* of the electrode on the card. As mentioned in Chapter 2 and in Section 4.3.9.1, this slope represents

the relationship between the conductivity of the solution to the impedance reading measured by the electrode. This is directly representative of the expected function of the electrode in commercial operation.

First, the card is flushed with 0.9 μS/cm solution and then allowed to stabilize as the peristaltic pump pushes the fluid at 50 uL/min. Once a reading is taken, the solution is allowed to clear into waste and the next is fed. This process is repeated for solutions of 4.5, 9 and 22.5 μS/cm.

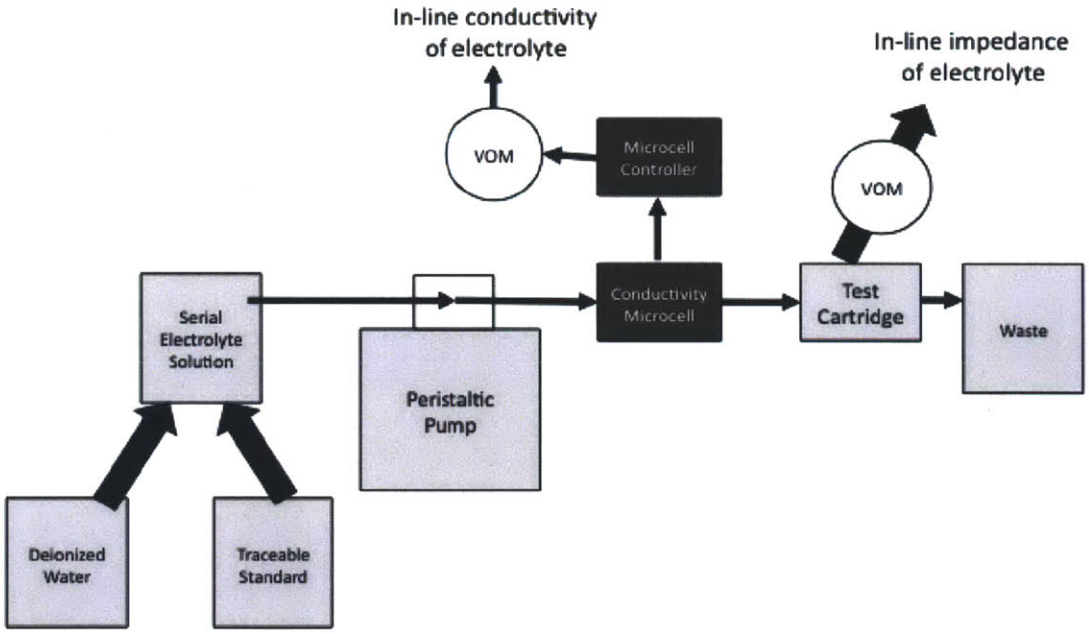


**Figure 5-4: The testing process for full-card impedance runs. [32]**

## 5.3 Gage R&R

A gage repeatability and reproducibility study was performed on this setup to measure the consistency of the measurement system. Due to external factors such as human and environmental interaction, variability can be introduced into the system itself. This study was not performed on the scale of a traditional gage R&R, but rather to obtain a quick quantified assessment of the setup. A single card was tested

ten times each by two operators, resulting in the run chart shown in Figure 5-5. Compilation of this data revealed an approximate 1.4% variation attributed to uncontrolled influences.

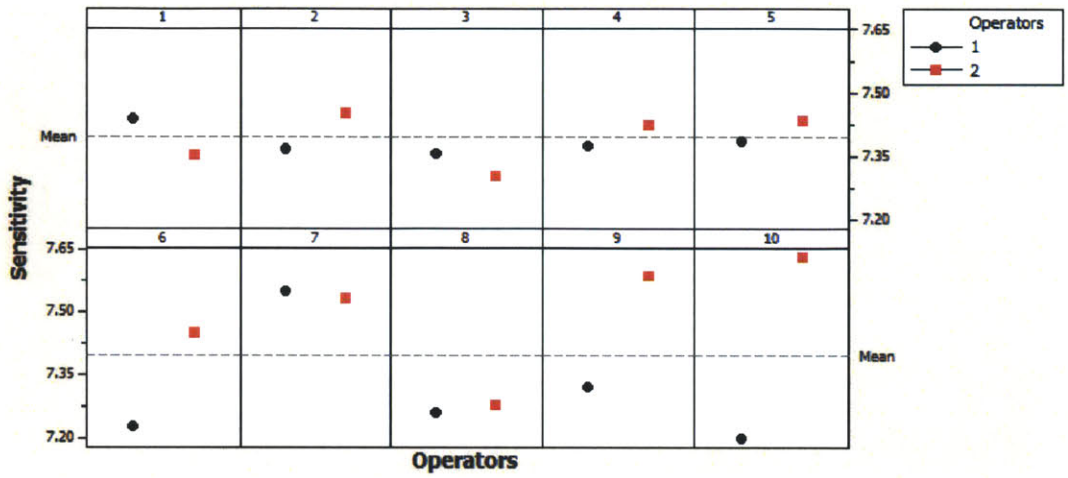


**Figure 5-5: A gage run chart showing the effect of externally caused variation in the test setup. A single card was tested 10 times by 2 operators, and the resulting sensitivity was calculated.**

## 5.4 Study on Temperature Effect

The effect of solution temperature upon impedance readings was also measured. Temperature variation can affect the geometry of the metallic deposit in the electrode, which can in turn affect the consistency of the test setup and reading. Three cards were each measured as solution flowed through the setup at temperatures from 14° C to 40° C. Results showed an approximate 3%/°C when using 10μS solution, a reflective midpoint conductivity level of the solutions used in testing. To remove this effect, solutions were allowed to stabilize at room temperature (22 °C) before the pump was connected.

## 5.5 OQ Part II Results

After collection, the data was compiled and organized using Excel and JMP software packages. This revealed important characteristics of electrode performance, as measured by sensitivity and variability.

### 5.5.1 Sensitivity

An effect test revealed the most significant factors when determining sensitivity. As shown in Figure 5-6, the speed (S) and energy (X) parameters have significant effects. The P values (right most column), which measures consistency between the results obtained in the trial, marking process anomalies as such, are within the threshold of 0.05 which corresponds to a predetermined confidence level. [18] In fact, energy, with a P-value ("Prob > F" column) of 0.0013, is very tightly correlated to the response.

In addition, the interaction of the two parameters, energy and speed, has an effect

**Effect Tests**

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| P | 1 | 1 | 0.0016525 | 0.0108 | 0.9183 |
| S | 1 | 1 | 0.7982257 | 5.2303 | 0.0345* |
| X | 1 | 1 | 2.2078010 | 14.4664 | 0.0013* |
| P*S | 1 | 1 | 0.0002863 | 0.0019 | 0.9659 |
| P*X | 1 | 1 | 0.0092866 | 0.0608 | 0.8079 |
| S*X | 1 | 1 | 1.3965859 | 9.1510 | 0.0073* |

**Figure 5-6: An effect test on Sensitivity response, indicating which parameters and interactions have a significant effect.**

on sensitivity. With increasing speed, energy input must also rise to maintain response levels.

These trends are shown graphically in the contour plots in Figure 5-7. Each plot, calculated at a different pressure value, reveals the average sensitivity of the electrode with respect to speed and energy. In all three plots, the locations of highest sensitivity were located around speed of 2 inches per second, with increasing response with increased energy input.

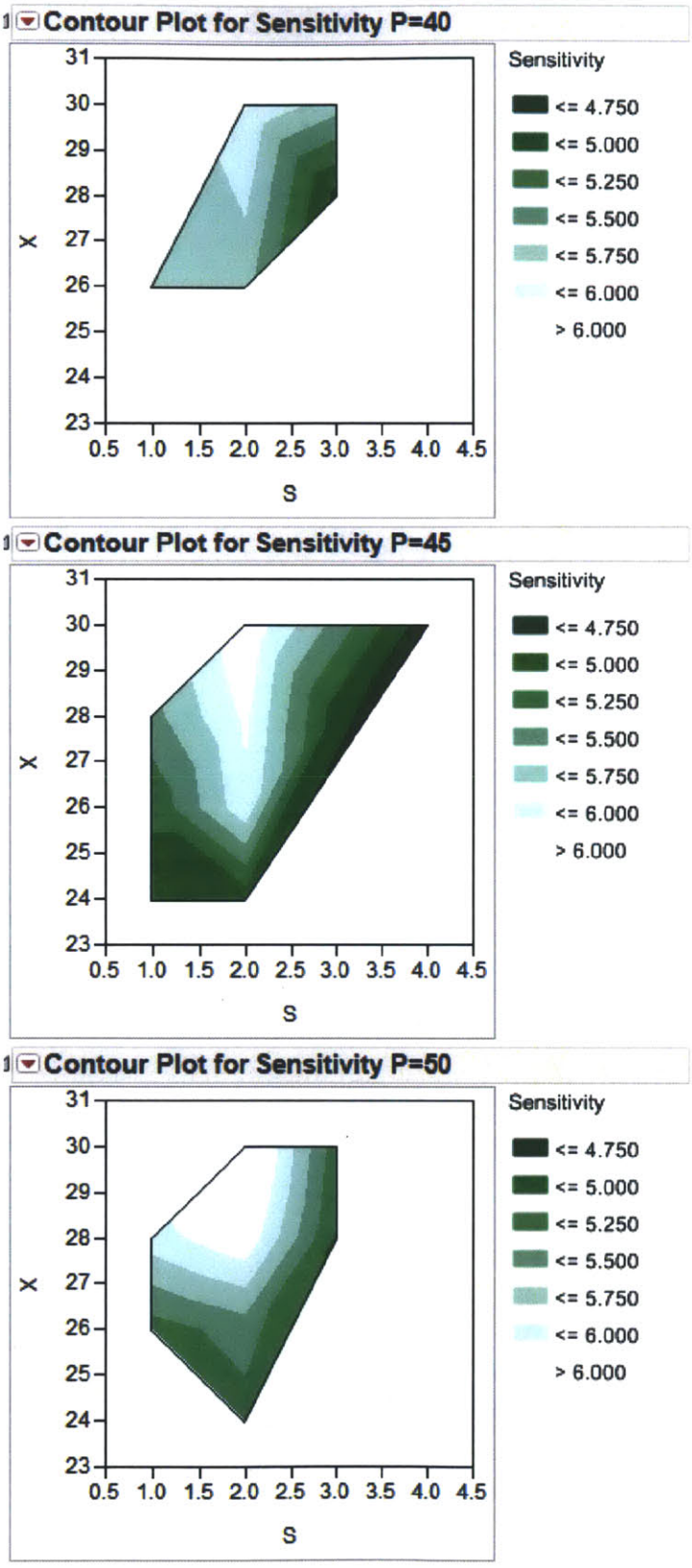


**Figure 5-7: Contour plots depicting the relationship of sensitivity response with respect to speed and energy at various pressure intervals.**

### 5.5.2 Variation

The effect test on variation revealed much different results. Variation was most significantly affected by the interaction of pressure and energy. As this parameters increased, the variation increased as well. Unlike sensitivity, have a low coefficient is desirable though. This trend is clearly revealed in the contour plots in Figure 5-8.

**Effect Tests**

| Source | Nparm | DF | Sum of Squares | F Ratio | Prob > F |
|---|---|---|---|---|---|
| P | 1 | 1 | 2.845271 | 1.2368 | 0.2807 |
| S | 1 | 1 | 1.000600 | 0.4349 | 0.5179 |
| X | 1 | 1 | 8.769876 | 3.8120 | 0.0666 |
| P*S | 1 | 1 | 3.496665 | 1.5199 | 0.2335 |
| P*X | 1 | 1 | 13.114835 | 5.7007 | 0.0281* |
| S*X | 1 | 1 | 3.052950 | 1.3270 | 0.2644 |

**Figure 5-8: An effect test on variation response, indicating which parameters and interactions have a significant effect.**

On top plot in Figure 5-9, a downward gradient is clear as energy is increased. Meanwhile, on plot bottom plot an upward gradient is clear as energy is increased. This could indicate that with low pressure, compensation with energy causes uneven metallic deposit because aluminum released from its carrier may not be placed directly beneath. It can be more easily displaced along the PMMA substrate. On the other hand, high pressure with lower energy may cause the aluminum to not settle on the substrate before being run past the head. In any case, this data indicates that to reduce variation, it is crucial to maintain a balance between these factors.

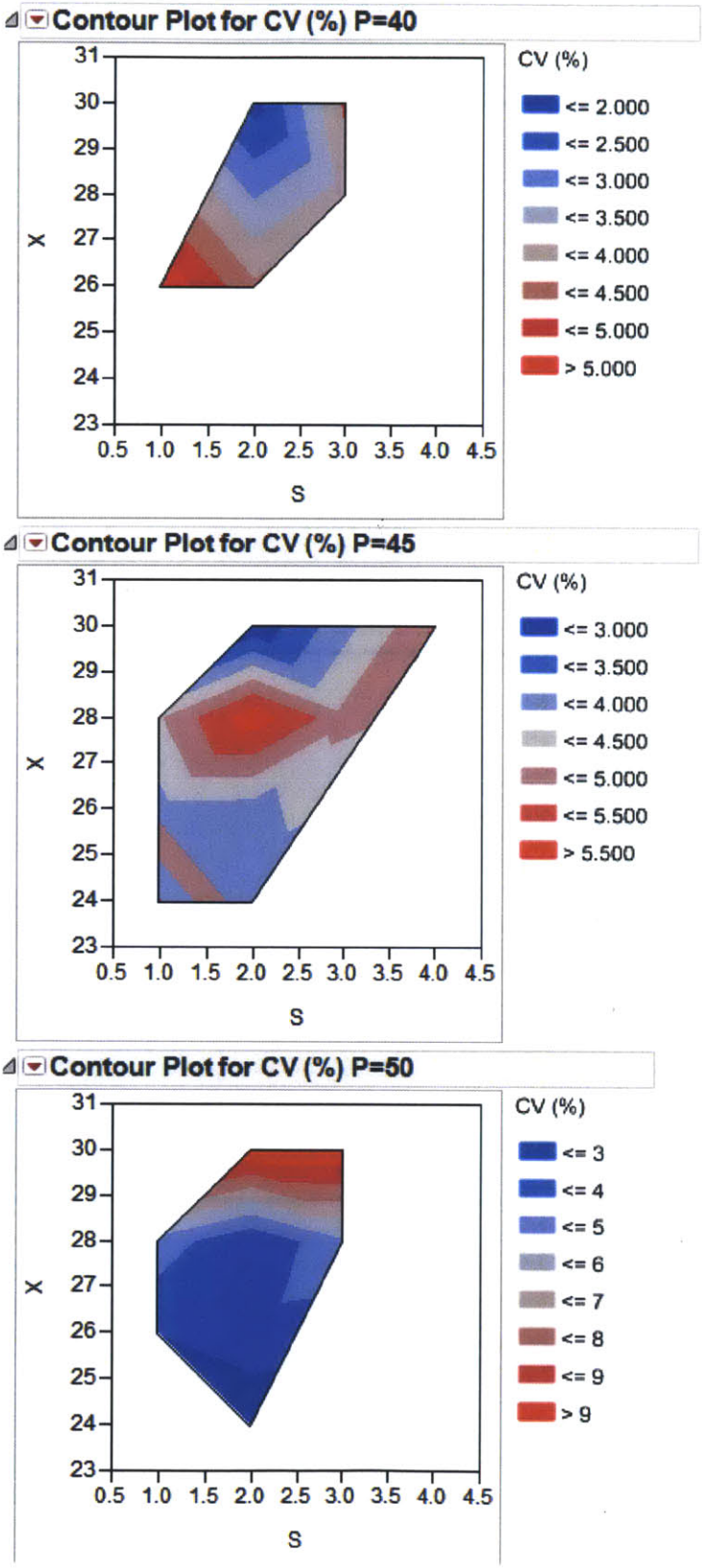


**Figure 5-9: Contour plots depicting the relationship of sensitivity variation response with respect to speed and energy at various pressure intervals.**

### 5.5.3 Card Failure

Because of the handling and energy intensive processes involved in welding electrodes to cards, previously working electrodes can become defective. Such occurrences can be random and difficult to control; however, non-random occurrences can be attributed to poor patterning during roll-to-roll printing. These might pass unnoticed during dip testing QC, but after exposure to harsh situations such as welding, acceptable electrodes may develop defects and become unusable product. Such occurrences are typically focused where the continuity leads connect the rail to the leads from the pad, because the weld line passes through these features. Figure 5-10 shows one reject card.

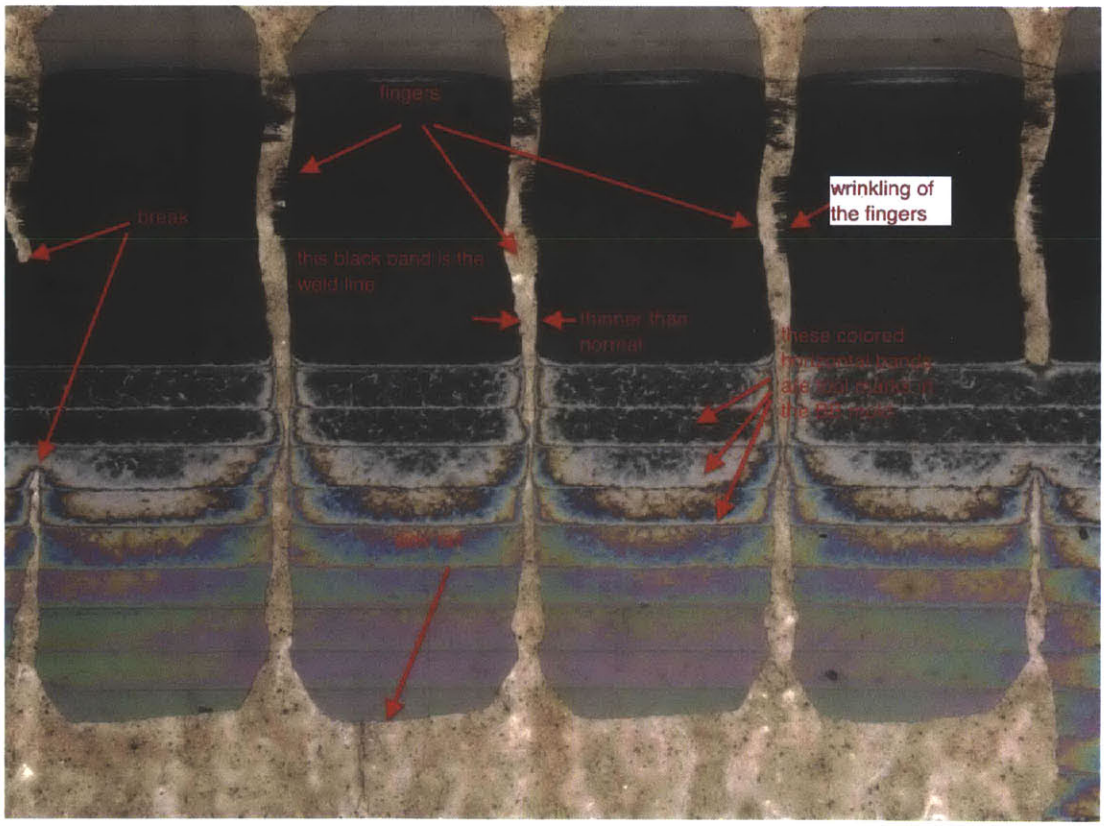


**Figure 5-10: An electrode with obvious defects after weld processing.**

### 5.5.4 Summary

It is clear from the results that a maximizing sensitivity and minimizing variability do not correlate directly. A balance of parameters, as also indicated through dip testing at an earlier stage, is necessary to achieve optimal performance.

A speed throughput of **2 in/s** balances the many intricacies of roll-to-roll printing- too slow, and heat buildup can affect print quality; too fast, and the metallic layer may not be properly deposited on the substrate.

The post-printing dip test indicated energy level of 26 and pressure of 45N along the pods created most desirable product. The full card test indicated more complex relationships between these parameters. This can most likely be attributed to the lack of control associated with the welding processing and handling involved with the utilizing a sourced contractor. Best results were seen when the parameters were balanced; when too much pressure or energy was placed on the printhead, one of the two key responses, sensitivity and variation, suffered.

The goal of this operational qualification was to determine the printing capability of the equipment by pushing the limits of designed production range, for one, and two, to refine the process parameters at low-resolution to find the nominal values for optimal outgoing material. While it is interesting and crucial to understand electrode production as a whole, in processes that occur outside of Daktari, a separate validation of these uncontrolled processes is required. Therefore, with a focus on the controlled process in-house at Daktari, the operational parameters proved to have best performance at an operational pull point of 45 N, 2 in/s, and 26 in energy input. These parameters yielded product that met design specifications, while proving robust for the entirety of electrode production processes.

# Chapter 6 Performance Qualification

## 6.1 Overview

In this last validation phase, the primary objective to prove the consistency in production under established operating conditions. During this lengthy run, variation typically occurs from both an environmental standpoint including temperature and humidity fluctuations, in addition to human and equipment effects such as stress and fatigue. For the electrode production process, key verifications include an assessment of thermal buildup at the interface between ribbon and substrate under the head as well as wear and debris buildup under the printer head. After such verification, the process can be determined as validated, and acceptable for commercial manufacture. [30]

## 6.2 Large Scale Run

Daktari's most imminent goal is to prepare for clinical trials, scheduled for the last quarter of 2012. This study will involve 4000-5000 cards, subject to human interaction and instrument testing. A full-length production run, as defined for the purposes of process validation, was a production goal of 5000 electrodes. The run involved the changeover of materials as one roll stock finished and another was loaded during the course of production. This action brought the production in-line with real-world commercial production which will arrive post-clinical trials.

**Figure 6-1: Two rolls of electrodes post-printing, ready for further processing.**

## 6.3 Test Setup

Much like the dip test performed earlier, the electrodes are placed in a conductive solution and measured resistivity is recorded. However, as production increases, quality control must be scaled to match the throughput. Instead of testing electrodes individually and manually, a test fixture was designed to quickly process a set of 8 electrodes.

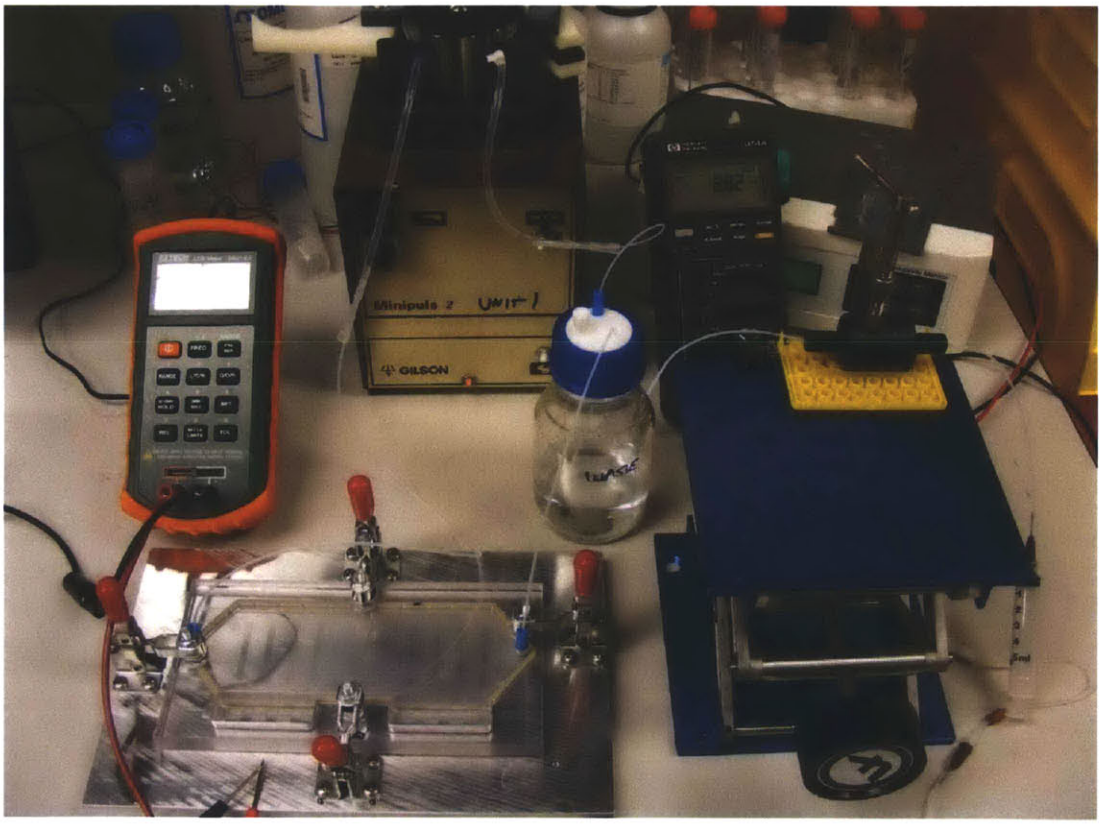


**Figure 6-2: The test setup is similar to that used in the OQ, except the electrode fixture, shown in bottom left, is designed to house 8 electrodes for each solution cycle.**

This fixture was designed with the intention of hold a volume of liquid over the electrodes, using a gasket to position the liquid and an inlet and outlet to allow for

constant solution mixing and refreshing. The electrodes are left exposed, allowing for quick contact measurements with a multimeter.

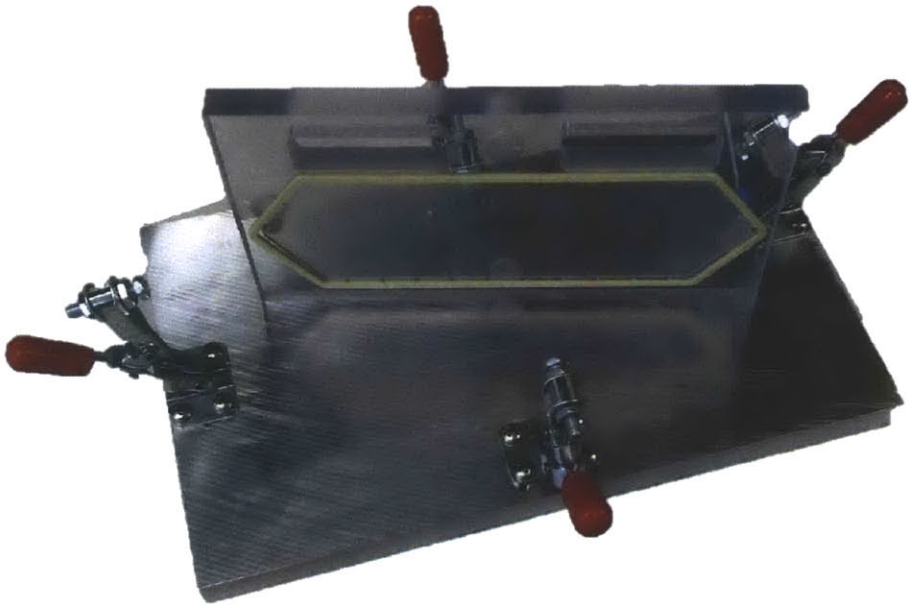

**Figure 6-3: The fixture for rapid testing of die-cut electrodes, with white-colored gasket for liquid-tight sealing.**

## 6.4 Test Procedure

First, standard solution was prepared at 9 μS/cm using the procedure outlined in Section 4.3.7.2. Then, eight electrodes were isolated at intervals of 500 along the production rolls. After cleaning with ISP wipes and careful drying, electrode sets were in turn placed in the fixture and connected to the Volt-Ohm meter, and resistances recorded.

Throughout the test, the conductivity of the solution was measured and recorded using the flowcell and conductivity monitor, as the solution was susceptible to drifting as it became increasingly dirty. Exposure to air and particles on the surface

of the electrodes has been shown to have a measurable effect on solution characteristics.

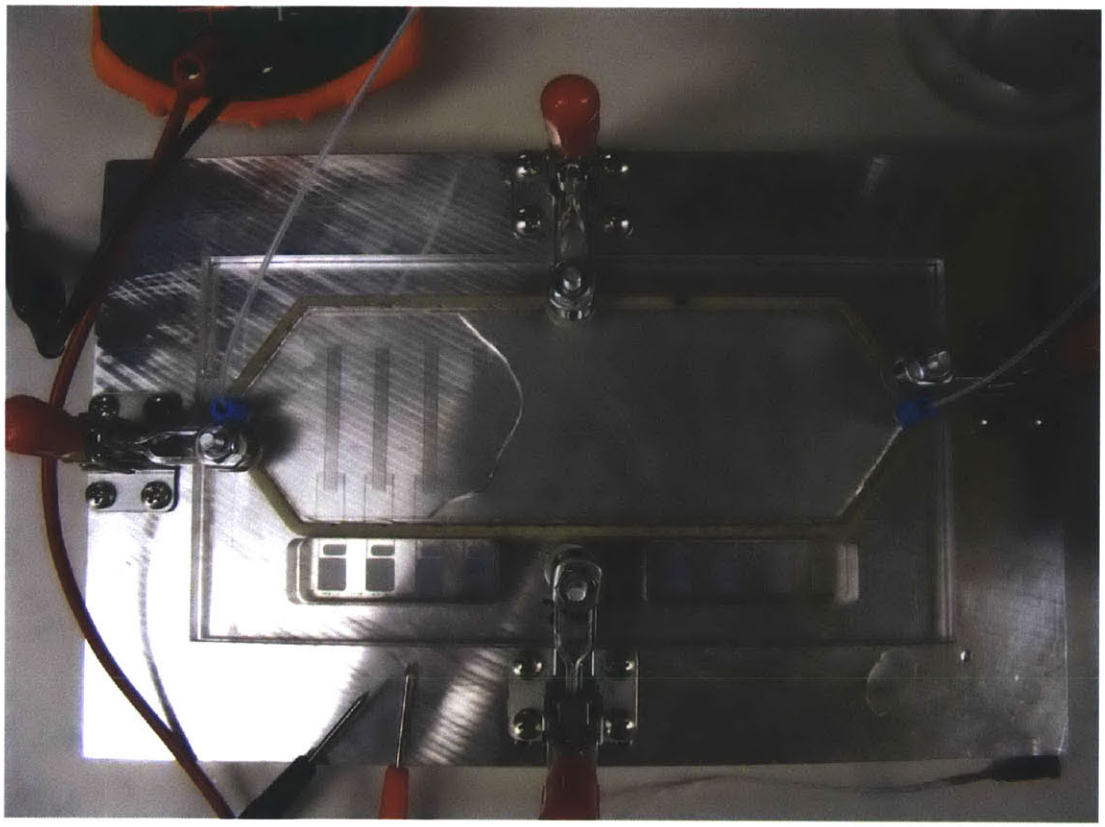

**Figure 6-4: Conductive solution, contained with a surrounding gasket, flows over the electrodes.**

## 6.5 Validation of New System

Due to the novelty of the test setup, it is necessary to verify use of the system has not introduced error or bias into measurements. Because this set up is a direct redesign of the dip-testing performed previously, a set of electrodes were analyzed using both setups.

**Table 6-1: A comparison between testing setups for die-cut electrodes.**

| Test Setup | CV (%) | Note |
|---|---|---|
| **Original "Dip" Test** | 1.17% | CV's differ by ~0.38%, less than 1% error seen from normal operation |
| **New Fixture Design** | 1.55% | |

Table 6-1 displays the results from the same set of electrodes with both setups. The recorded difference in coefficient of variation (CV) is only 0.33%. The setups were shown to have 1% variation solely through human and environmental interaction. Therefore, there setups proved to differ insignificantly and the setup was validated for whole-run testing.

## 6.6 Results

### 6.6.1 Overview

The large production run involved 5000 electrodes manufactured using two different substrate stocks. When the first spool was removed, a second was installed without tampering with ribbon and minimal cleaning.

Samples were then extracted at intervals of 500 along the run and tested according the aforementioned procedure. The results revealed two significant manufacturing characteristics.

### 6.6.2 Steady State Operating Conditions

First, there exists significant time required to reach a steady operating condition. As seen in Table 6-2, the electrodes at the first test point exhibited a noticeably higher average reading, at 18.1 reciprocal ohms. This is likely due to thermal feedback adjustments being continually processed by the print head controller. At the beginning of the run, the "smart" head is constantly making energy adjustments as

the desired print pattern is repeated. These changes are specifically included in the manufacturer's design to preserve the life of the print head. By supplying power only when and where it is necessary, the head can reduce component degradation through stresses cause by thermal buildup. The feedback sensory system imbedded in the printhead can therefore "learn" the repeating pattern and pinpoint optimal settings within the user-input energy level.

**Table 6-2: Single point calibration points at intervals along production run.**

| Single Point Calibration Points | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Location** | 500 | 1000 | 1500 | 2000 | 2500 | 3000 | 3500 | 4000 | 4500 | 5000 |
| **Sensitivity** | 18.06 | 16.93 | 16.12 | 15.31 | 16.14 | *** | 15.92 | 16.18 | 15.55 | 15.52 |

Indications from this study show that this production operation requires run-in time in order for the intricacies of the production to smooth out and perform consistently. From this study, it can be seen in Figure 6-5 that first 500 to 1000 electrodes fall within this varying period, after which steady state production occurs. This corresponds to approximately eight minutes of a production run of forty. While spending 20% of production time producing waste can seem inefficient and wasteful, when runs become longer with higher throughputs, the run-in time typically does not scale as well, meaning this waste becomes increasingly insignificant. Future studies of higher production run can confirm this.

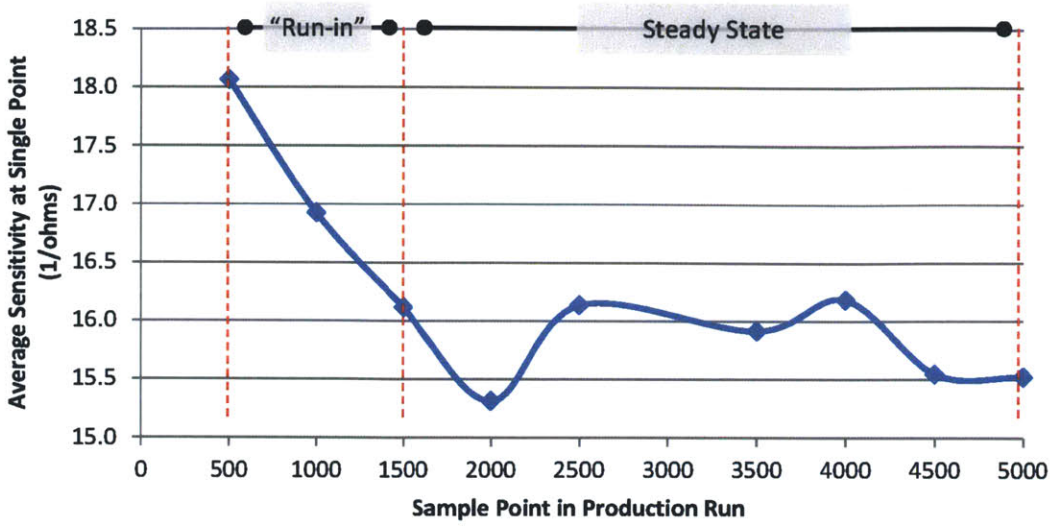


**Figure 6-5: Graph showing average reciprocal resistivity values along production run.**

### 6.6.3 Relationship of Coefficient of Variation and Run Position

The variation of sensitivity is the other important factor in characterizing electrodes; in this case, eliminating samples collected in non-steady stage regions, the coefficient of variation was 2.2% throughout the entire run.

At each individual test interval, the CV% was similar to the results obtained in the dip testing performed during operational qualification. As shown in Table 6-3, the variation at each point was around 1%.

**Table 6-3: Variation at intervals along production run.**

| Variation along Run | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Location** | 500 | 1000 | 1500 | 2000 | 2500 | 3000 | 3500 | 4000 | 4500 | 5000 |
| **CV (%)** | 1.11 | 1.48 | 1.55 | 1.51 | 1.48 | *** | 1.58 | 1.27 | 0.87 | 1.07 |

Also important is to observe that no general trend or drift is apparent. This is critical to ensure that the increased production, which brings long run times, does not cause deteriorating product. This production run on a scale ten times that ever run before, using new material and equipment setups, resulted in a small sensitivity variation that remained within Daktari specifications.

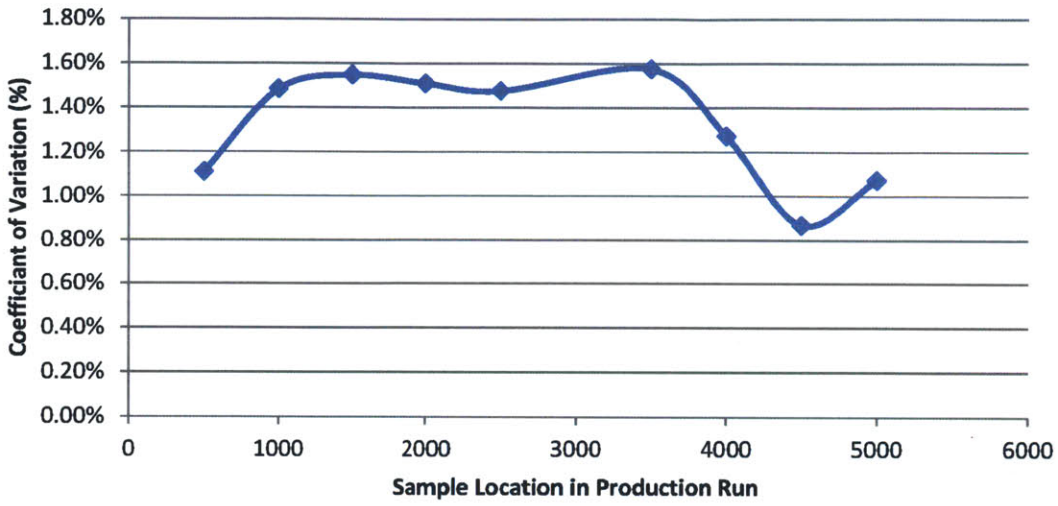


**Figure 6-6: Graph showing coefficient of variation values along production run.**

### 6.6.4 Production Anomalies

There were few production anomalies during the long production run. One in particular was particularly noticeable during testing though; around 3000 electrodes into the run, near the end of production on media roll 1, the tested electrodes had a relatively consistent jump in resistivity. Upon further examination, it was revealed that the rail on one side was broken about half way along the rail on dozens of electrodes. Figure 6-7 below shows the break in the electrode and notch in corresponding finger, taken under a microscope. This minor gap caused impedance levels to increase 50%. The cause of this issue is difficult to assess because the break did not occur all the way across the transverse axis of the electrode. It only appeared on one side, consistently.

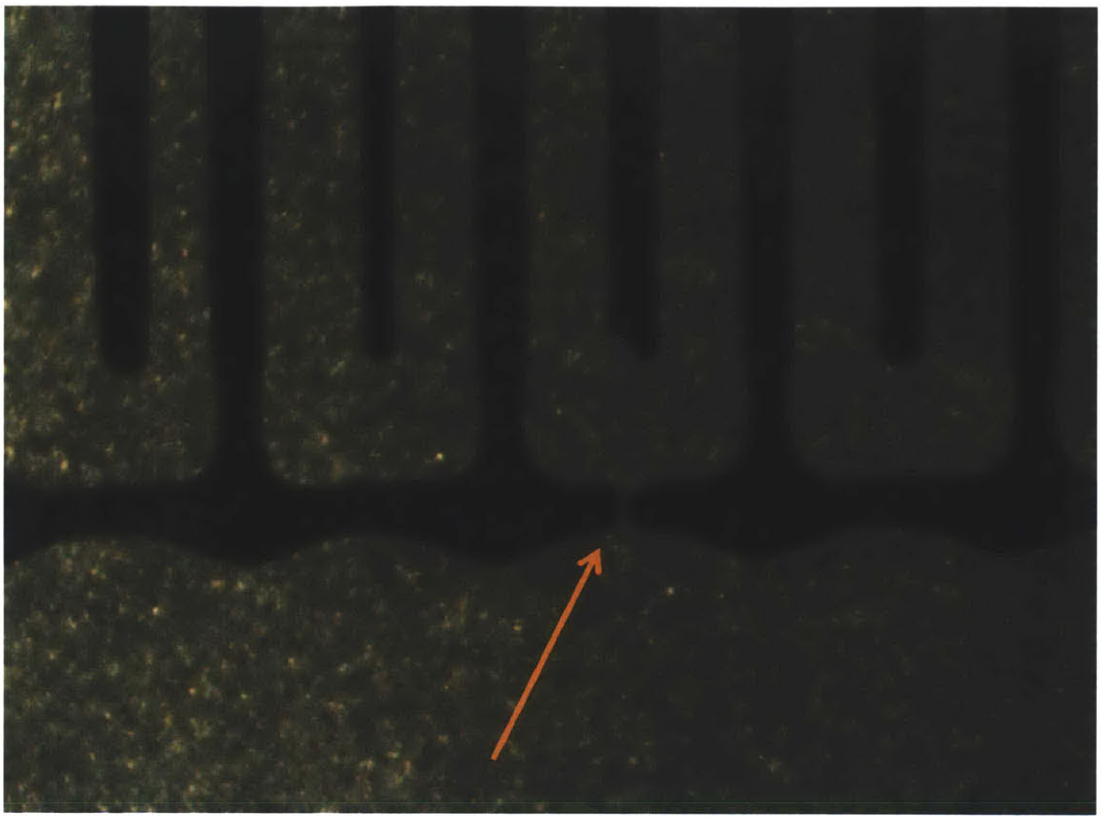

**Figure 6-7: A photograph of a break in the side rail of an electrode, causing inaccurate impedance measurements.**

Examination of the waste ribbon provided more information. In Figure 6-8, the large area at the bottom indicates the color of regions that were not printed. This color also outlines the fingers inside the rails. As the ribbon passes under the head, it heats the ribbon. This is indicated by the silver region just before the black. The black is the fully printed part of the region- the part of the ribbon that was deposited onto the substrate. At the point of the defect, there appears neither gray nor black.

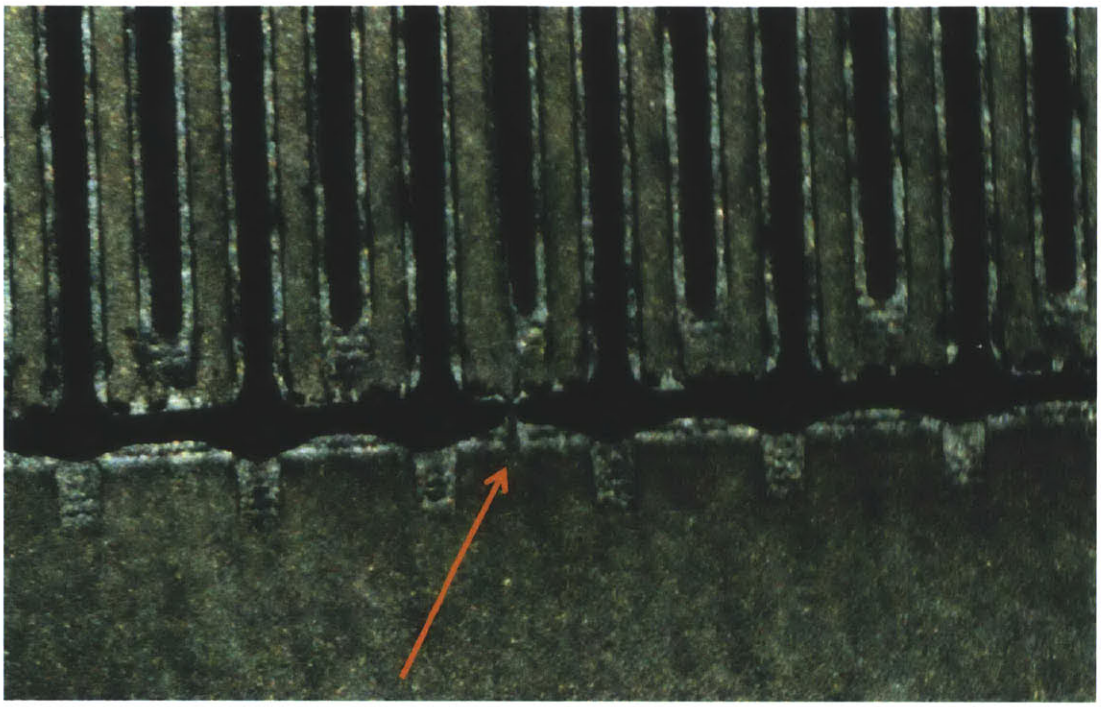

**Figure 6-8: The corresponding ribbon reveals heating issues at defective point.**

This evidence indicates that an issue occurred within operation of the print head, rather than stock material defects, or an unclean head. A material defect would exhibit still exhibit evidence heating, or graying of the ribbon. A dirty print head, perhaps due to deposit on a section of resistors along the head surface, would cause a cause a relatively continuous anomaly along the run. Instead, this defect appeared in specific points in particular electrodes. A further study of this behavior is necessary in order to verify it is a repeatable situation, and requiring of production redesign.

### 6.6.5 Summary

The evidence from this performance qualification shows that microscale electrode production for the purposes of performance impedance measurements in the CD4 system is controllable with specified limits at the 1000s scale. The conductive traces

proved to be consistent with only 2.2% variation in sensitivity through the run. While production anomalies existed, implementation of in-line conductive testing could mitigate this problem, leaving a manufacturing production line capable of meeting the stringent demands of required of the microscale electronic conductors in the Daktari CD4 system.

# Chapter 7 Conclusions & Future Work

The validation of electrode manufacturing at Daktari requires an extensive amount of studies due to the complex and novel nature of the process. Few studies have been performed on micro-scale thermal transfer printing with the metallic ribbons. Added to this is the erratic nature of plastic interaction. The specifications on outgoing material are detailed enough, without even the inclusion of added processing introduced by heat-intensive laser welding.

From initial experimentation through repeatability tests and now, full clinical trial production runs, the process must be carefully studied, analyzed, and diagnosed in order to have a better understanding of how controllable production parameters can optimize performance and how to minimize effects of the unavoidable and uncontrollable scenarios.

This study suggests that Daktari is prepared for production runs on the scale of thousands of electrodes, for clinical trials and beyond. And as the company scales

output to a level on a higher order of magnitude, careful testing must be performed to ensure quality product is maintained.

The following represent a list of important conclusions that can be constructed from this work, and improvements to be explored as Daktari nears product release and commercial production.

## 7.1 Print Parameters Balance

A balance of printing parameters is necessary for optimal performance. Overworking the head with high pressure can cause unnecessary wear. Meanwhile, high energy deposits more metal which can actually reduce performance- over deposit can cause shorting or even small issues such as non-uniformity in fingers- which can cause fluctuations in impedance measurements. Lastly, speed is clearly optimal at 2 in/s; higher speeds result in inconsistent small features, while low speeds can cause similar issues to that of excessive energy input.

In order to further refine the process, an additional, detailed study should be performed on the process. This study reflected a rough general outline of the operational ranges of the equipment, as well as a reflection on the quality of the electrodes within the acceptable range. Added firmware support for finer adjustments in speed, energy, amongst other parameters, would allow for a proper Design of Experiment (DOE) to be performed around the optimal nominal values uncovered in this study.

## 7.2 Quality of In-Process Testing

### 7.2.1 Solution Dip Testing

The introduction of a test fixture makes the process of testing electrodes post-die cut processing far more repeatable, and rapid. A statistical study showed that the new process differed insignificantly from the manual dip tank used previously.

Within minor equipment modifications primarily focused on pumping higher volumes of solution, the use of the fixture provided more instantaneous settled readings from the multimeter, thereby reducing operator error in impedance measurement.

### 7.2.2 On-Card Testing

The new method for making conductance solutions, using volumetric dilution, provided an accurate means to calibrate the system and perform electrode characteristic measurements on full cards.

In addition, a Gage R&R performed on the process, using multiple operators revealed repeatability and reproducibility values on the testing process as a whole. The indicated ~1% effect signified the theoretical limits on electrode production variation.

### 7.2.3 Introduction of In-Line Testing

Introduction of system utilizing the electrode pads to immediately detect shorts or clean breaks as the printing process occurs would be very useful. As exhibited during performance qualification where repeated breaking of a rail on each electrode occurred for a dozens of electrodes at a random point during the run, in-line real-time testing of the electrodes were simple anomalies would easily limit such behavior from destroying large quantities of outgoing material unknowingly. With simple audio or visual cues to indicate a defect, quick operator adjustments can be made to respond before waste builds.

## 7.3 Weld Effect

Comparisons between failure rates at the dip test and on-card levels revealed the detrimental effect of the welding process on electrode quality. More specifically, the intersection of metallic and weld lines proved to cause failures in previously accepted electrodes. However, electrodes manufactured using the ideal print parameters, as determined by the operation qualification, showed little evidence of

worsened performance after being attached to a card. The standard increase in CV, as has been shown to always occur when passing through Daktari's contractor, was the only apparent influence. With further optimization of the print process, the effects of laser welding might be mitigated ever further.

## 7.4 Aging Effect

The aging effect on samples has yet to be analyzed at this scale. After exposure to the environment, including thermal and humidity-related cycling, the electrodes could suffer from a quality perspective. Even minor drifting in sensitivity can throw off measurement accuracy. Therefore, future studies must be performed on the effect of time over the shelf life of the product.

## 7.5 Scaling Up

The most important aspect of this study has been on quantifying the variation in electrode production on a production scale ten times anything performed previously. With long run times, a more accurate assessment of the behavior of production can made. Thermal transfer printing involves many small intricacies that can affect outgoing material, especially due to the micron-level scale of the product.

It was seen during process qualification in this study that the process needs time to settle in before consistent product can be produced. Many processes seen throughout the industry and with applications far outside the medical field have exhibited similar behavior. Quantification of this need, including time need to settle, is important when planning production runs of even greater scale.

Daktari will scale up production to fulfill the needs of millions of people that will require CD4 cell testing in the future. But in order to properly meet the demand, from a quality perspective, it is crucial to understand the process at low thresholds. As production through-put increases, ensuring sensitivity and *variation* in sensitivity does not increase as well is vital.

**Figure 7-1: Several hundred samples in collected and saved from various electrode production stages.**

In order to do this, it is important that a more automated test system is implemented. As mentioned in Section 6.2.3, in-line quality control at the point of printed will prevent production of defective product from a macro scale. Visual defects such as breaks and especially shorting within the fingers, is simple to prevent at this processing point. However, more detailed testing, at intervals or even at the 100% inspection level is also necessary. Product that may appear acceptable from a visual perspective may be deviating from the expected values

electrically (resistively). The process performed in this study involved extremely time-intensive procedures and cannot be duplicated at higher throughput levels. Therefore, more automated processes must be developed to verify production at QC standards.

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