Point of Care Molecular Diagnostics for Humanity

Thesis by

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To my parents

The title of this thesis is in appreciation of people at Bill and Melinda Gates Foundation who provided resources and guidance for the development for this technology with the purpose of serving humanity. They enabled me to prove that high quality medical technologies can be low cost. I hope they will make it available where needed.

Acknowledgements

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Abstract

Diagnostics of disease at POC (point of care) has been declared one of the Grand Challenge by the Bill and Melina Gates Foundation (BMGF). Infectious diseases constitute a major cause of disease burden and cause more than half a billion Disability-Adjusted Life Years (DALYs) and millions of deaths each year. They have an especially large effect on children under 5 years of age. We have analyzed data from the GBD 2010 (Global Burden of Disease) project to emphasize the damage caused by infectious diseases, and highlight the opportunity of using diagnostic tools to rapidly identify and treat diseases. To motivate the work of this thesis, we quantify the expected impact of appropriate diagnostic technologies.

We have also analyzed the requirements that a diagnostic tool should meet to generate the maximal global impact. We present various existing TPPs (Target Product Profiles) from different organizations and suggest some additions to these existing TPPs. We explain the particular molecular pathology technologies which have the potential to allow deployment of functional products in the developing world for point-of-care pathogen detection, especially in low-resource settings.

We perform a detailed analysis on existing polymerase chain reaction (PCR) systems and describe the problems caused with thermal performance and optical interrogation. We list the requirements that disposable cartridges for such instruments should meet and suggest a metal base design with polymer top. After detailed FEA simulations, we demonstrate that the thermal response can be modeled using a one-dimensional (1D) lumped element system. We show improvements in thermal response due to using a metal base and the effect of fluid height. We also performed thermal-structural simulations to quantify the stresses on the adhesive bonds of metal/polymer cartridges.

Next, we explain fabrication of these cartridges. We show methods to dispense adhesive using a robot and a custom made jig to spread the adhesive during curing. The cartridge was tested with different PCR reagents and we obtained reaction efficiencies approaching those of the commercial real time PCR machines. Our fabrication technique is useful to join dissimilar materials and is production friendly. By developing custom software, we observed the cartridge performance in a continuous manner. We could see the thermal response of cartridges by continuous fluorescence monitoring, and used reflective aluminum which increase light collection efficiency.

We then present a simple and robust new way for thermal cycling. Robust thermal cycling has been a major challenge conducting PCR, especially in point of care situations. Here, we suggest a contact cooling approach, in which the cartridge rests on a thin metal plate with an integrated thin heater constructed from flexible printed circuit board (PCB) material. We use a solenoid to move a metal plate to cool down the sample cartridge during cycling. The metal plate then rests on a larger heat sink to disperse the shuttled heat. Our design is dust and water proof and was verified on a bench-top prototype.

A novel optical design for fluorescence detection during qPCR is also described. We suggest a lateral illumination waveguide geometry with prism coupling that eliminates lenses and is integrated into an injection molded cartridge. The light is homogenized using a light guide, and we quantify the sources of scattered stray light from the chamber edge by performing ray tracing simulations to optimize the precise geometry. The design is tolerant to misalignments and enables easy coupling of LED light into the chamber. As the light collection efficiency is high, the size of the chamber can be very small. We tested real PCR reactions using this concept and observed a rapid integration time, enabling very fast reading.

Sample preparation has been another challenge for all point-of-care (POC) lab-on-chip devices for many years. Here, we propose a new design which is robust, fast, flexible and simple, and uses a sliding seal to move the collected sample between various reservoir chambers. The sample moves on a slider sandwiched between seals that shuttles a DNA binding membrane between different reactions. Thus, size and volumes of reagents can be increased without increasing dead volumes. This design is easily automated, and positive displacement of fluids can work with many reagents without worrying about their characteristics such as foaming. The speed of the sample preparation protocols is high and complex protocols can be ported on this design concept, which we tested on real clinical samples and obtained impressive results. We designed and injection molded devices to test and verify this concept.

Finally, we focus on instrumentation and software required to allow our technology to be used at the POC. We describe our embedded electronics and describe the powerful micro-controller and various high performance ICs that are used to construct a fully functional for sample to answer instrument. We developed various versions of software. The developer software allows us to control our system and bench top setup. Our end user product includes a tablet and cell phone software interface. Software was developed for a windows 8 tablet, windows 8 phone and an Android based devices.

To conclude, we very briefly describe the POC systems that are under development: A portable qPCR system with a separate cartridge design, and a universal sample to answer system that performs qPCR, sample preparation and sample to answer protocols in one box depending on the cartridge.

As per best of our knowledge the cost of this technology is much lower than any other option in its class. The sample to answer instrument is expected to cost less than $500. The test cost is expected to be less than $5. The performance is not compromised. We hope that this work can help bring a transformative change in the practice of pathology especially in the developing world.

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1 Burden of Disease and Importance of POC Diagnostics

1.1 Introduction

In this section we will establish the importance of POC (point of care) diagnostics in healthcare and the merits of PCR based pathogen and biomarker detection. First, we will provide an overview of the burden of disease in the world and then we will show the expected impact opportunity of diagnostic technologies, especially focusing on the comparison between developing world and low income regions.

'Appropriate' diagnostics technologies have the potential to revolutionize healthcare in developing world immediately and in developed world eventually especially for infectious diseases. Molecular diagnostics (MDx) is also becoming very important in diagnostics and cure of communicable diseases such as cancer and cardiovascular disease. Everybody in the world will eventually die and nothing in the world can prevent this. It is estimated that 107 Billion have ever lived on Earth i.e., about 15 dead people for each living person at the present time. However, the cause of death varies among humans. The average lifespans also varies in different regions, and the prevalence of disease varies widely as do the average income levels. Most of these deaths are due to diseases of various kinds. Clearly, the impact of disease on human population is large, and while everyone will eventually die, diseases clearly lead to lower quality of life and productivity.

1.2 Burden of Disease

To develop a quantifiable metric describing the effect of diseases, the WHO has defined DALYs (Disability-Adjusted Life Year) [1] [2] [3]as a metric. DALYs are a measure of 'Burden of Disease'. In calculation of DALY, YLL and YLD (defined below), an ideal life expectancy is assumed and the years are counted if mortality or morbidity occurs before that ideal age. According to the WHO, the ideal case is that the entire population lives to an advanced expected age without death or disability due to disease.

The same life expectancy is assumed for all populations - irrespective of race or geography. This life expectancy from the GBD Study 2010 was assumed to be 86 years [2], and only age and sex are considered in these calculations. The disability weights (DW) are also the same for all populations.

The present estimated life expectancy of the global population in 2011 was 70 years, and the key metrics in relation to the burden of disease are defined in Table 1.1.

Table 1.1 Metrics to measure burden of disease

Metric	Calculation	
DALY : Disability-Adjusted Life Years	DALY = YLL + YLD	
YLL : Years of Life Lost due to mortality	YLL= N x L	N = number of deaths L = standard life expectancy at age of death in years
YLD : Years Lost due to disability due to morbidity	YLD = P x DW	P = number of prevalent cases DW = disability weight

The disease 'burden' groups the '**causes'** into the following categories.

- Communicable, maternal, perinatal and nutritional conditions
- Non-communicable diseases
- Injuries

Interestingly, problems caused to health by injuries are also included in the disease burden as injuries can cause death and disabilities and indeed burden the healthcare system. Here, we separate out communicable diseases (or infections) from the first group since these relate directly (in terms of immediate application) to our project. Communicable diseases are caused by pathogens, but it should be noted that many causes are interrelated. For example, nutritional conditions can compromise immune response, which in turn render a person more vulnerable to infections.

The actual number of infectious diseases is large. A comprehensive list can be found in 'International Classification of Diseases (ICD) 10[4] published by WHO (World Health Organization). Some of the most important infections categorized in the WHO database include ***Tuberculosis***, ***STDs*** *excluding HIV (Syphilis, Chlamydia, Gonorrhoea, Trichomoniasis, Other STDs),* ***HIV/AIDS***, ***Diarrhoeal diseases***, ***Childhood-cluster diseases*** *(Whooping cough, Diphtheria, Measles, Tetanus),* ***Meningitis***, ***Encephalitis***,

Acute hepatitis B, Acute hepatitis C, Parasitic and vector diseases*(Malaria, Trypanosomiasis, Chagas disease, Schistosomiasis, Leishmaniasis, Lymphatic filariasis, Onchocerciasis, Leprosy, Dengue, Trachoma, Rabies),* ***Intestinal nematode infections****(Ascariasis, Trichuriasis, Hookworm disease), Other infectious diseases,* ***Respiratory infections****(Lower respiratory infections, Upper respiratory infections, Otitis media, Maternal conditions and* ***Neonatal sepsis and infections****.*

1.2.1 Global Burden of Disease Project – a super human effort

To estimate the global burden of disease, a massive effort was made [5] [2] in the form of the Global Burden of Disease Project 2010 (GBD 2010). Data was collected for 291 diseases and injuries in 21 regions and for 20 age groups. 67 risk factors were also considered [6], and a large number of data sources were used to estimate the disease burden around the globe. The burden of disease is also strongly correlated with poverty[7]. Key resources to view data for this project are listed below.

Table 1.2 Sources of Data for Global Health

Items	Organization	Remarks
Data Tables, Publications	WHO	Data by cause, age, sex, WHO income groups, World Bank regions and UN Millennium Development Goals (MDG) regions
Publications, Analyses	**Lancet Global Burden of Disease Study 2010**	
Visualizations, detailed Data, Publications	Institute for Health Metrics and Evaluation (IHME)	Detailed Data by cause, sex, age, country - Search at http://www.healthmetricsandevaluation.org/search-gbd-data

1.2.2 Analysis of GBD 2010 Study

We have analyzed the GDB 2010 study to identify opportunities for the maximum impact of technological solutions on human healthcare. If each life is assumed to be of equal value, and there are limited resources, then such a study allows us to focus on some areas which are estimated to have a bigger impact on health than others. To be concise, we analyze DALYs and deaths due to different causes and in different regions and age groups. We also select WHO Income groups of our regions for analysis. The WHO divides the world in Low income, Low Middle Income, High Middle Income and High Income regions. We take the high income group as a reference for development and compare against it to find causes and categories in which there are large gaps that can be filled.

We use two major groups of causes for comparison. These are 'infections or infectious diseases' and 'noncommunicable diseases'. The leading causes of DALYs in the world are shown Table 1.3 whereas the major causes of death are summarized in Table 1.4.

2011				
Rank	**Broad Cause**	**DALYs (000s)**	**% DALYs**	**DALYs per 100,000 population**
1	Infectious diseases (incl. respiratory infections)	624141	22.7	8996
2	Cardiovascular diseases	378875	13.8	5461
3	Injuries	296836	10.8	4278
4	Neonatal conditions	231581	8.4	3338
5	Cancers	223539	8.1	3222
6	Mental and behavioral disorders	198370	7.2	2859
7	Respiratory diseases	134246	4.9	1935
8	Neurological and sense organ conditions	128613	4.7	1854
9	Musculoskeletal diseases	108401	4.0	1562
10	Endocrine, blood, immune disorders, diabetes mellitus	88211	3.2	1271

Table 1.3 Top ten leading causes of DALYs in the World

2011				
Rank	**Broad Causes**	**Deaths (000s)**	**% deaths**	**Deaths per 100,000 population**
1	Cardiovascular diseases	16586	30.4	239
2	Infectious diseases (incl. respiratory infections)	10066	18.4	145
3	Cancers	7870	14.4	113
4	Injuries	4971	9.1	72
5	Respiratory diseases	3881	7.1	56
6	Neonatal conditions	2420	4.4	35
7	Digestive diseases	2180	4.0	31
8	Endocrine, blood, immune disorders, diabetes mellitus	1901	3.5	27
9	Neurological and sense organ conditions	1351	2.5	19
10	Genitourinary diseases	1107	2.0	16

Table 1.4 Top ten leading causes of deaths in the World

Cardiovascular diseases now cause more deaths then infectious diseases, but infectious diseases are the leading cause of DALYs globally. The DALYs and deaths as per income regions are depicted in Figure 1.1 and Figure 1.2. We note that as the income increases, the ratio of infectious to non-communicable

diseases is reduced. In low income regions, infections cause more DALYs and deaths than non-communicable diseases even at the present time. In high income regions, only 4% of DALYs are attributed to infections, whereas in low income regions 43% DALYs are caused by infectious diseases.

These figures provide a good estimate of total numbers in the different income groups. They show that the total DALYs due to non-communicable diseases is a large number among all groups. However, the DALYs and deaths due to infections reduce significantly in higher income groups. Another view of same information is given in Figure 1.3, which provides a better view of how the DALYs change due to cause as income increases.

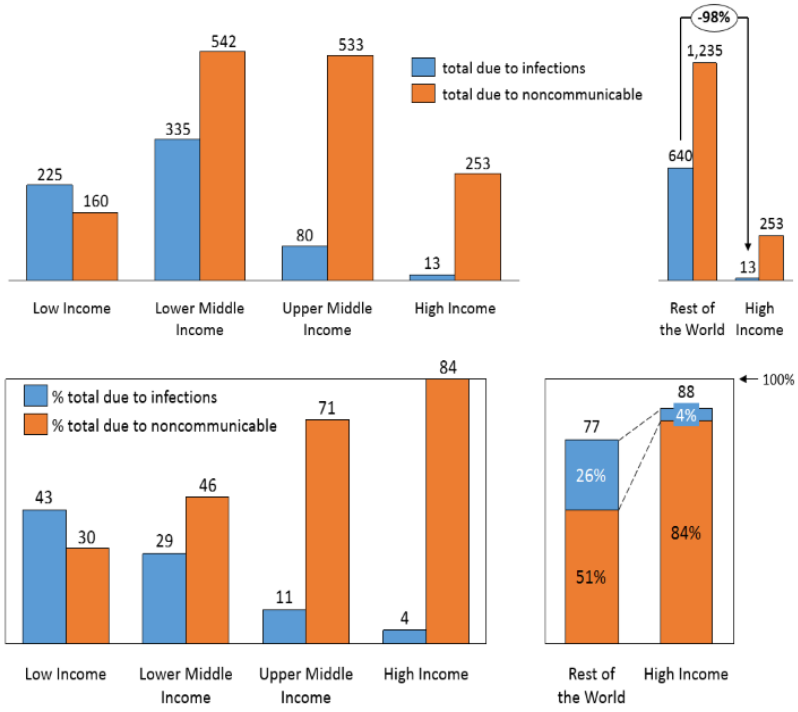

Figure 1.1 DALYs (total and % of total) as per income groups [millions]

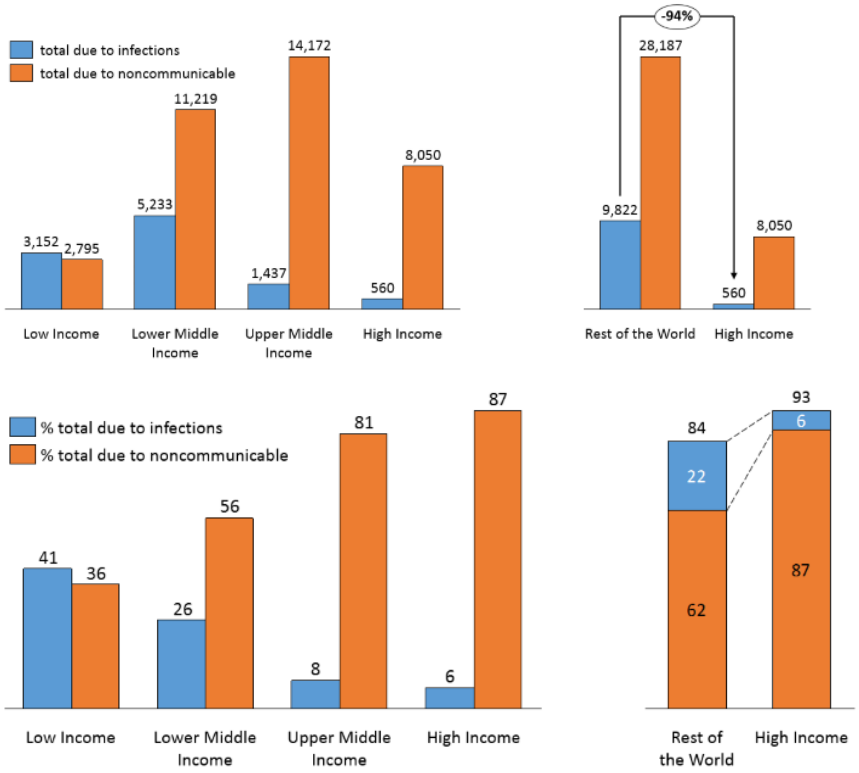

Figure 1.2 Deaths (total and % of total) as per income groups [millions]

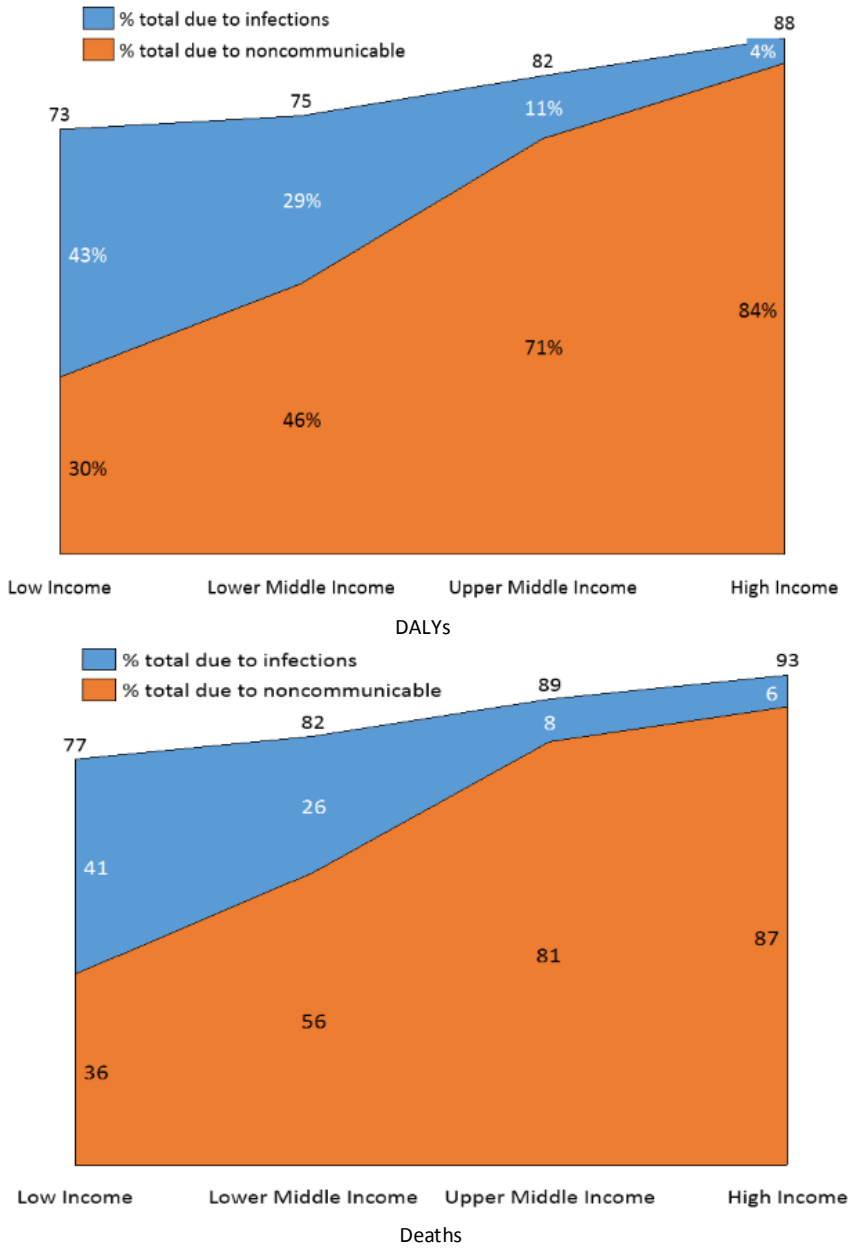

Figure 1.3 % total of DALYs and deaths as per income regions

DALYs and deaths normalized by population are given in Figure 1.4.

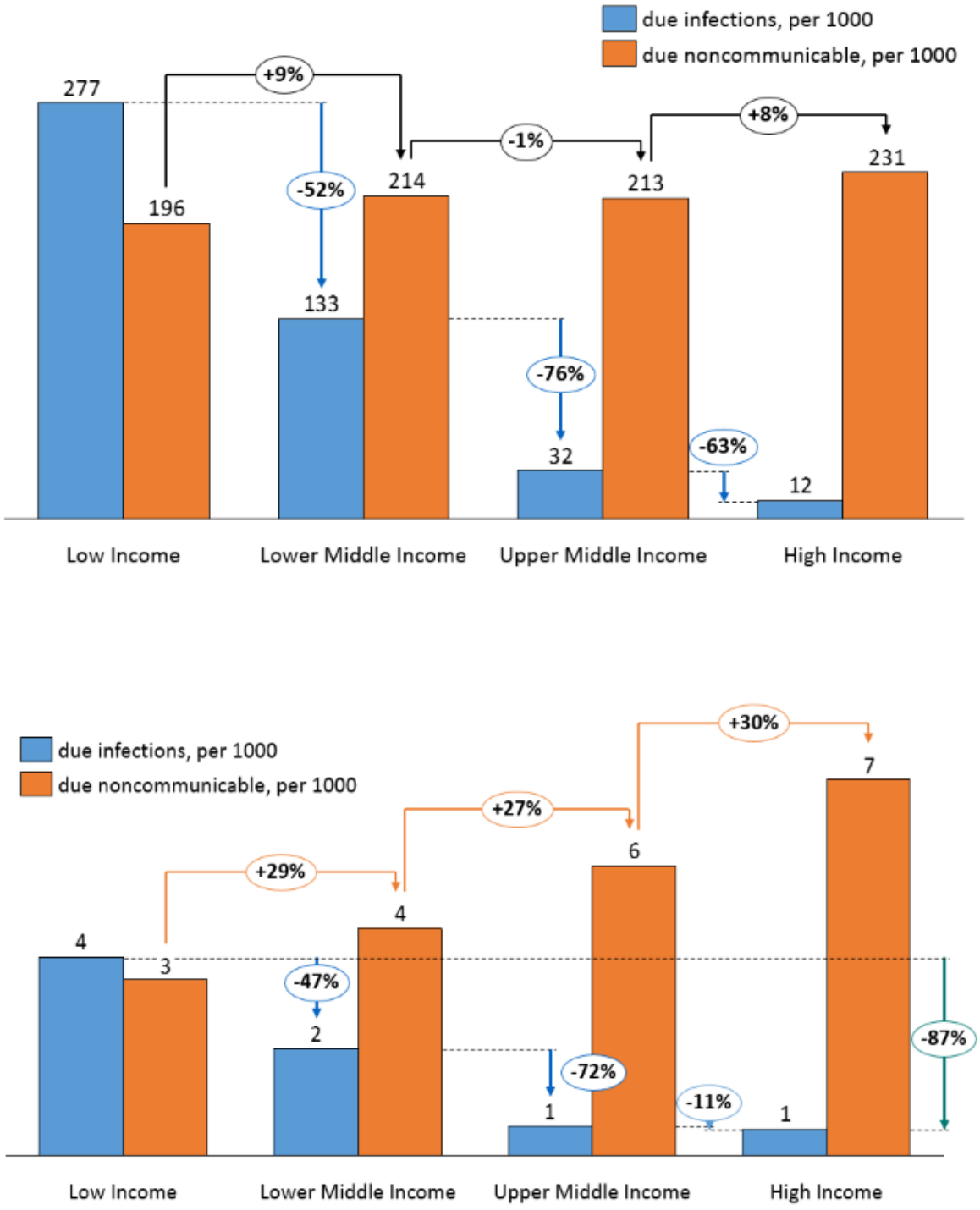

Figure 1.4 DALYs and deaths as per income group [per 1000]

Surprisingly, the DALYs due to noncommunicable diseases does not change much among all income groups! In fact the number of deaths due to noncommunicable diseases increases with income. However there is a marked decrease in both DALYs and deaths due to infectious diseases.

Although the ratio between DALYs caused by non-communicable diseases to DALYs caused by infections is becoming larger globally, there is a large variation of that ratio in different regions and changes significantly when going from low to high income regions.

Not surprisingly, people live longer in high income regions than in lower income regions. The population distribution is depicted in Figure 1.5. We notice that, for lower and lower middle income groups, the population normalized by total in each group reduces as age increase (implying more young people) while the opposite is true in high middle and high income groups. In the >70 age category, 35 percent of these people live in high income regions while only 5% live in low income regions. This shows the importance of careful analysis of the data. Above 70 year-old non-communicable diseases are very likely to be related to aging.

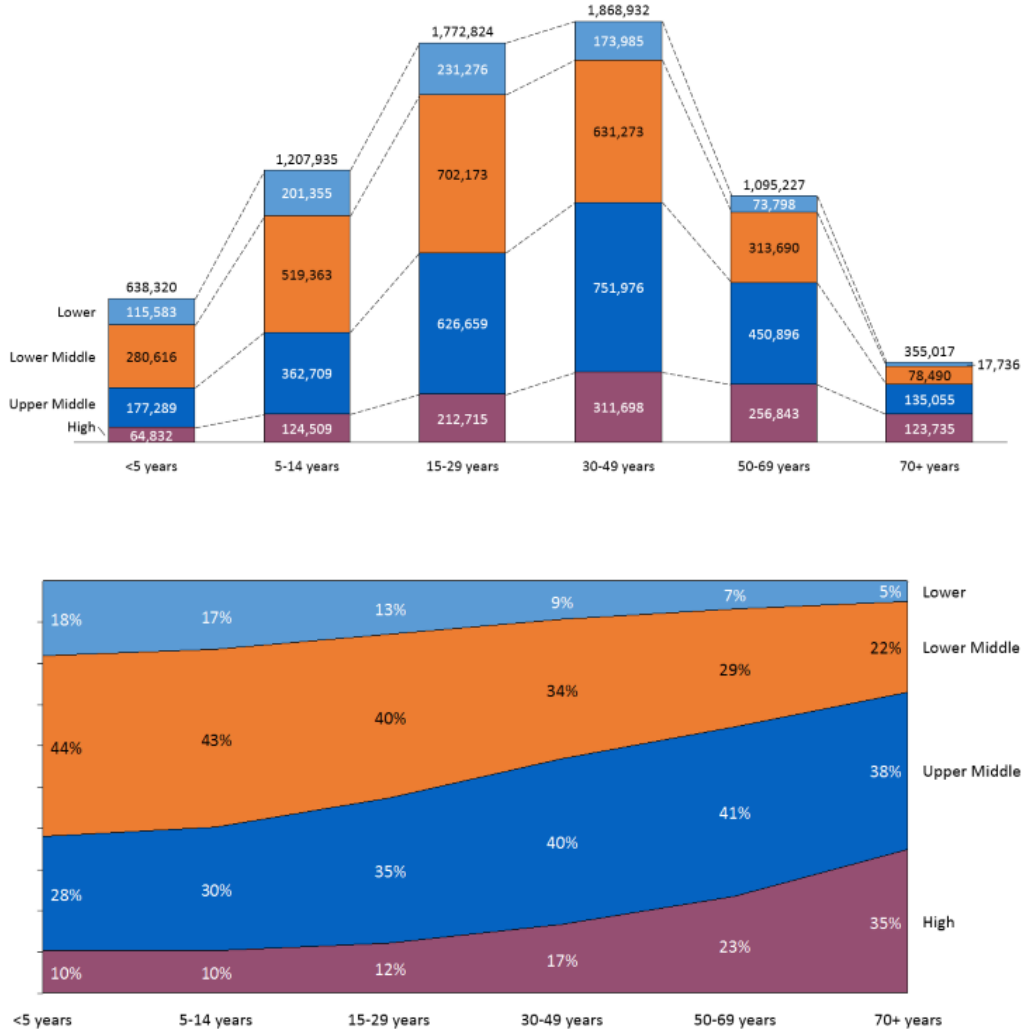

Figure 1.5 Population as per income regions and age groups

We thus establish that the variation in burden of infectious diseases is much greater than that of non-communicable disease. More importantly, we find that the infectious disease burden reduces with income level. This suggests that there is a proven and available methods for reducing the burden of infectious diseases that are available in high income regions and not in lower income settings.

We now focus attention to infectious diseases only. Figure 1.6 shows total and percent total DALYs and deaths within different income regions. We also compare high income (our reference) and rest of the world.

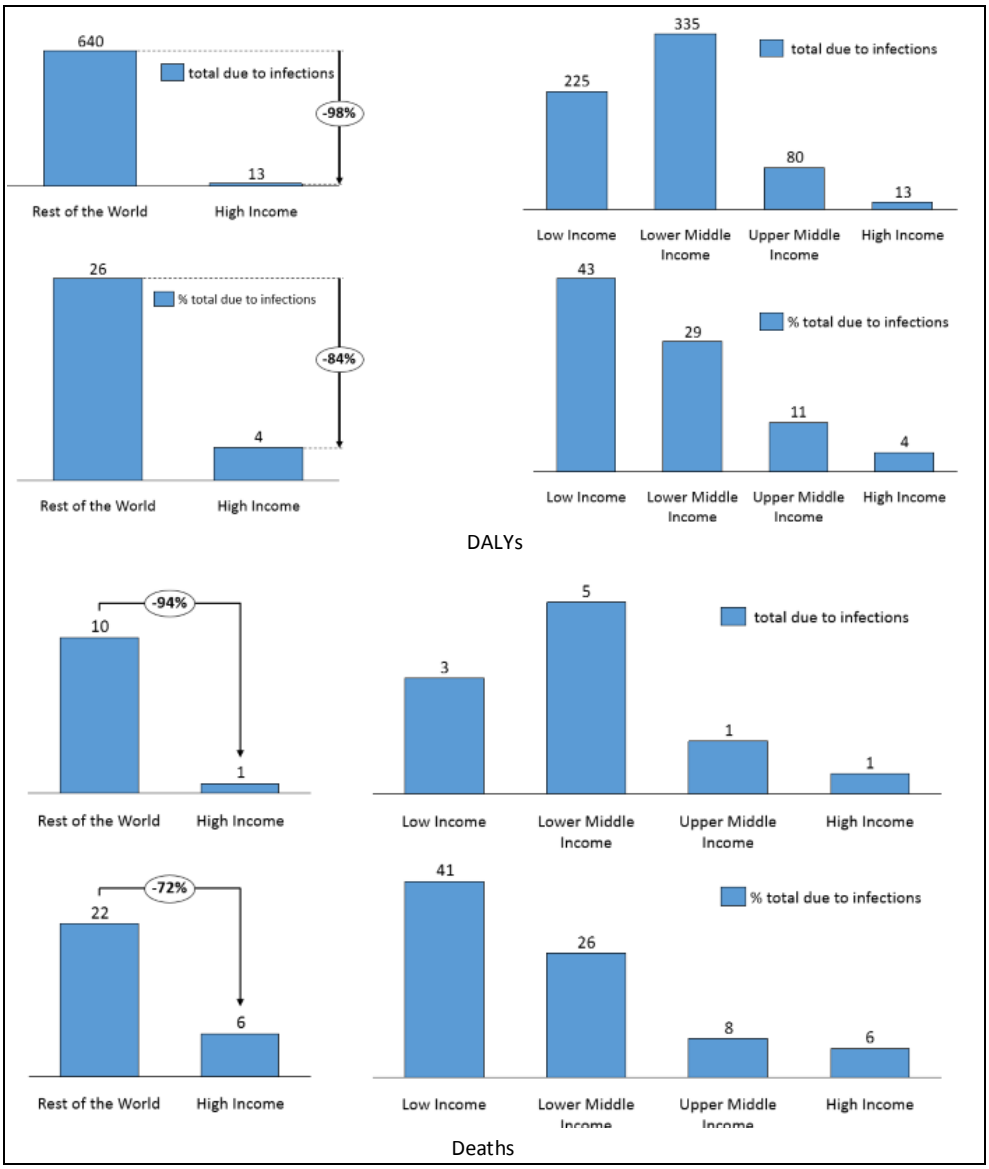

Figure 1.6 Total and % Total DALYs and Deaths due to infections as per income groups

The % total plots show a monotonic decrease in both DALYs and deaths as income levels increase. Also, we note the huge difference in both totals and % totals between high income regions and rest of the world. Next, we determine how the disease burden due to infections is distributed by age groups and income level. DALYs and deaths by income and age groups are depicted in Figure 1.7 and Figure 1.8 respectively. Totals and population normalized data are shown.

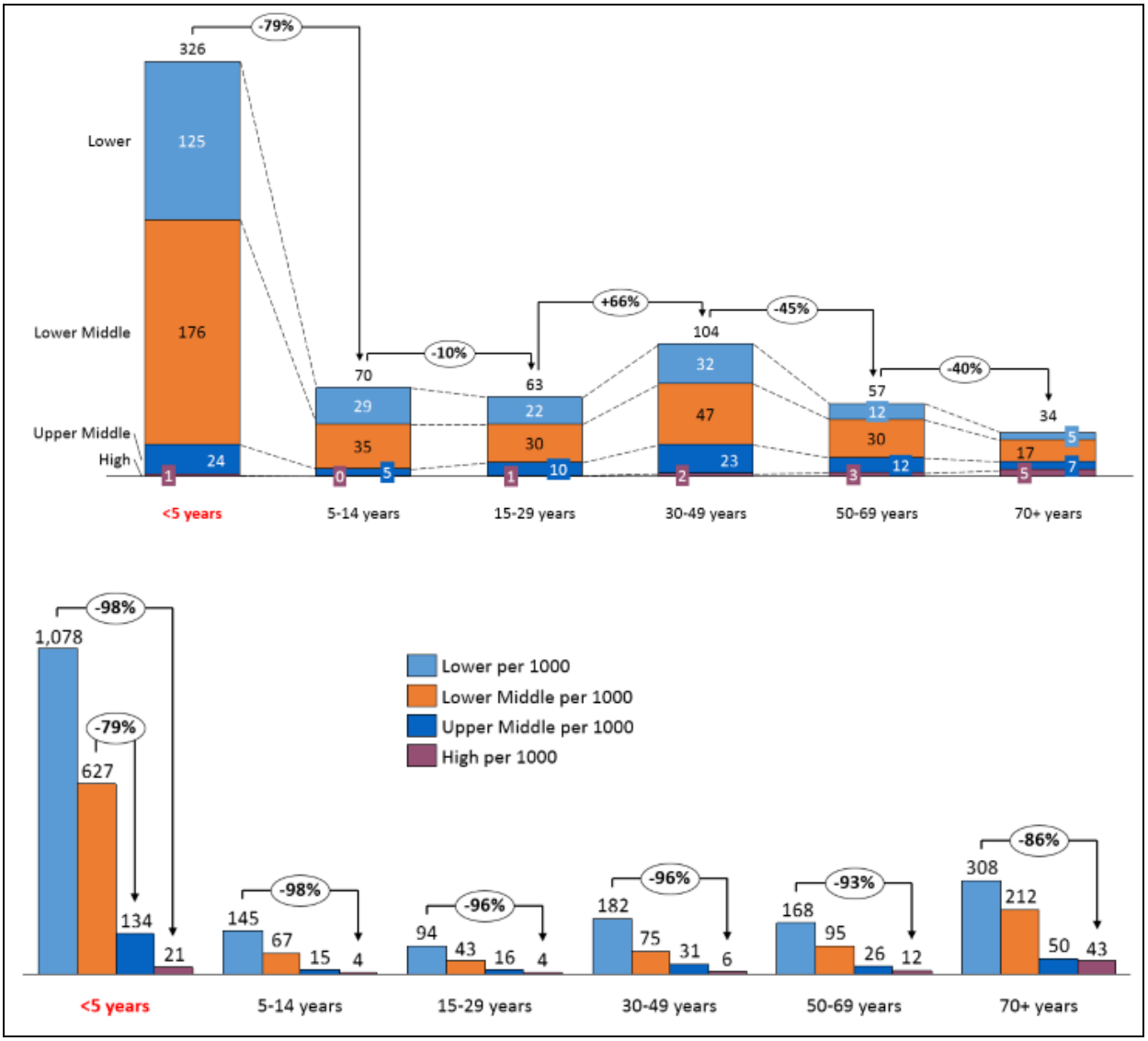

Figure 1.7 DALYs by income levels and age groups

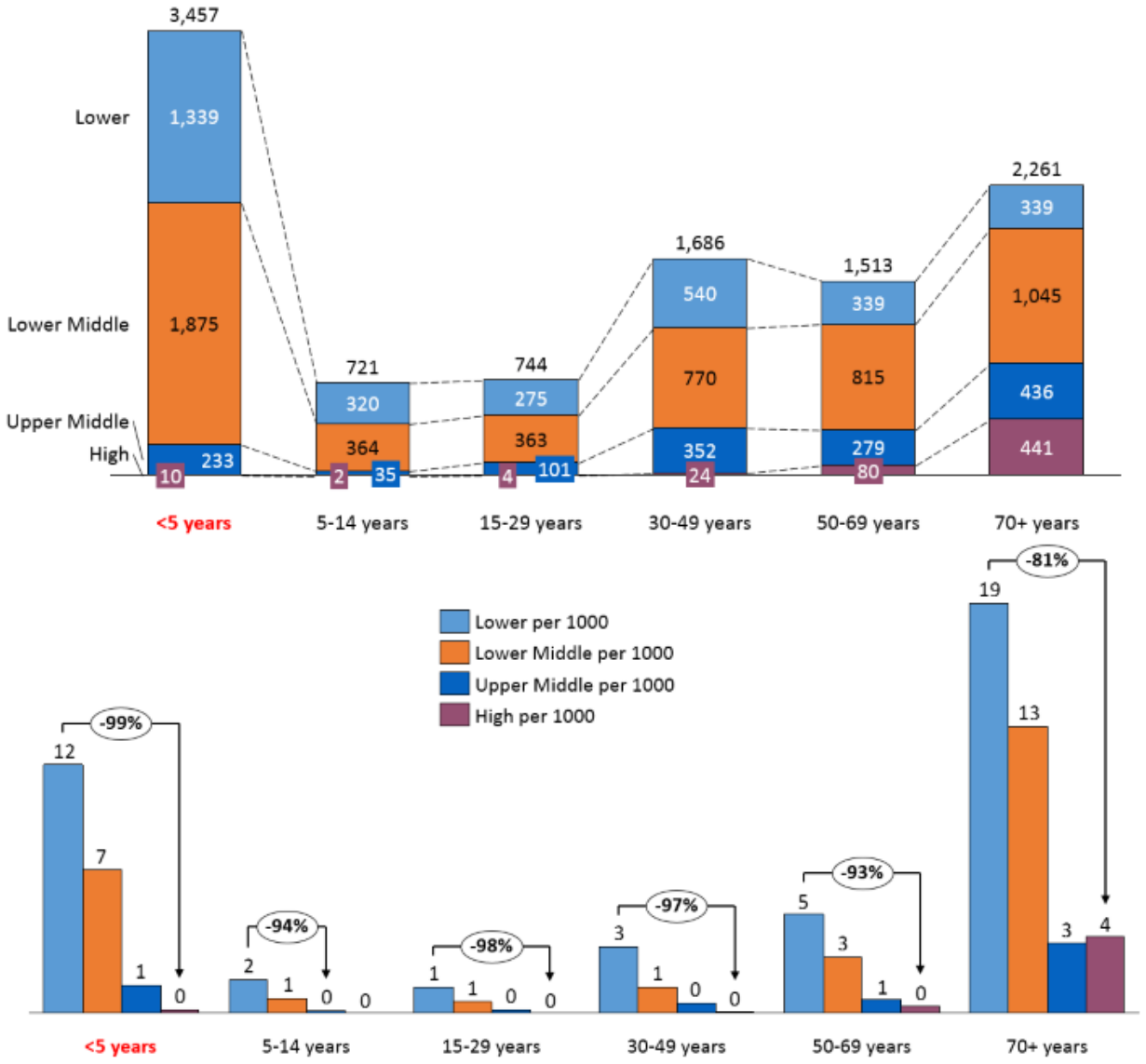

Figure 1.8 Deaths by income level and age group

These figures show that majority of DALYs and deaths occur in the age group of 0-5 year-old children, with a major share from lower income groups. The high income group shows 98% and 99% lower DALY and deaths in such children than the low income group. More children per 1000 under five years of age die than the people above 70 in low and lower middle income groups. We can specifically characterize the effect of top infectious diseases in different income groups, and DALYs and deaths caused by top infectious diseases are depicted in Figure 1.9 and Figure 1.10.

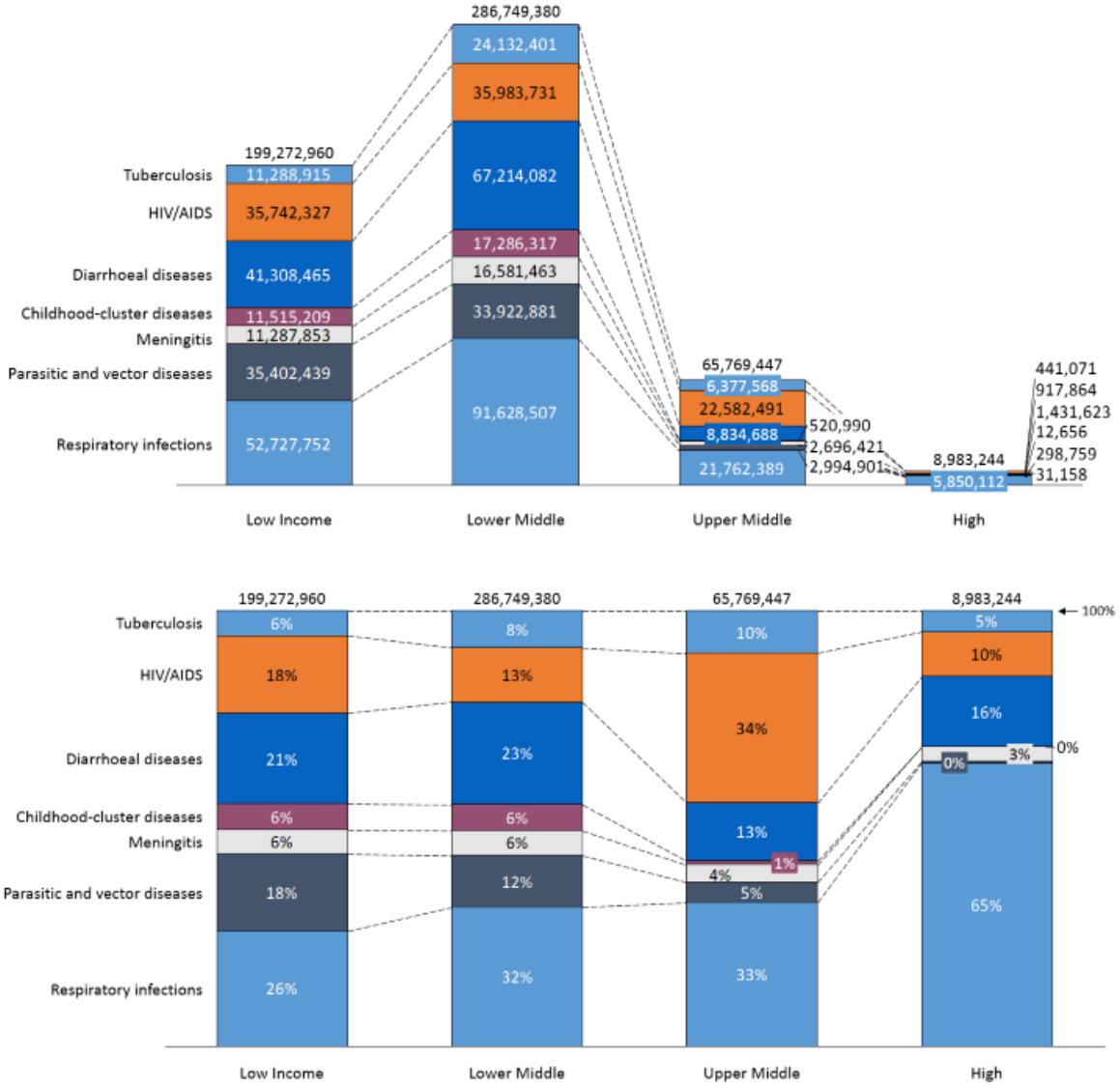

Figure 1.9 DALYs due to top infectious diseases by income region

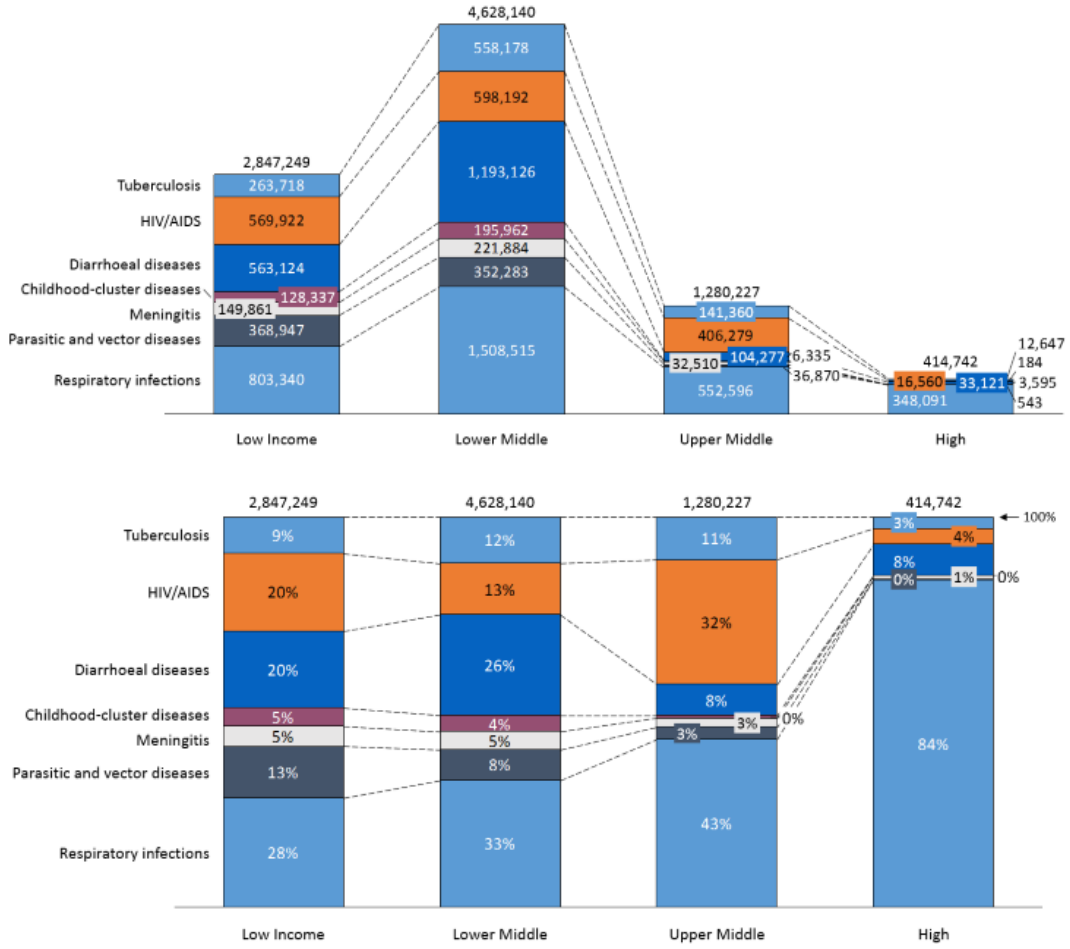

Figure 1.10 Deaths caused by top infectious diseases by income region

HIV/AIDS is one of dominant causes in higher middle income environments, while respiratory infections are major cause for high income groups in addition to low income groups. In all regions, respiratory infections are the major cause of DALYs and deaths. Moreover, diarrhea kills 2,195 children every day—more than AIDS, malaria, and measles combined [8].

1.3 Infectious vs noncommunicable diseases

If we use the high income region's healthcare as a reference of what is technically possible, then it is clear that the largest opportunity for technological impact on overall human health is in infectious diseases and not in non-communicable diseases, as non-communicable diseases are typically harder to prevent or cure than the infectious diseases.

The major non-communicable diseases are Cardiovascular diseases, Respiratory diseases, Malignant neoplasms, Digestive diseases, Musculoskeletal diseases, Mental and behavioral disorders, Congenital anomalies, Genitourinary diseases, Neurological conditions and Diabetes mellitus. The total and % total DALYs and Deaths caused by such non-communicable diseases are depicted in Figure 1.11. The DALYs for non-communicable diseases in high income region are much larger than in other regions. This is partly due to more expense on healthcare, which allows people with certain conditions to live longer. Note that although total DALYs are higher in high income regions, the death rates are lower. Thus, more people are living with disability in high income regions. It also shows that even high income regions have not been able to reduce DALYs in these kinds of diseases like in the case of infectious diseases.

The **causes** of many top non-communicable diseases, such as cancers and diabetes, are not exactly known. There are various risk factors associated with, for example, cardiovascular disease, but the onset of disease occurs statistically with age. Various non-communicable diseases are distributed towards higher ages and thus may be imminent. In contrast, many communicable diseases are preventable. One can say that non-communicable diseases do not affect other populations the way communicable diseases do. For example, a heat patient cannot infect another person, and the disease is more localized to each individual.

From reference [9] we quote “However, all of the most common causes of NCD death—heart disease, cancer, stroke, chronic obstructive pulmonary disease (COPD), and diabetes— are linked to unhealthy behaviors such as tobacco use, physical inactivity, overconsumption of alcohol, and diets that are low in fruits and vegetables and high in fats”. Therefore NCDs require social and behavioral changes in addition to technology[10]. The **importance of knowing disease** presence is very important for both types of diseases. Various cancers, if diagnosed early can be treated. However, if non-communicable diseases are not diagnosed in time the damage occurs to only the patient. In case of communicable diseases, knowing the disease can prevent its spread by taking precautions even for diseases that don’t have a cure (e.g. HIV). Both communicable and non-communicable diseases can stay in body for long times without much symptoms but the damage potential of communicable diseases is high because even in this state, these can be transmitted to other people.

Many of the non-communicable diseases are also much more **difficult to cure and treat**. For example, cardiovascular diseases might require surgery, which is much more involved than providing vaccination or medication in case of infectious diseases. Surprisingly, infectious disease which could be cured by a pill might kill a person faster than cardiovascular diseases if left untreated. Except for some diseases like

HIV, many infectious diseases are inexpensive to treat if the disease is known in time, and it is much easier to provide a drug than perform surgery. However, for some communicable diseases such as Hepatitis in advanced stage, liver transplants may be required which is a very involved operation. Even then, many of the leading infectious diseases, such as Malaria, TB and others, are relatively easy to treat.

The resources needed to cure, treat and manage non-communicable diseases are much more than for most infections. This is the reason that infections have much less burden in high income countries. Even with resources, non-communicable diseases remain a big problem. Thus, the "low-hanging fruit" is to reduce global disease burden by reducing infectious disease burden in in low income areas, as the resources required for this cause should be much lower than treatment of non-communicable diseases.

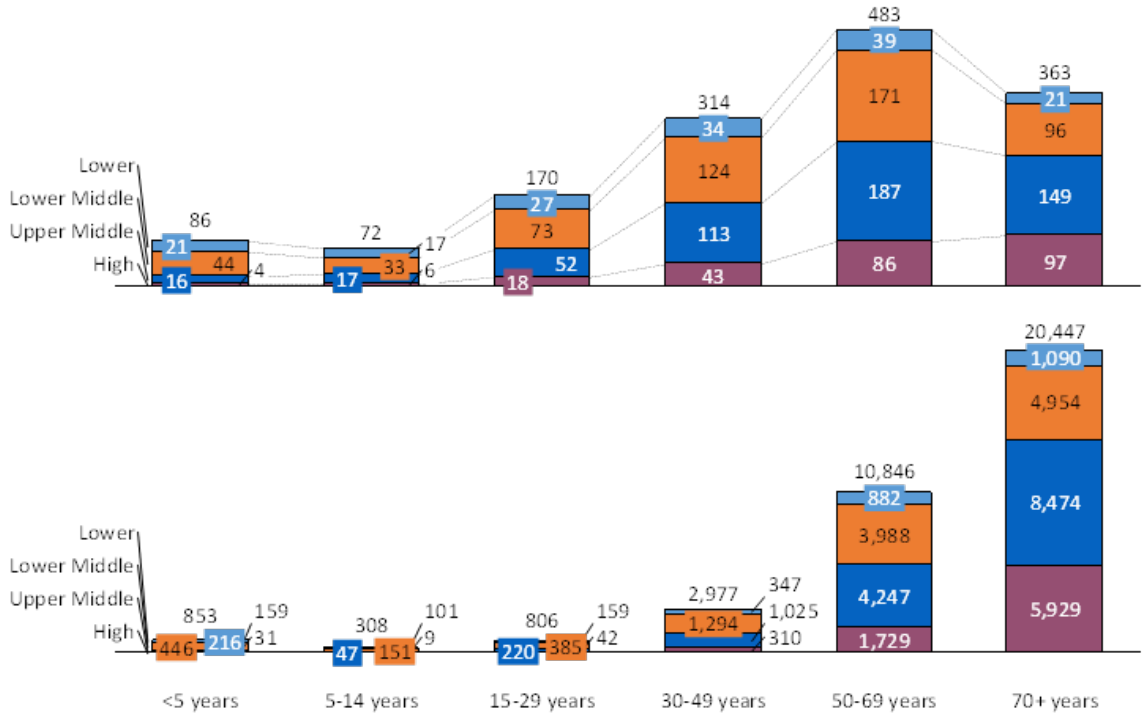

Figure 1.11 DALYs (millions) and Deaths (thousands) due to noncommunicable diseases

1.4 Importance of diagnostics

Now that we have identified the possibility of making an impact by reducing DALYs and deaths due to infectious diseases, we should consider various ways to achieve this goal. Technologies which can help developing world healthcare have been considered before [11], and diagnostics was ranked as the

number one biotechnology to have an impact – even higher than vaccine development. The top ten technology choices are listed in [11].

Table 1 • The top ten biotechnologies with scores based on rankings of the expert panel

Final ranking	Biotechnology	Final score
1	Modified molecular technologies for affordable, simple diagnosis of infectious diseases	288
2	Recombinant technologies to develop vaccines against infectious diseases	262
3	Technologies for more efficient drug and vaccine delivery systems	245
4	Technologies for environmental improvement (sanitation, clean water, bioremediation)	193
5	Sequencing pathogen genomes to understand their biology and to identify new antimicrobials	180
6	Female-controlled protection against sexually transmitted diseases, both with and without contraceptive effect	171
7	Bioinformatics to identify drug targets and to examine pathogen–host interactions	168
8	Genetically modified crops with increased nutrients to counter specific deficiencies	159
9	Recombinant technology to make therapeutic products (for example, insulin, interferons) more affordable	155
10	Combinatorial chemistry for drug discovery	129

Table 1.5 Top ten biotechnologies for developing world

Diagnosing disease has benefits of easier treatment at earlier stage, and can limit the spread of disease with a significant impact on disease burden. Various organizations, notably BMGF (Bill and Melinda Gates Foundation), have therefore placed an emphasis on the development of technologies for diagnostics in low resource settings. In a series of papers [12] [13] [14] [15] [16] [17] [18], they have estimated the massive impact of improved diagnostics in reducing the burden of infectious diseases especially targeted towards developing world. Another estimate for the role of improved diagnostics in given in [19] by Rand Corporation.

Appropriate diagnostics can save millions of DALYs. Figure 1.12 shows an estimate of DALYs which can be saved by diagnostics for few diseases[20].

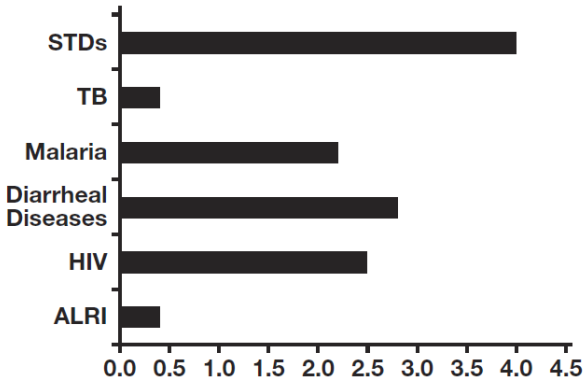

Figure 1.12 DALYs which can be saved with appropriate diagnostics

1.5 Other benefits of Dx at POC

There are many other benefits of diagnosing disease with confidence. Even ruling out certain candidate diseases is of great advantage, as appropriate diagnostics can save a large number of unnecessary resources spent on inappropriate treatment **[21]**, which can also lead to antibiotic resistance worldwide. Figure 1.13 shows unnecessary treatments in case of Pneumonia in the developing world.

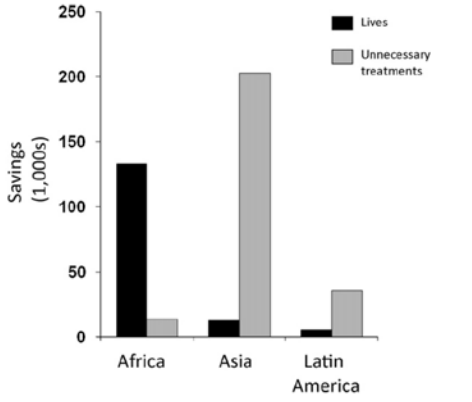

Fig. 1. Benefits of a new test for bacterial pneumonia in developing countries predicted by modeling the benefits of a new test for bacterial pneumonia for children under 5 (Girosi et al., 2006). The model assumes a population of 535 million children (based on 2004 estimates) and that each child has 5–6 acute respiratory tract infections a year. Black bars indicated lives saved from reducing disease burden and grey bars refer to unnecessary treatments saved.

Figure 1.13 Unnecessary Treatments

Antimicrobial resistance is now declared as a serious threat to public health by WHO[22]. A global report has been published recently in this regard [23].

Diagnosing at the right time has associated benefits in terms of economics – as many of these diseases are a result as well as cause of poverty. Democratizing healthcare therefore has many social, cultural and emotional benefits [24].

1.6 Importance of Nucleic Acid Testing and qPCR

Molecular diagnostics is a key segment of the diagnostic technology and is the technology which can have the biggest impact [11]. In this review of commercialization of microfluidic point of care devices [25], major technologies are compared for diagnostics. POC diagnostics from a drop of blood has been reviewed recently [26]. Point of care diagnostics technologies are covered in various papers [27-30].

Molecular diagnostics is a key segment of the diagnostic technology and is the technology which can have the largest impact [**11**]. In a review of commercialization of microfluidic point of care devices [25], major technologies for diagnostics are compared. Point of Care POC diagnostics from a drop of blood has been reviewed recently [26] and technologies are covered in various papers [27-30].

Diagnostics for detection of infectious disease is typically performed via bacterial or viral culture, Immunoassays and PCR bases assays. PCR based methods typically detect the RNA or DNA signatures of the pathogen. They can identify specific sequence variations and therefore the exact strain causing a disease and are specific and most sensitive. PCR based methods, especially quantitative PCR (qPCR), provide quantitative information which is useful in knowing the bacterial or viral load or to monitor therapy effect[31]. Another advantage of Nucleic acid tests is the longer life time of DNA. If samples are being collected to be transported to another place for running these tests, the lifetime of biomolecules becomes important. Typically, proteins degrade quickly and many immunoassays are not feasible on transported samples. However, nucleic acids can survive well, even on paper and paraffin samples. In fact, tissue samples can be transported and analyzed at another location. Due to its sensitivity, PCR can ideally detect a single nucleic acid molecule. This is very useful in early detection of disease or for screening. At the present time, PCR based methods are the gold standard for molecular diagnostics of infectious diseases and are the tests which one would ideally like to have.

Why Automation is Necessary

Automation is necessary in both developing and developed world. For the developing world, top reasons for automation including the shortage of trained manpower, Human error and chances of corruption, whereas in the developed world, a different set of limitations prevails. Typically, a shortage of physicians, a high cost of labor, human error, and making available relatively complex tests at POC (or pharmacy level) which need CLIA/FDA approval provide advantages for automated systems.

1.7 Need for New Technologies and Products

Products designed for the developed world or focused on developed world markets are not necessarily the best diagnostic tools for the developing world. Mature developed world medical instruments need to conform to complex regulatory guidelines, resulting from bureaucratic limitations and are focused on meeting reimbursement strategies that do not apply within developing world settings, where transportation, logistics and infrastructure are lacking. Many design options cannot be used in the

developing world, as complex instruments feasible for developed world laboratory applications might not meet the harsh environmental conditions encountered in the developing world. Conversely, designs rejected for developed world can be used in developing world. For example, there is no CLIA waived PCR test in USA to be used in clinics as commercial and political considerations presently do not favor the re-adjustment of tasks between physicians and registered nurses in diagnostic settings.

Diagnostics in the developing world clearly demands development of robust instruments that can survive harsh environment of low resource settings. However, unless a product is developed 'solely' for the developing world, it may not have much chance to make a major impact there. An entity producing and selling in the developing world will have much better understanding of the market. In this case incentives are also tied to the targeted market instead of using that market only as a stepping stone with little if any real interest to support a product in long term. More funding should also be dedicated for diagnostics technology. Figure 1.14 Money spent for development of technologies, and shows the distribution of funding for some major infectious diseases.

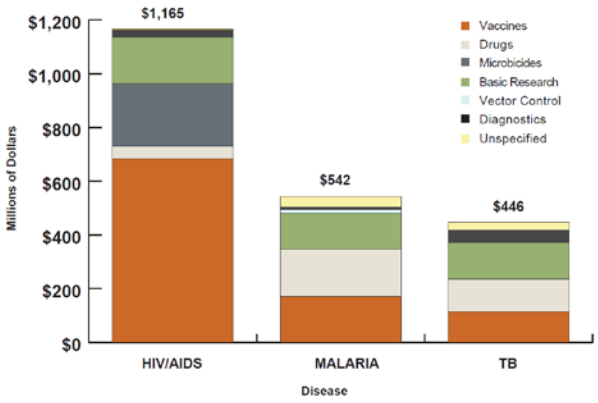

Figure 1.14 Money spent for development of technologies

1.8 Problems with Typical Commercialization

I believe that this thesis provides a very promising approach to low cost technology which can allow availability of high quality diagnostic in every part of the world. However, I learnt that the business models and practices are also very important to keep the cost low and availability high.

The usual approaches to commercialization will increase the cost and prevent spread of the technology due to commercial interests of some individuals. Owing to personal experience, I found various potential problems. Some executives think developing world as very low profit margin and hence not of sufficient interest for investors. Consultants in business planning have various problems with making costs lower. They mention that ministries of different countries are in contact and making cost low in one can make it difficult to sell for high cost in a richer country. Therefore, if a company with such executives has rights to such low cost technology for global operations than there can be serious problems. The typical business thinking will not like cost in even most poor countries to go too low. Even worse is that the company would actually not want to introduce products in such areas since they are both low margin AND can cause problems for charging high profits in other regions. Many of the executives are actually interested in getting acquired more than anything and operations in poor countries at low cost can cause problems in their actual goals.

1.9 Availability and Cost of Medicine

Typically, medicine is not expensive by nature. Many infectious diseases can be treated by drugs available at low cost. Medicine is becoming available for more infectious diseases such as HCV. However, new drugs can be very expensive. As a recent example, the Hepatitis pills Sovaldi from Gilead Sciences could cure Hepatitis in tens of millions of people, yet this drug is extremely expensive right now. It is very surprising to compare the expected cost of a drug that can be produced by a generic manufacturer at $1 per day for a patient to the price demanded by Sovaldi, which is $1000 per day. This constitutes a one hundred thousand percent difference in price versus a generic drug price. We can only hope that, eventually, generic drug manufacturers will find a way to reduce medication costs for developing world. There already have been very public international confrontations over pharmaceutical pricing in the case of HIV. Even with lower cost medicine, however, the diagnostics and screening is of paramount importance, and early detection can increase the effect of available medicines.

1.10 Accuracy of Disease burden Studies

Unfortunately, the studies to estimate disease burden seem to underestimate effects of disease. There are regions where epidemiology is not known. Very poor places have less reporting for diseases, and a

very recent study estimated the cases of new TB cases in children to be double than what was estimated earlier[32].

1.11 Expected Impact of POC Diagnostics

The real impact of our technology, if properly implemented, can be much greater than what is estimated. If a sample to answer qPCR instrument can be developed so that it costs less than $300, then many individual physicians can and will purchase it. Accurate diagnostics adds value to clinical practice. If such an instrument can be placed in every village and is as easy to use as a commercial consumer electronics instrument, then the impact can be even greater. Functional prototypes will enable pathologists all over the world to be able to identify many additional uses and add many more places for this technology to be clinically applied. The associated software can be used for many other purposes including patient registration, epidemiology, education and tracking of patients.

1.12 Economic growth is not enough to make the impact

Economic growth of a region or country is important for good health. However, within low-income and middle-income countries, macroeconomic growth is not sufficient to make an impact. It has been shown in large study that economic growth has a quantitatively weak impact on early childhood under-nutrition [33]. Thus efforts have to be made to really target the populations which suffer due to non-uniform distribution of wealth and resources. Intertwined global factors have to be addressed to deliver good health to most vulnerable sectors of society[34].

1.13 Political Domains contributing to health inequity within populations

Following are the political domains suggested that contribute to inequity[34]. According to authors, the arrangements to improve health are termed inherently political and for power and elites[35].

a. Finance
b. Intellectual property
c. Trade and investment treaties
d. Food
e. Corporate activity

f. Migration
g. Armed conflict

2 Target Product Profiles

2.1 Introduction

Now that we have identified the importance and possible impact of POC Dx, we have to define the requirements for our product. This is a complex job due to various factors[36]. First, the medical market is heavily regulated and it is not as easy to introduce new ideas and products into this market as in other consumer fields. This has caused a bias or predisposed beliefs about new technologies and methods. Many professional pathologists cannot believe that qPCR machines can cost less than $500, as we claim. There are other forces which want to maintain that misconception for their own commercial interests[37]. It is therefore necessary to challenge many of these misconceptions and to develop new engineering solutions through experience and intuition in determining what is possible and what should and can be done.

The US market is well understood and POC tests are targeted towards physician offices or at most to be placed in pharmacies. The statistics of such places are shown in Table 2.1. Although the number of pharmacies are much fewer than those of physician offices, the number of tests (and the business revenue) from these places can be significant due to customer base, and supermarket pharmacies are emerging in an attempt to cater to the customer needs for a one-stop shopping experience. However, there is no true portable rugged POC place in sight for companies developing molecular pathology instruments targeting this market. Moreover, the need for a skilled laboratory technician to prepare samples for such tests precludes the state-of-the-art instruments from being located anywhere except for centralized health centers and larger laboratories.

Type Of Laboratory	Number	Percent
Ambulance	3,894	1.59%
Ambulatory Surgery Center	5,517	2.25%
Ancillary Test Site	3,155	1.29%
Assisted Living Facility	2,551	1.04%
Blood Banks	449	0.18%
Community Clinic	6,475	2.65%
Comprehensive Outpatient Rehab	501	0.20%
End Stage Renal Disease Dialysis	5,654	2.31%
Federally Qualified Health Center	1,718	0.70%
Health Fair	663	0.27%
Health Maintenance Organization	654	0.27%
Home Health Agency	14,648	5.99%
Hospice	3,188	1.30%
Hospital	8,914	3.64%
Independent	5,856	2.39%
Industrial	1,786	0.73%
Insurance	49	0.02%
Intermediate Care Facility for Individuals with Intellectual Disabilities	1,282	0.52%
Mobile Laboratory	1,503	0.61%
Pharmacy	***9,740***	***3.98%***
Physician Office	***120,399***	***49.33%***
Other Practitioner	3,738	1.53%
Prison	404	0.17%
Public Health Laboratory	678	0.28%
Rural Health Clinic	1,774	0.73%
School/Student Health Service	2,098	0.86%
Skilled Nursing/Nursing Facility	14,949	6.11%
Tissue Bank/Repositories	61	0.02%
Other	22,266	9.10%
Total	**244,564**	100%

Table 2.1 Laboratory Types in US

The use of such instruments in the developing world is even more complex. In particular, the extreme variety of applications, diseases[38], placements, users[39], operators, geographical places and environments in which these instruments are supposed to be used[40] provides us with a formidable challenge. Epidemiology and other basic health facts are not known for large areas, and the placement of such instruments is envisaged to be at various levels as given in Figure 2.1. However, there are many other places where such systems are needed. For example, it should be possible to carry this system from home to home (or cottage to cottage) in many parts of the world, where the infection risk is high and travel difficult for patients. There are areas and diseases where this will be very beneficial. An

example is Leishmania in poor populations of India. An application like this is termed ‘Level 0’ or ‘Under the Tree’ health level by Gates Foundation.

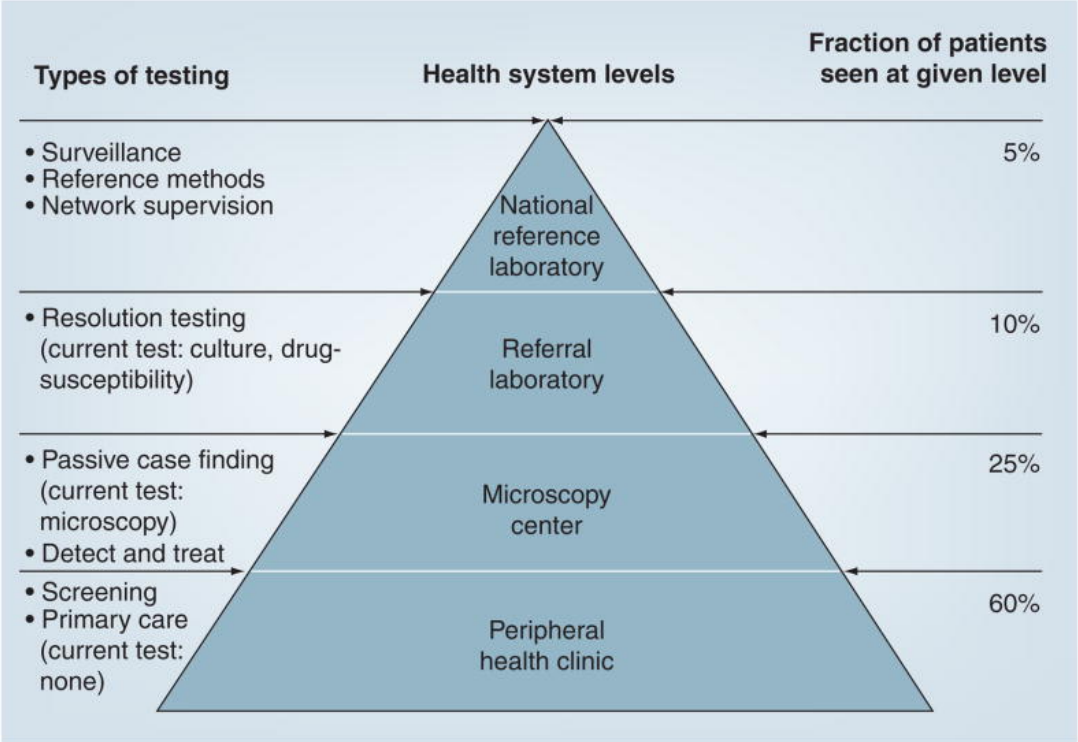

Figure 2.1 Health Level Systems [41]

It is suggested that a separate TPP might be required at the different levels of healthcare [40] depicted in Figure 2.2. The problem with this approach is linked to the traditional belief that complex nucleic acid amplification tests such as qPCR are not feasible at lower healthcare levels. Separate TPPs also imply that different healthcare levels need different diagnostic procedures and techniques and that high quality test may be screened out from the start to be used at the lower healthcare levels. For example, in Figure 2.2 qPCR based sample to answer tests are shown only in hospitals. This opinion is largely biased on past experience and technology available now. We must acknowledge that the best diagnostics tests are indeed needed at lowest health levels and this is what is actually required. Acknowledgement of this fact should be part of TPP even if TPP calls for lower standards due to development time and current technology. This will allow the developers and researchers not to loose sight of the ideal end goal.

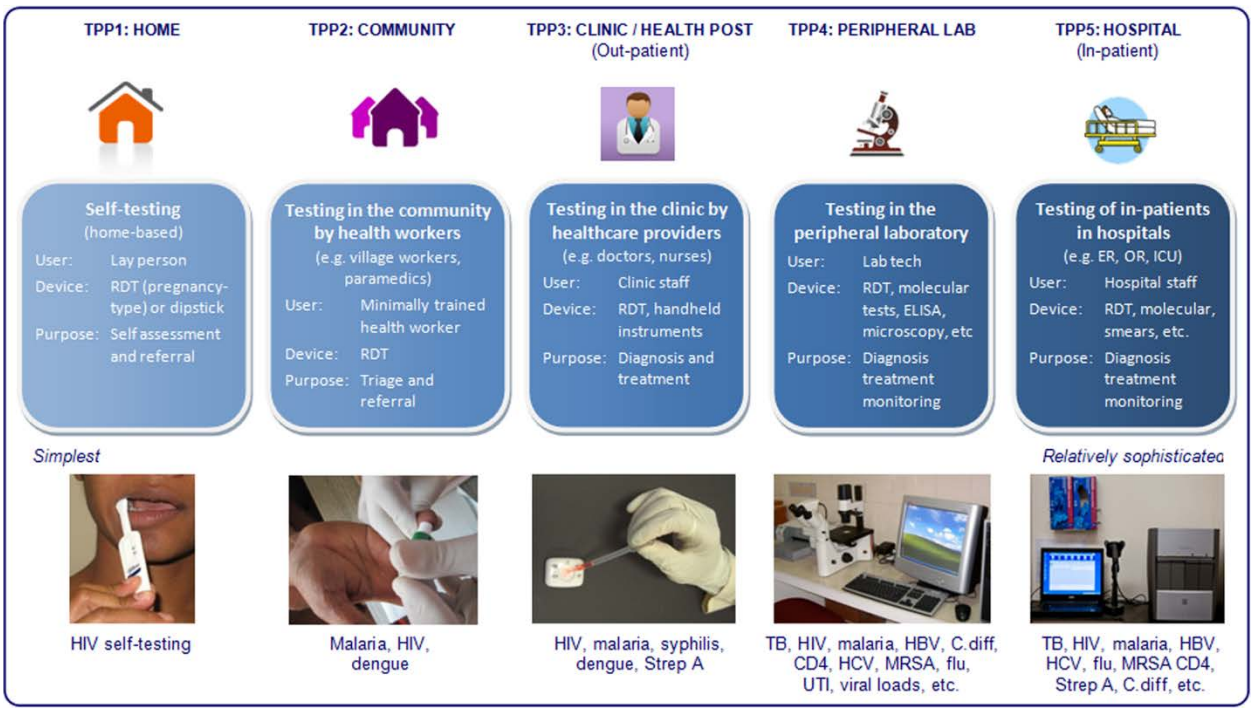

Figure 2.2 Various TPPs for Different Health Care Levels

Segregating the TPPs too much also makes a disruptive technology less applicable to many levels. It is our view that the lowest health level should be targeted from early on. The technology and instruments for such conditions [42] would have a high chance to be modified for upper health levels. Upper health levels typically would require more throughput but this can be achieved if the system is designed to be scalable by adding more random access modules. Developing for most health levels will also save reworks, modifications and duplication of effort. It should be much more economical in the long run.

2.2 Existing TPPs

With a foresight to challenge and add to any of the existing TPPs – their study is indeed a very useful starting point and reference. These have been prepared by people experienced in the field and after reviews by professional. Some have been updating for a long time.

The characteristics of ideal tests are often referred to as defined by WHO (World Health Organization). The acronym ASSURED [43] is well known. In fact this is a very concise set of requirements as give in **Error! Not a valid bookmark self-reference.**.

Table 2.2 WHO ASSURED for High Impact Diagnostics

ASSURED

Affordable by those at risk of infection
Sensitive (few false-negative results)
Specific (few false-positive results)
User-friendly (simple to perform by persons with minimal training)
Rapid treatment at the first visit and robust use without the need for special reagents
Equipment free (no large electricity-dependent instrument; portable handheld battery-operated devices are acceptable)
Delivered to those who need it

The Gates Foundation also performed a major effort to outline specifications of the desired diagnostic technology [42] and have funded various groups for POC Diagnostics over the past decade. The current TPP is now targeted to a level 1 system (community health clinics) with higher throughput. Initially the target seemed to be Level 0. The TPP is changing with time and is dependent on many parameters in addition to technology. Patents and intellectual property are also factors. PCR is attractive today since patents covering this technology are expiring soon (compared to isothermal amplification techniques). This TPP is unique in the sense that it's targeting a universal diagnostic platform (which they call PanDx). Gates Foundation envisages this as a platform to which assays can be added by multiple parties in future. As the cost of the envisioned platform is rather high ($5000-$15,000) the platform should also support different techniques such as cell counting and ELISA in addition to PCR. The idea is that by providing a large base of such instruments and engaging diagnostic instrument companies, there will be enough interest in developing and producing cartridges for different assays. A concept model is given in Figure 2.3.

Having a universal instrument for all cartridges (current and future) is technically very difficult to accomplish. Standardized interfaces between cartridge and instrument are tricky to define due to different domains (optical, mechanical, electrochemical, pneumatic and thermal) involved, as shown in Figure 2.5. As a result of the complex mechanical design, the large number of different elements and features, such an approach can severely limit innovation in cartridge design. From our experience, some innovations require tightly coupled instrument and cartridge functionality which is not possible when

many interfaces are fixed. For example if the optics in fixed in the instrument, new and novel optical concepts may become incompatible with the system.

Figure 2.3 PanDx Concept Model

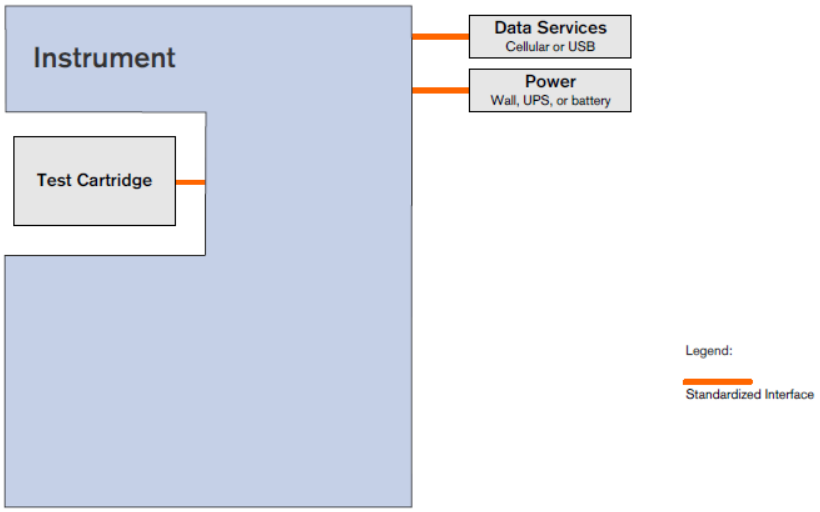

Figure 2.4 Concept developed by Stratos and Halteres for Gates Foundation

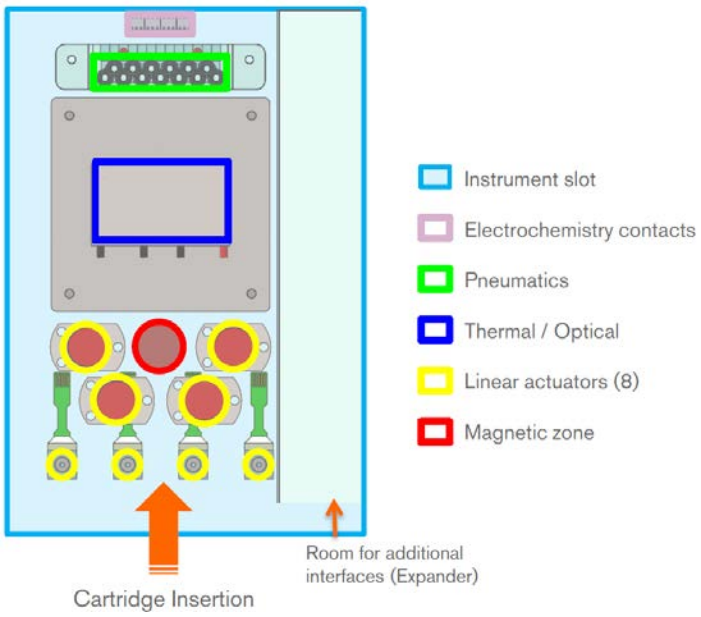

Figure 2.5 Instrument-Cartridge Interface

Fortunately, disruptive simple ideas can provide an alternative and modify the TPP. As a result of earlier experience [44], the Gates Foundation did not invite proposals using PCR amplification since thermal cycling is typically very power hungry and slow. We proposed a simple solution to the problem by using our electromechanical relay approach. A description of the requirements for diagnostic instruments deployable in the developing world, which includes our work as an example, is covered by [20]. A recent review suggesting additional criteria for point of care diagnostic tests for developing world is given in [45]. Further requirements are also discussed in literature[46]. The suggested revised criteria for ideal POC test in resource limited setting is shown in Table 2.3, and the corresponding paper also lists the diseases for which POC tests are available, which is a surprisingly small set.

Table 2.3 Suggested revised criteria for an ideal diagnostic point-of-care test in low resource settings

SUGGESTED REVISED CRITERIA FOR AN IDEAL DIAGNOSTIC POINT-OF-CARE TEST IN RESOURCE-LIMITED SETTINGS
Allows a quick clinical decision
Can be used at the clinical point-of-care by health workers
Affordable (low average cost per test)
Rapid (provides result during a clinic visit or within a reasonable waiting time)
Acceptable test efficacy (likelihood ratio times patient notification rate)

Cost effective

Another useful TPP is provided in [41] developed by PATH. This TPP is more focused on level 0. It also signifies the role of the disease targeted. This TPP is for TB where the idea is to treat as close to the patient residence as possible. TB spreads by aerosols and only 1-10 organisms are sufficient to cause infection. If people have to travel long distances, they can infect other passengers along the way. The nature of transport in developing world is different. Buses can be overloaded and the disease can spread during travel.

Bio Ventures focuses on point of care diagnostics for differential diagnosis of fever in children[47]. They have taken a well thought of incentive drive program approach (milestone based prize) to have a sustainable impact. The TPP is targeting Malaria, Bacterial pneumonia, biomarkers, TB and HIV. This TPP asks for a multiplex assay for a particular population. TPPs for neglected Tropical diseases is given in [48]. They consider mapping and surveillance.

Comments

All TPPs specify a completely integrated sample to answer system with minimum chance of human error. They have different focus on diseases and population groups but a properly designed product can be very close to satisfy all TPPs.

Another important fact is allowance of more options as a result of not targeting FDA approval. In US Cell phones most probably not be allowed as a device interface. However, cell phone is the only connectivity available in some places. Having an alternate way to evaluate products is very attractive and can also save huge amount which would otherwise be spent on FDA approvals. It's also very difficult to test for many global diseases in US due to their non-prevalence. For example, the United States has very small case numbers of TB, Malaria or Dengue.

In fact, Bio Ventures for Global Health [47] has put clinical studies at the actual usage sites and added CE approval in the plan. There is no mention of FDA approval. There are, however, a large number of applications of PCR instruments other than detection of infectious diseases. Our analysis shows that even subsystems, such as sample preparation and qPCR, are both useful within separate instruments for many applications. For example there are samples which can be directly analyzed in qPCR instrument [49] [50] [51] [52], and many applications of sample preparation to purify nucleic acids. With new

enzymes, such as heme-resistant enzymes[53] these applications may increase. Below, we list some of the applications.

- Blood Safety
- Organ donor testing
- Surgical Procedure for removing tumors
- DNA Cloning
- DNA sequencing
- Biomarker detection
- Cancer detection
- Forensics (genetic fingerprinting, Parental testing)
- Biosafety
- Gene expression
- Environmental biology
- Food safety
- Bio-pharma production contamination detection

Hidden Issues

Future applications of the technology and products are also very important to consider. The interface to medical record systems needs to be covered as well. Although the software development can be deferred, it would allow a better product to be designed if the final desired interface is know from start. The requirements for software are covered in a later section in this thesis. The standards for product ruggedness are also not defined in most instrument TPPs. There are applications of the product in which it will be subjected to rough use. Examples are severe vibrations during travel on unpaved roads, shocks during transportation, dropping and rough handling. Some guidance can be obtained from military standards (Milspec ratings) for vibration and temperature testing.

Caltech Proposal to Gates Foundation

Here, we show the technology to develop point-of-care qPCR systems that address all of the needs for developing world deployment. Such solutions must include sample preparation systems, covered in Table 2.4 and qPCR technology, covered in Table 2.5.

Quick time-to-answer	**15 minutes or less** (including qPCR assay)
Simple readout	yes / no / invalid test (embedded software interprets results) optional simple quantitative readout of pathogen load / species typing (determined automatically by the instrument)
Low cost to manufacture	**less than $120 for the complete POC instrument** (w/ qPCR module) **less than $1 per disposable assay cartridge** (includes reagents)
Excellent sensitivity/specificity	**within 1% of laboratory qPCR (diagnostic gold standard)**
Use by minimally trained personnel	**yes**, collect finger prick or heel stick blood sample or urine sample and insert cartridge into instrument
Wide operating environment	**up to 40°C / 100% humidity** (45°C possible)
Easy storage / transport	**no refrigeration required** for system or assay cartridges
No laboratory requirements	**no need for local reagents, water, nor additional equipment**
Detect multiple pathogens /Distinguish pathogens, strains and subtypes	**yes**, including **all the high impact diagnostics** (ALRI, HIV/AIDS, TB, diarrhoeal diseases, malaria, 3 STIs) and many others, via **blood samples** (finger prick or heel stick), urine samples, sputum, or swabs **Multiplexing capability** to distinguish strains/subtypes within one test **Multiwell capability** for fast, inexpensive tests in parallel
Compact size	< 10 X 10 X 20 cm^3 (complete POC instrument, incl. qPCR module)
Low weight	< 3 kg
Low power consumption	~ 100 assays per AA battery
Robust design / components	more like a *Land Rover S1* mobile phone than an *XYZCycler*

Table 2.4Target Platform Profile (TPP) for a complete POC instrument based on the proposed approach

Quick time-to-answer	**15 minutes or less** (including qPCR assay)
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Excellent sensitivity/specificity	**within 1% of laboratory qPCR (diagnostic gold standard)**
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Wide operating environment	**up to 40°C / 100% humidity** (45°C possible)
Easy storage / transport	**no refrigeration required** for system or assay cartridges
No laboratory requirements	**no need for local reagents, water, nor additional equipment**
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Low power consumption	~ 100 assays per AA battery
Robust design / components	more like a *Land Rover S1* mobile phone than an *XYZCycler*

Table 2.5 Target Platform Profile (TPP) for a complete POC instrument based on the proposed approach

The assays identified at that point at given in Table 2.6.

Infectious Disease	Pathogen	Sample Type / Volume	Sensitivity	Specificity	End-notes	Comments
ALRI (viral)	respiratory syncytial virus, influenza viruses, parainfluenza viruses, human metapneumovirus	whole blood (0.1-0.5 mL), nasal swab	95-97%	85-90%	i,ii	a multiwell strategy is low risk; potential also to multiplex
ALRI (bacterial)	Streptococcus pneumoniae, Haemophilus influenzae	whole blood (0.1-0.5 mL), nasal swab	90-95%	85%	iii	
HIV/AIDS	HIV-1 (+ HIV-2 in West Africa)	plasma or whole blood (0.1-0.5 mL)	90%	90%	iv	quantitative from plasma RNA; qualitative from whole blood DNA
Diarrhoeal diseases	C. difficile, G. intestinalis, G. lamblia, C. parvum, E. coli	stool swab	>95%	>95%	v	a multiwell strategy is low risk; potential also to multiplex
	E. histolytica, E. dispar	stool swab	>90%	>90%	vi	multiplexing within one sample reservoir is likely the best strategy
Malaria	P. falciparum, P. vivax, P. malariae, P. ovale	whole blood (0.3 mL)	>90%	>90%	vii	
TB	M. tuberculosis	sputum, stool	~90%	90-100%	viii	MDR/XDR detection is critical
Sexually	Chlamydia	swab,	>90%	>90%	ix	a multiwell strategy is

Transmitted Diseases	trachomatis	urine (0.5 mL)				best; multiplexing by wavelength will likely result in loss of sensitivity
	Neisseria gonnorrhoeae					
	Treponema pallidum				[x]	
	Trichomonas vaginalis				[xi]	
Hepatitis	HCV	plasma (0.3 mL)	>90%	>90%		lower limit of quantification is approximately 50 copies

Table 2.6 Assays of Interest and their parameters from literature

The tables for above mentioned TPPs are copied below from respective references for easy comparison and reference.

	Fever Panel Target Product Profile	
Disease / Pathogen	• Malaria: definitive and distinct diagnosis of Plasmodium falciparum and P. vivax. • Bacterial pneumonia: diagnosis of bacterial pneumonia by either definitive diagnosis of each of the three major pathogens causing bacterial pneumonia or diagnosis as a group of organisms through novel biomarkers: Streptococcus pneumonia, Staphylococcus aureus, Haemophilus influenzae B. • Supplemental pan-bacterial marker: One or more known general biomarker(s) for bacterial infection[24] to provide "rule-in" diagnosis of other bacterial infections that should receive antibiotic treatment or suggest referral for further testing even without definitive diagnosis, including atypical bacterial pneumonia and bacterial meningitis. A single biomarker or multiple biomarkers that comprise a signature are acceptable. • (Optimal) Supplemental pan-viral marker: general biomarker or multiple biomarkers that comprise a signature for common viral causes of fever, to confirm in patients who are entirely negative by the fever panel diagnostic that antibiotics should not be given. • (Optimal) Active tuberculosis: definitive and distinct diagnosis of Mycobacterium tuberculosis. • (Optimal) HIV: definitive and distinct diagnosis of human immunodeficiency virus.	
	Optimal	Minimal
Goal of Test	Differential diagnosis of the cause of fever for treatment, including in children <5	
Reference Test	Culture and microbiologic testing for bacterial diseases and microscopy for malaria	
Sensitivity	• P. falciparum – 95% • P. vivax – 95% • Streptococcus pneumoniae – 95% • Staphylococcus aureus – 95% • Haemophilus influenzae B – 95% • Supplemental pan-bacterial marker(s) that, in combination with pathogen-specific markers above, can identify all bacterial pneumonia with an overall sensitivity of 95%. • Supplemental pan-viral marker(s) – Best in class • Tuberculosis – Best in class • HIV – Best in class	• P. falciparum – 90% • P. vivax – 90% • Streptococcus pneumoniae – 90% • Staphylococcus aureus – 90% • Haemophilus influenzae B – 90% • Supplemental pan-bacterial marker(s) that, in combination with pathogen-specific markers above, can identify all bacterial pneumonia with an overall sensitivity of 90%.
Specificity	Each individual pathogen above, 85%	Each individual pathogen above, 80%
Reproducibility	>95%	>85%
Biological Principle	Not pre-determined	
Quality Control	Positive and negative control required	
Test Result & Interpretation	Visual readout that directs treatment without manual data interpretation	
Interfering Diseases	None	
Specimen / Sample	One of the following sample types: blood, saliva, sputum, mouth swab, or urine	One or more of the following sample types collected in a single patient visit: blood, saliva, sputum, mouth swab, urine
Sample preparation	None required (sample preparation/processing internal to device acceptable)	
Special Handling/ Equipment	None required	
Refrigeration requirements	None required	
Power requirements	Prefer none, renewable battery power (e.g., solar recharger) acceptable	
Stability	24 months at 55°C and 90% humidity	18 months at 45°C and 80% humidity
Water requirements	No running water required	
Training Required	Minimal: visual and intuitive interface and instructions; no language requirements to operate instrument; no more than 1 page of instructions	
Time to result	<10 minutes	<30 minutes
Duration of valid result	>72 hours	>24 hours
Precautions	Safe specimen / sample management	
Steps to Test Result	5 or less steps to result	
Patient Record	Patient identification required	
Test/Platform size	Handheld device; <5 lbs / 100 tests	Portable device; <10 lbs / 100 tests
Target Ex-Works Price[25]	$2-5, plus cost of device if one is required	$2-5, plus cost of device if one is required

[24] Such as procalcitonin

[25] Target ex-works price provided here should be considered a guideline. These target prices will be refined based on additional analysis prior to the launch of the incentive, as part of the process of validating the TPP. These prices are for the assay and do not take into account the cost of the device itself, should one be required.

Figure 2.6 Bio Ventures TPP

Requirements and example target product profiles for tuberculosis nucleic acid amplification testing systems used in remote microscopy centers and primary care settings of high-burden countries.

Characteristics of testing environment	Parameter	Minimal POC TB diagnostics requirements [106]†	Optimal POC TB diagnostic requirements†
Many patients in remote locations have no access to TB diagnostic methods, or to smear microscopy only. Active case finding with adequate clinical performance needed to initiate treatment and curb transmission	Clinical sensitivity	≥95% SSM+/C+; 60–80% SSM−/C+‡	≥95% SSM+/C+; 60–80% SSM−/C+ ‡
	Clinical specificity	≥95% SSM−/C−	>99% SSM−/C−
	Analytic sensitivity	Not specified	<250 CFU/ml
	Analytic specificity	Not specified	Not cross-reactive with NTMs and other relevant pathogens
Prevalence of MDR/XDR-TB varies by area, but increasing	Drug resistance screening	Not listed – focus on TB diagnosis only	Flexible platform, drug resistance testing optional as separate assays
Very limited financial resources – test needs to be affordable	Cost of all consumables per test	<US$10 after scale-up	<US$5 after scale-up
Purchase/maintenance of expensive instrumentation not possible	Cost of instrumentation	"Acceptable replacement cost", maintenance-free	Capital instrument cost integrated into reagent cost, maintenance-free
Consumables have to be provided to many remote sites with limited storage space	Reagents/ consumables	Self-contained kit with all reagents, sample collection device and water if needed	All reagents in one consumable, sample collection device provided, small consumable size
Remote sites can only collect noninvasive sample types	Specimen type	Sputum OK but not ideal, aim for other noninvasive sample types	Sputum and other liquid specimens, noninvasive samples
Additional equipment often not available, may break down or disappear, rendering the system nonfunctional	Additional equipment	Not specified	No additional equipment required
	Electronics/data analysis	Not specified	Integrated, no external computer needed
Ambient operating temperatures often high and uncontrolled, cold storage/ cold chain for reagent supply generally not available	Reagent kit stability	24 months at 30°C, higher for shorter periods of time. Stable in high humidity	24 months at 40°C, 70% humidity, plus stability for 48 h at 50°C, and daily thermal cycling at 25°C for 4 days, to account for stress during transportation and storage
	Operating environment	Works in tropical conditions	Operational at ≥10°C to ≤45°C, system tolerates 70% humidity
Testing systems transported to remote sites using basic means	Portability/ruggedness	Fits in backpack, shock resistant	Fits in backpack, resistant to shock, vibration, water, dust
Low operator skill level, low-infrastructure test environment leads to high risk of operator errors	Number of operator steps	Three steps maximum	Integrated, sample-in – answer-out
	Type of operator steps/ integration	No need for precision pipetting, no time-sensitive processes	Simple sample addition only, fully integrated platform
Tests performed by large number of low-skilled minimally trained healthcare workers	Training and required skill level	Training in 1 day maximum, can be used by any healthcare worker	Training in <3 h, seventh grade level education or equivalent, 'CLIA waiveable'
Patients often lost to follow-up	Time-to-result	3 h maximum, desirable <15 min	<15 min
Required throughput varies from <10 per day at remote primary care settings to >100	Throughput	At least 20 tests per staff member per day	Modular design adapts to various throughput demands, if needed ≥64 tests per staff member per day

Characteristics of testing environment	Parameter	Minimal POC TB diagnostics requirements [106]†	Optimal POC TB diagnostic requirements†
per day in some microscopy centers	Sample handling	Single sample processing (random access)	Single sample processing (random access), batch processing optional
Biosafety precautions minimal, high risk of infection	Biosafety	BSL1	BSL1, pathogen inactivated before sample introduction into device
Waste management challenging. Test site contamination with amplicons causes false positives	Waste management	Simple burning, sharps disposal, no glass, environmentally acceptable	Disposables volume kept to a minimum, sealed disposable, ideally amplicons destroyed after testing, biodegradable housing
Testing performed with minimal or no oversight – test results confounded by incorrect test execution, undetected reagent or equipment failure, use by unauthorized personnel	QC/EQA	Positive control in test kit, EQA/QC easier than for smear microscopy	Internal full-process positive control and negative control. EQA panel available to ensure operability at test site and to enable user proficiency testing, electronic tracking of reagent lots, user login, wireless connectivity links devices with central facility
Unreliable line power or no access to electricity	Power	Rechargeable battery	Rechargeable battery or solar power, AC or DC compatible
Results can be confusing, may not be read properly, have to be recorded for case notification	Result type Readout	Simple yes/no/invalid answer Easy to read, unambiguous, readable for at least 1 h	Simple yes/no/invalid answer Easy to read, unambiguous, electronic and printed, wireless transmission capable, including GPS

†Although the minimal POC TB diagnostic requirements listed here have been extensively vetted by many stakeholders, have been published [106] and frequently cited [9,17,60,105], the optimal POC TB diagnostic requirements included here are more hypothetical, based on the authors' opinions, personal communications with other stakeholders and opinions expressed in other publications [24–26]. It may not be possible for a system to meet all optimal product requirements.

Figure 2.7 PATH TPP for TB Diagnostics

Table 1. Proposed target product profiles for diagnostic tools for selected NTDs, mapping, and impact monitoring.

Characteristic	Lymphatic Filariasis	Trachoma	Schistosomiasis	Onchocerciasis	Soil-Transmitted Helminths
Intended use	Mapping, monitoring, and stopping decision	Mapping, monitoring, and stopping decision	Mapping, monitoring, and stopping decision	Mapping, monitoring, and stopping decision	Mapping and monitoring
Possible target population[a]	6–15-year-old children	1–9-year-old children (could be adjusted)	6–15-year-old children plus occupational groups	6–15-year-old children	6–15-year-old children
Possible sample types	Blood spot	Eye swab (other: mouth or nose swab, tears)	Blood spot or urine (avoid stool if possible)	Blood spot	Blood spot or urine (avoid stool if possible)
Ideal diagnostic marker	Parasite antigen	*C. trachomatis* antigen	Species-specific antigen OR pan-genus antigen	Parasite antigen	Parasite antigen
Ideal test format	POC or high throughput laboratory assay	POC or high throughput laboratory assay	POC assay	POC or high throughput laboratory assay	POC assay
Availability of ideal diagnostic marker	Available but not right format, low reliability, high cost, and temperature sensitive	Available but not right format	Not yet available	Not yet available. IgG4 antibody may be a reasonable proxy	Not yet available
Required performance characteristics	95% sensitive; *W. bancrofti*-specific	>50% sensitive, 99.5% specific	>50% sensitive, 99.5% specific	>50% sensitive, 99.5% specific	>50% sensitive, 99.5% specific
Comparator assay (current reference standard)	Night blood micro-filaraemia	Quantitative PCR	Kato-Katz (multiple slides and multiple days) and/or urine filtration	Skin snips to detect micro-filariae	Kato-Katz (multiple slides and multiple days)
Possible sampling strategies	PBPS/LQAS, school based, sentinel sites	PBPS/LQAS, home based, sentinel sites	PBPS/LQAS, school based, 50/school, increasing with control	PBPS/LQAS	PBPS/LQAS, school based

Table 2. Proposed target product profiles for diagnostic tools for selected NTDs, post-elimination surveillance.[a]

Characteristic	Lymphatic Filariasis	Trachoma	Schistosomiasis	Onchocerciasis
Intended use	Post-elimination incidence of infection	Post-elimination incidence of infection	Post-elimination incidence of infection	Post-elimination incidence of infection
Possible target population	Children born after transmission interruption	Children born after transmission interruption	Children born after transmission interruption	Children born after transmission interruption
Possible sample types	Blood spot	Blood spot	Blood spot or urine (avoid stool if possible)	Blood spot
Ideal diagnostic marker	Antibody	Antibody to a conserved species-specific epitope of MOMP	Antibody	Ov16 antibody
Availability of ideal diagnostic marker	Not available	Libraries available	In development	Available, but additional validation needed
Ideal test format	High throughput laboratory assay	High throughput laboratory assay	High throughput laboratory assay	High throughput laboratory assay
Population infection thresholds (for stopping MDA)	1%	Not defined	10% of school-aged children	1/3,000
Probable sampling strategy	PBPS	PBPS	PBPS or school surveys (or sentinel occupations)	PBPS

Table 3. Immediate research priorities.

Disease	Research Goal	Feasibility (0–10[a]: 0, Impossible; 10, Inevitable)	Impact if Achieved (0–10[a]: 0, None; 10, Massive)
Lymphatic filariasis	Development of antigen tests to usable/reliable format	9	8 if ≤USD 0.50
	Development and validation of tests (e.g., IgG4-subclass antibody detection tests using recombinant Bm14, BmR1, WbSXP, and *W. bancrofti*-specific antigens [20] or PCR-based detection of parasite DNA in homogenised mosquitoes [21]) useful for post-elimination surveillance, with accompanying standardised survey methodologies	9	8 if ≤USD 0.50
Trachoma	Development of a test for ocular *C. trachomatis* infection [22] able to maintain specificity at high temperatures and low humidity [23]	9	8 if ≤USD 0.50
	Development of eye/nose swab-, saliva-, or blood-based anti-*C. trachomatis* antibody test and exploration of the impact of successful trachoma control on antibody profiles in endemic populations	3	5
	Development and validation of a school-based survey protocol (need threshold minimum school attendance)	7	8
Schistosomiasis	Development of antigen [24] or antibody [25] isotype combination(s) useful in high and low transmission intensity environments, able to distinguish current from past infection	8	9
	Development of antigen or antibody isotype combination(s) to distinguish between different species	8	4
	Development of serum markers of morbidity	6	8
Soil-transmitted helminthiases	Development of reliable blood- or urine-based assays for detection of current infection	4	9
	Development of serum markers of morbidity	6	8
Onchocerciasis	Development of a quantitative antigen test for use in endemic areas in Africa and validation of Ov16 antibody test for demonstrating interruption of transmission in Africa	5	8
	Development of a test for loaiasis	5	9

Figure 2.8 TPP for NTDs

Table 2.7 TPP for Gates Foundation POC Dx Program

Characteristic	Optimal Target	Minimal Target
Intended Use		
Target Setting	• Moderate Infrastructure Settings (e.g., Level I Health Centers / Community Health Clinics)	• Same
Target User	• Staff with minimal technical training • Staff with some formal healthcare expertise to understand sample handling requirements, use of results and similar	• Same
Operating Environment	• Large temperature range (5-40C) • Broad humidity (to 95% non-condensing) and altitude ranges • Direct sun light to low light • Dusty conditions • Intermittent water and electrical access	• Same
Device Characteristics		
Platform	• Modular-based instrument approach with common user interface for all technologies	• One - three instruments acceptable so long as they meet performance and cost targets • Simple set of user interfaces
Design Considerations	• Table top instrument (50 x 75 x 50 cm) or smaller • Cartridges containing all required test reagents; no reagents on instrument • No requirement for water • Primary power source is UPS on local 110-220 AC power with rechargeable battery backup (>8 hrs testing with <2 hrs recharge)	• Other design approaches that meet assay performance specs and throughput requirements will be considered • Backup battery capacity sufficient to complete run(s) in progress (e.g., up to 90 min)without loss of result

Technologies Employed	• Qualitative and quantitative protein (Lateral Flow and ELISA equivalent) • Qualitative and quantitative nucleic acid (PCR or equivalent and isothermal qualitative) • Quantitative chemistry panels (e.g., routine enzymes) • Quantification of cells (e.g., CD4, CBC)	• Qualitative protein (one technology) • Qualitative and quantitative nucleic acid (one technology) • Same • Same
Demonstration Menu and Initial Product Offering	• HIV Therapy Initiation Panel • HIV 1/2 rapid serological test • Quantitative determination of HIV viral load • Quantitative HIV CD4 • Quantitative Liver and Kidney panels • Qualitative detection of MTB infection and determination of resistance to first line Rx • Quantitative CBC determination • Qualitative detection of malaria infection, including species distinction	• Same
Expanded Menu	• Reasonable technical path to full menu (appendix 1) • Tiered approach in no more than 3 waves of release	• Same
Clean Water Requirements	• None	• Same
Sample Handling		

Sample types, volume ranges	• Whole blood (20 µL - 100 µL) • Dried blood spots (20 µL – 100 µL dried onto matrix) • Sputum (1-2 mL) • Saliva (100-2000 µL) • Urine (50-1000 µL) • Stool (20-200mg)	• Whole blood (20 µL - 1 mL) • Dried blood spots (20 µL – 1 mL dried onto matrix) • Sputum (1-2 mL) • Saliva (100-2000 µL) • Urine (50-1000 µL) • Stool (20-200mg)
Sample Prep	• Integrated on cartridge • Time equal to no less than the difference between testing time and 1 hour • No specialized equipment, containment or skills required	• Simple off-board sample processing (e.g., concentration or filtration, especially for urine or stool samples); included within <5 steps by operator • Time equal to no less than the difference between testing time and 2 hrs.
Stability of Valid Sample (collection to test initiation)	• 24 hrs without refrigeration	• 8 hours without refrigeration
Performance and Functionality		
Test Performance	• Comparable to equivalent FDA approved (or other established gold standard products if FDA approved product does not exist)	• Same

Analytical Specificity	• No result altering reactivity with potential interfering substances	• Minimal detection of non-target analytes that does not alter clinical decision • Minimal result altering reactivity with potential interfering substances
Reagent Stability	• 24 mos at up to 40°C, 95% humidity from date of manufacture • Include shipping stress (48hrs at 50°C) • No cold chain required	• 12 mos at 40°C, 70% humidity from date of manufacture • Include shipping stress (48hrs at 50°C) • Cold chain permissible until product is shipped from manufacturer
Time to Result	• Including sample preparation: • <30 min for NAT • <15 min for rapid tests • <5min for chemistry tests	• Including sample preparation: • <90 min for NAT • <30 min for rapid tests • <10 min for chemistry tests
Throughput (tests per day)	• 50 tests with reported results in 6 hours per module, or 250 tests per 6 hours per system • No stat or batch testing within a module • Max capacity based on avg throughput of typical assay methodology mix, not highest throughput needs of molecular-only tests	• Total combined throughput up to 250 tests per day • Same
Workflow	• Target ≤ 3 operator interface steps • Not including sample prep	• Target ≤ 5 operator interface steps • Same

EQA, Process Controls and Surveillance Measures	• System satisfies local EQA requirements • Built-in assay process controls and instrument surveillance mechanisms • Only valid results to be reported; but all results should be stored for failure tracking	• Same
Information and Communications Technology (ICT)		
Data and Communication	• Data will be available locally and remotely • Must permit entry of patient information as required by the interpretation algorithm and allowed locally	• Same
Instrument User Interface-Hardware	Alphanumeric Touch Screen • Partially configurable so that assay workflow can be parameterized (including switching on/off functions or steps); • ≤ 5 GUI (graphical user interface) pages Alphanumeric Keyboard Barcode Reader Wand Audible Beeper LED Status Indicators	• Same
Physical Electronic Data Communications Interfaces	• USB (1): Removable Storage/Software Update • USB (2): Printer Attachment • Local Ethernet Port (1) • Bar Code Wand Port (1)	• Same
Wireless Data Communication Interfaces	• Mobile Cellular Network (GSM/GPRS) • GPRS should be able to use Internet File Transfer Protocol	• Same but no satellite communication requirement

	• M2M Satellite Modem (fallback) • Local Bluetooth • RFID Reader	
Data Import	Internal Bar Code Reader • Cartridge Label 1D/2D Data External Wireless/USB • System software update • Install new assays process • Reject disapproved Lots External Bar Code Wand or RFID reader • Patient ID	• Same
Data Export	Electronic Activity Record & Reports (wireless/printer); for example: • Dx Test ID # • Date & Time Stamp • Result • Patient meta data • Lab ID # Instrument Operational Status Instrument Maintenance Codes QA Information HL7 Compatible Compatible w/ Health Information Systems/DHIS2 Implementation	• Same
Data Storage	• TBD	• TBD
Security/Privacy	• HIPAA compliant (to make it attractive to developed world integrators)	• Same

Distribution, Support and Training		
Cold Chain	• None at any point in supply chain or storage	• No cold chain required once kit leaves manufacturer
Maintenance, Service and Support	• Instrument / module swap out model • Maintenance/calibration performed in field by user without specialized training or tools or requirement for internet access • No annual preventative maintenance needed requiring authorized personnel or specialized training • Electronic alert sent to support center if issues arise • Mean time to failure of at least 18 mos • Remainder of instrument will continue to function if one module (other than control module) fails	• Same, except: • Internet or specialized training might be needed for maintenance or service • Use of reference standard tool provided by manufacturer • Mean time to failure of at least 12 mos
Training	• < ½ day • Trained lab technician not required to operate; can be properly operated by healthcare worker with minimal training	• < ½ day • Minimally trained lab technician required for operation
Biosafety	• No need for biosafety cabinet • Direct disposal or incineration of consumables	• Same
Cost Considerations		
Cost of Consumables (COGS at scale)	• <$5 USD per assay, for example:	• <$10 USD per assay

	• TB identification - <$5 • TB Resistance profile <$5 • Malaria with speciation <$4 • HIV VL <$5 • Chemistries <$3	
Cost of Instrument (COGS at scale)	• <$5,000 USD (base system – one console plus one advanced processor module • <$15,000 USD (complex system composed of one console and up to 5 advanced processor modules)	• <$20,000 (total cost for all instruments required to satisfy TPP)
Regulatory Considerations		
Regulatory Requirements	• Manufactured pursuant to GMP, ISO 13485:2003; authorized for use by a member of the Global Harmonization Task Force (GHTF) • CLIA waiveable or equivalent	• Same

2.3 Additional Points

We added following points for our development.

- Develop systems that can be used at level 0 and also provide a way to combine them for higher throughout at higher level.
- Allow most sample types at least blood, urine, stool, plasma, swabs, dried blood spots, sputum, and saliva. Consider tissue, forensic, soil and food related samples.
- Compare well to commercial PCR machines in terms of performance like detection limit.
- Allow all major protocols for sample prep and qPCR e.g., HRM, multiplex, MC so that assays can be easily ported.
- Develop software concurrently for better feedback on the whole solution.

2.4 Conclusion

Understanding the requirements is critical to have greater chances of success and scale up after the product in introduced in the market. It's not trivial to develop a TPP which will serve such a diverse need.

3 Overview of POC Instruments and Disposables

3.1 Introduction

This chapter provides an overview of the final systems which use various innovations of our work. The details of sub-systems or components are explained in later sections. Here we provide an overall view of the technology and how things fit together.

There are various technologies which are unconventional. Explaining the final systems which combine these technologies without first explaining each technology in sufficient detail makes it difficult to explain the entire system in a concise way. Nevertheless, we have attempted to provide a system level description of the system in this section. For system designers having expertise in the field it should provide a good overview. It is recommended to review the individual technologies in following chapters in case more explanation is desired while reading this section.

3.2 Overview of Sample of Answer Technology and Innovations

To build a sample to answer system, various components and technologies must function in harmony. A high level view of the steps involved in such a system are show in Figure 3.1. The accompanying innovations are shown in red callout boxes. It is important to keep in mind that a full solution is required for significant impact. Even for trials and field testing, many components which are typically ignored in academic research are very important. Software development and ergonomic design are examples of such components.

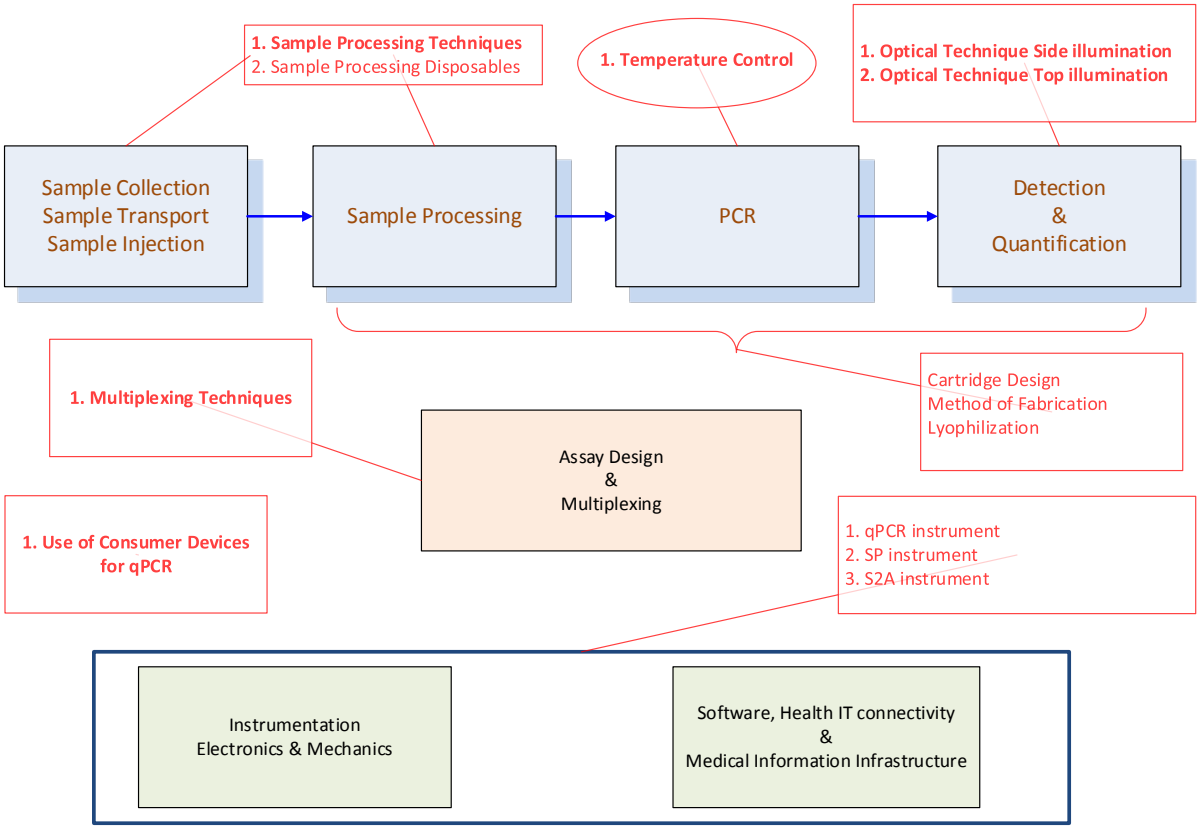


Figure 3.1 Sample to Answer Steps and Innovations

The technical work areas are shown in Figure 3.2. By its nature, this is a multi-disciplinary effort involving diverse fields. Various processes which effect the design down the line have to be considered from start. Large scale production is a feature which is particularly important to consider early in the design. Any design which does not allow rapid scale up of production can be a serious problem in later stages of the project.

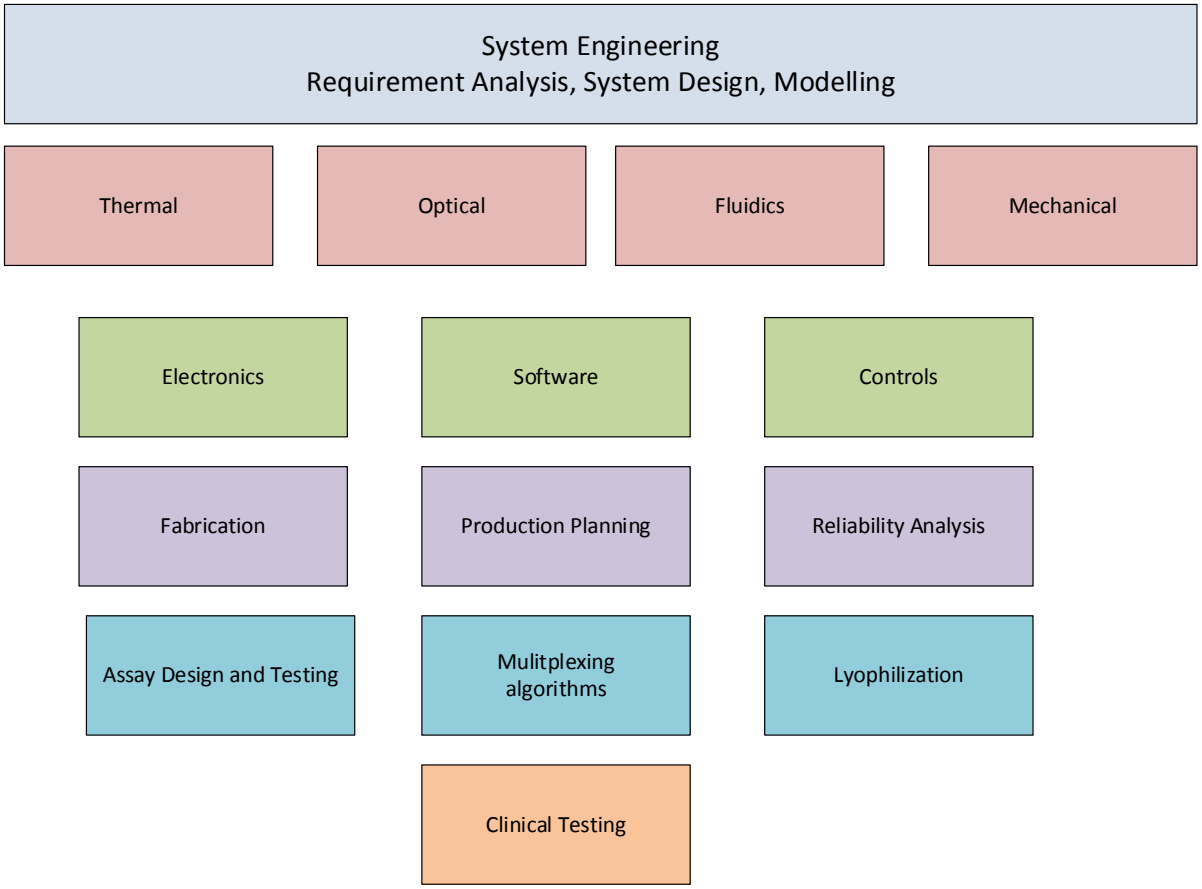


Figure 3.2 Technical Work Areas

There are various challenges to meet the desired requirements. We present a brief summary of the problems and our suggested solutions in Table 1.1.

Table 3.1 Challenges and Associated Solutions

Domain	Outstanding Problem	Solution

Thermal	• Power hungry
	• POC issues
	  • Dust, moisture
	  • Clogging
	  • Insertion, contact, housing
		• Contact cooling
		• Metal base cartridges

Optical	• Lens based optics
	  • Expensive
	  • Ruggedness issues
	  • Alignment
	  • Cartridge alignment
	  • Opto-mechanics
	• Expensive filters
		• New integrated TIR and light guide design
		• Metal base

Sample Preparation	• Valves, pumps, vacuum • Robotics not amenable to POC • Robustness issues • Truly closed system very difficult	Sliding/rotating design for wide variety of samples with high flexibility and multiple samples
Usage	• Sample injection • Connectors for POC • Cartridge placement and alignment	Contact cooling Metal base cartridges

Assay Cost	• Probe based assays
	  • Expensive
	  • Fragile
	• Probe based multicolor
	  • expensive
		New technique and algorithms

Multiplexing	Complex Optics	New optical designs allow low cost multiplexing Simple multi-well designs possible
Instrumentation	Expensive, complex, power hungry	Innovative and simple but robust and reliable instrumentation
Automation	Low performance electronics design	World class electronics design
Production	Complex and expensive production methods like laser welding, solvent bonding	Low cost well established production method compatibility.
Flexibility and Future Proofing	Most designs don't work if assay parameters change	Design allows unprecedented flexibility in assay operations and use of current and future assays.

The interaction of innovations, problems solved and advantages is given in Figure 3.3.

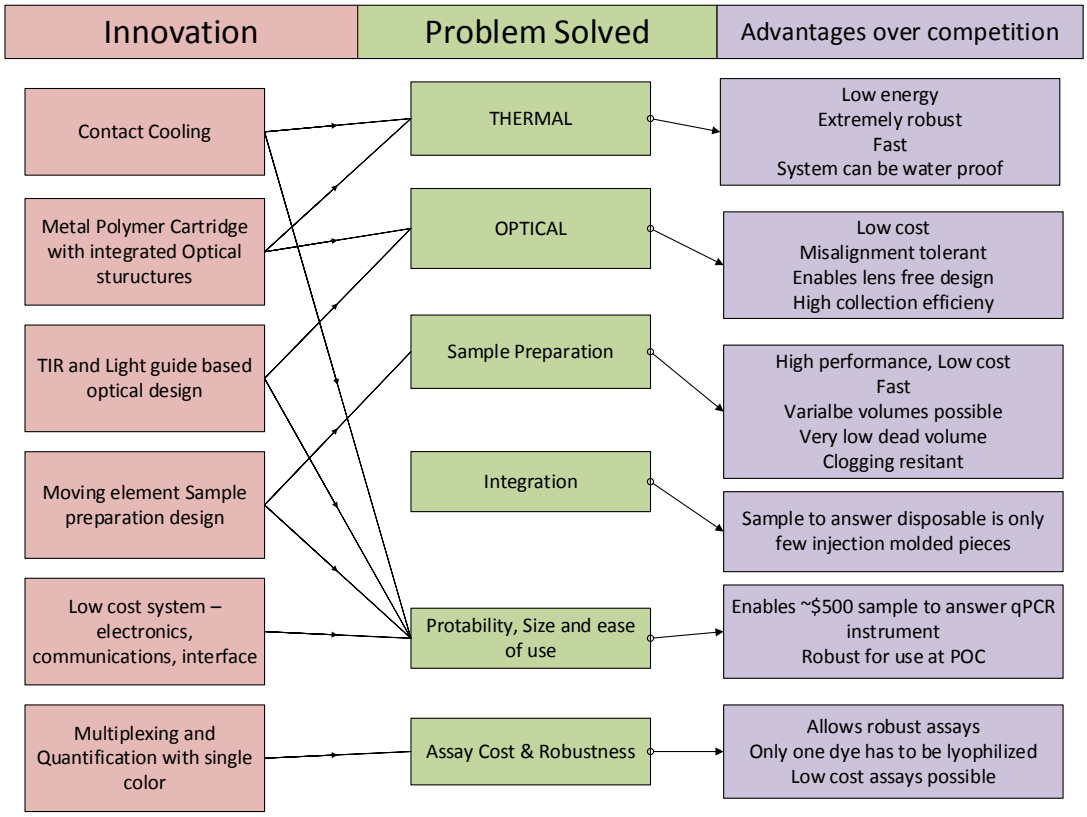


Figure 3.3 Innovations, problems solved and advantages

3.3 Product Configuration Concept

Based on these innovations, the design is based on independent modules which can be stacked together. The sample to answer (s2a) module is a core module which can accept cartridges to run various assays. Each s2a module can be used as a standalone module as well. We thus have a scalable solution which can server from Level '0' (no infrastructure) to reference labs. The base module has elaborate user interface and communication elements. However, even in case of failure of base module, each s2a module is functional. S2a modules can have multiple interfaces to control and view results. They can be controlled via base module or mobile devices like cell phones and tablets. A personal computer can also be used to control them. The s2a module can have limited display and buttons so that it can still be used independently. The concept is shown in Figure 3.4.

Figure 3.4 Health Center System Concept

The s2a module can be taken to remote locations to act as a level ‘0’ module. It can also be used during travel due to the design of the disposable and the instrument. This can be useful for screening and diagnosing during travel. We present an example deployment in a village or dispersed community. The central dispensary or health center can have the few modules stacked with base module which are being used for screening and regular diagnosis. However, there are cases where the patient should be diagnosed in his living place. Examples are old, very sick or TB patients who can transmit the disease during travel. It’s very useful to easily carry the system to the patient. The s2a module can be stacked with a battery and optional display. Therefore, we don’t need different kinds of systems for different uses. The example portable system is shown in Figure 3.5.

Figure 3.5 Portable System Concept

3.4 qPCR instrument demonstrator

We developed qPCR cartridge and some of the associated technologies earlier than sample preparation. Various instruments were designed and fabricated.

The qPCR cartridge has a metal base and polymer top. The polymer top has an integrated prism to couple excitation light. The detection is performed on the top. The cartridge is shown in Figure 3.6.

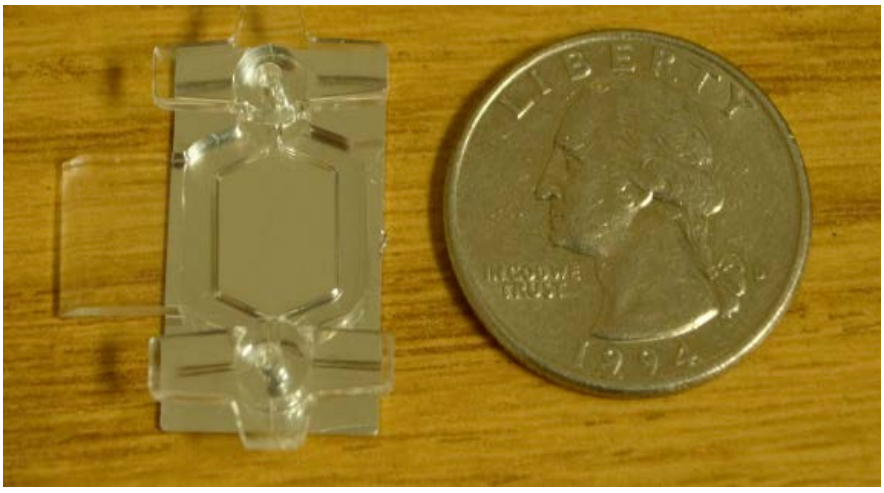


Figure 3.6 qPCR Cartridge

The housing was largely made using 3D printing. We used Thorlabs cage system brackets and lens tubes to hold optical components. This is useful as the optical components can be changed and different optical assemblies can be put in the box without chaining it. The opto-mechanical components from Thorlabs are already black and have screw threads which help in reduction of stray light. There are also a variety of detectors and sources which are available to be assembled in this case. We designed various items to fit in this scheme. We designed circular PCB's which can hold high brightness LEDs. The light guide as mounted using laser cut supports which fit into the lens tube system. For detector we designed PCBs to have one or multiple detectors. Multiple detectors are useful in multicolor real time PCR.

We used two bi-stable solenoids of two types. ERB35 is a bi-stable latching solenoid. This solenoid has a spring which is useful to apply pressure when the moving plate is used to cool down. ERDI115 is a bi-stable reversible linear solenoid in which plunger is retained at both position by a permanent magnet. This solenoid required better tolerance design than ERB35.

Figure 3.7 shows CAD diagram and pictures of one of our designs. The design used our embedded electronics which has a socket for Wi-Fi or Bluetooth module.

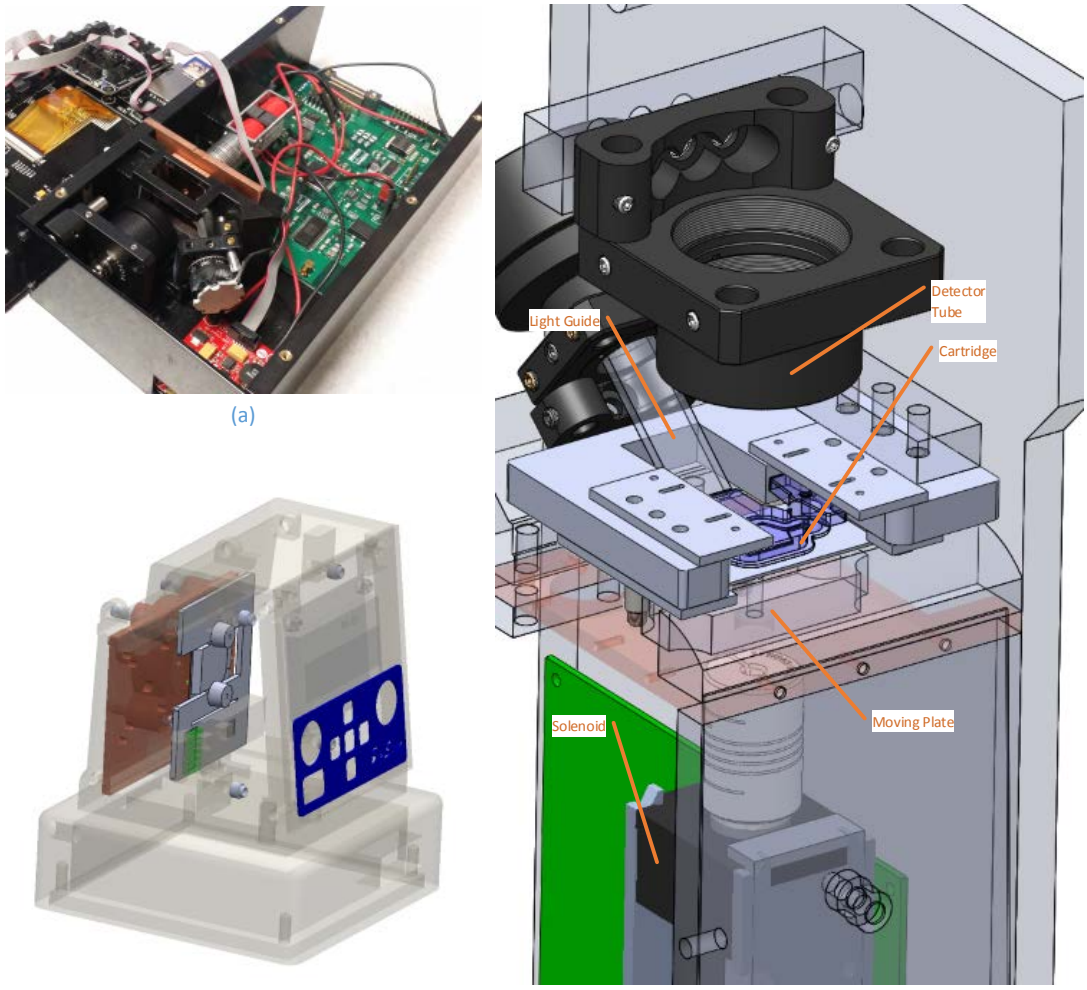
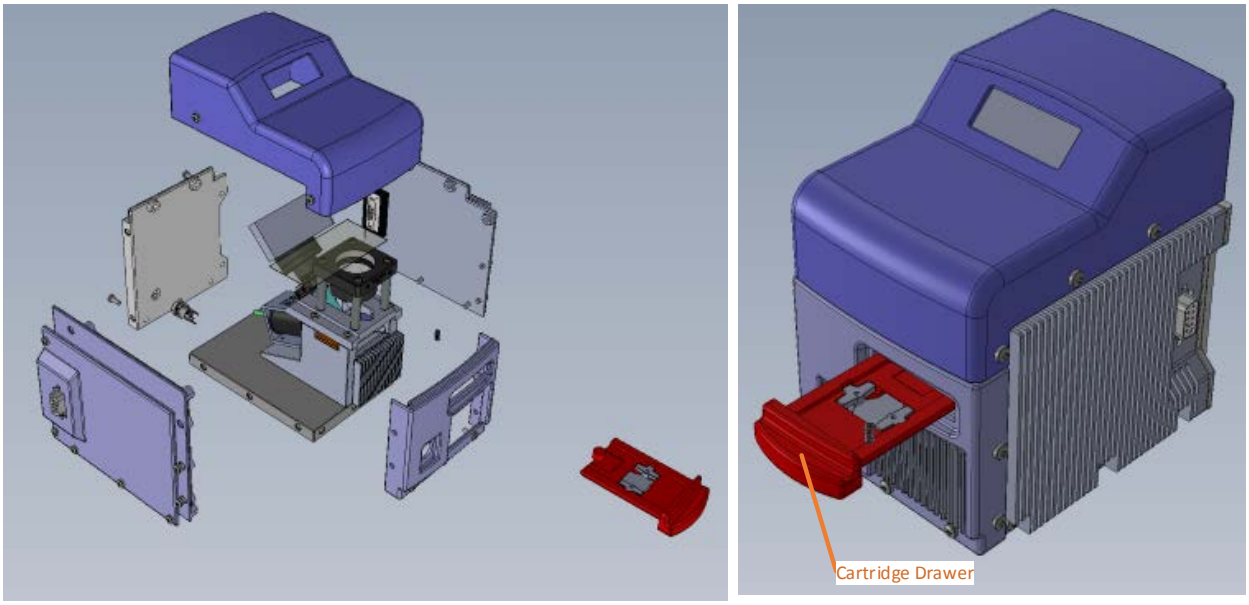


Figure 3.7 Prototype qPCR Instruments

To design a system which can used in the field or at least in lab setting by a third party, professional designer was hired. The system is shown in Figure 3.7. The system uses a metal wedge which moves to make a contact with the fixed heater plate. The fixed heater plate has an inbuilt heater. The cartridge is placed on the heater plate under pressure. This mechanism is achieved by springs integrated into the cartridge drawer mechanism. Good contact is crucial for thermal performance of the system. The moving wedge is always in contact with instrument base and has a low thermal resistance paths to heat sinks open to atmosphere. When the wedge is in cooling mode, it's in contact with both the heater plate and the instrument base. Hence the wedge's thermal mass is not the limiting factor and the heat can flow to much bigger thermal mass of the instrument base and casing.



(a)
Exploded Assembly View

(b)
qPCR Box

(c)
Side View

(d)
Top View

Figure 3.8 Latest qPCR System

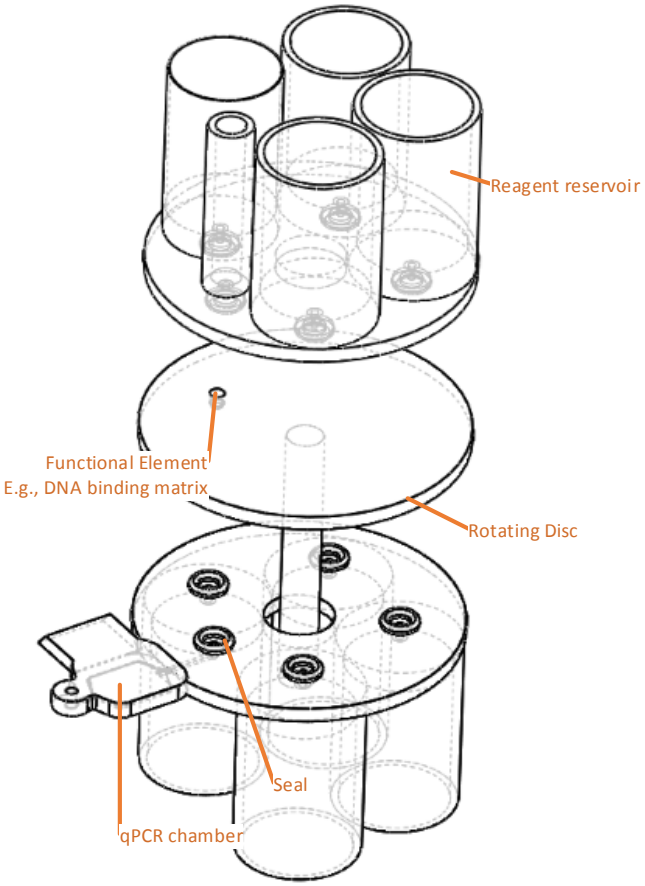
3.5 Universal instrument Design

The universal instrument is supposed to allow three kinds of cartridges. It can accept qPCR only, sample prep only and sample to answer cartridges with appropriate adapters if needed. The concept layout is shown in Figure ***3.11*** . It uses a rotary design and employs a single chamber for qPCR. A motor rotates a disk and a linear actuator. The linear actuator can move syringe plungers.

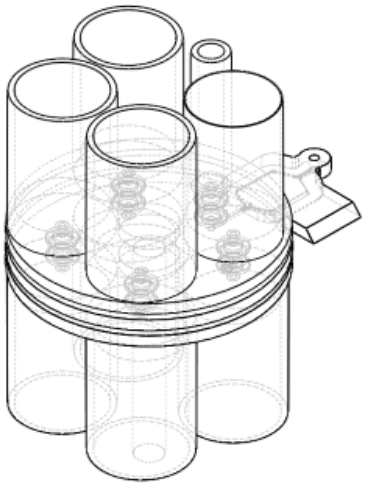
An example s2a cartridge for nucleic acid test is shown in Figure 3.9. The top and bottom parts have syringe type reservoirs which can hold various reagents. The bottom part has the qPCR chamber directly under the elute buffer cylinder. A disc acts as a seal and is compressed by flexible seals between top and bottom parts. The disc contains functional elements. In our example only one functional element is shown i.e., DNA binding matrix. A silica membrane can be used. Rotation of the disc can bring the functional element in line with the ports of each syringe type cylinder. It may be easier to explain the operation of the cartridge by depicting it in a linear configuration as in Figure 3.10. This has an additional tilted syringe type structure with a filter. Such design can be used to get plasma into the first vertical cylinder after the user puts full blood in the slanted cylinder.

In a typical bind-wash-elute protocol, the sample can be introduced in one cylinder via a port in it. The cylinder already has lysis and binding buffer. The dna binding membrane is aligned to this cylinder. By moving the plunger up and down, the binding efficiency is increased. We also get mixing due to moving the sample through membrane. Next the dna binding membrane is aligned to cylinders containing wash buffers sequentially. The membrane then aligns to drying cylinder where it is dried. Finally the membrane aligns to elute chamber. Here the elute buffer fills the cartridge which has lyophilized reagents while bringing the bound dna into the chamber. Thus all the elute volume is used for PCR.

Figure 3.11 shows the cartridge and cartridge-instrument elements. The qPCR chamber interface to the instrument is similar to the one explained in qPCR instrument. A motor can rotate the disc and frame on which linear actuator is mounted. The linear actuated can move syringe plungers. The syringes reservoirs can be incubated by using heaters.



(a)
Exploded Assembly



(b)
Concept S2a Cartridge

Figure 3.9 Example sample to answer cartridge

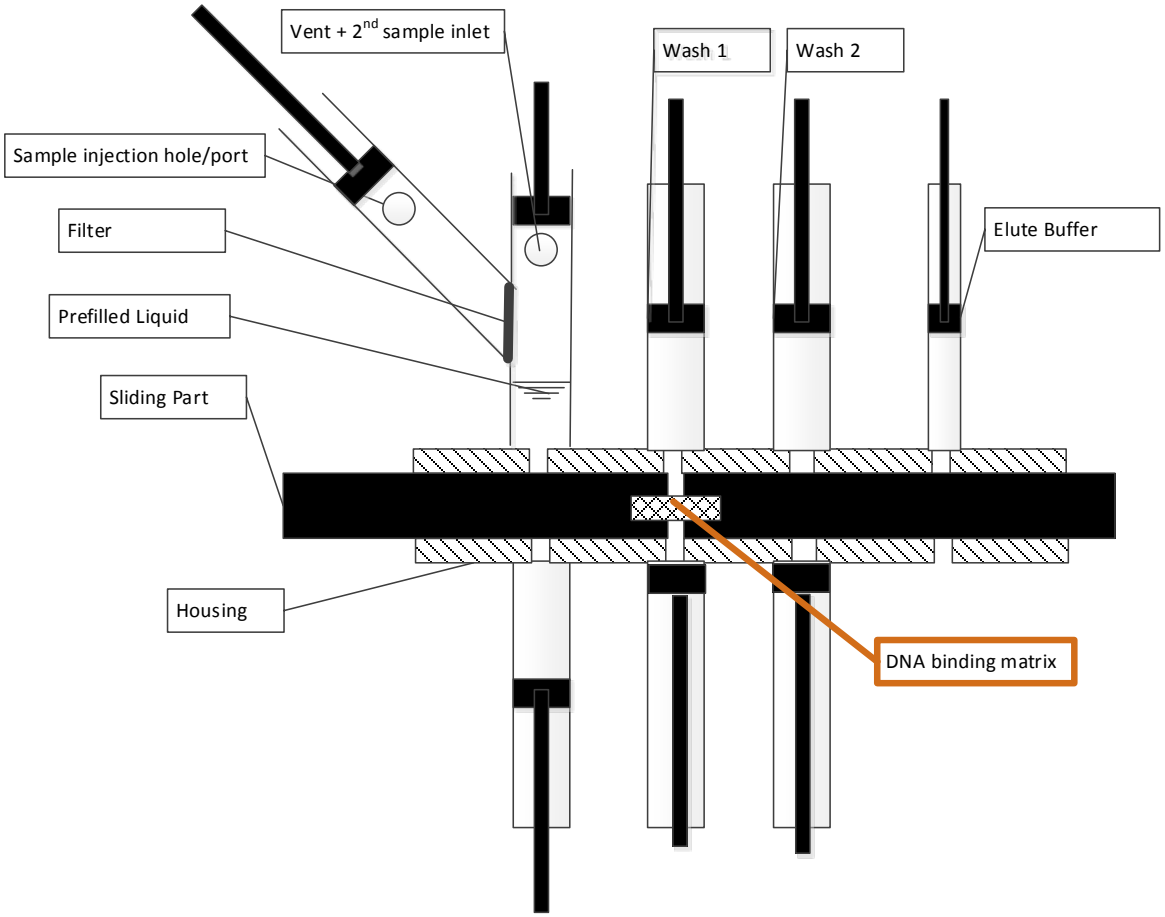


Figure 3.10 Sample to Answer Cartridge Depicted in Linear configuration

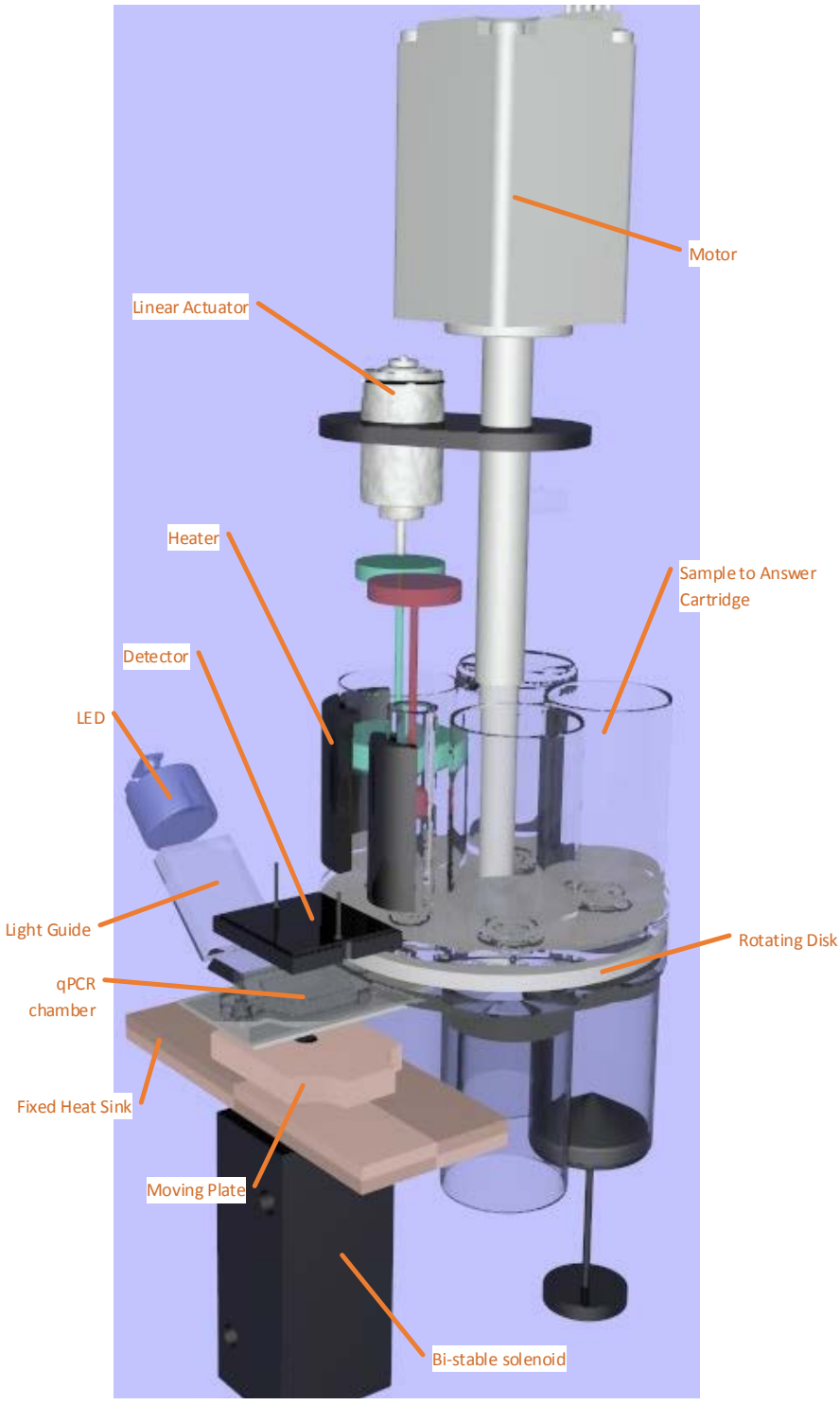


Figure 3.11 Concept Layout of Sample to Answer System

3.6 System Level Benefits and Comparison with Other Technologies

3.6.1 Cost

The system is lower cost than any other sample to answer systems with similar capabilities. We have tried to reduce cost for all major components. Low cost takes care of 'installed base problem' of expensive instruments. It also opens up new markets like individuals involved in healthcare.

3.6.2 Flexibility

It's important to allow a wide variety of assays in the same instrument. Various protocols should be supported. Our design offers flexibility in number of stages, operations and fluid movement. The system is also highly configurable. It allows multiple wells (channels) and multiple detection wavelengths. Very complex protocols are possible. Examples are multi-sample and multi-protocol disposable. It's also possible to have nucleic acid and protein tests in one cartridge, however the complexity increases and cost analysis should be performed for such designs. Very difficult samples are process able in the system.

3.6.3 Reliability and Low maintenance and calibration requirements

The things which can go bad after repeated use or get out of calibration are avoided. For example pneumatics is a common way of moving fluids. However, the pneumatic interface from instrument to cartridge can be non-trivial. The seals can leak after repeated use.

3.6.4 Robust

The technology allows waterproof and dustproof designs. Owing to syringe assisted fluid movement, operation is possible during travel. Examples are use during travel in a bus in developing world.

3.6.5 Gold Standard techniques and Fast Assay development and porting

The technology uses proven gold standard techniques. Some systems simplify the underlying techniques so that the system can be used at POC. However, many of these techniques are inferior in performance.

Another advantage of the system is fast adaption of existing assays to our platform. Commonly used spin column based extraction is very close to our system. Automated liquid handling equipment is available which can be used in assay development. Protocols used in robotic liquid handlers are similar.

Thus porting of existing protocols and development of new protocols can take use mature liquid handling technology. This is not the case with designs which have difficulty in mapping their protocols to existing liquid handling instruments.

3.7 Features of Sample Preparation Technology

The sample preparation technology provides a new type of liquid handling platform which is portable and robust. The dead volume does not change much with reagent volumes and number of reservoirs. Same physical cartridge can be used for different assays as the volumes of reagents can be varied by syringe tip position. This can also be used to have programmable operations depending on sample input volume or other conditions. The operation is closed and does not need venting ports open to atmosphere. There are less restrictions of fluid characteristics. The system can work with harsh chemicals and foaming liquids. Unconventional functional elements like mixers, chopper and homogenizers can be integrated into to the system for a wide variety of operations. Multiple passes from the same functional element are possible in a robust way. Increased binding efficiency is expected by using this feature. The fluid flow rate and dispense amounts are precisely controllable.

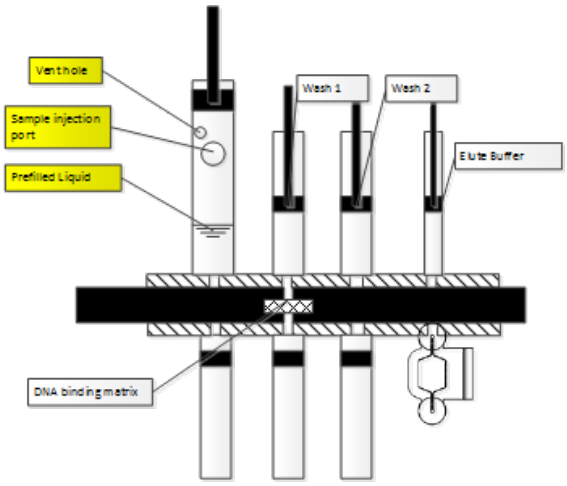
3.8 Features of Reaction Chamber

The reaction chamber is tightly coupled with optical and thermal design. However, it has significant advantages to be used alone as a component in lab on chip systems. The volume of the chamber can be varied by changing height of the cavity only. Thus interface to instrument including optics can remain the same. The chamber can be used for qPCR, ELISA, immune-PCR and other detection methods as well. Rapid thermal response and large optical interrogation areas are provided. Rapid and low cost manufacturing is possible since the plastic parts are produced by injection molding and bonding is achieved using adhesives. The design offer mechanically robustness as well. A variety of substrates can be used owing to the bonding methods. Lyohpilizaition can be efficiently done due to flat surface and metal substrate.

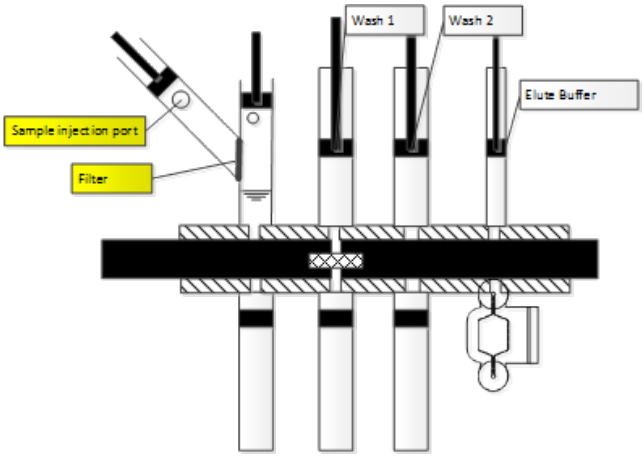
3.9 Example disposables for different assays

We briefly describe few sample configurations for the disposable to show the flexibility of design. Figure 3.12 (a) shows a cartridge for a sample which does not need filtration and can be directly added to lysis

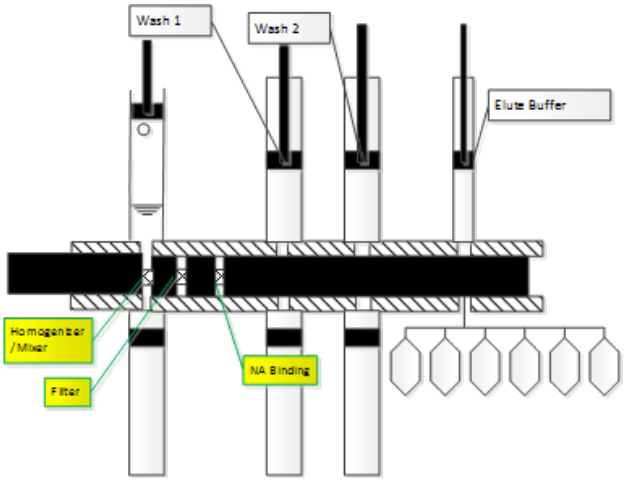
buffer. Example of such an assay is STD assay using urine as a sample. Figure 3.12 (b) shows cartridge which used whole blood as the sample but separates plasma to continue analysis. Various pathogens including Dengue, Malaria, HCV, HBV and many others can be detected using this design. Figure 3.12 (c) shows a candidate design for stool sample for polio detection. This design is based on assay be CDC (Center for disease control) which utilizes six separate reaction chambers. The cartridge can accept stool as a sample. Due to robust fluid movement, stool can be homogenized or mixed into liquid buffers by using a homogenizer type functional element. Then the filter functional element can allow only supernatant to pass to only one cylinder. Next the usual cycle of bind-wash-elute can continue.



(a) Urine Sample – STD Assay



(b) Blood Sample – Malaria, Dengue, HCV, HBV



(c) Stool sample – Polio Assay

Figure 3.12 Example cartridges

4 Metal-Polymer Cartridge for Quantitative Real Time PCR at Point Of Care – Design and Analysis

4.1 Abstract

We report the design, fabrication and testing of metal-polymer hybrid disposable cartridge for qPCR with capability to perform other biochemical reactions. In the first part we focus on the POC (Point-of-care) requirements, concept, design and analysis of the cartridge. Practicality is given special weightage in concept and detailed design. We perform coupled transient FEA simulations to get more realistic data for the both thermal and structural design. The goal is to find parameter space which gives predictive view of cartridge robustness in large scale production. We extract important information from FEA simulations and develop a lumped transient model which can be simulated and optimized by wide variety of tools. Such detailed simulation and analysis give accurate limits of performance of the fluid domain and the advantages of the concept. There are compelling advantages of using metal in lab on chip devices. Metal provide better thermal and optical performance. It also allows ruggedness and ease of use in POC settings without sacrificing performance. The chip can be placed directly over a flat plate cycler (slide cycler) without need of special adapters. The polymer part is injection molded with integrated pillars to allow air gaps and easy filling with a pipette. A novel seal design is presented. Alternatively the chip can be sealed with an adhesive tape easily due to the designed air gaps and pillar structure. We show ways to achieve six sigma by design approach to design of POC diagnostic cartridges. Use of metal allows heating and cooling on the metal side to be much more effective than in the case of polymer layers which sometimes would need heating and cooling from both top and bottom for best

performance. The design also allows freeze dry procedures before bonding and since bonding can be done at room temperature the reagents do not have to be exposed to solvents (in case of solvent bonding) or extreme temperatures. The design and analysis provides a guideline for robust design of rapid cycle PCR cartridges which undergo stresses in short time. The design provides large optical interrogation area while still having high quality thermal performance. The design lends itself to production processes like injection molding and adhesive bonding which are both widely used in industry and are lower cost than many other alternatives.

4.2 Introduction

PCR cartridges have been made in a variety of materials, volumes and shapes. They have been reviewed in [54-64]. Various types have been developed including self-contained [65-69], with analyzers [70, 71] [70], and with different features[72-81] [82]. Various microfluidic for PCR chips are reviewed in [83].

4.2.1 Applications of PCR cartridges

The PCR cartridges are also useful in themselves without sample prep integration for many applications. They can be used with other instruments like flatbed cyclers, hotplates and plate readers available at various locations. There are various regions and places where one can find sufficiently trained manpower to do sample preparation as the equipment needed to do that can be inexpensive. However, PCR requires controlled timing which need an instruments. Providing an instrument or even a cartridge which can use existing equipment can have significant impact.

The samples which don't contain inhibitors or if the chemistry is available for amplification from raw sample, then the cartridge itself is sufficient as a full independent system. Some examples are full blood enzymes[52], [84]and assays using direct samples[50] [51].

If the cartridges provide better speed, cost and ease of use they can also be used in research especially for labs which require low throughput or need faster speeds than typical commercial instruments. There are many commercial applications of PCR chips or PCR chips with associated instruments. Such systems can be used in pharmaceutical manufacturing, cancer surgery and for samples which don't need much sample processing.

4.2.2 Why PCR cartridges instead of capillaries and tubes

PCR and qPCR reactions are typically performed in PCR tubes in research and clinical labs. There are many manufacturers to offer PCR and real time PCR machines of various kinds.

Here we are not considering the robotic and automated sample to answer systems. These systems are very expensive and not many labs can afford them.

Many commercial qPCR machines have been approved for diagnostic tests. The sample preparation for diagnostic tests typically involves centrifugation and many other manual steps. However the detection occurs via qPCR (quantitative Polymerase Chain Reaction) in PCR tubes. These tubes are low cost and widely available. Some suppliers have tubes available with lyophilized reagents as well. However, most of these are not FDA approved.

There are some fundamental problems with PCR tubes.

i. Due to the shape of the tube, the thermal response is slow. The tube has small surface to volume ratio. The tubes have to be robust enough so they can be pressed against the wall of thermal block. Thus the plastic cannot be made very thin. As plastic has very low thermal conductivity, the thermal response becomes slower.

ii. Since the instruments lump at least 48 wells together the energy required to heat and cool becomes huge even if a single tube is being used for the reaction. A single test is thus very energy inefficient. The whole metal block in which tubes are inserted has to be heated and cooled. Some models like Qiagen Rotogene, BioFire Lightscanner and Lightclcler2.0 use rotary mechanism and air flow for temperature control. These also are designed to handle multiple capillaries and need large amount of energy. None of these systems is amenable for POC individual reaction based systems.

iii. The shape of the tube or capillary limits the design options for optical design. The tubes are mostly queried from top from a transparent cap. Typically only a small fraction of fluorescence is captured. In configurations like ‘optics shuttle’ in Bio-Rad CFX or Light scanner which query each reaction vessel individually, the time on each well can be fast (as is 20ms in case of Light scanner) but still a number of the chambers have to be queried increasing the total time.

iv. The design of instruments for multiple tubes is not easily translated for integration to sample to answer systems for POC. POC systems should be able to conduct single test with high energy efficiency and small footprint.

4.3 Thermal FEA to find thermal response of existing Technology

The research literature does not show accurate thermal response for tube and cartridge based systems which are available. To have a clear idea we modelled the Bio-Rad CFX and Cepheid cartridge for having thermal response data. Bio-Rad CFX is a popular machine with fast thermal cycling capability in a typical multiwell qPCR machine using tubes while Cepheid is the most prevalent POC type instrument.

First we list key properties of materials which we will encounter in design and modelling.

4.3.1 Key Properties of Selected Materials

Material	**Thermal conductivity (k)** $\frac{W}{m\ K}$	**Specific Heat** $\frac{J}{\mathrm{kg}.^{\circ}C}$	**Thermal diffusivity** ($\alpha = \frac{k}{\rho.c_p}$) $\frac{m^2}{s}$	**Co-efficient of thermal expansion** $10^{-6}\frac{m}{m.K}$	**Specific Gravity**
Water	0.61	4182	0.143×10^{-6}	NA	1
Aluminum	205	897	8.418×10^{-5}	22.2	2.72
Copper	401	385	1.11×10^{-4}	16.6	8.79
COP 1420	0.15	1500	0.0986×10^{-6}	70	1.014
Polycarbonate	0.19	1170 - 1250	0.144×10^{-6}	70.2	1.2-1.22
Polypropylene	0.1-0.22	1920	0.096×10^{-6}	100 - 200	0.946

Table 4.1 Thermal Properties of Selected Materials

A Comparison of properties is given in Table 4.2. All materials are compared to Water except for co-efficient of thermal expansion which is compared to Aluminum.

Material	**Ratio of Thermal conductivity (k)**	**Ratio of Specific Heat**	**Ratio of Thermal diffusivity**	**Co-efficient of thermal expansion All vs Aluminum**	**Specific Gravity**
Aluminum	336	0.21449	588.6	1	2.72

Copper	657	0.092061	776.223	0.747	8.79
COP 1420	0.245	0.358	0.6895	3.153	1.014
Polycarbonate	0.311475	0.29890	1.00699	3.162	1.2-1.22
Polypropylene	0.327	0.45911	0.67132	4.504-9	0.946

Table 4.2 Comparison of Thermal Properties

4.3.2 Bio-Rad CFX

The Bio-Rad low profile PCR tube for qPCR was measured to obtain precise dimensional data. The CAD file was obtained as a result of this service. Following procedure was adapted to have the required answers.

i. The simulation is transient thermal only. We don't consider any material dimensional change.

ii. Boundary Conditions: The walls of the tube were given a temperature boundary condition. Thus we assume there is no air gap between the thermal block and the tube which is the best possible case. We also assume that the thermal block temperature at every position is equal at the boundary of the block and the tube. It is also assumed that thermal block thermal mass is much bigger than the PCR tube and we model the boundary as a constant temperature assuming that any amount of heat can be provided.

iii. As the reaction occurs in a closed space, we neglected air convection from top of liquid to the surroundings. This is a valid assumption due to low thermal conductivity of the tube and presence of large air gap inside the tube between the liquid surface and the cap.

iv. Natural convection of air is neglected from the outside of the tube which is not in contact with the thermal block. This is the upper portion of the tube. Natural convection can speed up heat transfer. This is however a symmetric system and heat is being applied from sides as well as bottom.

v. Natural convection is neglected inside the fluid as well. Natural convection can speed up heat transfer. This is however a symmetric system and heat is being applied from sides as well as bottom.

vi. The lid is heated to 105 ° C typically so that condensation of vapor does not occur on the PCR cap. The effect of lid heating is neglected due to the large air gap. The primary purpose of heated air lid is to counter condensation of the tube cap.

vii. Heating simulation is conducted from 55 to 95 ° C. initially the system is assumed to at initial temperature of 55 ° C. this models the condition that the cycler has been at 55 ° for a while so all elements reach 55 ° C.

viii. The walls of the tube are given a boundary condition of temperature jump from 55 to 95 ° C. The temp is then held at 95 ° C. The speed of the system may be limited by ramp rate of the metal block but we are focusing on the liquid reservoir for comparison to other systems.

ix. For cooling simulation similar procedure is adapter with temperature jump from 95 to 55 ° C.

Following results were obtained

Operation	Volume	Time for liquid to equilibrate
Heating	25ul	~20 seconds
Heating	50ul	>13 seconds

To find out the effect of plastic tube on the heat transfer performance, we change the material of the tube to Aluminum. Following results are obtained.

Operation	Volume	Time for liquid to equilibrate
Heating	25ul	~12 seconds
Heating	50ul	>23 seconds

We determine the following

- The fluid in the tube takes considerable time to equilibrate even with metal walls. Thus this is not the optimum geometry if speed has to be increased.
- The polymer wall makes heat transfer considerably slower. Changing walls to metal can save significant time.

The simulation setup and results are shown in Figure 4.1.

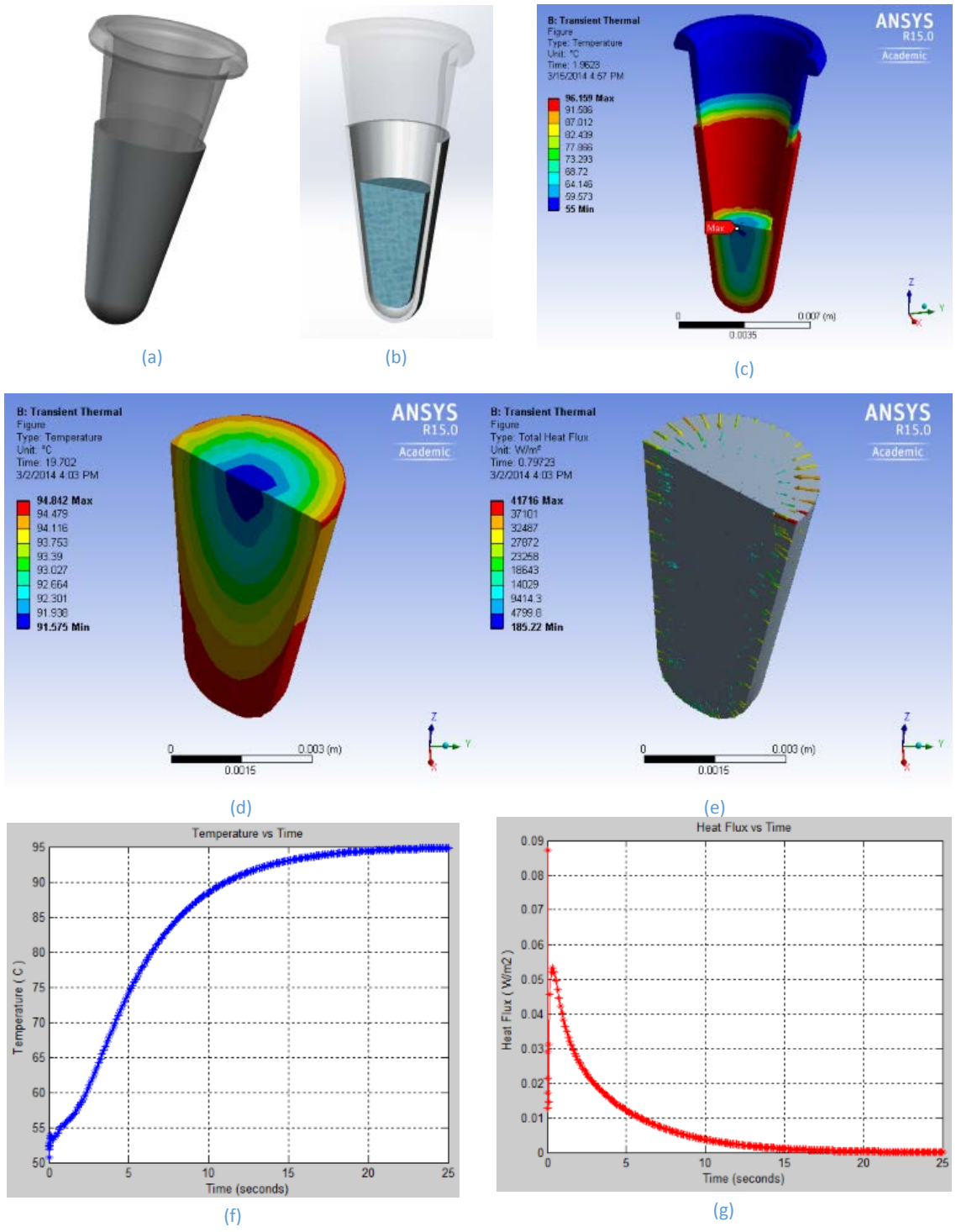


Figure 4.1 CFX Tube Simulation Results

4.3.3 Cepheid Smart Tube

The smart tube has extremely thin walls (about 30um) polypropylene. The polypropylene is laser welded to the cartridge. The cartridge is heated from both sides. This limits the optical interrogation to be possible only from sides. The concept is provided in Figure 4.2. The system uses ceramic plate heaters. To maintain good contact and may be for other reasons the cartridge is designed so that its pressurized when the cap is closed. This is probably to bulge out the walls so that a good contact is made with the flat surfaces of the heater. This shows another serious consideration of thin wall plastic design i.e., a way to have good contact with the heating surface.

Heating from both sides is important for any fully plastic based design. Since the Cepheid wall thickness seems to be thinnest possible practically, it is worthwhile to test effects of heating from both sides versus a single side. In fact the question of heating from both and single sides has already been discussed for another geometry with thicker polymer substrate.

The procedure for heating for both sides is as follows.

i. The simulation is transient thermal only. We don't consider any material dimensional change.
ii. Initial temperature of the system is set at 55 ° C i.e., all elements are set to 55 ° C.
iii. Both walls of the cartridge are given temperature boundary conditions. The temperature is set to 95 ° C. This assume that heaters can be at constant temperature and provide the heat without changing temperature.
iv. The initial condition for the whole system is 55 ° C for heating and 95° C for cooling. This is a valid assumption due to the configuration of the system. The cartridge is in between two heater plates and surface to volume ratio is large.
v. Internal convection inside cartridge is neglected. Raleigh number for cartridge is given in Table 4.4.
vi. Convection to air from side walls is neglected. Two of the walls are very close to the optical excitation and readout parts of the instrument. The thickness of the cartridge is also small compared to its length and width.

We also check the performance by heating from one side only. The procedure is the same except following

i. For heating, entire system is given initial condition of 55 ° C. Thus we assume the air around the cartridge is at 55 ° c and the system has been in this state for long enough to equilibrate with the surrounding. We keep the temperature of surrounding air at 55 ° C. we don't consider heating of the air due to heating of the plate. We also run the simulation by neglecting the convection to air from top surface.

ii. Natural convection inside cartridge is neglected as Raleigh number is not sufficiently high.

We obtain the following results

Operation	Volume/Tube Type	Time for liquid to equilibrate
Heating from Two side	25ul	<5 seconds
Heating from Two Sides	50ul	>13 seconds
Heating from One Side	25ul	>15s seconds
Heating from one sides	50ul	>25 seconds

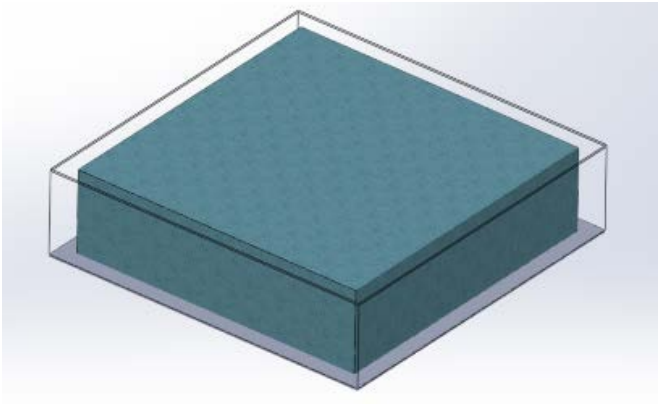
We make the following observations

i. Heating and Cooling from one side is much slower than from both sides.

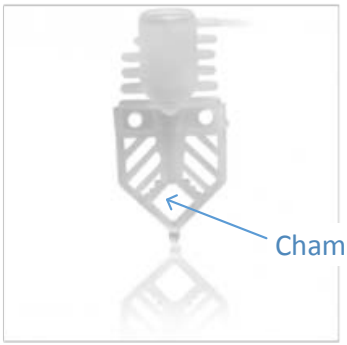
ii. The temperature gradient does not go away even after considerable time if we heat only from one side.

iii. If the cartridge has to double in volume while keeping the sides length same then the time increases considerably. Therefore the design is not flexible to increase optical interrogation area if volume is increased.

Figure 4.2 shows simulation results in the case of heating form one side while Figure 4.3 shows the results while heating from both sides.



(a)



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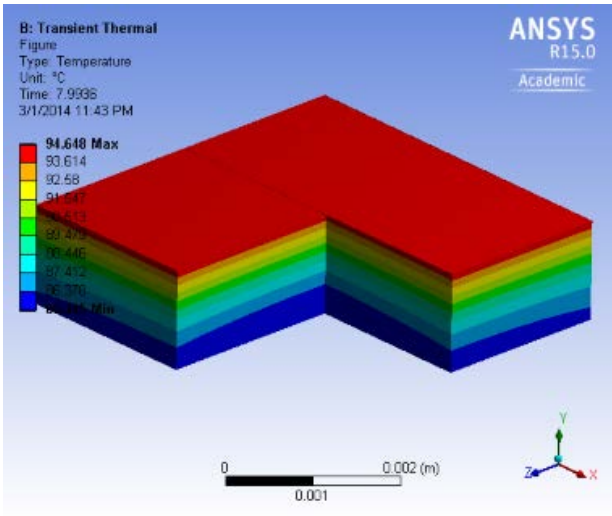

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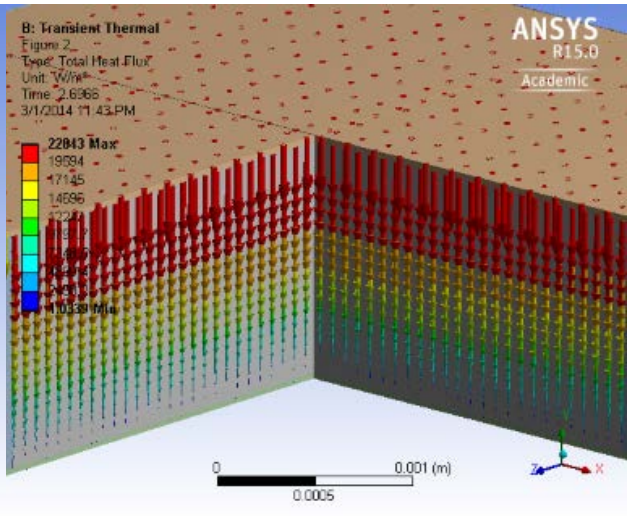

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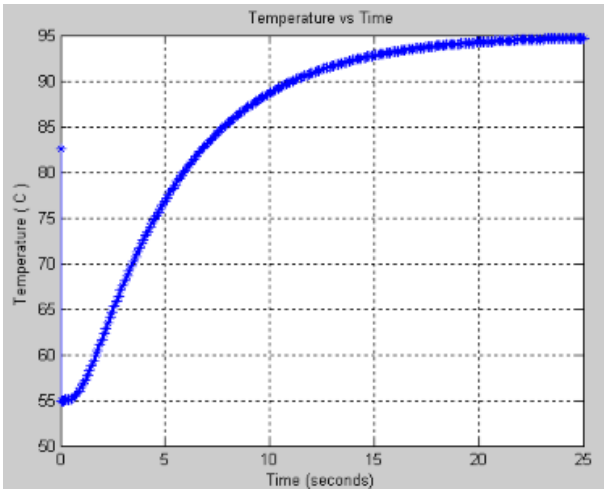

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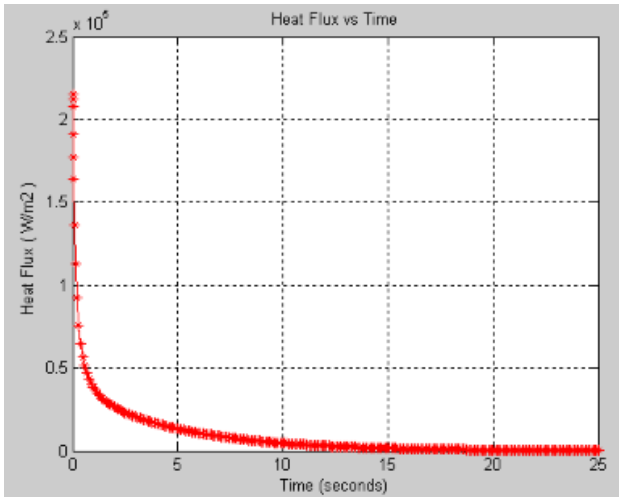


(f)

Figure 4.2 Cepheid Tube, Heating from One Side

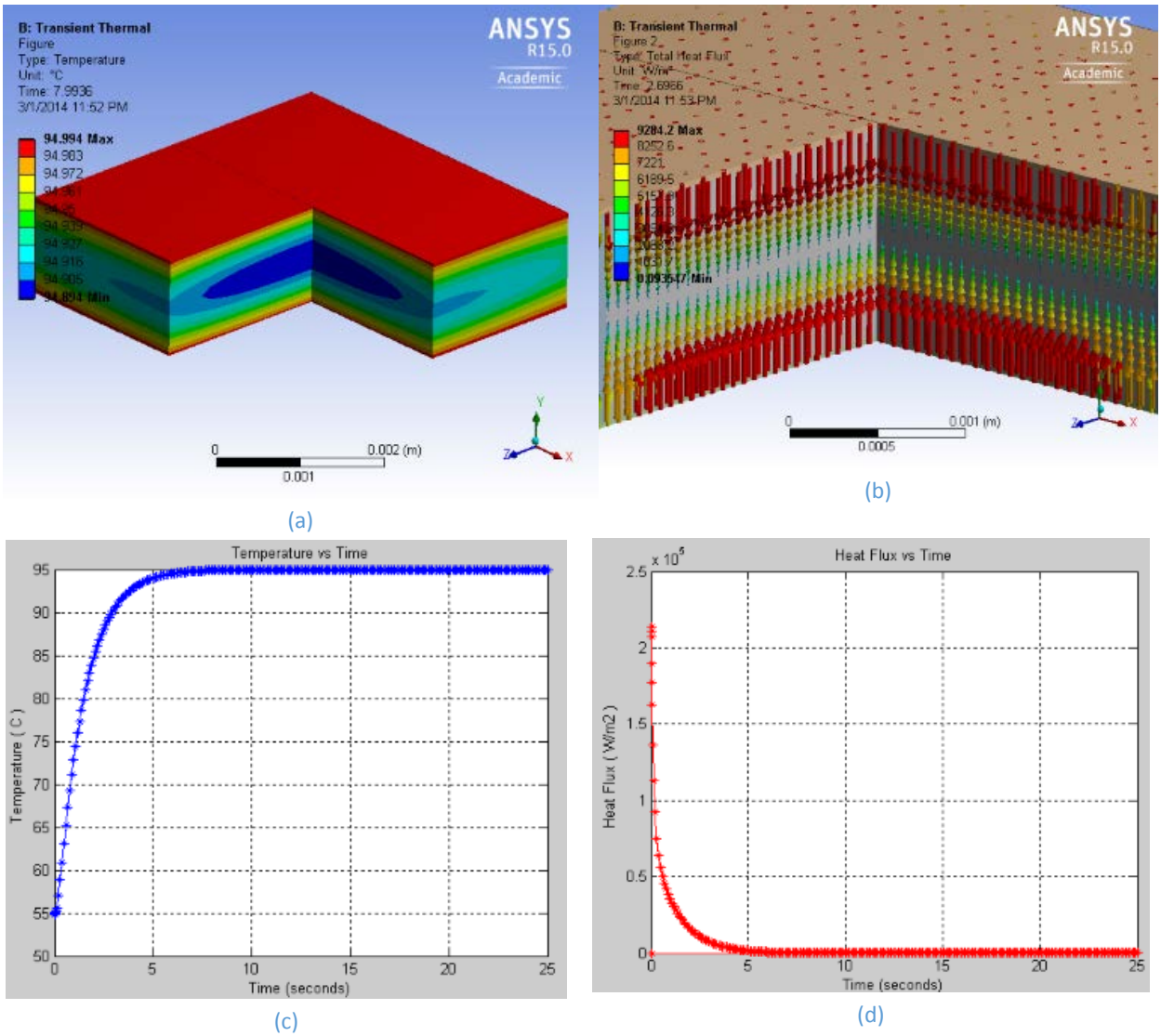


Figure 4.3 Cepheid Tube, Heating from Both Side

This cartridge cannot be used in a flatbed cycler which is much more readily available and requires to fit precisely in only the manufacturer's instrument. Thus it is tied to an expensive system. Surprisingly the cost of instrument is a major part of the expense and cannot be neglected as in a razor blade model of economics. Also the features of instrument become very important in addition to the cartridge alone.

The cartridge is very sensitive to bubbles and partial filling is not allowed. It also needs a centrifuge to get rid of bubbles. This is not desirable at POC as it adds an additional instrument.

4.4 Existing Cartridge Based Systems

There are few commercial systems that use PCR cartridges in non-integrated fashion. They are listed below in Table 4.3.Figure 4.4 shows images for these systems with associated disposables.

Company	Cartridge Type	Remarks	Cost
Veredus	Microchip	Too complex	High
Epistem	Tube like	Three tube, qualitative results	Low
DxNA	Tube Like		
Meridian	Tube		
Cepheid	Flat chamber	Limited by cooling rate Compatible only to Smart Cycler Limited optical interrogation area	Very expensive
BigTech	Cartridge	Speed is not high	
Ustar	Tube like		Low
Mobidiag	Tube Like		
QuantumDx	Cartridge	Non-optical detection	

Table 4.3 Existing commercial PCR systems

There are only few commercial instruments which use cartridges instead of PCR tubes. In case of Cepheid the advantage is performance. In case of Bigtec it's the portability and cost. However the speed is not much faster and one can argue if there is some real advantage of having such a machine instead of an instrument which accepts a single PCR tube with modified dimensions. Both of these instruments don't use Peltiers which are very power hungry and one of the main limitations of bringing PCR to POC. The advantage of BigTec design seems to have lower power consumption then using a peltier. However, looking at Epistem POC instrument which uses tube like element and Peltier- and stills seems very portable, the question of using expensive and difficult to fabricate cartridges arises. Epistem mentions their system to cost $400, the consumable around $0.5 and 30 cycles in 17 minutes. A low cost optical system can do qualitative tests. With respect to cartridge design, Cepheid and Bigtec systems do not seem to give major advantage. Rather Cepheid system is very complex and its penetration into developing world POC is unlikely. Same is case with Bigtec. The cartridge looks more complex than desired. IP issues seem to drive these designs as well and many options are avoided and various approaches adapted not on technical merit but IP issues.

One advantage of Cepheid in a clinic is the flexibility and scalability. Individual's reactions are independent and can be run independently at different times. This can be an advantage in a busy clinic. All wells of normal PCR machines are 'booked' even if one reaction is running. There are some companies which have two or three independent blocks but not individual well scalability like Cepheid.

Out of these systems a very attractive approach would be to have a system implemented like Epistem for normal or modified qPCR tubes and try to reduce the cost of optics to have a quantitative capability.

Figure 4.4 Existing Commercial PCR systems

One advantage of Cepheid in a clinic is the flexibility and scalability. Individual's reactions are independent and can be run independently at different times. This can be an advantage in a busy clinic. All wells of normal PCR machines are 'booked' even if one reaction is running. There are some companies which have two or three independent blocks but not individual well scalability like Cepheid.

Out of these systems a very attractive approach would be to have a system implemented like Epistem for normal or modified qPCR tubes and try to reduce the cost of optics to have a quantitative capability.

4.4.1 Heating and Cooling from One Side

Heating and cooling from one side vs both sides in considered in [66]. Two thermocouples were inserted at the centers of floor and ceiling of the chamber. The chamber height is 750um while the top and bottom layers were 250um each. The gradient in the chamber was measured and found to be significant. A thermal guard was used to have better temperature control [85]. A window was made to allow optical query from the top. Both of these are shown in Figure 4.5. We note that the optical interrogation area is reduced while chip placement becomes more involved. Polymer chips should make good contact with heating surfaces which makes system more complex.

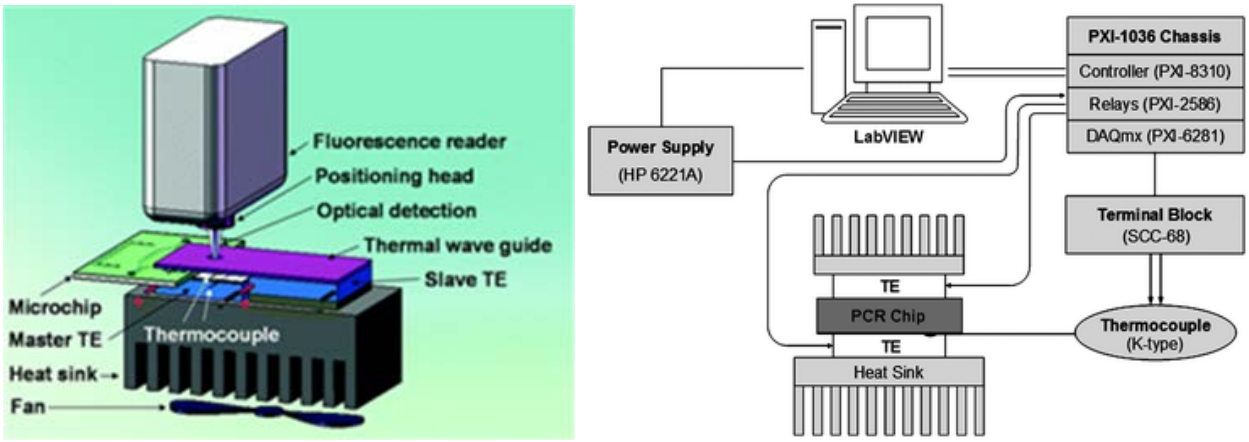


Figure 4.5 Heating from Both Sides in Literature

4.5 Review of Earlier Work in Research Literature

There have been various PCR cartridges reported in literature [55, 63, 65-83, 86-97], however most of them have a polymer substrate with heating applied thorough a polymer. The first problem is that to have efficient heat transfer the walls have to be thin. This makes the walls fragile. For example Cepheid has the thinnest polymer wall (30um) but each tube is tested individually for pressure seal. Thin walls may have caused the production cost to go up and the reliability to be a problem.

The second problem is the reduced heat spreading and uneven heating across the surface in some designs. Especially in peltier based designs the thermal gradients across the peltier surface will persist due to polymer wall. A heat spreader has been used in some cases []. However, the energy required to heat and cool then must also take into account the heating and cooling of the spreader. Since a thin polymer wall will bulge due to pressure developed inside the PCR chamber, a rigid support is needed. There will be concern of heat transfer and the temperature control of this support as well. For example in Cepheid cartridge, the cartridge is supported on both sides by rigid plates which does not allow it to be optically queried from top.

An overlooked feature is the mechanical implementation needed to hold the chip. A fragile chip needs a rather complex mechanical design to hold the chip, align it and apply pressure to have efficient heat transfer. In case of optical interrogation sometimes it would need serious alignment requirements (as in case of Cepheid).

Another neglected area is world to chip and ease of sample injection. Many 2D designs like made from Silicon substrates inherently are 2D. To have sample injection by untrained personnel outside lab environment can be very error prone and difficult. A related problem is sealing the cartridge. A PCR tube has an integrated cap which be pressed to make a seal, however, most of cartridges in lab don't have a sealing mechanism to be used at POC. This is in part related to the limitations of design. A sliding mechanism [86] was used a design to seal the design which could be filled with a pipette. However this design used several discrete components which can make it expensive in production.

4.6 Problems to be solved

We find that following problems need to be solved

i. Heating from two sides or thermal guard approach reduces interrogation area and makes cartridge design and related instrumentation complex.

i. Using films or flat substrates have problems with sample injection. Separate connectors have to be used for interfacing to pipettes.

ii. Bonding methods. Both laser welding and solvent bonding are relatively complex. Solvent bonding puts quality constraints on the flatness which requires expertise in injection molding. Solvent bonding can make it difficult to use lyophilized reagents.

iii. Effect of bonding method on lyophilized reagents.

iv. Lyohpilizaition methods: Ideally the reagents should be lyophilized on some substrate before bonding. Else there can be lyophilized palettes. Lyohpilizaition once the chip is assembled and starting from liquid phase is not a good idea. The surface area for lyohpilizaition process is this case is severely reduced. Note that in case of PCR tubes the surface area is still relatively large due to wide opening at the end of the tube. However, most chips have narrow channels for filling which will limit the surface area of lyohpilizaition process. The chip would undergoes extreme temperatures which can put addition design constraints on its structural integrity.

4.7 Checklist for PCR cartridge design for POC

To have something useful in POC settings new requirements are desired. Overlooking these requirements can provide a large number of options to be considered. Using these requirements as a checklist can help weight an idea very early before putting time and effort to make it work.

i. Cost of production and design
ii. Robustness- integrity of the cartridge should be maintained by relatively rough handling
iii. A way to have lyophilized reagents already stored
iv. Ease of sample injection (world to chip), preferably should be easy to fill using a pipette
v. Some solution to bubble problem during filling and other operations
vi. Cartridge insertion and ease of placement in the instrument
vii. Preferably can be used with low cost commercial instrumentation of easy to make setups like flatbed cycler

viii. Ease of monitoring – for optical interrogation
ix. Easy sealing.
x. Testing of new assays should be easy to include more diseases in future
xi. Does not need special equipment like centrifuges etc.
xii. Variable volume design should not be limiting factor.
xiii. Variable volume filling is desirable like a PCR tube

Many technologies which work well in lab will not work reliably in harsh and changing conditions at POC and especially in remote areas in developing world.

4.8 Design and Analysis

Now that we have a good idea about limitations of common PCR cartridges and the problems which need to be solved, we go forward to solve some of the problems.

4.8.1 Concept

The fundamental ideas of our cartridge design can be summarized as below

i. Use a metal substrate. The metal will offer very low thermal resistance. A very thin coating on a metal can also act very close to being a metal only substrate as far as thermal performance in concerned. Metal also acts as a heat spreader.
ii. Use a polymer top for optical viewing and interrogation.
iii. Integrate various structures in the polymer and design for injection molding. Integrate ports for filling and seals for sealing into the design. Also integrate structures to physically handle the chip.
iv. Use adhesive bonding. This is cheap and fast. Adhesive bonding puts less constraints on quality of the injection molding parts like flatness. Adhesive bonding and even UV adhesives can work by masking some area where lyophilized reagents are present.
v. Do lyohpilizaition on metal substrate which is flat and has very good thermal characteristics for lyohpilizaition process thus speeding up production speed and reducing time and energy required.

4.8.2 Design

To have a robust design we employ state of the art modeling approaches. The cartridge has to be designed to withstand stresses due to thermal mismatches, retain optical performance and allow fluidic filling. There are various parameters which need to be optimized or considered on the bases of ease of use and practical fabrication constraints as well as availability.

4.8.2.1 *Shape*

The basic design consists of a polymer top with metal base. The heating and cooling is done from the metal side. After considering a various options, the polymer top is injection molded with cavity. The height of the cavity is 0.5mm. The bonding area is at the periphery of the cartridge. There are pillars and sealing structures explained later. There have been 2D designs mentioned in literature which use approaches like LOM (laminated object manufacturing) and lithography. However they have the difficulty of world to chip connections. If a separate connector is used, the problems of bonding and cost arises. There are limited commercially available options for connectors which interface easily to pipette tips and can be sealed easily. The cavity is a rhombus. This is a shape to allow filling without bubbles. Our design need only one bonding step and still allows 3D features.

There are various options to implement the basic metal-polymer design. Few options are shown in Figure 4.6.Figure 4.6 (a) shows the cavity etched in metal, (b) shows the cavity in polymer and (c) shows the cavity in the intermediate layer. In the last case the cavity layer is separate from the polymer top so there is one additional layer in this case. This can be implemented by using a patterned tape, for example. However tape adhesives are generally weaker than a cured adhesive in general.

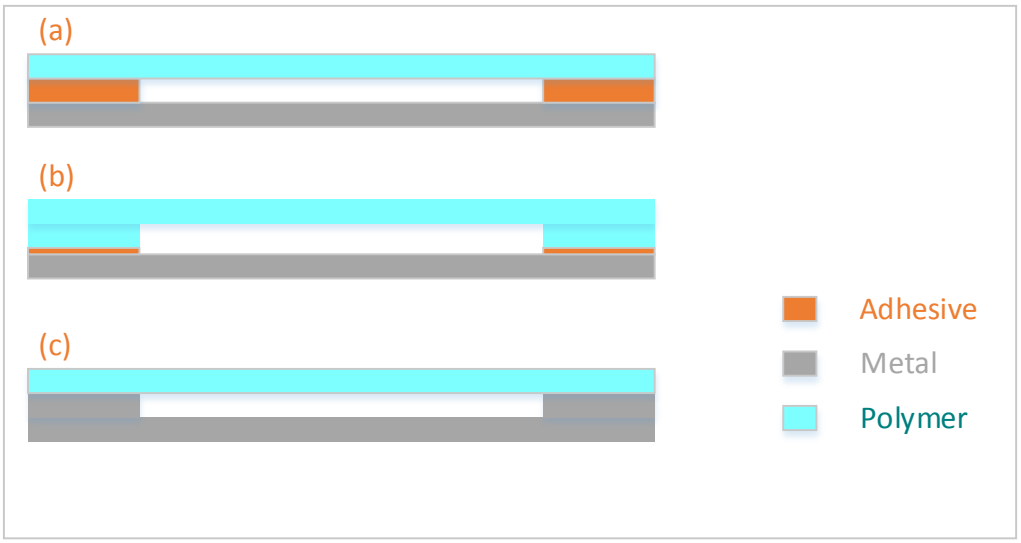


Figure 4.6 Options for Metal-Polymer Cartridge. (a) Cavity is defined by Adhesive Thickness. (b) Cavity in Polymer. (c) Cavity in Metal.

The cavity in the metal can be made using etching, embossing and various other processes. Etching was tried using Aluminum etchant. However, the mirror finish is not retained as a result of the process. Recovering the mirror finish needs additional processing. In this case the metal has to be at least as thick as the depth of the cavity. This is a disadvantage as opposed to other two cases in which the metal can be very thin and whole thickness of metal is used in heat spreading and robustness. It is not trivial to obtain mirror finish in the lab. Polishing in an etched cavity is more difficult than a flat surface. Amonet used PVD (physical vapor deposition) to achieve a highly reflective surface. It's easy to get polished Aluminum in flat sheets. It's also easy to cut polished metal in desired shape while retaining flatness by laser and die cutting. Metals in simple shapes are very cheap.

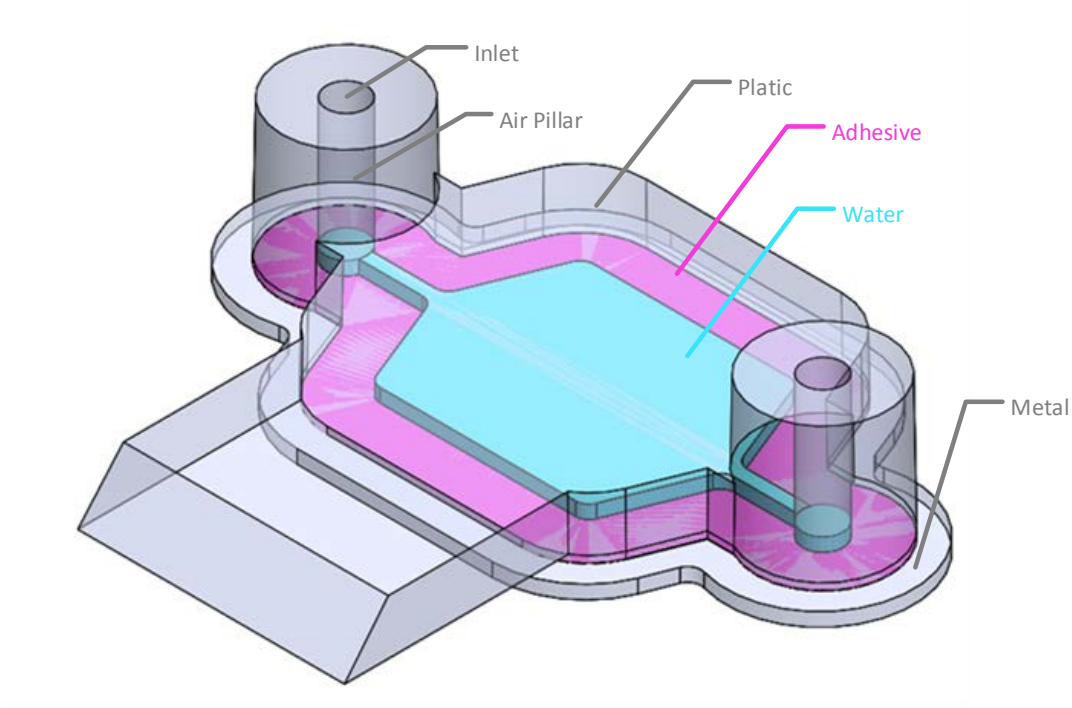
Using a separate adhesive layer can be implemented by using double sided adhesive tapes. However, they are typically not as strong compared to cured adhesives. There is also the need to align and hold under pressure the polymer top, adhesive and the metal. The important point is that the adhesive need to be as thick as required height of the cavity. In our case the height is 0.5mm. Thus the sides of adhesives which are exposed to the fluids offers a relatively large as compared to a very thin adhesive layer. There are ways to circumvent that problem by making the top polymer shaped to reduce adhesive exposure, however they make the top more complex. It is also more complex to automate the process of alignment of the adhesive layer. Another important problem with this approach is with optical design which is explained later in this document. The adhesive adds another layer and interface. The adhesive should ideally be non-fluorescent but most of the adhesives have auto fluorescence.

It’s easy to get mirror finished Aluminum sheets at a reasonable price. One can get a single material polished sheet or a sheet with coatings

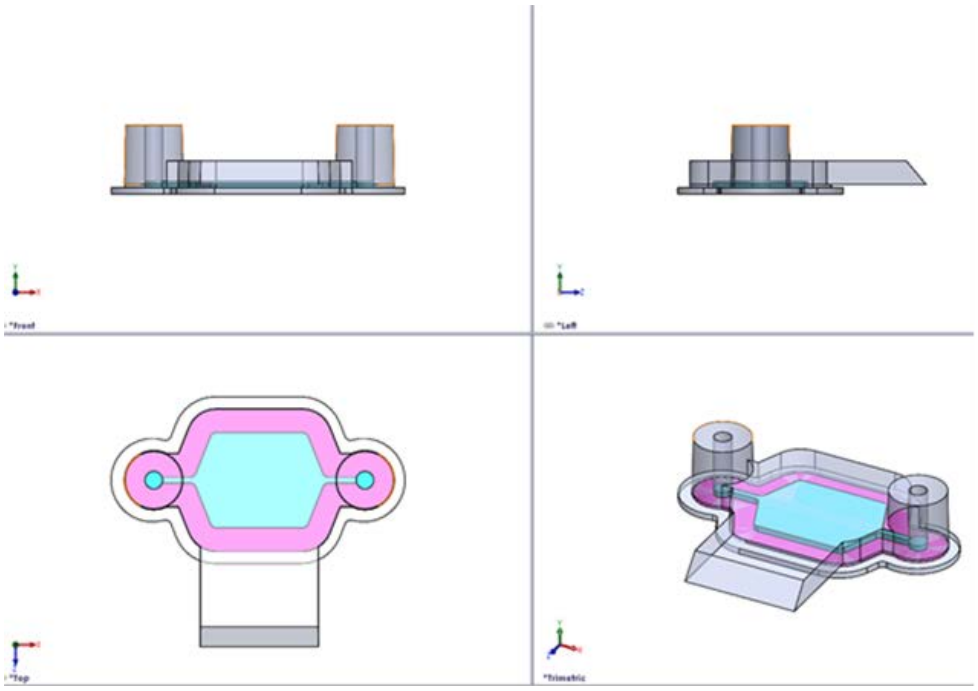
4.8.2.2 Thermal – use of metal

It is intuitively obvious that metal will offer lower thermal resistance as compared to a similar thick sheet of polymer. However there are some other important advantages. Metal also acts as a heat spreader so the entire surface is at uniform temperature. Another important advantage is that metal acts as a local heat supplier. With polymer base, a separate heat spreader might be necessary. Heat can be provided to the PCR solution even with a thick metal due to very low thermal resistance. This allows the solution to reach the temperature of the metal quickly even with one sided heating which is not easy to achieve with polymer based systems. This fact has been overlooked in many designs. Since the publications normally do not quantify the thermal gradients in the cartridge, such an artifact can remain hidden but could still have negative effects on PCR reactions or its efficiency. In some cases the cartridge was heated on both sides by peltier devices[66]. However such an approach is energy expensive and normal flat PCR machine cannot be used. Heating from both sides also constraints the region of optical readout and puts mechanical design and space issues into consideration. Metal also allows a rugged design.

The cartridge CAD diagrams is shown in Figure 4.7 while the picture is provided in Figure 4.8 .



(a)



(b)

Figure 4.7 PCR Cartridge

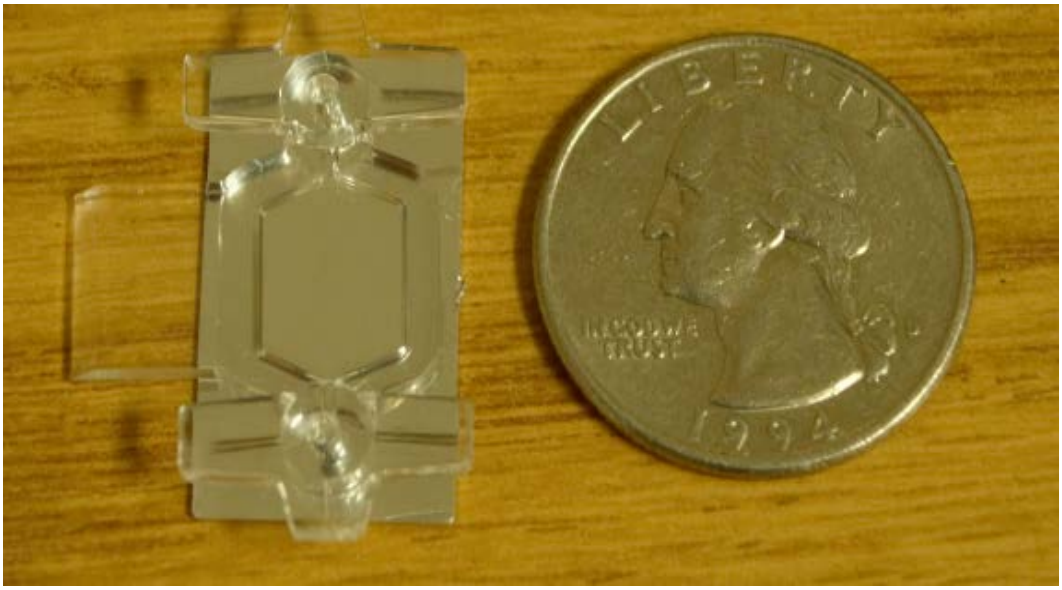


Figure 4.8 PCR Cartridge Picture

4.8.2.3 *Key Features of Different PCR Chambers*

Different feature of different PCR chambers are given below in Table 4.4.

Chamber Type	Heating Surface to Volume Ratio	Rayleigh Number (Ra)
Sphere of 25ul	0.5	
Sphere of 50ul	0.251	
CFX PCR Tube – 25ul	1.57	
CFX PCR Tube – 50ul	1.544	
Cepheid Smart Tube – 1 sided heating	0.81	474
Cepheid Smart Tube – 2 sided heating	1.62	
qPCR Cartridge, 0.25mm thick, 25ul	4.24	7.42
qPCR cartridge, 0.5mm thick, 50ul	2.12	59.36

Table 4.4 Key Features of different PCR chambers

4.8.3 FE simulations - Thermal

We run parameterized FE simulations first to know thermal response of the system. We then run structural simulations to know the specifications needed for adhesives and its thickness.

We compare following cases

i. Metal substrate with fluid thicknesses of 25ul and 50ul
ii. COP 1420 substrate with 100um thickness. 100um is considered to be thinnest feasible substrate thickness for practical applications.

The simulation is setup as following

i. The simulation is transient thermal only. We don't consider any material dimensional change for thermal simulations. Since the dimensional change is very small this is a valid assumption.
ii. Initial temperature of the system is set at 55 ° C i.e., all elements are set to 55 ° C.
iii. Bottom surface of metal substrate is given temperature boundary condition is 95 ° C.
iv. The initial condition for the whole system is 55 ° C for heating. This is an estimate of what would happen between thermal cycles.
v. Internal convection inside cartridge is neglected. The Raleigh number for this cartridge is much lower than that required for onset of convection [98] [99].
vi. Convection coefficient of 5 $\frac{W}{m^2}$ is assumed for polymer surfaces. The ambient temperature of air around cartridge for convection is assumed to be 25 ° C.

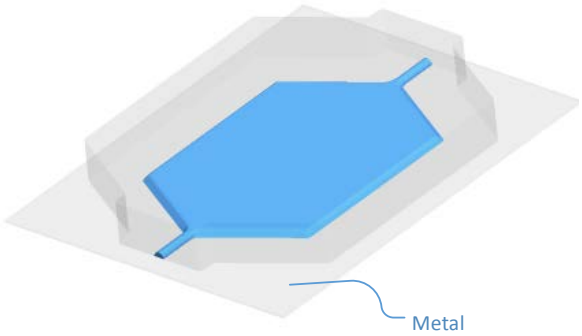
Figure 4.9 Shows simulation results for cartridge with metal substrate while Figure 4.10 shows results for cartridge with polymer substrate of 100um thickness.

We note the following

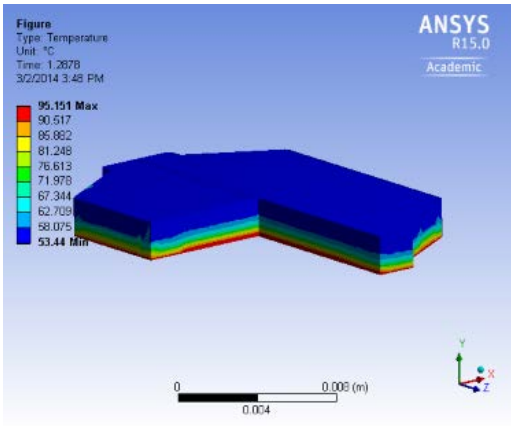
i. There is marked difference is liquid approaching the set temperature in case of metal and polymer substrates. It shows problems caused by thin polymer substrates.
ii. Heat flux also shows lower heat flowing into the fluid because of polymer substrate which acts as a resistance to heat flow.
iii. As shown in Figure 4.9 (b), the higher thermal conductivity of water helps in letting the fluid equilibrate to the temperature of the plate. In contrast this advantage is lost due to polymer substrate as shown in Figure 4.10 (b).
iv. Heat flux in both cases is essentially in vertical direction.
v. Even in case of metal substrate there remains a gradient for long time in the fluid. This is since heat is still flowing into polymer. However in metal's case we have an advantage of having thermal insulator on top. The more thermal resistance, the better. Fortunately PCR

process is robust to these small temperature variations as it's a kinetic process. The gradient is much smaller in case of metal substrate.

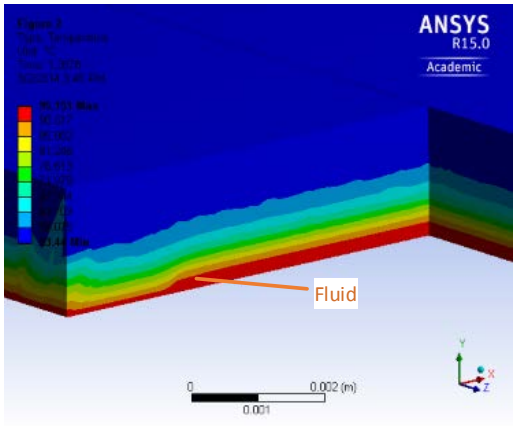
vi. Thermal uniformity in horizontal direction is very uniform and it can be approximated that the layers of fluid are uniform temperature.



(a)



(a)



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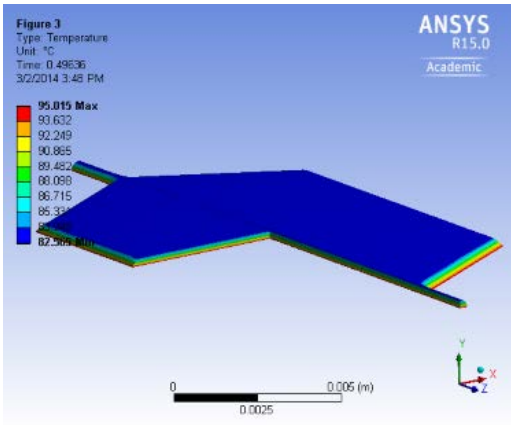

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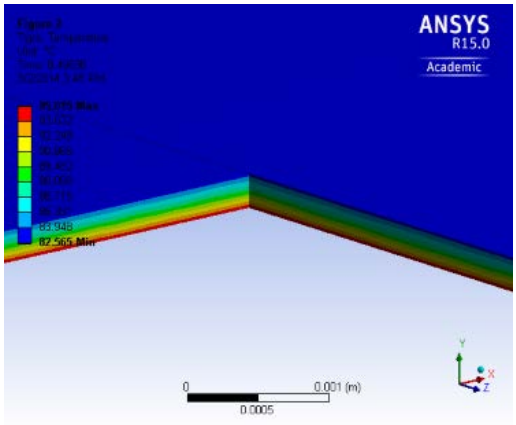

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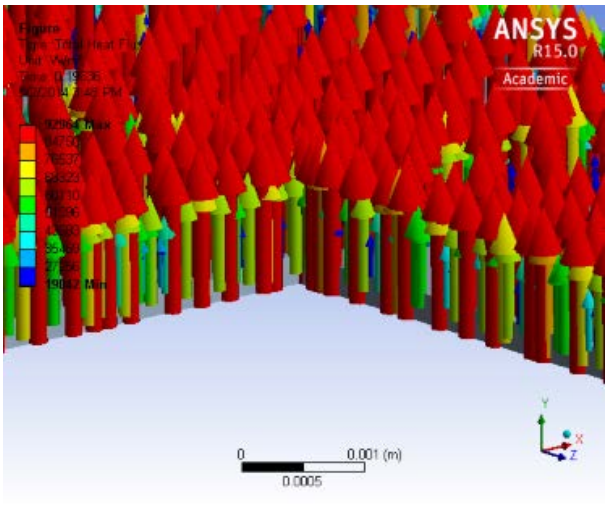

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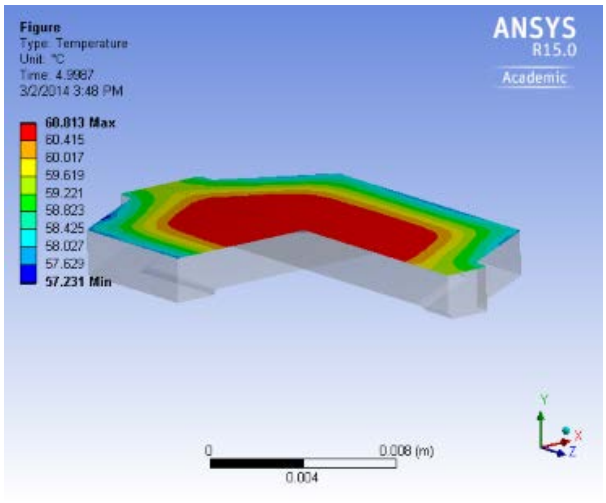

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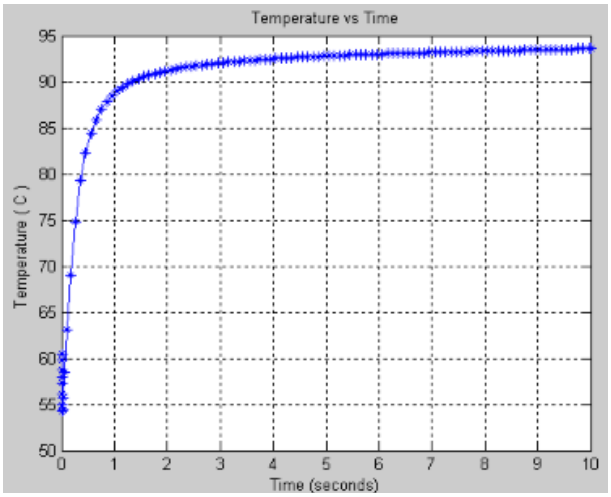

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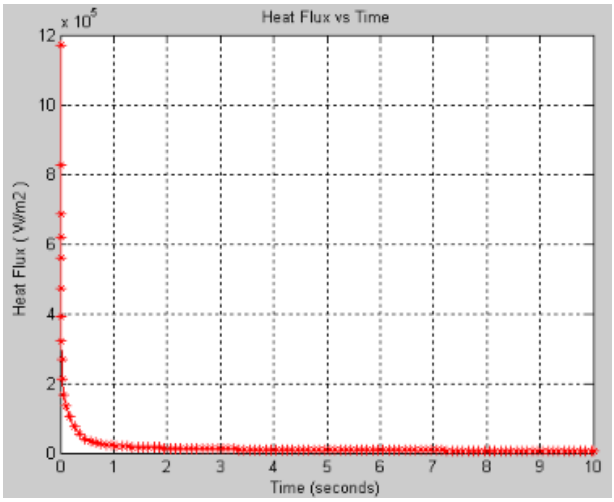


(e)

Figure 4.9 Simulation for PCR Cartridge with Metal Substrate

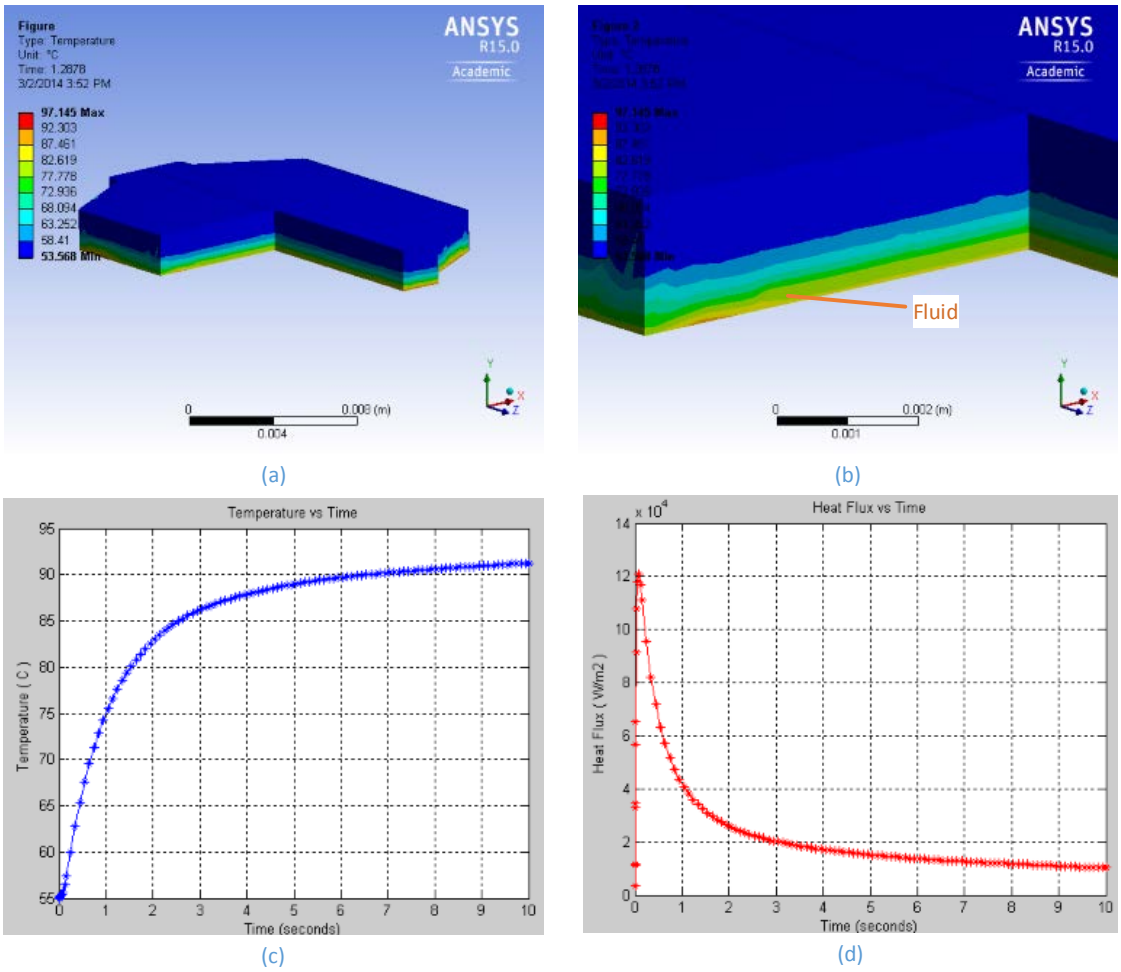


Figure 4.10 Simulation for PCR Cartridge with Polymer Substrate

As can be seen the polymer causes significant delay in reaching equilibrium.

4.8.4 Analysis of Data

In Figure 4.11 the results of transient simulations are presented. The temperature for qPCR cartridge is at its center and at fluid-polymer boundary.

First we compare the cartridge with fluid height of 0.25mm with Cepheid cartridge which is heated form both sides. The temperature in our cartridge rises faster. However it retains small slope. This is due to heat transfer into the top polymer. Thus there will be a temperature gradient in the cartridge. The gradient can be reduced by waiting longer. However, if the reaction is robust to small differences in temperature then the time can be reduced. For example if 2 °C difference inside the chamber can be tolerated then the time to reach desired temperature is same for both Cepheid and qPCR cartridge.

However, in our case our cooling can be almost as fast as heating. In Cepheid, the cooling is significantly slower. Also the effect of polymer base is shown. The polymer base increases the rise time.

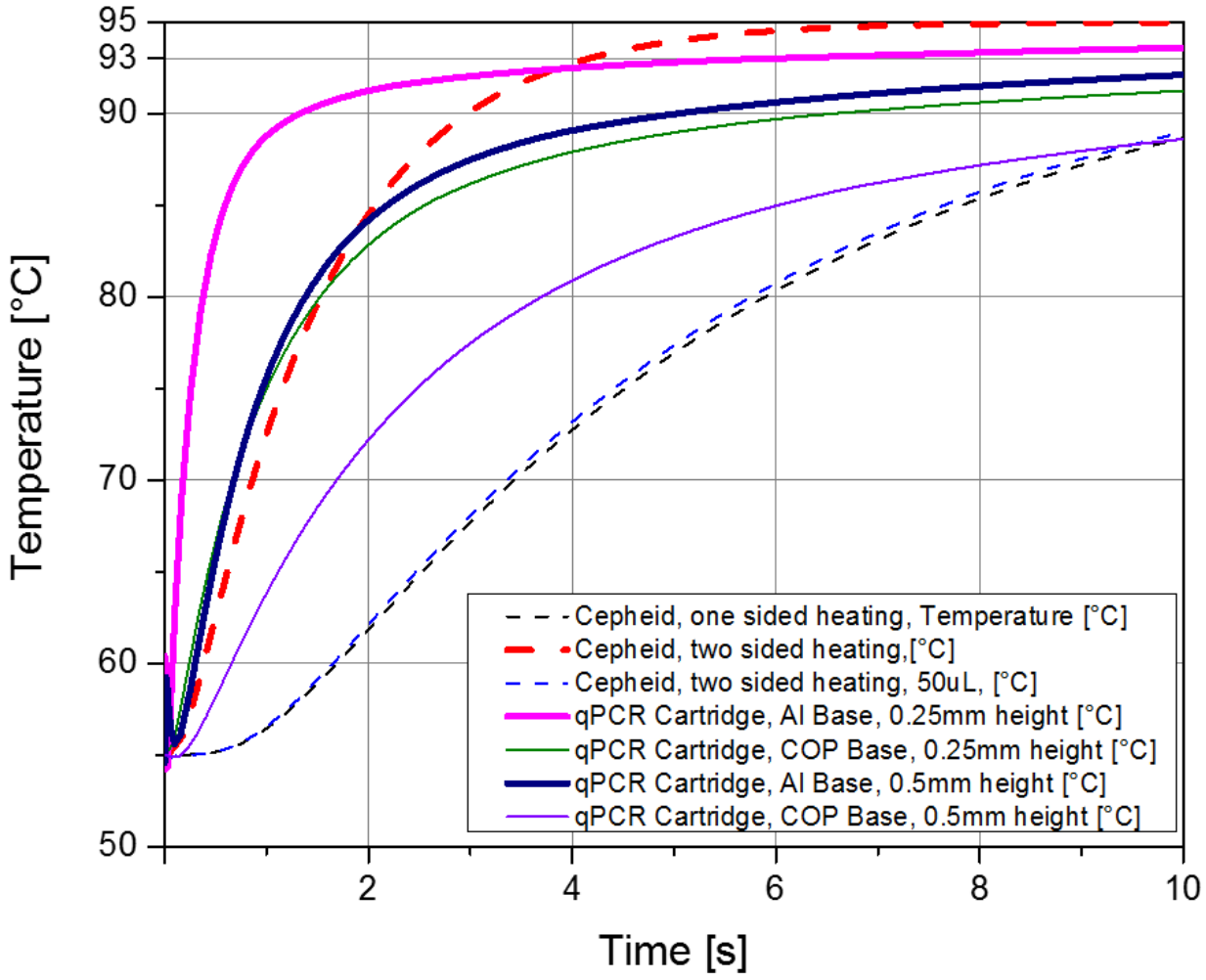


Figure 4.11 Comparison of Transient Thermal Response

4.8.5 Thermal 1D lumped models

We have verified that a 1D model (in vertical direction) is valid for thermal simulations and modelling by our 3D simulations. There are various tools to have lumped element modelling applied to our system. The advantages of such 1D modelling is much higher speed and thus getting results faster for parametric design.

The parameters were thickness of the fluid, thickness of metal and thickness of the top polymer layer. Since we need interlayer thermal profile we divided each of the elements into multiple layers. Various tools were evaluated to conduct this study. We compared Matlab, maple and others. Although there are

tools especially designed for thermal modeling, Matlab has features additional to such modeling such as optimization so it was preferred. Physical modeling toolbox allowed generating various layers and models of conduction and convection. A script was written for analysis. A block diagram of the model is shown in Figure 4.12. The system is thus a high order dynamical system. Heat flow or temperature can be used as excitations. These were applied to the bottom of metal plate as would be done in real situation. Each material layer has multiple lumped elements thus providing information inside the layers. We can thus see the thermal gradients in various materials. This model can also simulate additional elements. For example we have added a heater to the base of metal. This is a model of very thin heating layer applied to the base of the metal and bonded to metal using an adhesive.

The lumped model allows various configurations of integrated heating and cooling be tried out in much shorter time than FE analysis. Another advantage of lumped element modelling and using Matlab® is that the control systems can be tried and optimized to have best possible thermal response. The power of Matlab for control system design is well known and integrating the cartridge with Matlab in a model is a very powerful approach to optimization of the whole system. By using numerical optimization techniques, the ultimate performance which can be achieved via such system can be known. This is significant since various simple strategies like PID controller can be evaluated against the upper bound to find out their efficacy and whether it's appropriate to apply more advanced controllers or not.

There have been various controller strategies applied to improve thermal response of PCR in cartridges, but most of them were without an upper bound to know the real merit of such techniques.

Using such models allow us to apply arbitrary waveforms as excitation to the system as well. They can also model the measurement uncertainties, noise and time lags.

The rich theory of optimal and model based control and modern estimation theory can be used in the design of such systems.

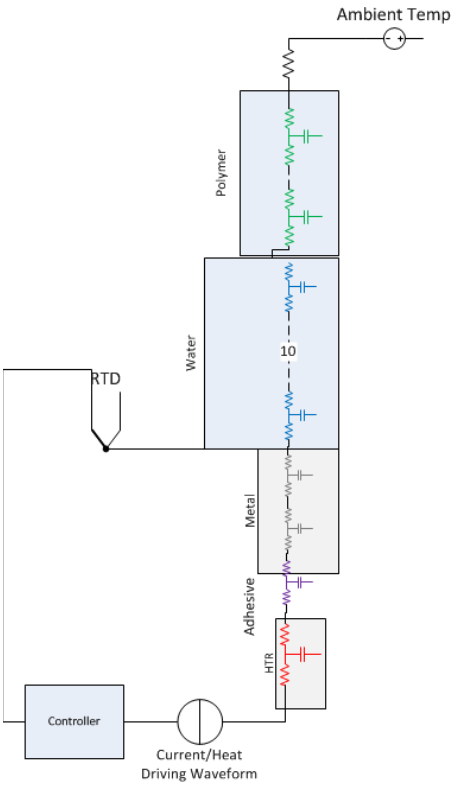


Figure 4.12 1D Lumped Model for thermal Design

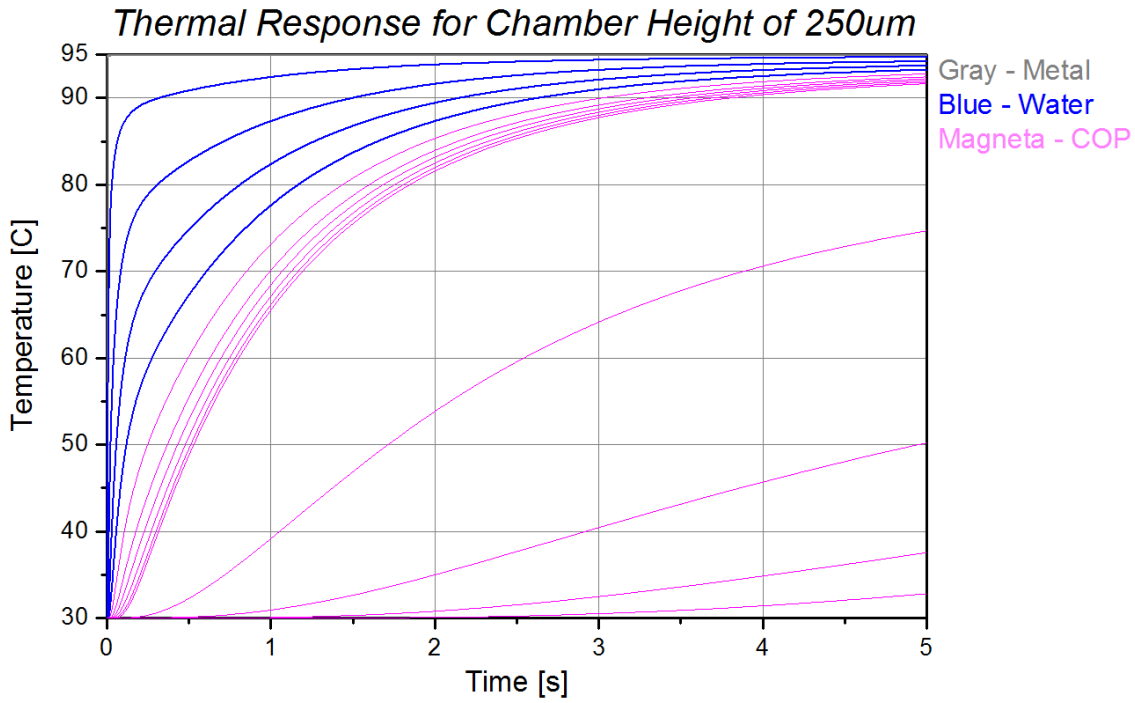


Figure 4.13 Lumped Model results for Fluid thickness of 250um

Temperature spread obtained from1D simulations are show in Figure 4.14.

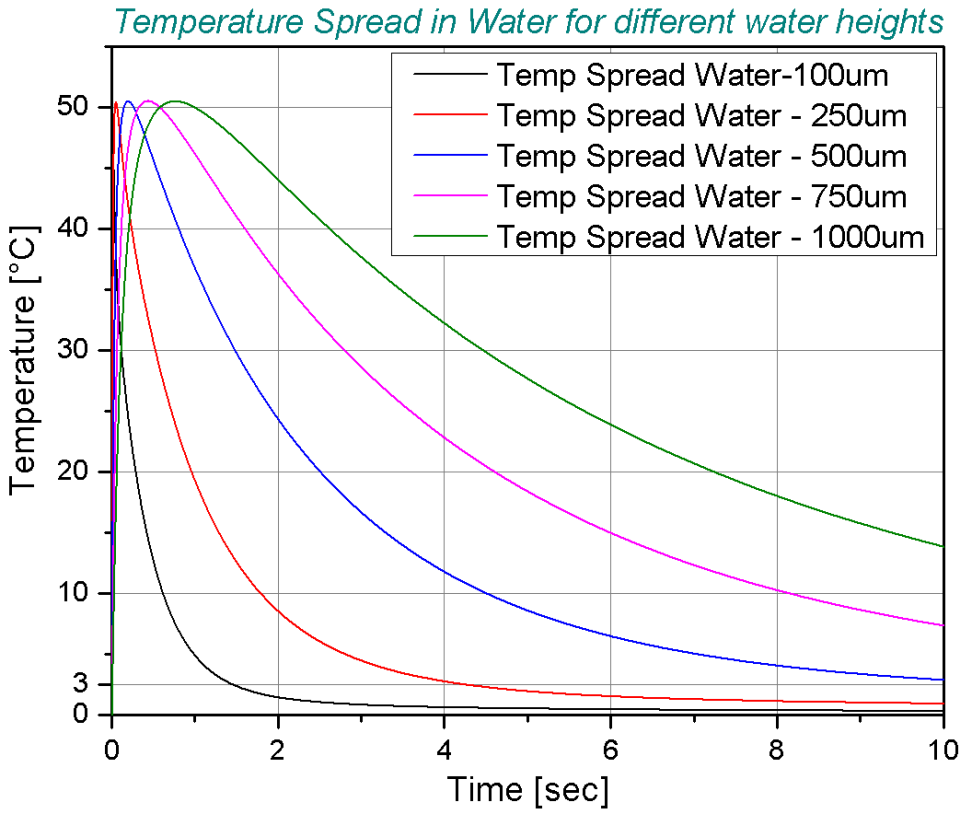


Figure 4.14 Temperature Spread in Water

The important observations from the results are as follows

i. We can know the time it takes for all fluid layers to come close enough in temperature. Although we are measuring temperate at the bottom metal surface (or its equilibrated there), there is still time for all fluid layers to reach the set temperature. To measure this experimentally is very difficult.

ii. Metal acts as a local heat supplier. Therefore if the temperature is monitored at metal it's enough. To briefly explain these effects consider that the top layer of metal is in contact with the fluid. If heat is applied the entire metal changes temperature much quicker than the fluid. The heat contained now in the metal can move from layer to layer to the top layer which is in contact with the fluid and equilibrates with it. If a similarly thick block of polymer was set at the temperature this would not occur since heat cannot be transferred so quickly from layer to layer.

iii. Having different thicknesses of metal changes the energy required for the whole cartridge. This becomes important if the chip has to go in a POC instrument which needs to save on energy. However thicker metal plate increases thermal uniformity horizontally as well in case the heating and cooling excitation are uneven as in case of TECs.

iv. Heating element can be attached to the metal like a thin film heater or conductive ink. Thus we would require low energy while achieving thermal uniformity and mechanical robustness. This approach can also be very fast. However, it makes the substrate more complex and more expensive than simple metal base.

v. In case of forced air convention cooling (using a fan for example) metal convection coefficient has an advantage. Therefore metal substrate is better even for air cooled systems.

vi. The top layer on the top is insulating and cannot supply or pass heat to atmosphere due to high thermal resistance between layers. Thus it can be made thick as well. Making it thick will increase mechanical robustness. Since materials like COP have very low auto-fluorescence, such a design is both mechanically robust while being able to be queried optically for real time PCR or high resolution melt type analysis.

vii. We would retain the advantages of metal even when we a very thin coating (biocompatible or having some other function). When the coating is very thin the advantages of metal would be relevant. Thus we can have an extremely thin polymer coating (having the surface chemistry of it) which still not contained to make it thick as a substrate layer.

4.8.6 FE simulations – thermal- Structural

We have analyzed the thermal performance of the cartridge and its dependence on various parameters. However, the cartridge should also maintain structural integrity while the temperature is cycling. This becomes very important in case of metal substrate as polymers and metal have different thermal expansion coefficients. Mechanical stresses can place different constraints on the selection of parameters.

Since we want to use adhesive bonding, the stresses on adhesive become very important. The adhesive has to sustain large temperature differences. There is a wide variety of adhesives available. Testing all of these adhesives in not very practical. Thus getting some quantitative information by simulation is very

helpful to screen various adhesives in a predictive manner. It also allows robust design by calculating the margin of safety for operations.

Surprisingly, we could not find a full thermal-structural simulation for PCR cartridges. However, there may be many designs which are marginal and do not quality for six-sigma design for production and safety.

The simulations also provide information about high stress points and areas which can guide in the iterative design of cartridge.

To have a realistic design, we first used thermal simulations as described above. The thermal load (temperature at each point in space) is applied to a structural simulation. In this way realistic temperature distribution is applied to various bodies.

There is a complication in this approach due to fluid in the system. Although the thermal simulation can use fluid as it treated all bodies as incompressible due to small displacements, the structural simulation has to take into account such deformations. In actual system, the fluid can flow and it's not a solid body. As can be seen in Figure 4.7 , the air pillars have air entrapped which will allow fluid to move up as air is easily compressed in those pillars. Thus the pressure applied by fluid all along can be approximated by a constant pressure. The pressure can be approximated as vapor pressure of water. We can thus neglect fluid body by making its relevant parameters for structural simulation such that they don't affect other bodies. Ansys® allows to perform this operation. Figure 4.15 how the basic mechanism for such calculations.

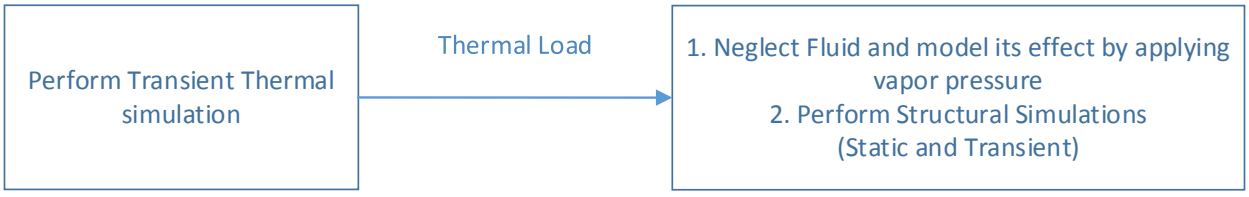


Figure 4.15 Thermal Structural Calculation

One of the problems is to apply correct structural boundary conditions to the cartridge. In actual use we want to put cartridge over a flat surface. The cartridge is not firmly fixed and is free to move and deform at a very small scale. However, if we want to somehow constrain the cartridge to disallow rigid body motion during simulation. This problem is solved by observation of transient thermal simulation. We note that top of pillars remain at almost the same temperature till end of simulation time. We apply a

'Fixed Support' boundary condition to top of one pillar. Applying this boundary condition to both pillars is not realistic to the actual use.

One of the main parameter in structural simulations is the adhesive thickness. Intuitively, a very thin adhesive layer has to undergo more stress. It is important to know how adhesive thickness changes the requirements on adhesive performance.

The stresses on adhesive give us quantitative information about required performance. This is of great advantage since now instead of testing each adhesive on the cartridge, we can get adhesion performance between simple shaped substrates and use our simulation data to find out which adhesives are suitable for our application. If a new geometry has to be used, same measured data can be used. The data for various adhesives which quantifies their performance in not readily available and may have to be measured. However, tensile strength tests and similar common techniques are useful to get this data. Flat and small substrates (typically 1inch by 4 inch) are used to get this data. Once measured the data for a set of substrates can be used in simulation and significant changes can be made to design without worrying about bonding issue.

The bonding issue in PCR cartridges, especially mass produced are notorious to cause leaks. We thus provided a predictive way for robust design circumventing lot of expense, time and experimentation.

The results for simulation are shown in Figure 4.16. Note that the cartridge has to withstand deformations due to forces generated by thermal loads.

Getting correct models for adhesives in not trivial. Both the adhesives mentioned above have glass transition temperature lower than 95 °C. Thus the correct model might need viscoelastic parameters. This data is not readily available. Nevertheless, the simulation allows to figure out trends and values with estimated parameters.

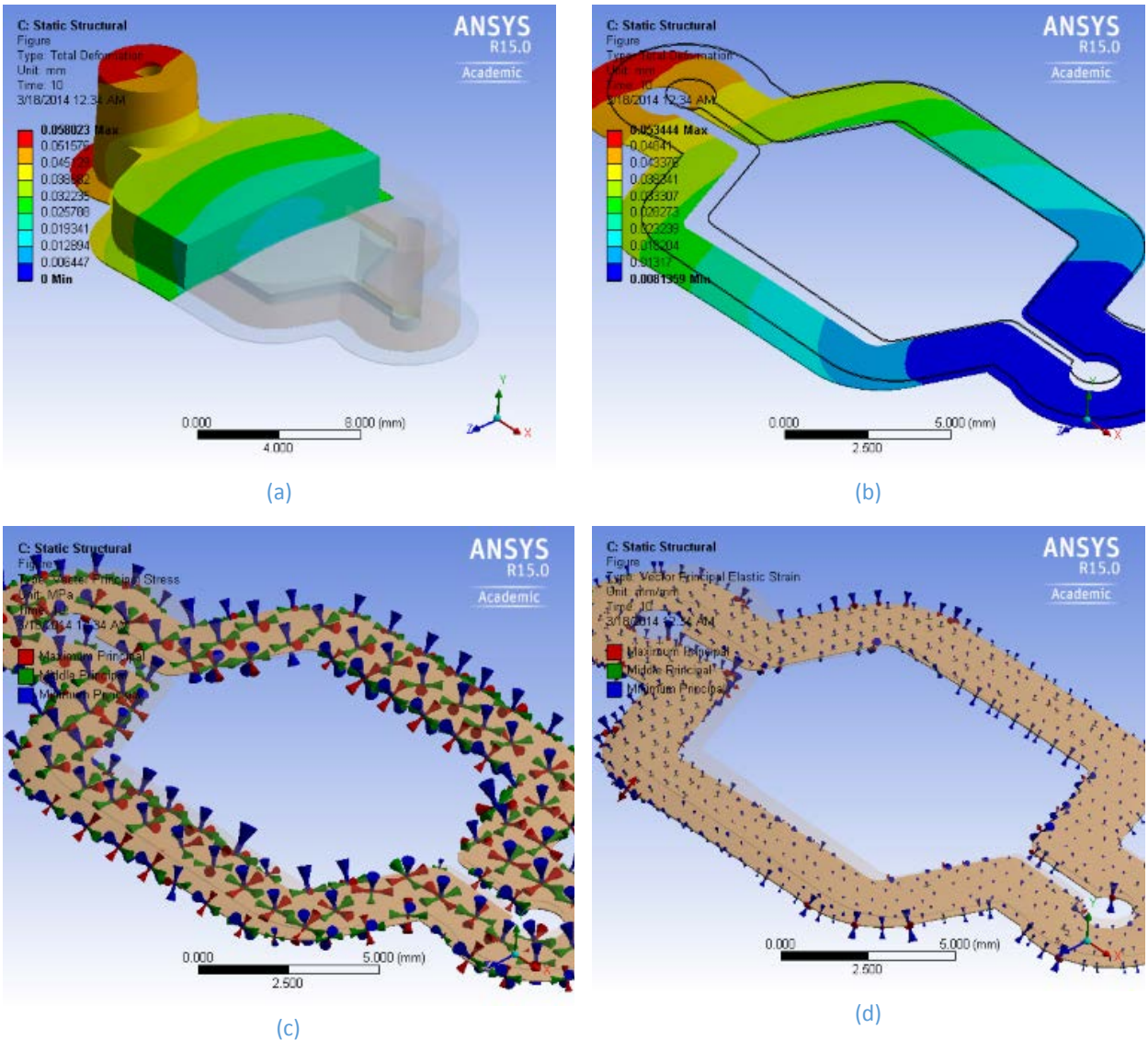


Figure 4.16 Thermal-Structural Simulation Results

Figure 4.17 shows the shear and normal stress as well as deformation in the adhesive as a result of thermal loads. Both shear and normal stress decrease with increasing bond line thickness as expected.

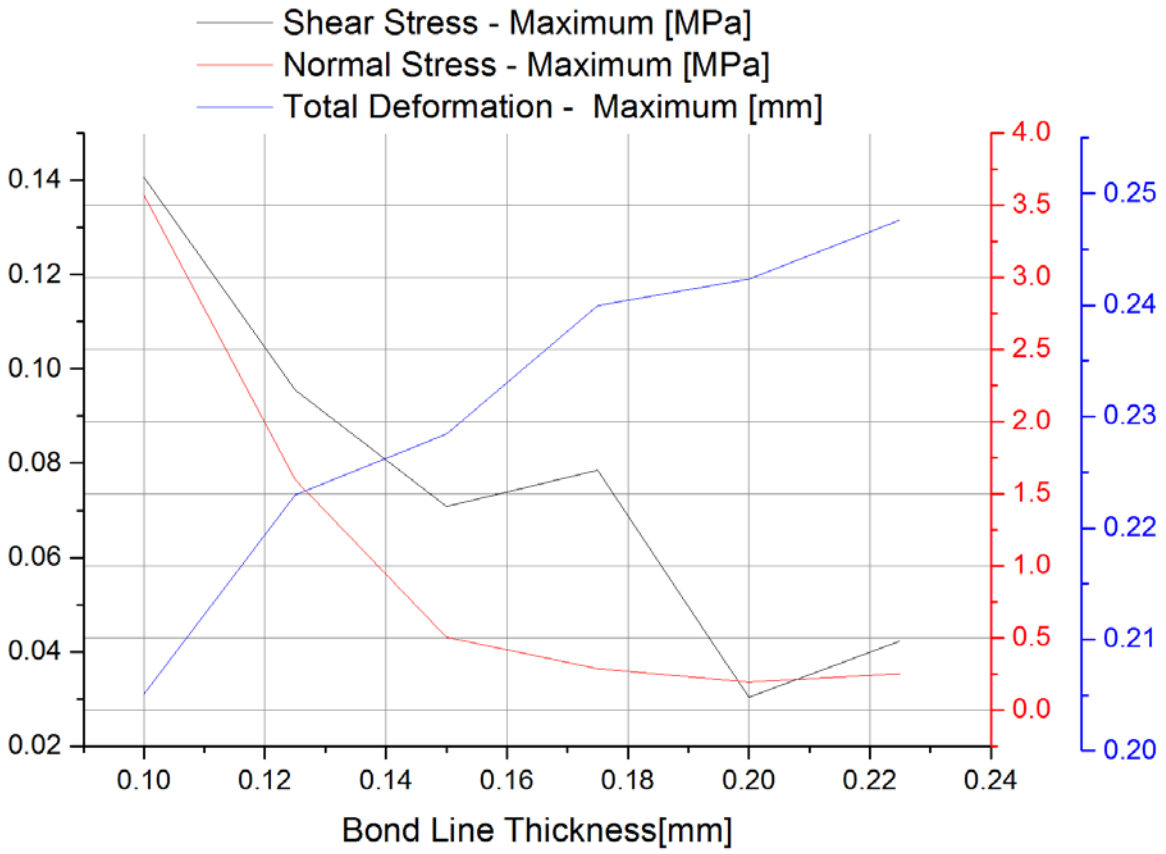


Figure 4.17 Stresses and Deformation in Adhesive

4.8.7 Choice of COP and its Properties

There are many polymers which have been used in PCR cartridges. Some examples are Polycarbonate, polypropylene, Topas or COC (cyclic olefin copolymer) and COP (cyclic olefin polymer). COP is about 3 times as expensive as Polycarbonate. However, it has some excellent properties to be used for bio-diagnostic purposed. COP has low water absorption, excellent optical clarity, low auto-fluorescence, low surface energy, and low protein binding and good injection molding properties.

COP thermal properties are not readily available so we have provided them here. We notice that many parameters change with temperature. As temperature rises, the heat capacity increases while thermal resistance decreases. As thermal time constant is the product of thermal resistance and the heat capacity, it's interesting to see that overall thermal time constant increases with increase of temperature. There is about 10% increase in thermal time constant from 20 ° C to 100 ° C. This non-linear change has been considered in the simulation.

Table 4.5 COP 1420R Specific Heat

Temp C	**Cp(J/kg K)**
51.15	1378.81
80.15	1574.61
110.15	1754.47
119.15	1825.96
128.15	1942.99
135.15	2074.11
142.15	2145.5
170.15	2273.41
205.15	2410.7
240.15	2536.1
275.15	2643.11
310.15	2701.66

Table 4.6 COP 1420R Thermal Conductivity and Resistivity

Temp C	**K(W/m K)**	Rth (K m/W)
31.15	0.161	6.211180124
48.15	0.158	6.329113924
80.15	0.172	5.813953488
111.15	0.181	5.524861878
141.15	0.206	4.854368932
171.15	0.215	4.651162791
201.15	0.218	4.587155963
231.15	0.228	4.385964912
261.15	0.235	4.255319149
291.15	0.245	4.081632653
321.15	0.263	3.802281369

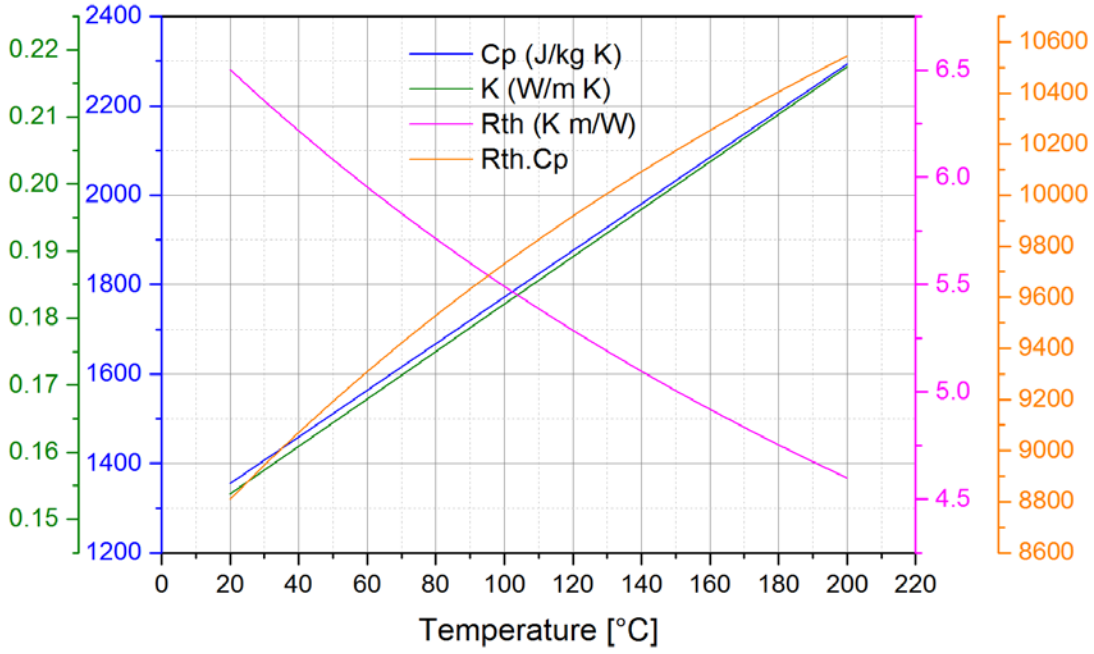


Figure 4.18 Thermal Time Constant vs Temperature of COP 1420R (Interpolated data)

4.8.8 Bonding

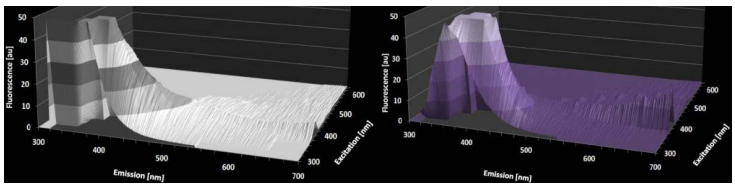
Since metals and polymers have different CTE's there will be stress on the adhesive bond. This will also change as a function of the properties of adhesive and its thickness. Metals and the polymers are solids. However due to the pillars and entrapped air the fluid will move. To model this fluid modulus was set to extremely low value so that artificial effects of its expansion are not there. A vapor pressure of 13 psi was used in the chamber which is the vapor pressure of water at 95 °rees.

Such modeling helps in screening adhesives which would be useful for this application and the methods of their application. It is difficult to find out the stresses which would be developed by other methods. Figure 4.16 shows the stresses developed which the adhesive bond must survive.

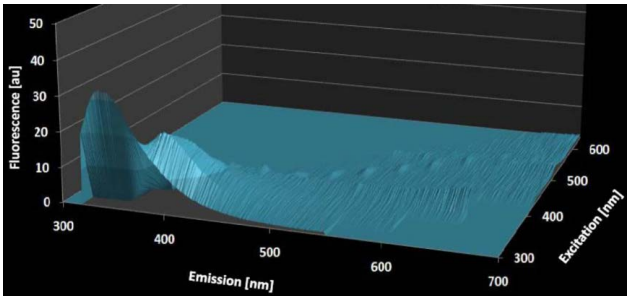
4.8.9 Optical

To enable optical detection for real time PCR, COP has excellent optical properties like very low auto-fluorescence.

The surface is metal is desired to be smooth and reflective. This is additional advantage of metal. If polymer substrate is coated with metal then a similar optical performance can be achieved. This allows the fluorescence which is directed in the downward direction to be reflected upwards.



Polystyrene, 1.5mm *Topas 8007 COC*



ZEONOR 1420R, 2mm

Figure 4.19 Auto-Fluorescence of COP 1420R vs Topas and Polystyrene

Key features of selected polymers are given in [100].

	Acronym	Water absorption (%)	Optical tranmissivity Visible (%)
Cyclic Olefin (co)polymer	COC/COP	0.01	>92
Polycarbonate	PC	0.12-0.34	90
Polypropylene	PP	0.1	~50
Polyethylene	PE	0.01	low

4.8.10 Design Features of Cartridge

We have established that a thick top does not compromise thermal performance. As such thick top is easy to be produced via injection molding. Having thin and long features are a problem with injection molding process [101, 102]. Having very thin walls are thus not much feasible with injection molding and hence the cost of such designs can be more.

Injection molding also provides us with many desirable features while lowering production and assembly cost. Injection molding can provide complex 3D features which are not suitable to be produced via silicon micromachining including lithography techniques. Various other techniques for microfluidics like LOM (laminated object manufacturing) also don't provide 3D thick features and to add additional elements can make the chip much more complex [103].

From our previous experience with building cartridge with adhesive tapes, we appreciated the importance of connectors and the difficulty of putting them as a separate component, as is done with nanoport connectors.

We thus added ‘pillars’ on each side of cartridge. The pillars act as inlet and outlet. The diameter is around 1.5mm which fits well to pipette tips. The inlet has slight fillet to allow docking and sealing of pipette tip. There is flat area (support structure) on the top side of pillars. These are useful to apply seals, holding cartridges and during processing and fabrication of cartridges.

The support structure on top of pillar is convenient for sealing and acts as a useful place to hold while in operation and processing. We used these in the jigs for vacuum metallization, plasma cleaning and adhesive bonding jigs.

4.8.10.1 *Sealing*

Although the cartridge can be sealed using adhesive films conveniently we designed a custom seal which can slide over the cartridge. There is an option of making the closing part an integral part of the cartridge like a PCR cap. This can be done in production but would make the tooling expensive during prototyping. However with sufficiently large quantity this is an attractive option and the cartridge can be sealed like a PCR tube on both or one ends. The seal compresses the rubber. Figure when it slides on the supports of the pillars. The seals can be made suing a 3D printer. The seals as given in[86] don’t have a pillar structure for variable fill volume and air volume. Also this design covers some of the top portion thus reducing area of optical interrogation or optical observation during filling or operation. This can be reduced by having long channels but that increases the dead volume. Our seal design does not block area of optical interrogation.

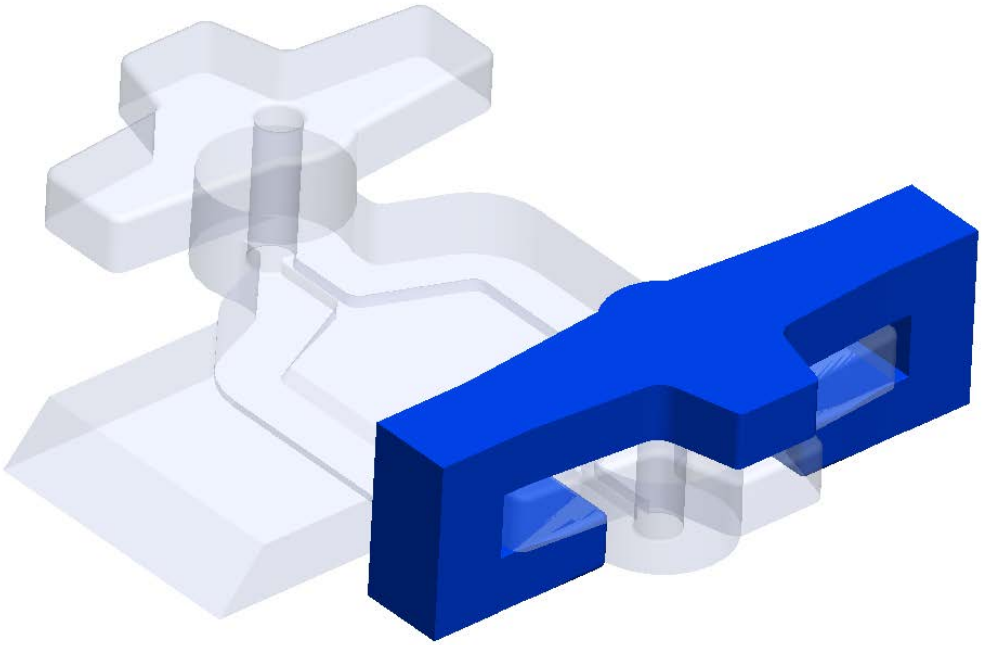


Figure 4.20 Cartridge with Seal

4.8.10.2 *Sample injection*

If the chip is flat (2D) then one has to be very careful putting in right amount of fluid. The excess fluid or even the correct amount can come out of the other side due to pressure applied by pipette. The thermal expansion will put additional stresses if there is no air in the chamber. The pillars not only serve as convenient connectors but also allow air in them to be compressed thus reducing stresses. Air also acts as insulator.

The chip allows easy sample injection using a pipette. By injecting the right volume, the fluid fills the cavity without bubbles. There is an air gap between the fluid and the seals due to the integrated pillars. This design is more forgiving of casual filling as would be a fully planar chip. Also the temperature at the seal is reduced due to insulating properties of air.

4.8.10.3 *Lyophilized Reagent Storage*

The lyophilized reagents on a polymer substrate has its problems. Polymers have varying degrees of moisture absorption and reagent absorption as well. To circumvent this problem aluminum deposition is normally done to reduce the absorption for long term storage of reagents on chip. However in our case since the lyophilized reagents can reside on the surface of Aluminum, long term storage is effective. The

metal plate in a tray lyophilizer would allow very fast heat transfer and lyohpilizaition can be performed directly on the Aluminum plate before being assembled.

4.8.11 Cartridge Filling Simulation

The cartridge shape should be such as to allow full filling. The contact angle on Aluminum and unprocessed COP is different. We have to design the chamber shape to allow for variations in parameters so that filling is reliable in operation.

Following are brief specifications for the simulation

- We used VOF (Volume of Fluid) Methodology to model multiphase flow
- The fluid domains were water and air
- k-e Turbulence model was used with Implicit body force enabled
- Gravitational acceleration was considered
- Surface Tension with wall adhesion is modeled (this can be changed as parameters to ensure filling with varying specifications)
- Adaptive time-stepping was used

Figure 4.21 shows results of the simulation in animation form.

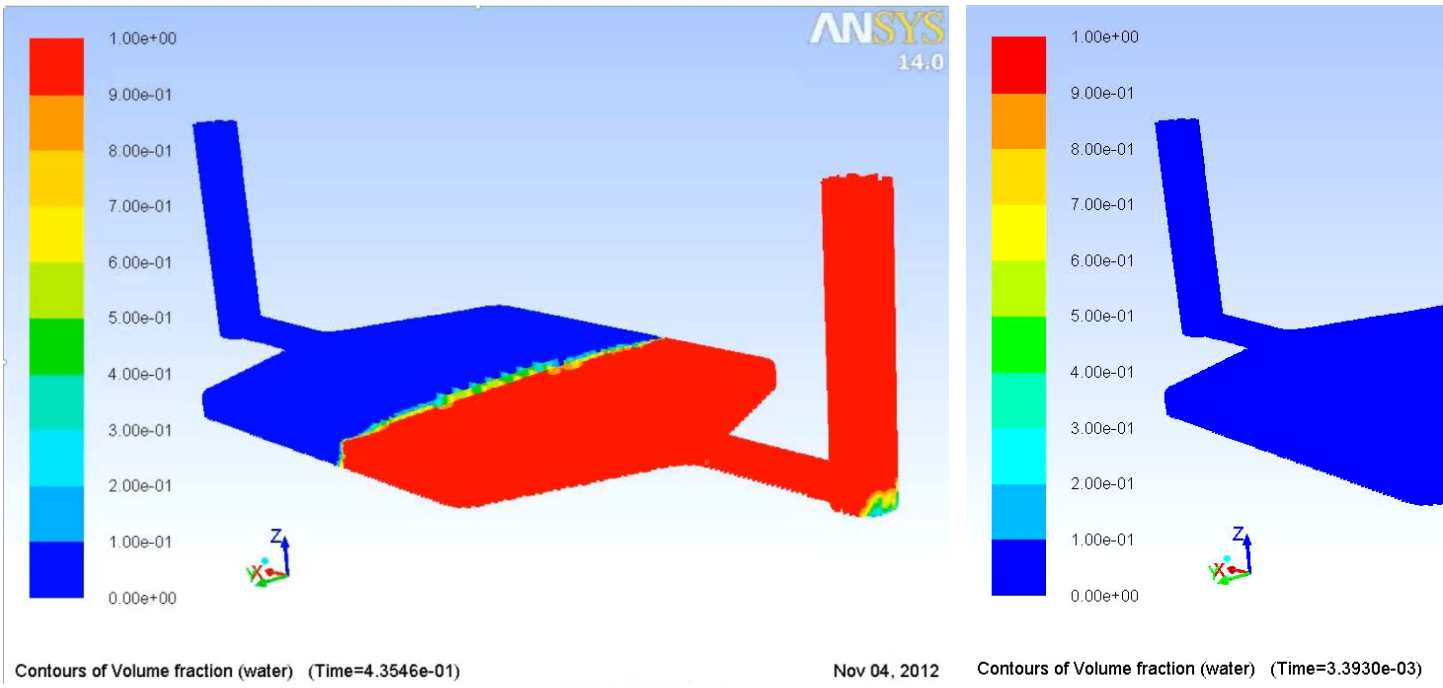


Figure 4.21 Cartridge Filling Simulation

4.8.11.1 *Conclusions*

We have studied the limitation of existing systems by accurate simulations. We listed the key requirements which point of care qPCR cartridges should meet. We then present our concept and do a simulation guided design. We first do thermal simulations. We used the thermal load to calculate the stresses which the adhesive has to withstand. We have provided a predictive way to find the correct adhesives for such applications. The cartridge was also added with many useful design features like integrated fluidic connectors, supports and sealing ports. By using COP as the polymer material we hope to have excellent optical performance.

Our design provides a solution to many longstanding and hidden problems. We have provided a way to heat and cool from one side while still having a mechanically robust design with large optical interrogation area. Our design is amenable to lyophilized reagent storage as well. Due to mechanically robust and rigid cartridge, the placement is much easier. Due to metal substrate the heat transfer performance is expected to be much better as compared to polymer based systems.

5 Metal-Polymer Cartridge for Quantitative Real Time PCR at Point Of Care – Fabrication and Test

5.1 Introduction

In the first part, we performed simulation based design of the cartridge including simulations for adhesive bonding. However, it should be mentioned that there are other ways for bonding as well which can be employed [100].

5.1.1 Bonding Method Selection

We can divide these techniques into polymer to polymer and polymer to metal for our purposes. We consider polymer to polymer as well since we can coat the metal with a polymer if that is the only feasible way. Polymers can be joined in a wide variety of ways including direct bonding (adhesive bonding) and direct bonding (thermal bonding, solvent bonding, laser welding, ultrasonic bonding, surface treatment and modification) and mechanical methods (seals under pressure) [104].

Adhesive bonding has the advantage of joining dissimilar materials as is sort of a **universal method** provided appropriate adhesive can be applied. Adhesive bonding is also widely used and is one of the lowest cost methods. Adhesive bonding can be fast and there are easily automated for production. For our purposes, adhesive bonding can preserve our design intent of having a simple metal substrate with reflective surface. Metals in simple shapes are cheap and easily available.

Direct methods can require much more control may be difficult to automate especially when it comes to small sizes encountered in lab-on-chip devices. Thermal bonding requires precise control [105-108]. The process parameters have to optimized and the surface quality can still suffer [106]. The minimum

feature size can also be a limit in this process. Thermal bonding of COC is also studied and the process parameters are described [109]. Thermal bonding has another major problem in our case. If the substrate already has lyophilized reagents, thermal bonding may not be feasible due to the temperature which the reagents have to go through. Thermal bonding also seems very slow in production and not feasible for out purposes. Thermal bonding also requires special pressure controlled jigs or presses. The pressure and temperature may have to be controlled over time and many have to follow a profile. Given we are interested in producing a large number of cartridges, this is a very expensive preposition.

Solvent bonding[110] has been used for various substrates including polycarbonate(PC), Polymehtylmethacrylate (PMMA) and cyclic-olefin copolymer (COC) [103]. Various companies now offer solvent bonding services. Solvent bonding protocols for COC are given in [103].

Solvent bonding has various problems. It can cause surface roughness. We want to preserver optical quality of our cartridge due to optical design which will be discusses later in the thesis. Solvent bonding also requires very high quality surfaces. Thus the injection molding has to be high quality. Solvent bonding also requires pretreatment especially vacuum drying which is not needed for adhesive bonding. Special precise equipment is needed for solvent vapor or liquid application and special jigs have to be made to keep the exposed substrates under pressure and temperature profiles. As such it has few of same problems as thermal bonding. It is also not compatible with our idea of having lyohpilizaition done before assembly on the substrate. The bond strength of solvent bonding can be very high and an approach that of bulk material. Solvent bonding has also been shown to preserve narrow channels. For optical applications, another advantage of solvent bonding is that there is not issue of auto-fluorescence of adhesives. Solvent bonding can be slow since the parts may have to stay in special jigs till fully bonded. Solvent absorption in the polymer is another potential problem of solvent bonding. Additional drying may be needed to get rid of absorbed solvent but this may not be desirable is the cartridge houses lyophilized reagents.

COC and COP are one of the toughest polymers to bond via adhesives due to their low surface energies. Table 5.1 shows surface energy of various substrates. Note that Aluminum and Copper have very high surface energy. It may look counter-intuitive that mirror finish Aluminum is easier to bond then plastic but adhesive action is mostly chemistry which happens at molecular scale.

Substrate	Symbol	Surface Energy (dynes/cm) or (mN/m)
Aluminum	Al	840
Copper	Cu	1103
Glass		250-500
Polycarbonate	PC	46
Polydimethylsiloxane	PDMS	42.1
Polypropylene	PP	30
Polymethylmethacrylate	PMMA	41
COC/COP		30
Polyethyleneterephthalate	PET	43
Teflon (Polytetrafluoroethylene)	PTFE	19
Polyvinylchloride	PVC	41.5

Table 5.1 Surface Energies of Various Substrates

There is a large variety of adhesives available. Adhesive business runs into billions of dollars annual globally. We are mostly interested in medical adhesives. At its simplest adhesive tapes were considered. Such tapes are also available in PSA(pressure sensitive adhesive) formulations [111].However there are multiple problems with this approach. In general these tapes don't have bond strength which one can get using applied adhesives which are later cured. There is inflexibility in thickens of tapes especially at the lower end. The second is optical consideration. Such tapes add more interfaces to the cartridge. The overall cost of such a structure may be larger than applied adhesive.

We thus focus attention to clear adhesives for medical applications. Certain figures of merits were defined. Optical clarity is required. Temperature of operation must sustain PCR process. Water absorption should be low. The adhesive should be compatible with PCR process. The auto-fluorescence should be low. In general auto-fluorescence data is not available for these adhesives and hence this has to test in the lab. However, with optical design, it's possible to mitigate the effect of auto-fluorescence of the adhesive.

For our cartridge design it's important that adhesive bonding works. If adhesive bonding works with COC/COP then we can be very confident that various substrates with similar geometry can be used and we thus have a fast and universal method to bond similar and dissimilar materials. Adhesive bonding is also forgiving of the surface flatness and does not demand strict constrains on injection molding process. Adhesive bonding also allows a low cost and well established production process.

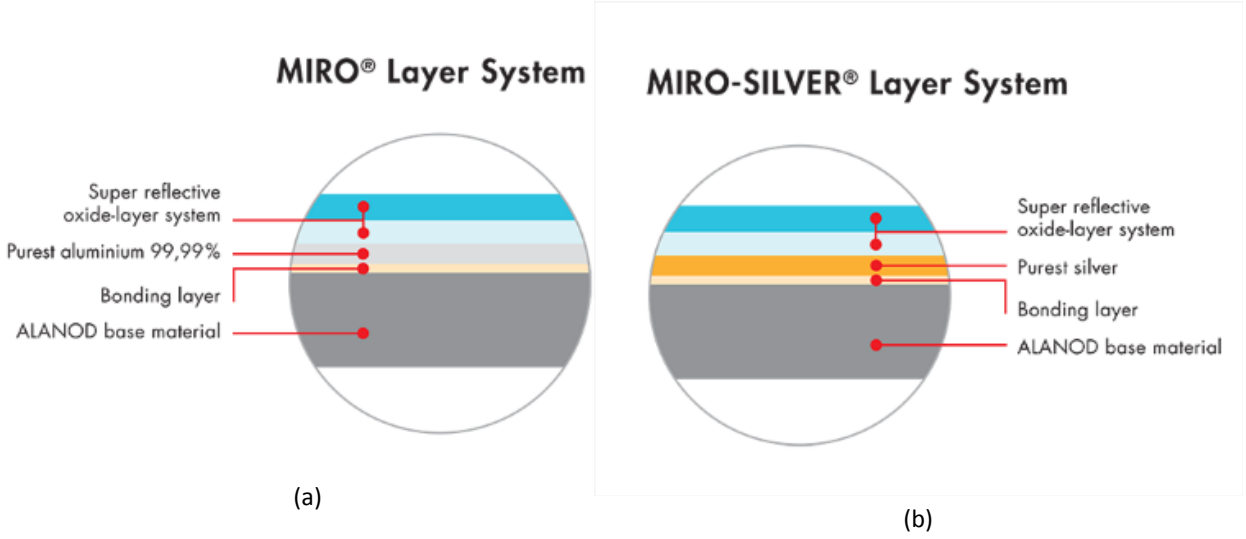
5.2 Materials

5.2.1 Metal

We tried various kinds of metals – mainly Aluminum due to its reflectivity in all visual range. Aluminum is low cost and widely available. It's not trivial to polish Aluminum in a typical research lab. However, reflective Aluminum has various uses in optical applications. For example they can be used as reflectors in lamps. Over the years and due to widespread use of Aluminum, mirror finish Aluminum is easily available.

We used multiple substrates. . Alloy 1101, mill finished, 0.016" thick were bought from McMaster. Mirror finish alloys were bought from alumina (Canada).

Of particular interest was reflective Aluminum procured from Anomet®. This company supplies reflective Aluminum which have categories of anodized or electro polished, PVD (physical vapor deposition) Aluminum enhanced and PVD silver enhanced. The electrolytic anodizing process generates a hard oxide surface and provides reflectivity of about 87%. With their PVD processes and application of multiple coatings, reflectivity reaches to about 95%. The surface is exceptionally durable with a 25 year warranty. Even then the cost is relatively low. Table 5.2 shows the layer system of these Aluminum sheets as obtained from website. Note that top layer is an oxide layer system.



(a) (b)

Table 5.2 Reflective Aluminum Coatings

Surface flatness has to be maintained for the use of these sheets as substrates for our cartridges. Various methods were tried including waterjet, steel rule dies, and die cut with special shear die and laser cutting. Die cut in normal tonnage machines bends the metal. Flattening the metal using a plenisher deteriorates the optical quality. These sheets are other reflective sheets typically come with a plastic cover sheet. Using waterjet removes the sheet and also degrades optical finish, most probably due to high speed water with abrasive. Laser cutting is attractive except that the cover sheet is removed due to heat. The sheet was cut in desired shape by a shear die which retained the cartridge. This method is extremely low cost and fast. It also retains the cover film and is better than laser cutting in speed and cost. Part received after this process did not need much cleaning due to intact cover film which can simply be removed. It should be mentioned that Aluminum is susceptible to adhesion by organic compounds due to its high surface energy and cleaning can become an issue if the process of fabrication lends itself to deposition of organics to the surface. Parts as received by various processes are shown in and the die to be used in our lab is shown in Figure 5.1.

Figure 5.1 Dies for Metal Cutting

5.2.2 Polymers

We first tried to fabricate cartridges using hot embossing in our lab using a simple mold and by pressing palettes between platens of a carver press. This technique has been successfully applied to formation for many devices including channels. However, our cartridge has thick areas as well and voids were observed as shown in Figure 5.2 . Another problem with this approach is long time needed to produce the desired quantity. Also note that this is not suitable for making pillars and support structures which

provide connection to the outside world and many useful features.

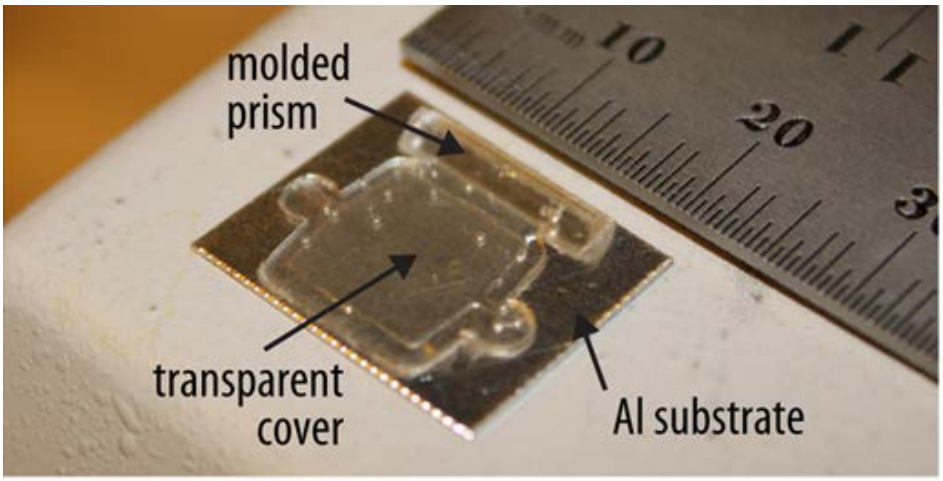
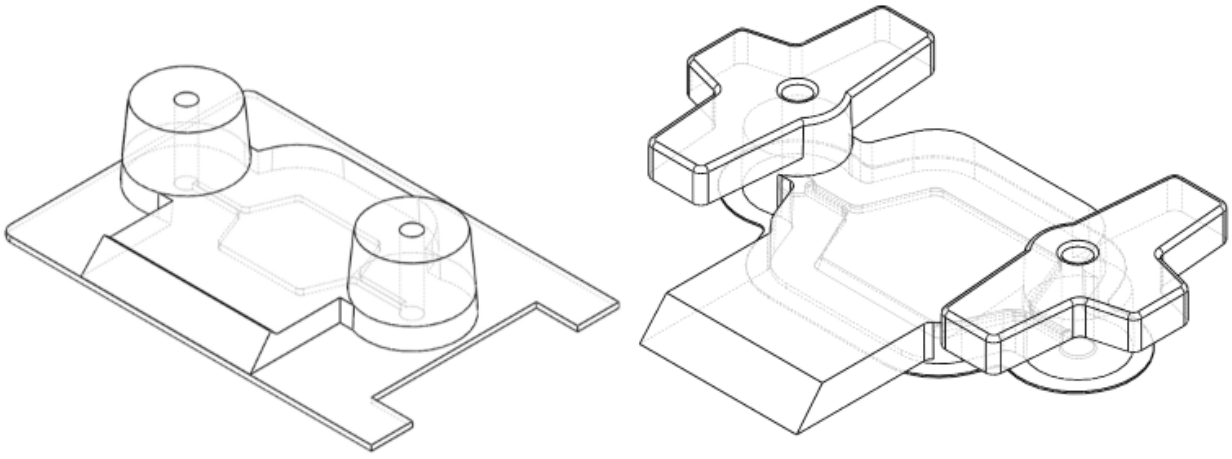


Figure 5.2 Cartridge fabricated by Hot Embossing

Injection molding was done in following substrates. The same molds can be used for different polymers if designed accordingly and if found feasible.

i. Polycarbonate
ii. COC, Topas 5013
iii. COP, Zeon Chemicals 1420R

The two versions of cartridges are shown in Figure 5.3. We worked primarily with second version due to its design and injection molding quality. The first version did guide us towards improvement to reach the second.



(a) (b)

Figure 5.3 Cartridge Versions

5.2.3 Adhesives

The selection of adhesive is one of the main efforts in fabrication and design of cartridges. We could not find an earlier example of this concept for PCR application. Adhesive bonding of COC with metal was studied in [112]. However the polymer was vacuum coated with Aluminum and thus it was a metal to metal bond. Metallization of plastics is common process. In our case we had to deposit Aluminum on the bonding area as shown in Figure 5.4 Bonding Area. A special jig was made the cartridge was metallized at the desired area. Metallization has further benefit that the adhesive is under the metal and hence auto-fluorescence problem is reduced. Nevertheless this is an additional step in the manufacturing of cartridge and it would be more desirable if we can bond polymer directly to metal base or coated metal base.

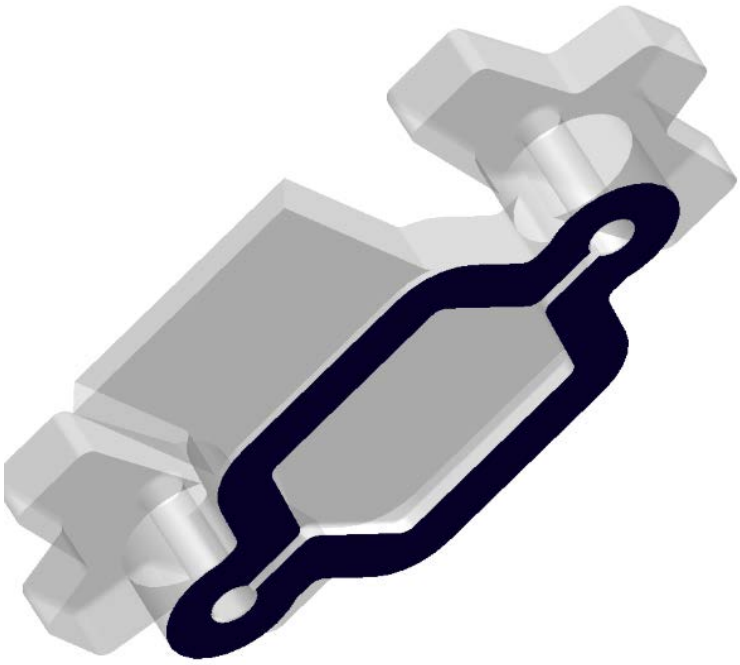


Figure 5.4 Bonding Area

A rather long list of shortlisted adhesives was selected and many were tried. We describe two of the most successful ones. They are as follows.

i. Loctite M31-CL Medical Epoxy two part
ii. Dymax 1072-M Medical Light/UV Cure adhesive

Both adhesives are optically clear. Dymax 1072-M is a new adhesive introduced in 2013 so we worked with Loctite M31-CL for first few years. Dymax 1072 is specially mentioned as COC/COP bonder but has a long list of compatible substrates including aluminum and many plastics. It's exceptionally flexible. I has low auto-fluorescence. It flexibility and rubber like characteristics in cured state are very useful for our purposes.

To test biocompatibility of both adhesives, we cured them inside PCR tubes and then added PCR reaction mixtures over them. We then ran them in commercial PCR machines. We did not found any appreciable change in amplification performance. Commercial real time machines also give rough comparison between auto-fluorescence of different adhesives. Positive displacement pipettes can be used to control the dispensing of viscous adhesives.

UV adhesives are very attractive in production due to very short cure time. They also have almost unlimited working time.

Two part epoxies have working time limitations. Once mixed the adhesive viscosity starts changing and for our deposition technique, we have to cater for it over time.

5.3 Fabrication

5.3.1 Cleaning and surface Treatment

Cleaning is very important step in adhesive bonding. The aluminum surface also requires thorough cleaning. Very thin layers of organic material can deposit which is visually hard to see. To clean Aluminum substrates we used oxygen plasma for 5 minutes. Thus the substrates received though shear die cutting have a major advantage as they can be put in plasma just after removing the cover film. They don't have to be washed and then put in ultrasonic cleaner. For other cases we put the metal substrates in water-isopropanol mixture for 15 min in ultrasonic bath. However in production, retaining the cover film has a major advantage. We also had successful bonding without any cleaning in the case of die cut parts. However, plasma treatment is recommended. The inline atmospheric plasma built for production lines are a feasible option for large scale production.

The polymer parts were received from injection molding process. It is possible that there is some form of organics like mold release compounds still on the surfaces. The parts were put in ultrasonic bath for 15 min in water-isopropanol solution. The parts were air dried and then put in oxygen plasma for 5 min. the plasma cleans the surface and makes it hydrophilic. Thus adhesives can have a better action. Plasma treatment changes the contact angle on the surfaces.

We also used plasma treatment on injection molded pieces without washing and achieved bonding.

5.3.2 Patterning

One of the most challenging aspects of fabrication is the adhesive patterning. Various approached have been used for adhesive bonding. However our case is different in the respect that the bonding area is much smaller than the cavity. Adhesive printing was used in [113]. Interstitial bonding was used in [114]. Adhesive bonding has been reviewed in []. There are various books on the subject as well []. However to the best of our knowledge this application is not given a clear solution. We compared various techniques for this purpose

i. Manual application
ii. Screen printing
iii. Spinning (lithographic techniques)
iv. Stamping
v. Pad printing
vi. Cylinder based printing
vii. Robot dispensing
viii. Under fill process

To have a repeatable process the bond line thickness should be consistent. The amount of adhesive should be same between cartridges. As can be seen in Figure 5.4, there is a large flat area on which adhesive has to be dispensed but the thickness can be very small.

Initially we tried putting adhesive by a tooth pick and then joining putting the polymer part on top of the metal with some clamping mechanism. This had low yield and the method was not repeatable.

Screen printing could not apply a thin enough adhesive. There is a minimum thickness of the mesh used in such operations. Also the technique is not convenient to automate at small scale. The control on amount of adhesive is also much more difficult. When using adhesives with limited working time, the mesh would become clogged or has to be cleaned repeatedly.

The adhesive has its own fluidic properties like surface tension and viscosity. Even if the required amount of adhesive is dispensed on the bonding surface the adhesives don't remain flat.

In silicon processing applications, epoxies like SU-8 and retain high quality of flatness[115]. However, our shape does not permit spinning to control thickness unless special adapter are prepared. This will however, make operation much more complex. Also we want to use adhesives commonly available without special changes to viscosity. SU8 has been used as an adhesive [116].

Stamping, pad printing and cylinder based printing are adhesive transfer methods.

Pad printing is a widely used stamping techniques which is very fast and accurate. The pad printers are optimized for inks not adhesives so cleaning of the adhesives after each run is to be ensured in case of adhesives with finite working times. There is not much flexibility in the thickness of adhesive applied. For good control the depth of etch on the plates has to be varied. However pad printing is very fast and precise and deposits the adhesive in one stroke in contrast of robot moving across a path. The plates can

be prepared by etching into steel for long life. There are laser pattern able plates also available which can be used for rapid testing. They are available for different thicknesses of the polymer layer. The polymer layer controls the thickness of the etch cavity (and hence the adhesive) since the cavity is ablated by laser. However, viscosity of the adhesive comes in to play. Typically the shapes of pads will make full contact with the polymer surface if the adhesive is being applied to it. This many not be desirable so a hollow pad might have to be developed for this purpose. We tried PAD printing in our effort. However, it was difficult to control adhesive flowing out at undesirable places. This is a serious problem with M31-CL. Technique similar to PAD printing has been used to bond PMMA chips with channels [113].Figure 5.5 shows the pad printer and various plates. The Steel plates for the cartridge are also shown.

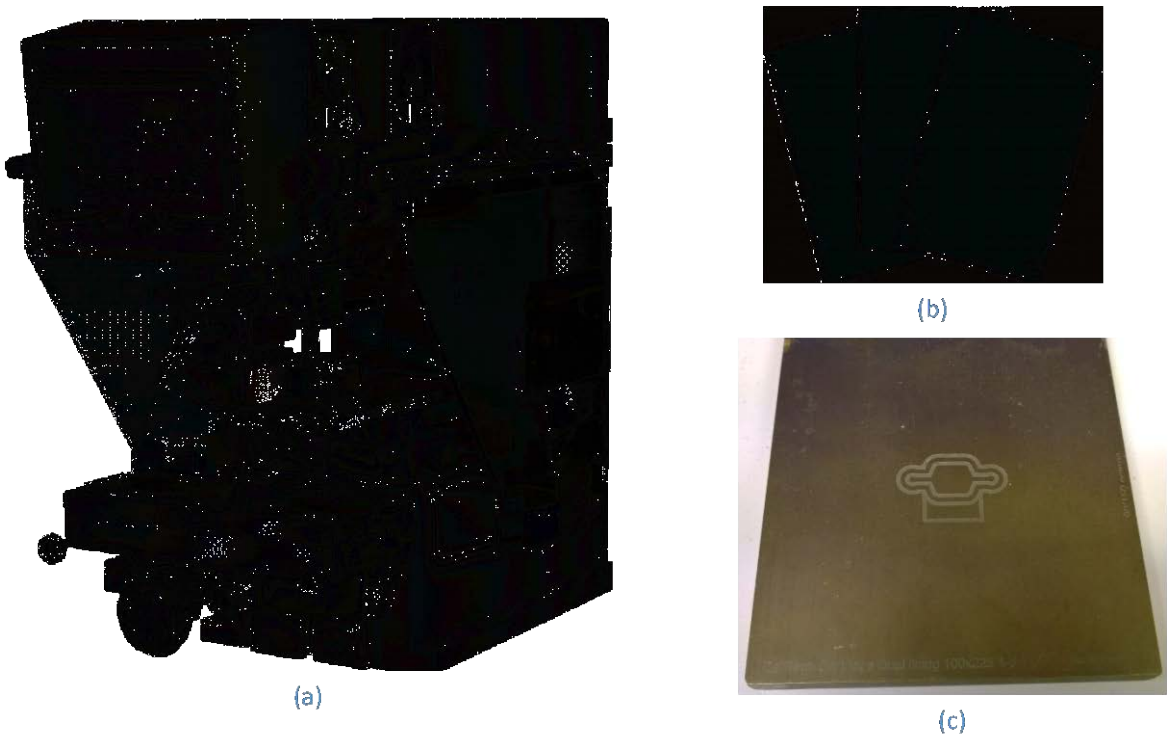


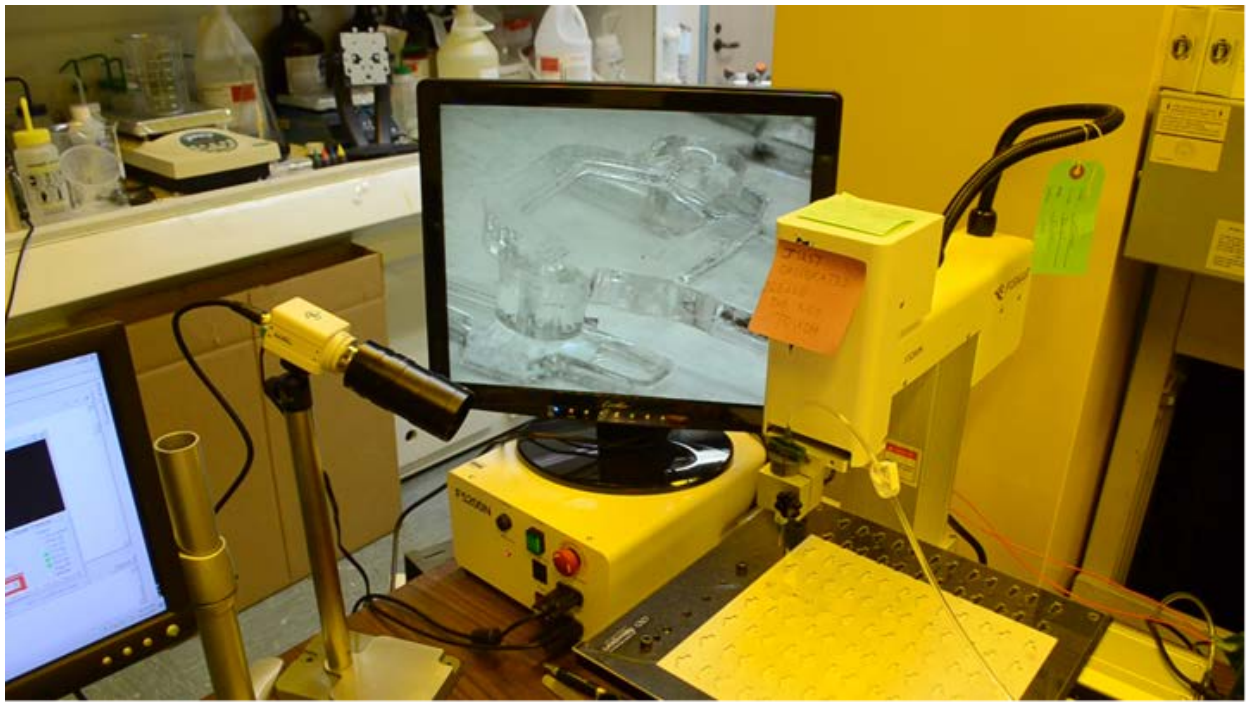
Figure 5.5 Adhesive application by PAD Printing

Cylinder based application of adhesive is an interesting option [117]. However, this requires an elaborate setup and in not amenable to tested in a typical research lab. Also the flexibility to change the amount of adhesive is limited. For low viscosity adhesives, there may be a problem if one uses this technique.

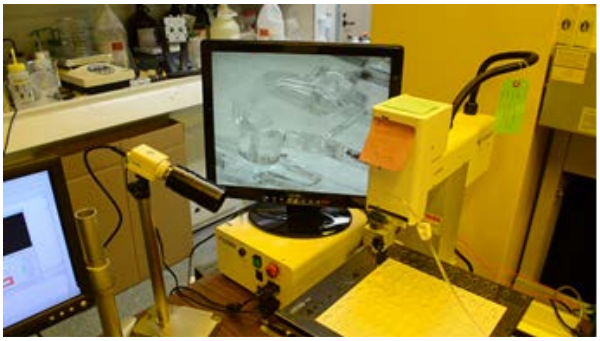
For quickly and accurately controlling the amount of adhesive, electronically controller dispensers are an attractive option. They are widely used in industry. Both air pressure driven and positive displacement types are available. The amount of adhesive can be controlled by regulating the air pressure. The amount can also be controlled by timing. Thus they provide great flexibility to control adhesive quantity for adhesives of various viscosities. One can also change the parameters for adhesives which change viscosity during working time to have consisted amounts dispensed.

However, it's still a challenge to apply adhesive on the entire area in a consistent fashion manually. Therefor a 3D fluid dispensing robot was used to have a repeatable amount of adhesive on the cartridges. The robot also controlled the dispenser to start dispensing at correct locations. Various pressures, tip sizes were tried to reach a set value. This setup gives lot of flexibility in quickly changing the amount of adhesives dispensed without changing the setup. The robot was programmed to dispense in the center line between boundaries of the bond area. The tip was brought close enough to the cartridge so that the adhesive bead touches the surface. This is important since adhesive has a tendency to climb up the syringe tip and hence not being deposited. By having speed, pressure and distance controlled the dispensed adhesive kept on joking the already transferred adhesive and hence the whole bond line was covered. We used cameras with zoom to see the quality of dispensing in operation. The system has to be calibrated after change of syringe only. This is also aided by the camera setup. We changed the air pressure with time for M31-CL. However with UV adhesives, things remained constant.

Figure 5.6 shows the setup for adhesive dispensing. The technique is very repeatable and fairly quick. Automatic image processing based inspection is possible during production. Image processing tools can also help in automatic registration after changing tips.



(a)



(b)

(c)

Figure 5.6 Robot based dispensing setup

Underfill process is also used in semiconductor industry and is an interesting technique [118]. This is used in flip chip assembly as well. In our case we can have some bumps injection molded as a part of the polymer part of the cartridge. In under fill process the robot dispenser can dispense in continuous way to dispense jets along the boundary of the cartridge. The concept of underfill in show in Figure 5.7. However, in our case the adhesive should only cover bond line and not proceed further into the cartridge. Since our cartridges had flat surfaces underfill process was not tried.

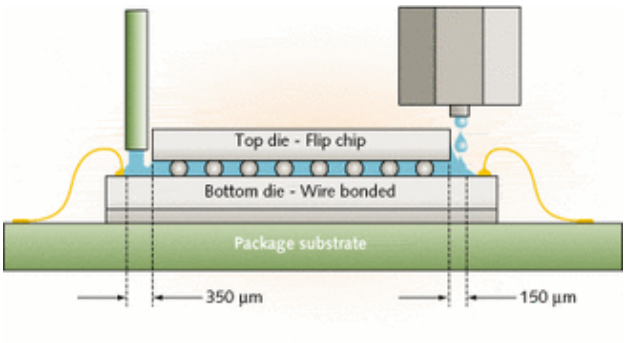


Figure 5.7 underfill process

5.3.3 Jigs

In most cases even after application of adhesive by dispenser or pad printer, the adhesive layer is not uniform. Surface tension of adhesive and method of application does not allow a sufficiently flat surface if left in this state. This may not be a problem in underfill process but to try different thicknesses of bond line, we need some way to fill the bond area with adhesive. The adhesive has to be cured in this state.

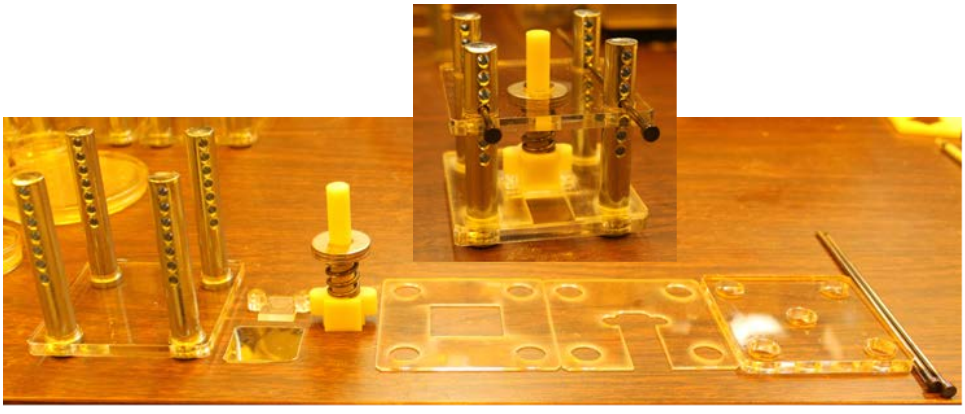
It was observer that when pressure was applied after application of adhesive the bubbles would go away and the adhesive would stop flowing further after the boundary within some pressure limits. This can be explained using lubrication theory.

To be consistent we designed a series of jigs to hold polymer and metal together under pressure. The pressure can be changed. Shows first two types of jigs. The first two types are for M31-CL as they don't allow UV light to pass through due to plunger. However, the 3rd type uses a quartz plate to apply pressure and can be used with UV and non-UV adhesive. The first type of jig uses opto-mechanical parts. Cage plates were used. A plunger was designed to put pressure on the cartridge. A precision compression spring was used. Various cutouts were made using CO2 laser to align cartridge and metal. In operation after putting the metal and cartridge with dispensed adhesives, the top cage plate was pressed and the set screws were tightened. However, after repeated operation the set screws started giving problems. It was also inconvenient to tighten set screws each time. Also the repeatability of pressure is questionable. Pressure was approximated by pressing by hand and could vary. The pressure can be made consistent to some extent by cage rods with dimensional markings, however the problems with set screws is still there. The parts were relatively expensive. To make the jig simpler we used clevis rods which are much cheaper. We used die pins to hold them at a particular position. However, these parts are not as well made as opt mechanical parts and there was variation in the pressure over space.

Also note that the base in this case was made of laser cut acrylic. During curing of adhesives, we used to put them in an oven at 75° C. However, since acrylic was under pressure it bent. We put glass slide at bottom surface to mitigate this effect. Dimensional tolerances and the shape of the clevis pins were a major problem to cause inaccuracies.

Both the above mentioned jigs are not suitable for automation as they have to be joined in a complicated way. For automation, a robotic arm should be able to perform the operation of jig in a rather simple way. We also wanted to have the next jig to allow UV bonding. Figure shows the jig which allows UV and non-UV bonding. Optical stages are used in this design. They allow excellent perpendicularity and a very repeatable pressure. The optical stages are available with springe of different stiffness's and hence different pressures. A quartz plate was machined to allow good UV and light transmission. The base was made with optical parts. The stencils were of metal and were laser cut. Repeated use of intense UV with plastics stencils is not a good idea. This jig is high performance, easy to use and very consistent. The design also shows way to automate the process in production.

(a)



(b)

Figure 5.8 First Two Types of Jigs

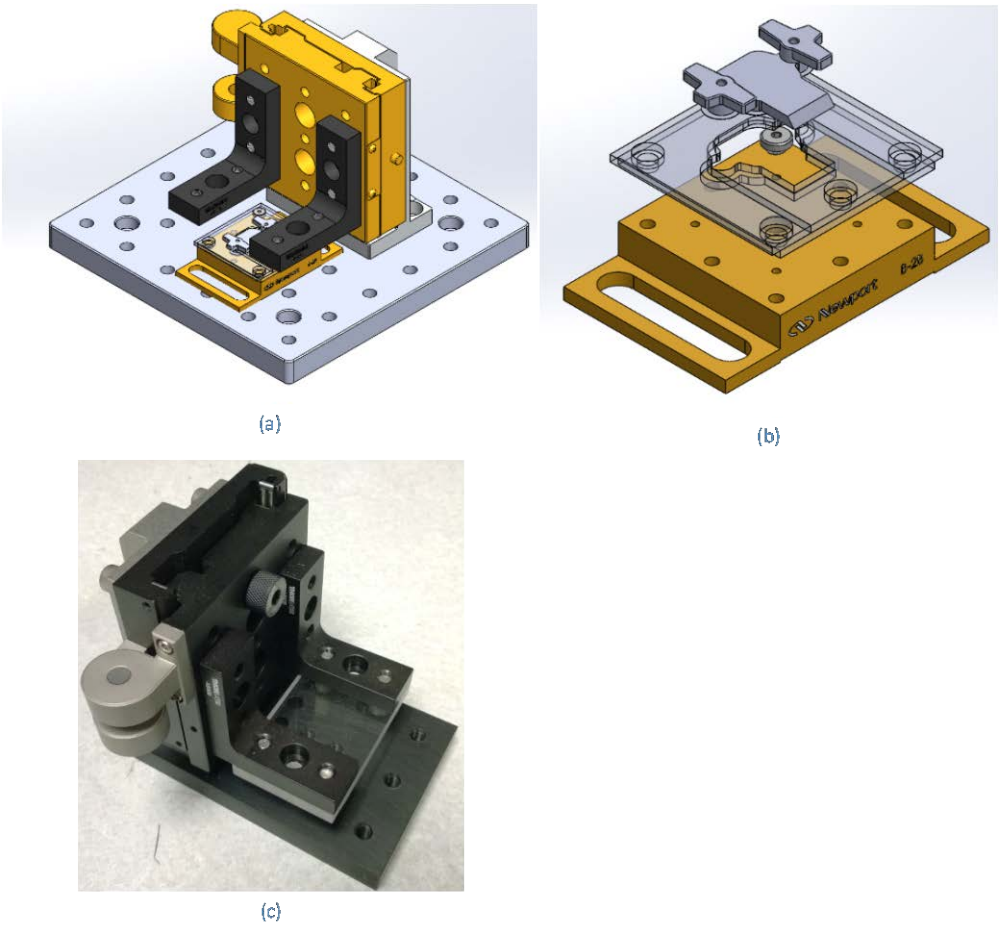


Figure 5.9 UV adhesive Jig. (a) Shows CAD diagram of the assembly. (b) Shows the stencils and base to align metal and cartridge. (c) Shows a picture of the actual setup

5.4 Test Setup

The cartridge was initially tested in a flatbed cycler (GenePro Thermal Cycler, Bulldog Bio). However, the speed of such systems is extremely slow for our purposes. We also wanted to execute complex assays like touch down PCR and easy changes in protocols. Building such an application with tools like LabView is not very productive. Also the real time nature of the system required various sensors to be synchronized. We thus resorted to writing the code in Microsoft Visual Studio. We selected C# as programming language. We tried different thermal controllers with profile capability and ramp and soak features. These controllers are typically made for much slower systems. They do work for our application but the control algorithms were not in our control and synchronization of other sensors like optics has to ensure. We thus wrote code for thermal control as well. The software ran on a PC under Microsoft Windows environment. The great benefit of such a system is its ease of trying different

algorithms. Commercial controllers like Watlow are typically PID based only. There are advanced controllers available from other companies which interface to some of these controllers but this makes the system complex and can add more hardware. Thus synchronization can become a bigger issue. Having PC as real time controller also has a big advantage of getting graphing and other libraries to speed up development.

Figure 5.10 shows the hardware setup for testing the system. This is an elaborate setup to have detailed information about the cartridge operation. High speed sensor interface modules were used (USB 2414 from measurement computing and TC-08 from Picolog). These provide both Thermocouple and RTD inputs. USB 2414 can also measure resistance and thus a custom RTD sensor can be used. To generate PWM, USB 1608G from measurement computing was used. The same module was used to route the PWM signal so that both heating and cooling can be achieved via the H-bridge. The H-bridge was implemented by high speed solid state relays with integrated heat sinks.

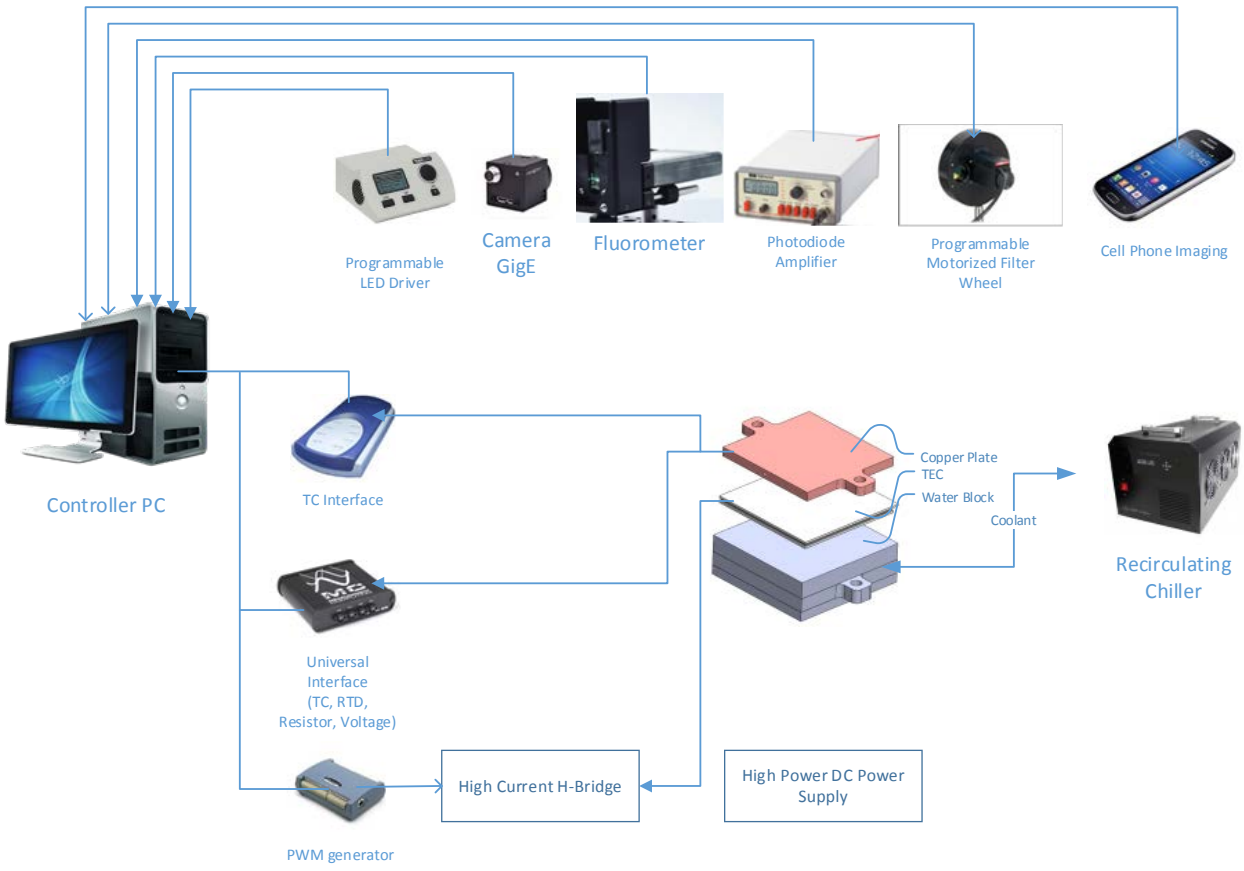


Figure 5.10 Test Setup

We wanted the actual heating cooling surface to be under darkness. We tried Peltier cyclers with fans and heat sinks. However this makes the assembly bulky. Also the whole assembly was supposed to be under a black box on an optical table. We used a water block with circulating chiller. This keeps the assembly small and quiet. The performance is also very good. Only pipes need to go into the light blocking enclosure. A picture of the setup is given in Figure 5.11.

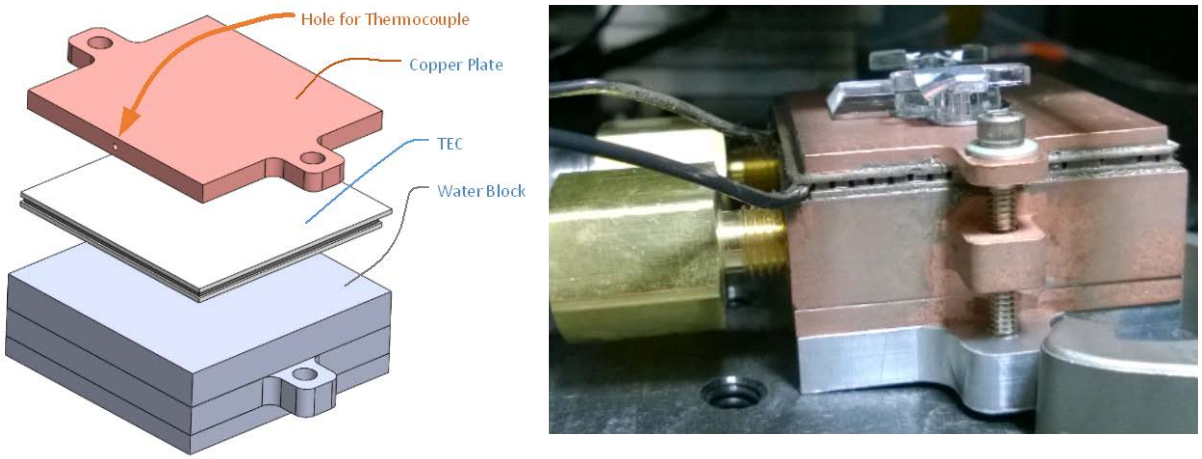


Figure 5.11 Thermal Setup

There are problems if we put the cartridge directly on the peltier surface. The temperature profile on the surface is not uniform. Also we have to somehow attach a sensor. We thus got a metal plate machined in Copper. The thickness was 2mm. A small whole (1mm diameter) was to allow a thin thermocouple to reside inside. It's advisable to apply thermal grease to make good contact with metal surface inside the hole. Thus the temperature is measured and the whole top surface is available to place the metal base cartridge. 2mm is a good thickness for heat spreading if copper is being used. The three components were joined by first placing thermal grease and then using screws with breville washers to keep all parts in good contact. Figure 5.11 shows the assembly.

The software allowed synchronized information collection from variety of optical sensors. We showed that Qiagen ESE log can indeed be used for real time. Earlier it was mentioned that it can be used for end time detection [69]. That may be probably not integrating the instrument with software. In that setup the cartridge was suggested to be put on a flatbed cycler and fluorescence can be read by ESE log. However, our setup is much faster and cheaper than a flatbed cycler with full real time capability. It also does not need an adapter and takes much less bench space. It only uses one peltier while the flat bed cyclers typically use four elements.

We used PID controller. The software allowed assays with arbitrary number of steps. Melting curve could also be obtained.

The camera was also synchronized to the software. The software can take a picture at annealing time and thus a history of full images labelled by cycle number can be obtained. The software can also trigger picture capture via an android phone. An application was developed to take picture at the right time for the phone. Getting pictures via camera is a good diagnostic tools to track fluid motion, bubbles and bonding failures if any.

5.5 Reagents

We tested following assays on our system

i. STD assay with multiplexing via melt curve
ii. Multiplex Dengue and Malaria Assay though our collaborators at Stanford. This was a probe based assay.

Following are the assay details

5.5.1 STD Assay

In order to test the thermal and optic system a multiplex real-time PCR assay using melting curve analysis for identification, designed and characterized at Dartmouth by Ahmad N. Abou Tayoun, was used.

Sexually Transmitted Disease (STD) panel was tested and optimized using clinical samples and then tested in our system using synthetic targets. The real-time PCR primers for the identification of trichomoniasis, chlamydia, gonorrhea, and an internal control, their synthetic target sequences, and their melting temperatures (Tm) are shown in Table 5.3. All oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, Iowa).

PCRs controls were conducted with the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). The same reaction mixture and conditions were then replicated using our in house POC system. The PCR mixture contained forward and reverse primer concentrations of 100nM CT (chlamydia), 175nM of NG (gonorrhea), 125nM TV(trichomoniasis) and 150nM IC2 (internal control) as

well as 2× SsoFast™ EvaGreen® Supermixes (Bio-Rad Laboratories, Hercules, CA), UltraPure™ DNase/RNase-Free Distilled Water (Invitrogen, Carlsbad, CA) and each template DNA at 4uM in a 50µl.

	Primer Target		Sequence (5'→3')	Tm (°C)
Primers	*C. trachomatis*	CTn8F	TCTGAGCACCCTAGGCGTTT	58.6 ºC
		CTn8R	CGTAACTCGCTCCGGAAAAA	55.3 ºC
	N. gonorrhoeae	NG3F	CCGGAACTGGTTTCATCTGATT	55.2 ºC
		NG3R	GTTTCAGCGGCAGCATTCA	56.6 ºC
	T. vaginalis	TV1F	AAGATGGGTGTTTTAAGCTAGATAAGGT	56.1 ºC
		TV1R	CGTCTTCAAGTATGCCCCAGTAC	57.3 ºC
	S. oleracea [a]	IC2Fa	GGTCGTTACTGGACAATGT	52.1 ºC
		IC2Ra	GAATCCAATGATACGGATGAA	50.1 ºC
Target		CTn8T	TCT GAG CAC CCT AGG CGT TTG TAC TCC GTC ACA GCG GTT GCT CGA AGC ACG TGC GGG GTT ATC TTA AAA GGG ATT GCA GCT TGT AGT CCT GCT TGA GAG AAC GTG CGG GCG ATT TGC CTT AAC CCC ACC ATT TTT CCG GAG CGA GTT ACG	
		NG3FT	CCG GAA CTG GTT TCA TCT GAT TAC TTT CCA GCG TGA AAG TAG CAG GCG TAT AGG CGG ACT TGC TGT TTT GAC TCG GAA CAA ATT GAA TGC TGC CGC TGA AAC	
		TV1FT	AAG ATG GGT GTT TTA AGC TAG ATA AGG TAT TTT CCG AAG TTC ATG TCC TCT CCA AGC GTA AGT ACT GGG GCA TAC TTG AAG ACG	
		IC2FaT	AAG ATG GGT GTT TTA AGC TAG ATA AGG TAT TTT CCG AAG TTC ATG TCC TCT CCA AGC GTA AGT ACT GGG GCA TAC TTG AAG ACG	

Table 5.3 STD Assay

5.6 Results

We ran the assay in our cartridges and Bio-rad CFX 96 deep well real time PCR Machine. We cannot increase the speed beyond a certain point in the machine. Also the machine does not allow continuous fluorescence monitoring which we found very useful for our purposes.

Figure 5.12 and Figure 5.13 shows the amplification and melt curve for SsoFast Reference Assay (Bio-rad) for an intercalating dye. We also record the continuous time fluorescence and a portion of it is shown in Figure 5.14. It is interesting to see that fluorescence change can be observed even during first cycle.

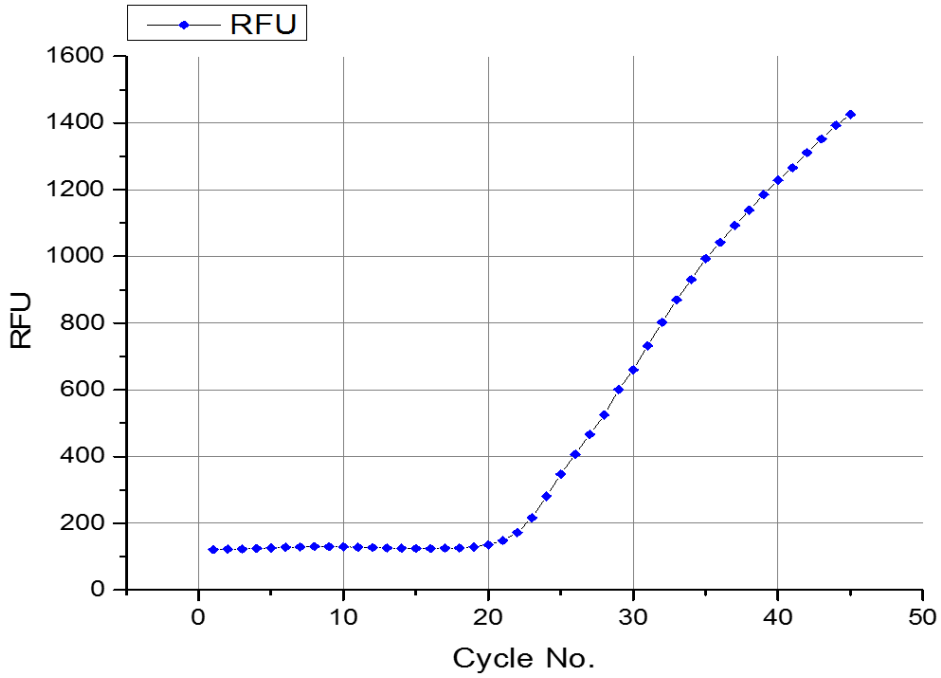


Figure 5.12 Amplification Curve

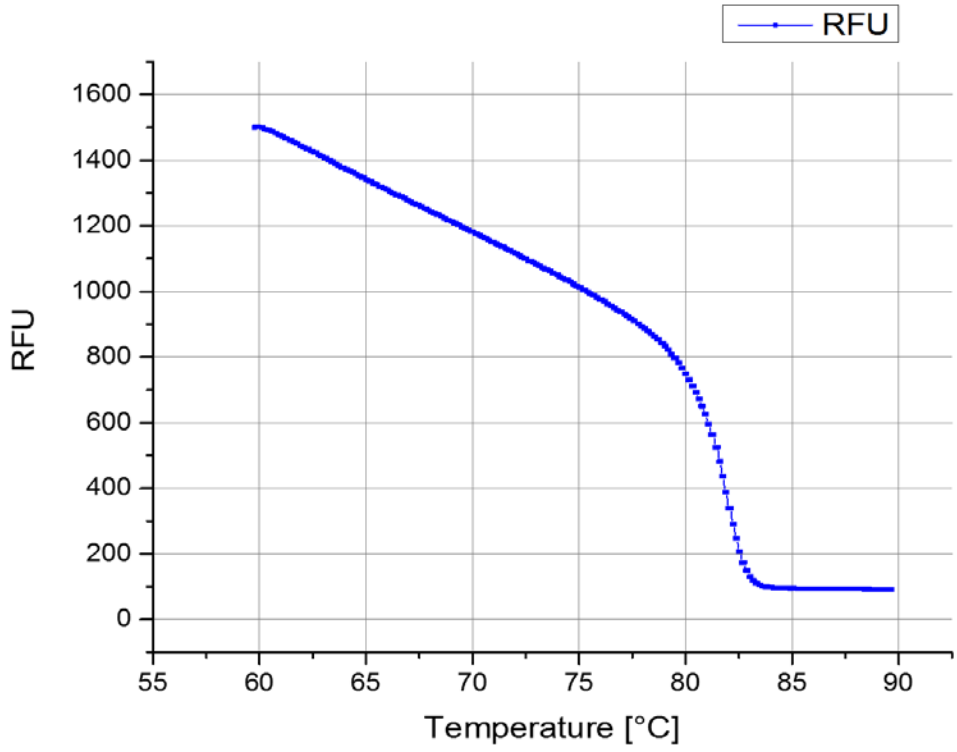


Figure 5.13 Melt Curve

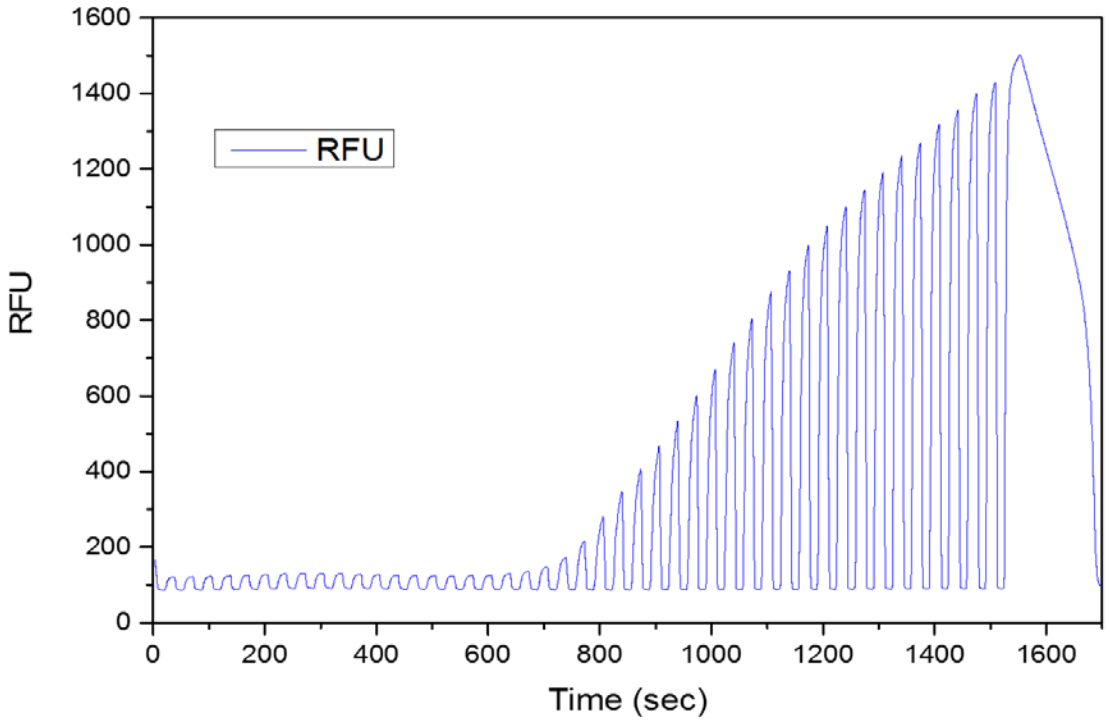


Figure 5.14 Continuous Fluorescence Curve

To verify our modelling we observe the continuous time fluorescence graphs in the plateau phase. This is show in Figure 5.15. It is interesting to note that the fluorescence increases in a similar pattern as predicted by modelling. The change is fluorescence gives a good measure of the speed of heat transfer. In our case it clearly shows the role of liquid and polymer to dictate the thermal speed. Also shown is the graph of quantity (100 – Temperature) to have a clear view of delays between temperature and fluorescence measurements.

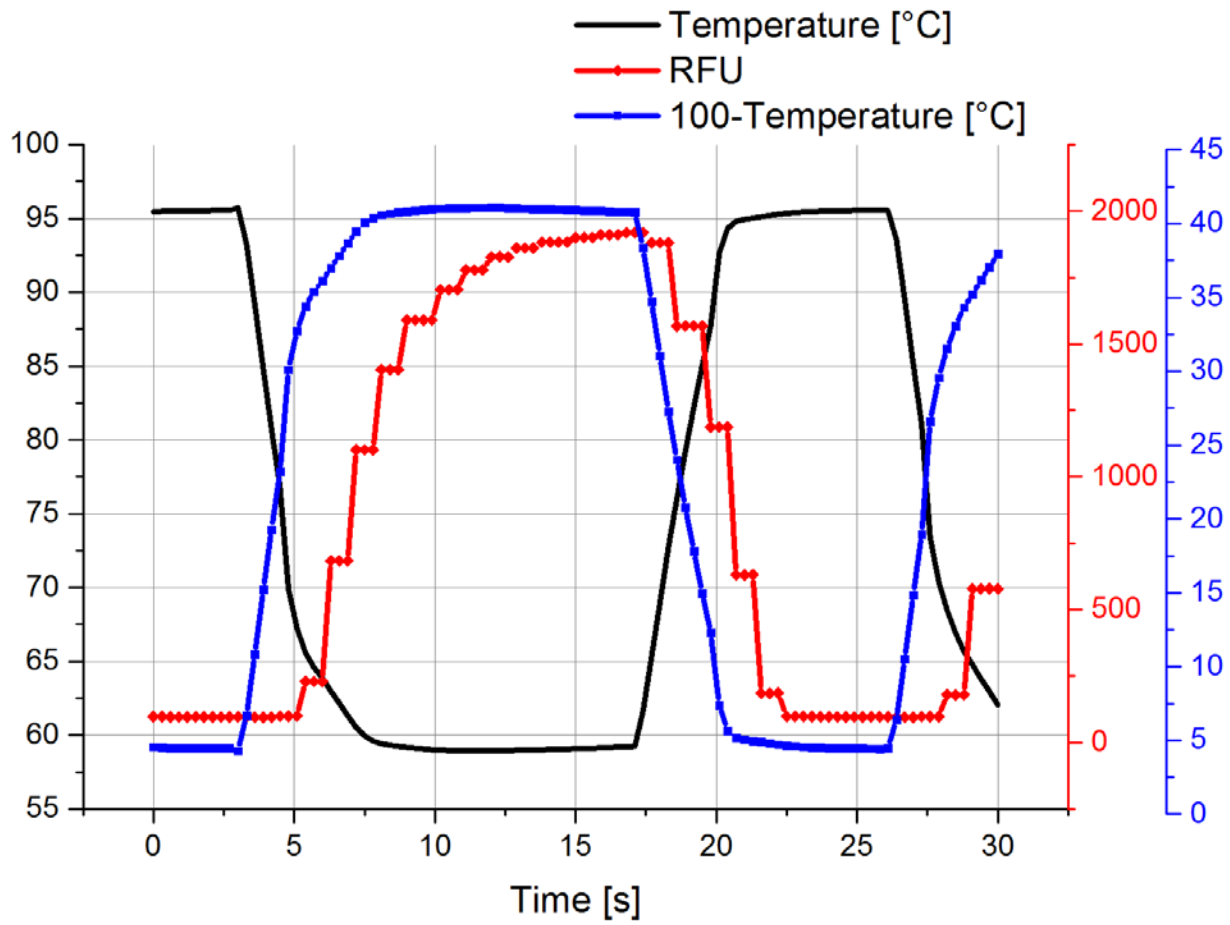


Figure 5.15 Thermal Response of Cartridge

5.6.1 Tests with Different Metal Bases and Efficiency Calculations

We tested with three different types of Aluminum bases. For efficiency calculations we did single rue efficiency calculations. We found efficiency approaching a value to 2 (that is ideal) in case of Amonet Miro IV substrates.

Figure 5.16 shows amplification curves for ssoFsat reference Assay (Bio-rad). The efficiencies for S1, S2, S3, T1, T2, W2 and W3 are 1.83 1.89 1.94 2.02 2.02 1.86 1.89 respectively. This is similar to what we obtained for same reaction in Bio-rad CFX. Calculating efficiency by single curve methods has the advantage of coping with various issues which can cause conditions to change in the experimental setup.

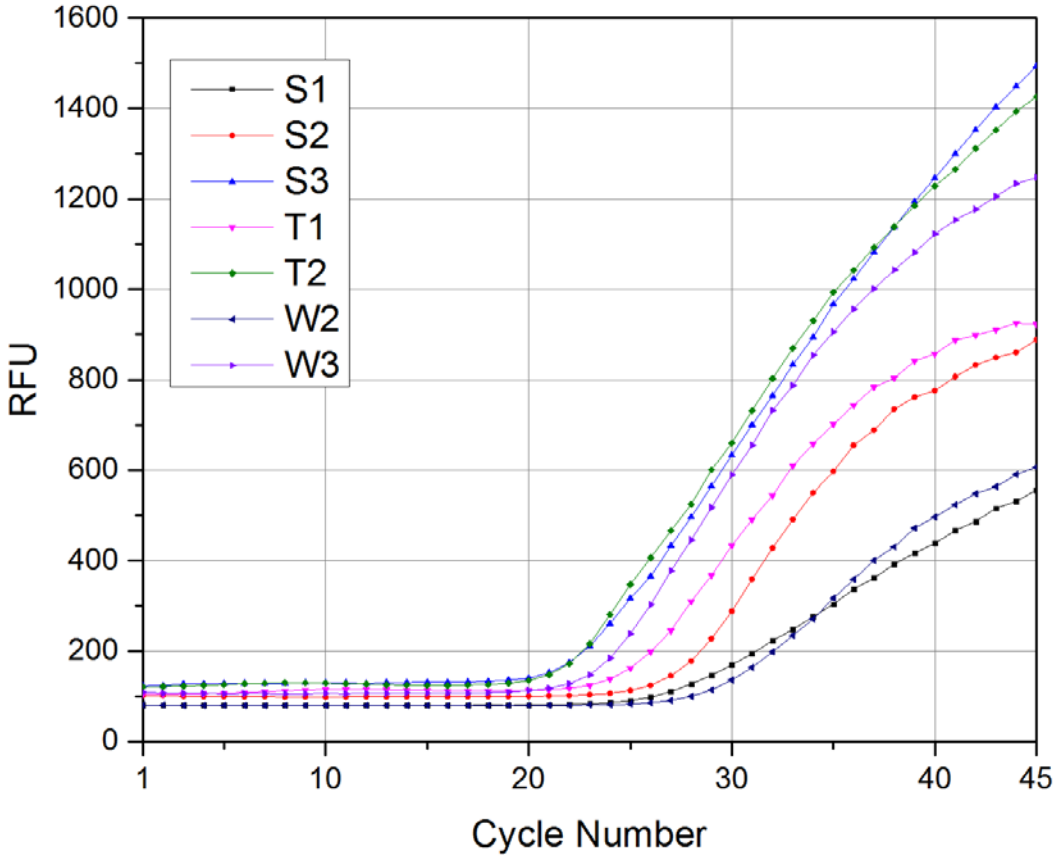


Figure 5.16 Amplification Curves for various metal substrates

5.6.2 Efficiency Calculations

5.6.2.1.1 Baseline Correction

Baseline is the background noise level before a significant amplification occurs. In order to find the correct amplification efficiency this background noise must be subtracted from the fluorescence signal. The algorithm used to find the baseline is the modified version of the algorithm used in LineRegPCR.[119]

This modified algorithm use the same comparison of Supper and Slower. Only difference is that instead of following the algorithm to increase or decrease the tentative baseline value based on the slope value, we calculate the slopes on all the baseline values ranging from the minimum value in the fluorescence signal to the start value of exponential region.

5.6.2.2 *Amplification Efficiency*

Amplification efficiency is completely based on the algorithm used for LineRegPCR.[119, 120]

$\log(N_C) = \log(N_O) + \log(Eff) \times C$ [120]

Where N_C and C are measured fluorescence data and cycle number, respectively.

The algorithm to calculate efficiency is shown in Figure 5.17.

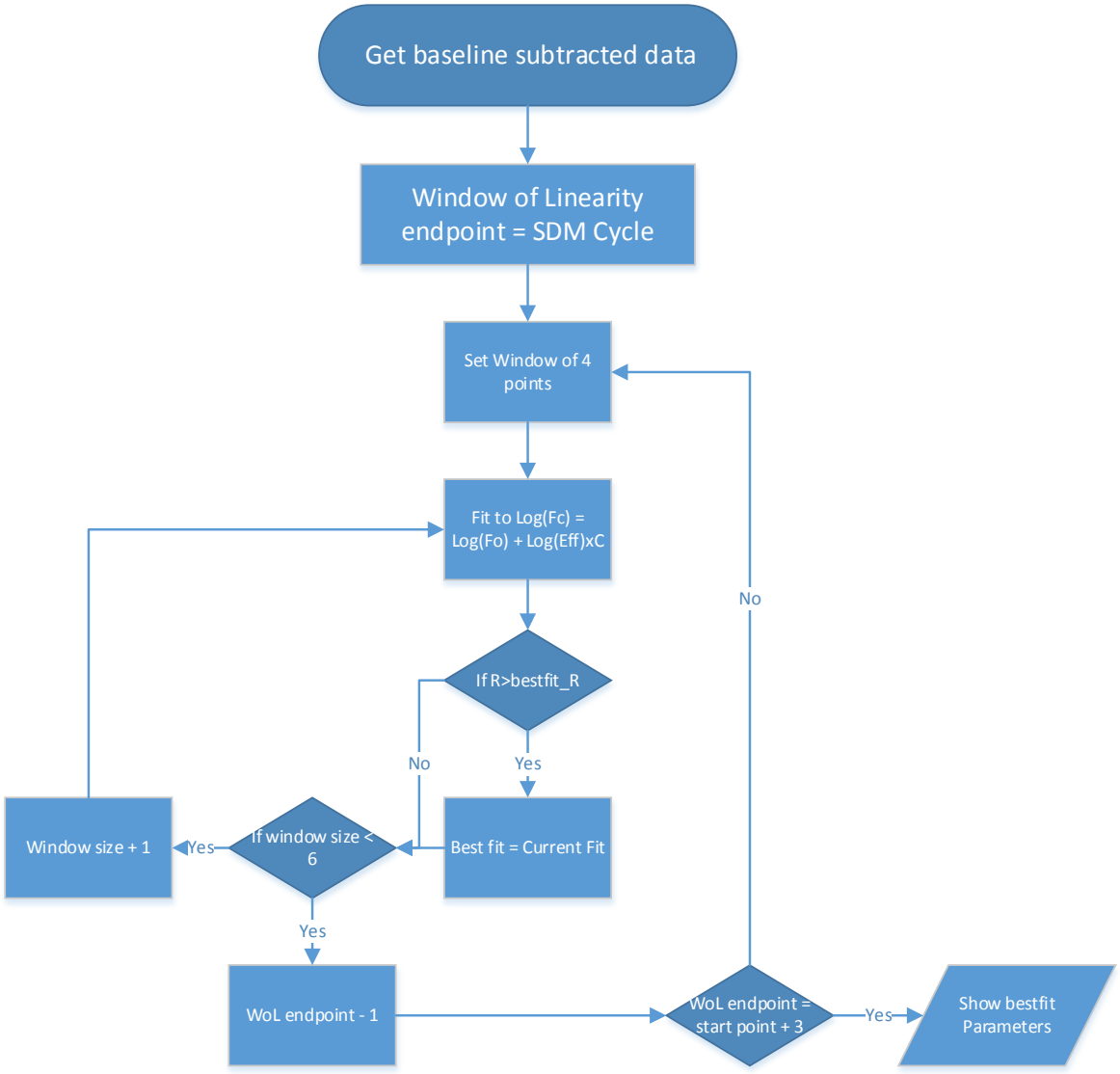


Figure 5.17 Efficiency Calculation Method

5.6.3 Partial filling

We also partially filled the cartridges to see variable volume capability of our cartridge. The camera based record is a good tool to track fluid movement for such purposes.

5.6.4 Bubbles

Left over bubbles did not cause much problem. The bubbles coalesce on sides over time. However, if the cartridge is in a vertical position we hope strongly that bubbles will rise to the top.

5.7 Conclusion

We have shown amplification in a polymer metal cartridge. The thermal speed of cartridge is fast. The cartridge is robust and easy to handle and place. A large optical interrogation area is available. We showed reliable bonding using adhesives and suggested a path to high speed automation of the process.

6 Low Cost and Low Energy Thermal Cycling For POC PCR

6.1 Abstract

We present a novel contact cooling based method for temperature control which is fast, simple, low cost, low energy and robust. Our technique is especially useful for PCR in Point of care applications. The methods provides ways to speed up the thermal response. It also allows large volumes to be handled. Neither the disposable cartridge, nor the fluid in the cartridge needs to be moved. The method allows complex protocols and melt curve analysis to be performed which many other techniques don't provide. We show a proof of principle implementation and various options to further improve the systems.

6.2 Introduction

Thermal cycling is fundamental to PCR [121, 122]. There are many other biochemical methods which require temperature control. Being able to quickly change and control temperature has a wide variety of applications for lab on chip devices[103]. However, thermal cycling is the application where temperature has to be rapidly change for many cycles. It was shown many years ago that the speed of biochemical reaction is not the limit [123, 124] [125] [126] [127]. However, the thermal speed of available instruments has been limiting the smallest reaction times and not the biochemical reaction itself. The speed of the instrument in linked to the geometry of the chamber in which PCR reaction resides. We have already shown that the fluid can greatly affect the thermal speed[128]. We have already compared the effect of plastic walls and substrates on the thermal response. One of the early systems for rapid PCR was Light Cycler [129] which allowed amplification in 15 to 30 minutes.

Another important thing to note is that PCR is a kinetic process. During assay design annealing temperature sometimes needs to be adjusted for improved efficiency. Many modern PCR machines allow gradient capability i.e., the temperature across wells varies and the fluorescence can be measured thus providing a comparison of the amplification by changing temperature.

Although the fact that thermal system is the limiting factor in PCR speed, it's surprising that even the modern machines have not been able to overcome this limitation in genera. It shows the difficulty in satisfying various requirements for use of PCR in lab and in clinic.

6.3 Earlier work and problems

The thermal techniques for various commercial systems are covered in various books[124, 130]. The reasons for limited speed for PCR instruments is elucidated in [124]. The techniques used for commercial real time PCR machines can be summarized below from [130].

Technique	Example Systems
Peltier	ABI 7500, Bio-rad CFX
Heated Air	Roche LightCycler
Resistive Heater with air cooling	Qiagen rotorgene
Peltier with thermal conductive fluid	Illumina Eco
Direct Heating in combination with electrically conductive polymer	Biogene InSyte

Figure 6.1 Thermal Design of Commercial Machines

Except for direct heating approach by Biogene, other techniques use a large amount of power which is not entirely directed the reaction mix.

For POC systems, energy consumption is also of paramount importance. Interesting examples are systems from Epistem and Bigtech. Shows the design of Epistem POC systems. Interestingly it still uses a Peltier with a heat guide to a fan. There are designs which use resistive heater and a fan to cool down. In case of Epistem the fan can be external. If fan has to blow air over the reaction vessel, several problems arise. The first is to design for routing air in an efficient way to cool the system. The second is that the system is not entirely dust and water proof. Fans do wear out and become clogged. For a low resource setting environment, this is a major problem. The problem of fans is still there with systems with peltiers as a lot of heat has to be dissipated causing noise and power consumption.

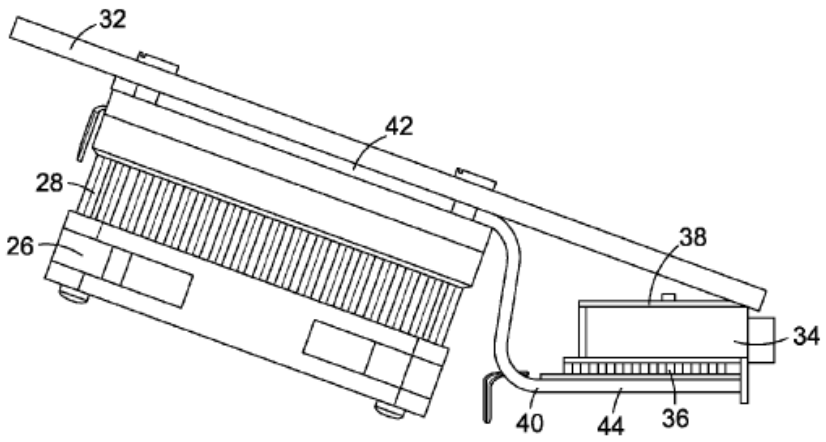


Figure 6.2 Epistem Thermal System Design

Thermal cycling at POC has been a major problem. For this reason there has been lot of focus on isothermal approaches [95]. However, isothermal approaches have major problem of not being truly quantitative. There are a large number of assays already developed for real time PCR and isothermal will not use them directly. The patent protection is also an issue. qPCR patents are expiring soon and it makes much more sense to follow PCR path for widespread adaptation in low income places.

Various designs have been tried in literature to improve thermal speed of the PCR reaction. We feel that for optimal design both the thermal mechanism and the cartridge design has to be taken in to account. However, it is observed that many of these techniques work for only very small samples. Thus the techniques are not scalable for diagnostics at POC.

The techniques to do thermal cycling can be divided into two broad categories. One is the convective PCR which has been employed by many researchers. Both natural and forced convection has been used. However, this technique has problems of moving fluid with boundary wall effect and it is more difficult to control temperature profiles. It's not clear how one would do a melt curve analysis using this technique.

Other interesting techniques uses are shown in *Figure 6.3*. *Figure 6.3* (a) shows a chip rotating over three heat blocks. This technique requires there separate heat blocks[97]. Also melt curve analysis is not possible. Since the chip is plastic, it may be advantageous especially during heating to apply predictive control. That will sometimes require raising the temperature of heating surface higher than the set temperature. However, this design does not allow this. *Figure 6.3*(b) shows a very fast system[131].

However this is not amenable to Point of Care as a droplet is covered with oil. This technique is also not useful for large volumes. *Figure 6.3*(c) show a rotating disc is used to move the tubes between three stages are different temperatures. Each individual tube can have its own temperature. The system is very complex due retraction of the tubes and then their movement. Examples of very fast thermal systems are shown in *Figure 6.3* (d) [125, 131] and *Figure 6.3* (e). Both of these use external pumps and move fluids to heat and cool. They are complex and bulky for point for care applications.

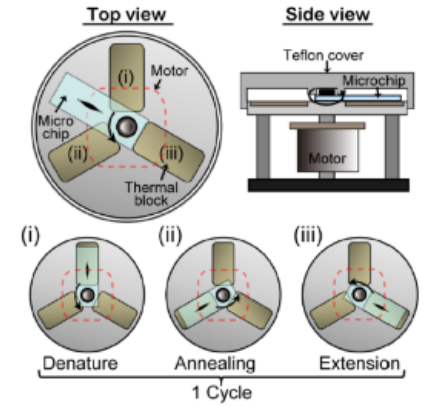
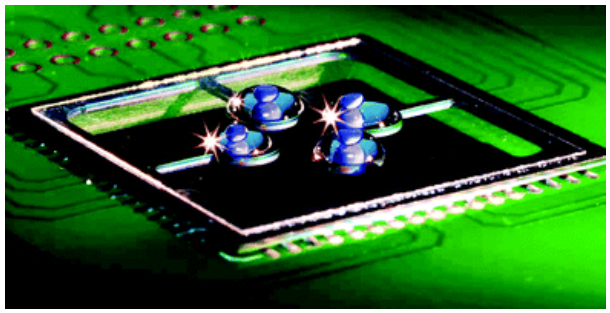


Fig. 1 Schematic illustration of a Rotary PCR Genetic Analyzer which consists of a microchip, three heat blocks, and a stepper motor. Top view (left) and side view (right). Rotary PCR is performed by rotating a PCR chip to the (i) denature (94 °C), (ii) annealing (58 °C), and (iii) extension (72 °C) heat block subsequently (bottom panel).

(a)



(b)

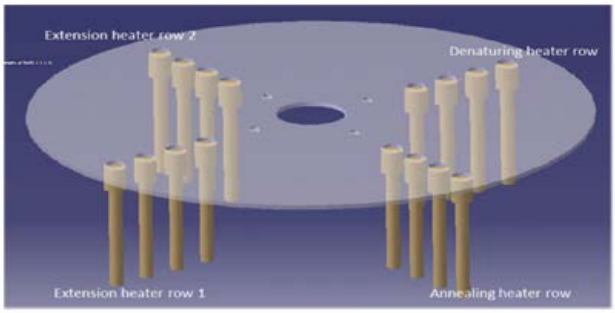
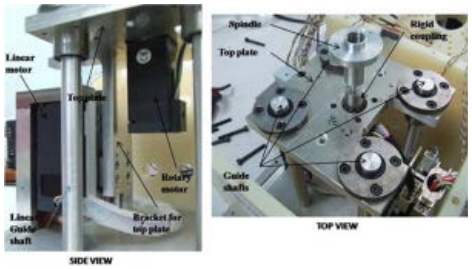
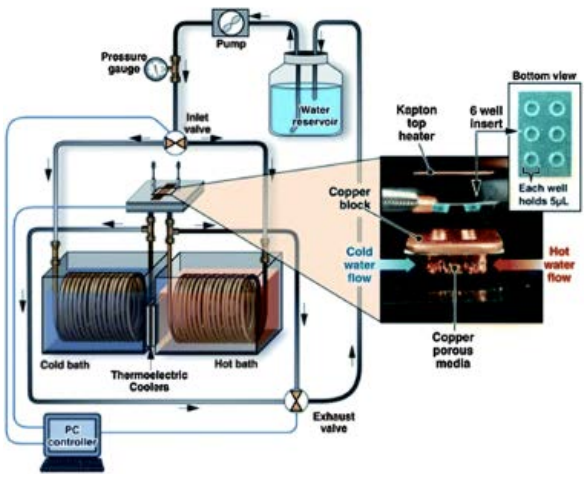


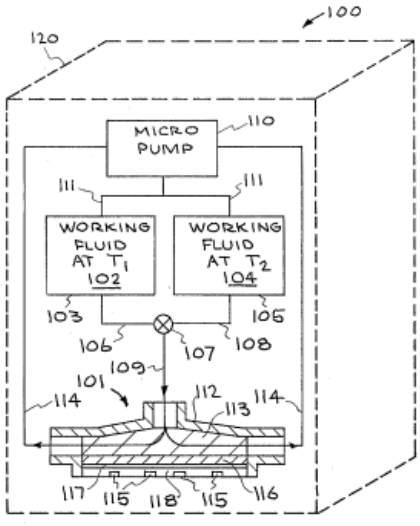
FIG. 1. Schematic illustration of the PCRDisc and heaters.

(c)





(d)



(e)

Figure 6.3 Example Thermal Control Systems

Liquid metal has been used in to have fast thermal response as well[132].

6.4 Thermal control by PCR - Requirements

To have a versatile instrument which can run all or most of the protocols, we have to meet certain requirements. Several techniques described above are not flexible to meet all the demands.

The first requirement is to have the capability to run complex assays with many steps. One example is touch down PCR [133] which can require changing the annealing temperature many times during the amplification steps. Convective PCR and fluid heat exchangers are not easily adapted to this application.

The second very important application is the melt curve analysis. Melt curve analysis has various uses including confirmation of the product[134] [135]. Multiplexing based on melt curve temperature is also a useful applications. HRM (high resolution melting) is used for genotyping and SNP (single nucleotide polymorphism).

Programmable ramp rates are provided in most commercial PCR machines. However, by appreciating that PCR is a kinetic process, its use for POC assays is not critical. A high ramp rate looks to be good enough to meet the requirement. However, if the ramp rate is too slow, it may cause broadening of gel bands. Faster ramp rates also lower the total time of PCR amplification.

Although the design of thermal system is closely linked to the design of the cartridge, it would be helpful if the system allows modern control theory to improve the speed. The design of thermal fluids and convective heat transfer always fixes the set temperature to which the fluid in the chamber equilibrates. Thus by newton law of heat conduction, the heat transfer slows down once the temperature differential is reduced. Electronic controls cannot be employed to improve the response.

6.5 Basic idea

It was decided that Peltier should be avoided to save cost and energy. Peltiers also have reliability issues. They have to be protected from moisture and have limited lifetime.

It was also appreciated that heating can be well controlled using resistive heating. Current can be easily controlled using electronics. A resistive heater is efficient and can be implemented by a variety of ways.

However, it does not have an equivalent in terms of cooling. A peltier has a big thermal mass as compared to a thin resistive heater or a heater embedded in to chip.

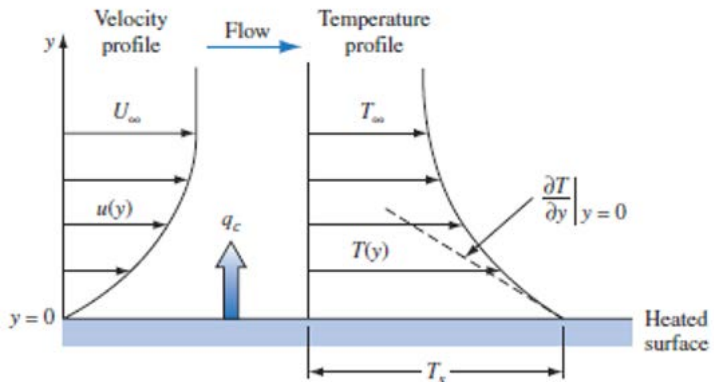
We also note that all the temperatures in PCR cycling are above the maximum operational temperature of 40 ° C ambient. Thus we conclude that a resistive heater can be used in an energy efficient way for precise thermal control. The main challenge is to get thermal energy dispersed to the surrounding in an efficient way.

We consider the primary ways of heat transfer – conduction, convection and radiation. Radiation is not feasible for cooling our system. Thus we must employ either conduction or convection. Convection can be further divided into forced or free convection. Shows basic concept of heat transfer with such mechanisms.

Table 6.1 Heat Transfer Modes and Expressions

Mode of Heat Transfer	Governing Law	Thermal Resistance
Conduction	$q_k = -kA\frac{dT}{dx}$	$R_k = \frac{L}{Ak}$ for a wall
Convection	$q_c = -k_{fluid} A \left\lvert \frac{\partial T}{\partial y} \right\rvert_{y=0(\text{int}\,erface)}$ $q_c = -\bar{h}_c A \Delta T$	$R_c = \frac{1}{\bar{h}_c A}$
Radiation	$q_r = A_1 \sigma (T_1^4 - T_2^4)$	

We note that convection is due to similar phenomena as conduction i.e. molecular collisions. Also from boundary layer theory the fluid in contact with the hot surface has zero velocity and hence at the interface heat is being only transferred by conduction. Shows a typical velocity and temperature profile[136].



Heat moves to lower temperature fluid by molecular collisions. If the fluid was static (like a solid body) then the rate of heat transfer will be slow. Both air and water have low values for thermal conductivities. Shows thermal conductivities of various materials. (Include liquid metals). The best efforts for this purpose has been to use liquid metals. However, this is not an attractive approach for robust systems at POC.

	Thermal Conductivity at 300 K (540 °R)	
Material	**W/m K**	**Btu/h ft °F**
Copper	399	231
Aluminum	237	137
Carbon steel, 1% C	43	25
Glass	0.81	0.47
Plastics	0.2–0.3	0.12–0.17
Water	0.6	0.35
Ethylene glycol	0.26	0.15
Engine oil	0.15	0.09
Freon (liquid)	0.07	0.04
Hydrogen	0.18	0.10
Air	0.026	0.02

Figure 6.4 Thermal conductivities of Materials

As per newton law of cooling the heat transfer is proportional to the temperature differential. Convection improves heat transfer due to the movement of fluid near the hot surface. In case of natural convection buoyancy is responsible for fluid movement. However in case of forced convection we move fluid by external mechanism. However, in both cases we want the temperature differential to be high. We achieve that by replacing the molecules which have absorbed heat by molecules which have not.

If we see from the hot body perspective, all what we are doing is to provide another material at a lower temperature. Convection keeps the temperature of that material lower so that heat transfer is faster. Eventually the heat has to go to atmosphere. Thus our target is to get heat eventually to atmosphere which can be modeled as an infinite heat sink at a fixed temperature. In convection based designed we use fluids and let them or make them move to provide a low thermal conductivity path from our heated object to the material accepting the heat and later dispersing to atmosphere. Thus the heat transfer from the object to the material and from material to the surroundings can decouple to some extent from a design point of view.

We note that physics works in favor of getting heat of our heated object to the surroundings. What we want is to accelerate this process by providing a path from this object for heat to flow to the surroundings.

We want to have a material which takes in heat and keeps its temperature low. We also want that material to have good thermal conductivity.

This can be realized with an electromechanical relay like concept. An electrical relay forms a low conductivity path between two points at different potentials. If we use analogy of current to heat, voltage to temperature then all we need is a thermal relay. A thermal relay is in fact a low thermal conductivity part which can make a contact between the hot object and an object of lower temperature. The difference in the electrical and thermal domains is that the heat can be stored in this contact as well. In this concept we don't need any convection. We are providing a very high thermal conductivity material to absorb heat.

Figure 6.5 shows the basic concept. Notice that thermal contact will also have thermal capacitance in addition to thermal resistance. This thermal capacitance can be used in our favor. The thermal contact can either make a path for heat to flow or act as a heat sink itself.

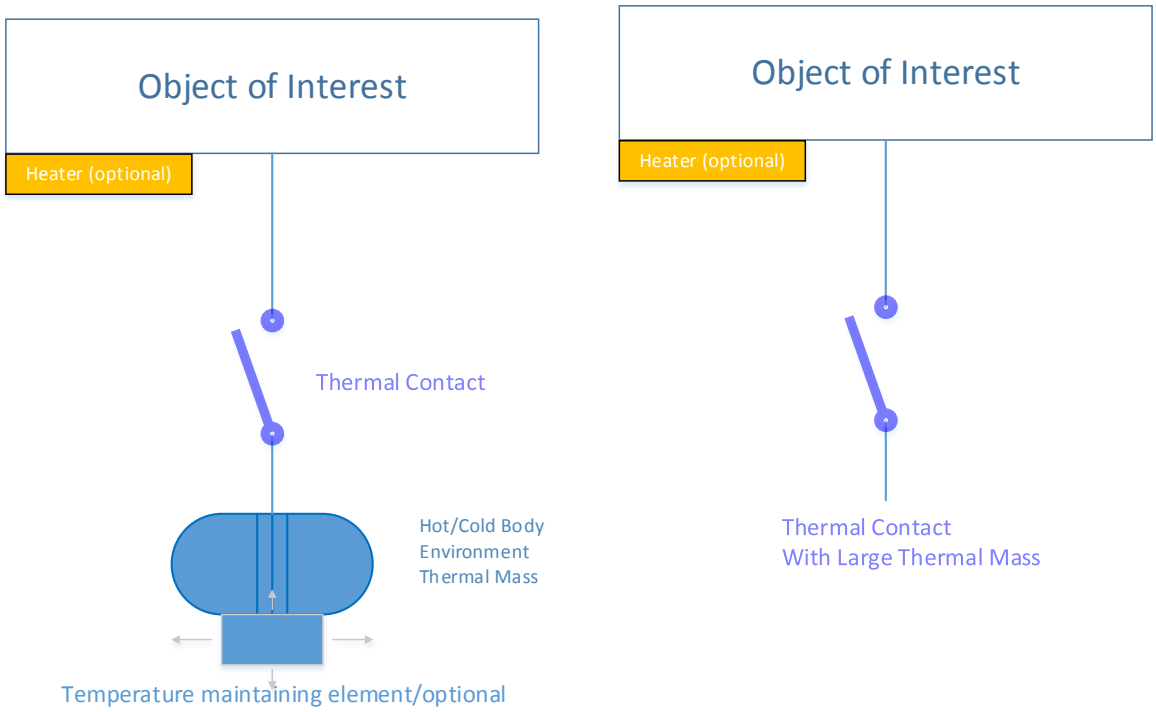


Figure 6.5 Basic Concept of Thermal Contact

The thermal contact is best realized with a moving metallic structure due to its very high thermal conductivity. The moving metal provides a material in which heat can flow rapidly. Shows typical convection transfer coefficients.

	Convection Heat Transfer Coefficient	
Fluid	**W/m² K**	**Btu/h ft² °F**
Air, free convection	6–30	1–5
Superheated steam or air, forced convection	30–300	5–50
Oil, forced convection	60–1,800	10–300
Water, forced convection	300–18,000	50–3,000
Water, boiling	3,000–60,000	500–10,000
Steam, condensing	6,000–120,000	1,000–20,000

Figure 6.6 Order of magnitude of convection heat transfer coefficients

For our purpose we are interested to lower temperature of cartridge from 95 to 55 ° C. We calculate heat transfer in the case of unit area as follows.

> $\Delta T := 95[\![C]\!] - 55[\![C]\!]$

$= 40\ [\![C]\!]$

$A := 1\left[\!\left[m^2\right]\!\right]$

$h_c := 100\left[\!\left[\frac{W}{m^2 C}\right]\!\right]$

$q_c := -h_c \cdot A \cdot \Delta T = -4000\left[\!\left[\frac{kg}{s^4 A}\right]\!\right]\left[\!\left[C\right]\!\right]\left[\!\left[m^2\right]\!\right] \xrightarrow{\text{units to SI system}} -4000\left[\!\left[W\right]\!\right]$

The heat transfer rate is thus -4000 Watts for an area of 1 meter square. This is only the initial rate as it will drop with the temperature of hot body. The resistance value for a thermal circuit in this case is $\frac{1}{h_{c,hot} A}$ which comes out to be $10^{-2}\ m^2 \frac{K}{W}$.

The heat transfer rate is not easily calculated for the case of conduction via a contact due to the presence of contact resistance which is non-trivial to model. The contact resistance changes with pressure and surface finish as well [136, 137]. There are no satisfactory correlations found and each situation has to be dealt with separately[136]. Contact resistance can be lowered w°ith an interracial fluid. Using a soft metal foil is another feasible option.

Show typical contact resistances for various interfaces under various conditions.

	Resistance, R_i(m^2 K/W × 10^4)	
Interface Material	**Contact Pressure 100 kN/m^2**	**Contact Pressure 10,000 kN/m^2**
Stainless steel	6–25	0.7–4.0
Copper	1–10	0.1–0.5
Magnesium	1.5–3.5	0.2–0.4
Aluminum	1.5–5.0	0.2–0.4

Interfacial Fluid	**Resistance, R_i(m^2 K/W)**
Air	2.75×10^{-4}
Helium	1.05×10^{-4}
Hydrogen	0.720×10^{-4}
Silicone oil	0.525×10^{-4}
Glycerin	0.265×10^{-4}

[a] 10-μm surface roughness under 10^5 N/m^2 contact pressure.

Contact resistance changes markedly with contact pressure. There is no way to predict the change in contact resistance quantitatively.

Nevertheless we note that contact resistance can be lower than what is offered by high quality forced air convection with many advantages.

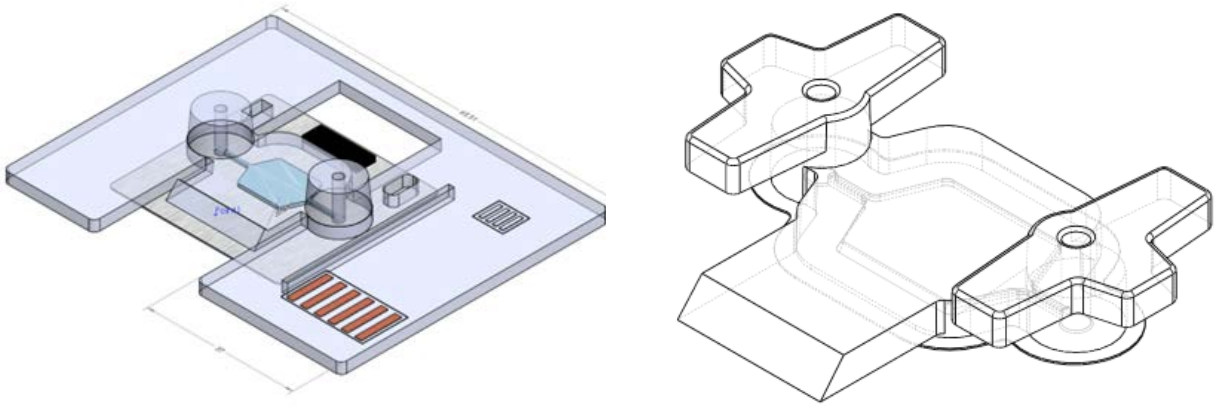
In case of forced convection the temperature of cooling fluid is typically kept close to the desired temperature. In our case this temperature will be 55 ° C. Thus the thermal resistance for convection heat transfer will be between the temperature of our cartridge and 55 ° C. however, in case of contact based cooling the temperature at other end of the contact can be much lower.

6.6 Configurations

To apply the basic idea to PCR cartridges, several choices have to be made.

The first is placement of the heaters. First we consider the case of cartridge with integrated heaters. These heaters should be attached to the metal base in our case. They can be placed on sides of the polymer part or under the metal plate. To have a first order approximation of the time constant for equilibration we derive the thermal time constant of a rectangular layer to be $\tau = \frac{1}{\alpha} height^2$. We note that the time constant increase linearly with thermal resistance but quadratic with the height. In this case height is the direction of flow of heat. Thus with few millimeters the time constant can be in range of seconds even for Aluminum with α of $8.418 \times 10^{-5} \frac{m^2}{s}$. There will be uniformity issues as well. Therefore, the heather should be placed under the metal substrate. The time constant will be very short and the temperature uniformity can be very good. In putting the heater under the metal substrate thee is a disadvantage. The heater also has to be cooled the thermal contact. Hence the mass of the heater and its thermal resistance to the fluid should be as small as possible.

Another important choice is whether to integrate heather to the cartridge or have it attached to a metal plate in the instrument. Integrating the heater to the cartridge has the advantage of removing one temporary contact. However, it can make the cartridge more expensive and complex to fabricate. Disposable cost should be low and adding the heater can add significant cost. *Figure 6.7* Shows both cartridge types as designed.



(a) (b)

Figure 6.7 Cartridge Types. (a) Shows cartridge with integrated heater. (b)Shows bare cartridge

6.6.1 Contact types

A wide variety of contacts can be used for this concept in general. The technique can work at different size scales as well. Some examples are

- Relays
    - Electromechanical Relays - all types
    - Relay (solid-solid)
    - Mercury relay
    - Mercury wetted relay
    - Vacuum relay
- Contactor
- Electrical switch
- MEMS switches - electrostatic, electromagnetic, pneumatic etc.
- Actuator based contact which can use following actuation
    - SMA (Shape Memory Alloys)
    - Electromagnetic
    - Electrostatic
- Manual
- Gravity assisted contacts
- Liquid metal - solid contact
- Modifications to contacts

  - CNT coated
  - Nano-structured to improve performance /increase contact area
  - Latched
  - Sliding
  - Liquid Flow
  - Variable pressure, distance
- Custom types
  - A syringe type filled with liquid metal can work as a thermal contact as well. When the plunger goes forward the contact is made, when it moves away no contact.
  - Patterned /grooved metal contacts to increase contact area
  - Sliding metal surfaces with or without wetting
  - Controllable contact area, pressure and distance.
- Radiation Assisted/Controlled
  - If one body is at a very different temperature then by changing distance or by blocking radiation by a screen can help in heat transfer. Note that this allows heat transfer without mechanical contact.
- Convection Assisted
  - In this case the hot/cold body may be kept at a temperature by flowing liquid (e.g., a natural fountain or pump) and a solid contact connected to OOI makes a contact to this stream.

It is to be realized that the contact resistance reduces with increasing area of the contact. We initially tried electrical relays with and without modification. Shows one implementation. However, the mechanical design is not flexible for these parts.

6.7 Contact Actuator

It was decided to move a flat metal plate to contact the base of cartridge (in case of integrated heater) and the back of fixed plate (in case of cartridge with metal base without integrated heater). Keeping in view the size of the plate, various actuators are readily available. Solenoids and motors can be used. To conserve energy, we shortlisted solenoids which would only need energy to move but not while holding. They are available in both rotary and linear configuration and are called bistable solenoids. One type has a holding magnet on one end and a spring to move to other end when the effect of this permanent

magnet is cancelled by an electrical impulse. The other type has permanent magnets on both ends. The spring helps in applying pressure and gives much easier mechanical tolerance for the design. Rotary solenoids are also available in bistable configuration. Therefore the energy required for cooling is drastically reduced. Each cooling cycle only required two electrical impulses for operation. For bistable solenoids, 100ms is enough for actuation.

Thermal contact can be made to multiple temperature sinks for fast cycling as well. This has been done but for many applications like melt curve analysis a heater is more appropriate.

We can increase the speed

6.8 Advantages

The system allows fast speed yet the design is very simple. The fluid does not have to move. Nor does the cartridge has to move across contacts or to get convention to its advantage. A real water and dust proof design is possible as the mechanism can be enclosed and the heat can be dispersed to the casing.

The reliability of system is very high. Solenoids have lifetimes for millions of operation. The lifetimes of peltiers is in the range of 10 thousand cycles.

The cost of solenoids is much lower. The electromechanical relay has been developed a century ago and is a widely used component. Custom designs are possible.

Another advantage is the tuning of response. Even with best convention based systems, the heat transfer slows down when the differential reduces. However, if high speed is desired the contact or the heat sink can be set at much lower temperature. For example if the heat sink is at zero ° C, heat transfer will increase. One can think of using ice or other mechanisms to cool down the heat sink.

Accurate temperature control is not needed for heat sink or contact. If we measure the temperature at cartridge metal surface or the fixed plate, we don’t need to know the temperature of the contact or heat sink. As PCR temperatures are higher than ambient, this is a useful feature. Both heat sink and contact can be let equilibrate to the ambient.

In short we are using physics to accelerate a process which is favored by a low energy technique which is both simple and reliable.

6.9 Design and analysis

To have quantitative information for predicting performance and for design, we use lumped element modelling. We used Matlab physical modelling toolbox. We have already established that a 1D model can be used in design of our flat metal based cartridge.

6.10 Lumped element analysis for cooling

We are primarily interested in cooling performance. The performance of cartridge has been covered already. We want to study effect of ambient temperature and thermal contact on system performance. Such modelling shows us the distribution of temperatures inside fluid chamber and the effect of parameters.

We consider various cases. The models are shown in *Figure 6.8*.

i. Air convection cooled
ii. Thermal contact cooling for cartridge with integrated heater
iii. Thermal contact cooling for a bare cartridge with heater on a fixed plate.

In the last case there are two thermal contacts in the system.

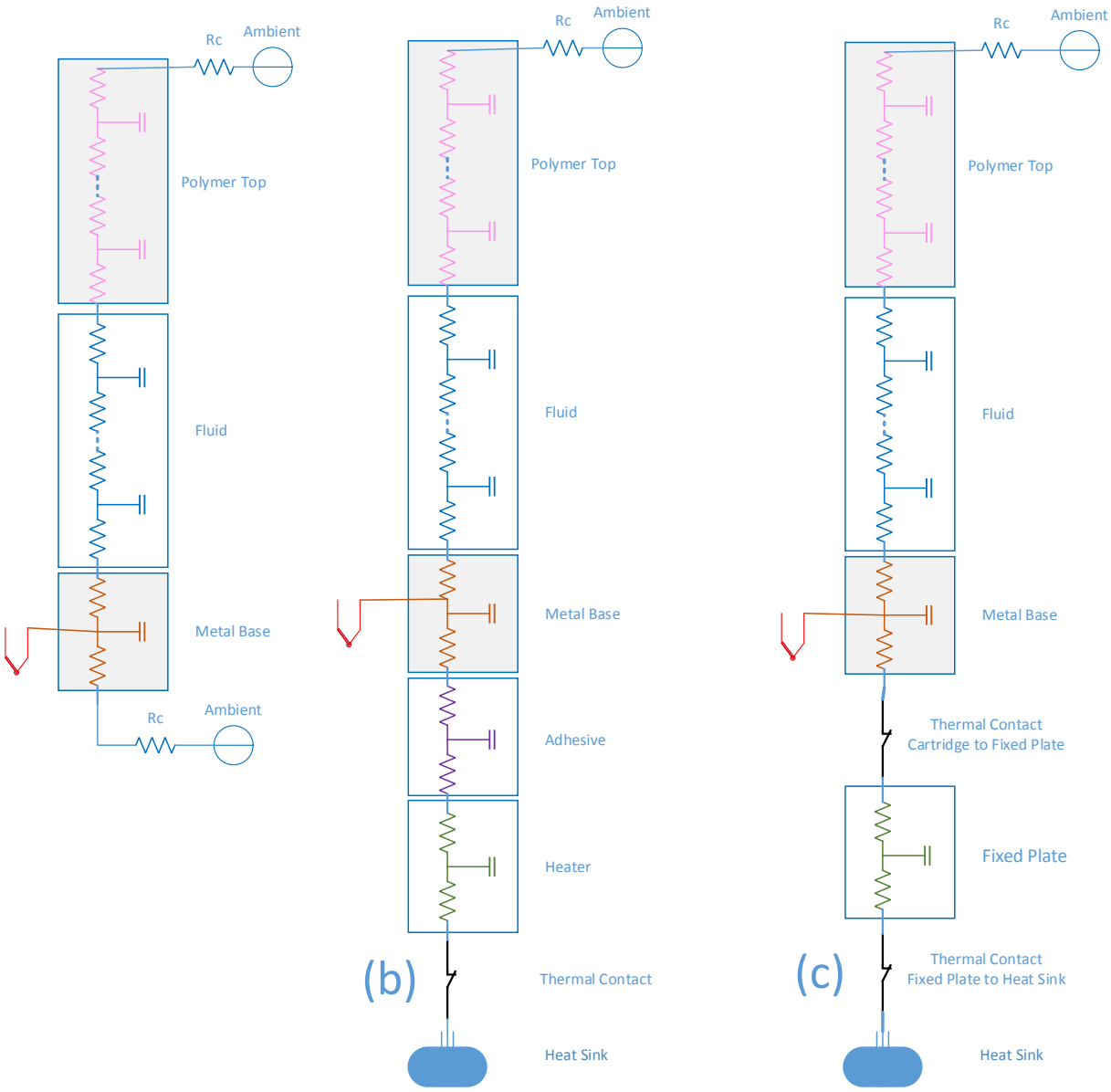


Figure 6.8 Lumped Models for Various configurations. (a) Shows convection cooled. (b) Shows cartridge integrated with heater (c) shows bare cartridge with a fixed heated plate

First we compare the thermal resistances of convection and a single contact. Assuming

$$h_c = 100 \frac{W}{m^2 K}$$

$A = 10^{-4} m^2$, where A is the area of chip of sides 10 mm x 10mm

Then $R_c = \frac{1}{h_c A}$ is $100\frac{K}{W}$.

Similarly, we calculate the range of contact resistances we can expect. *Figure 6.9* shows the expected range for comparison. Thus from a first order estimate, contact cooling can give similar or better performance.

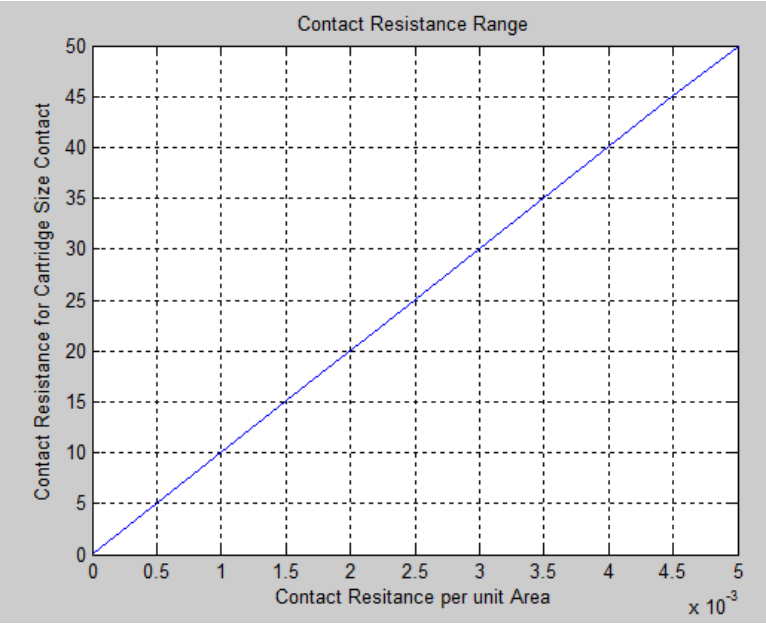


Figure 6.9 Expected Contact Resistance Range for Cartridge

Such resistances are useful for getting broad ideas about design, however for transient analysis various other factors are also important so a full simulation gives more information.

6.10.1 Effect of ambient temperature

The technique is dependent on ambient temperature to some extent. Shows the cooling time as a function of ambient temperature.

6.10.2 Contact resistance

6.11 Contact modification

The contact can be modified in a number of ways. Interstitial fluid has been used. A think metal foil can also help to increase contact area. Coatings can be applied and surfaces can be structured to improve performance.

6.12 Options for HTR plate

A fixed heater place has a distinct advantage of making cartridge simpler and lower cost. The heat transfer through the system is largely dependent upon the heater as metal offers very low resistance to heat flow.

There are various ways to implement heater functionality. The figures of merit are thermal uniformity and least thickness. We especially want to reduce thickness of any polymer layers. The available flex heaters or kapton heaters have typically two layers of insulation guarding the heater element. This offered substantial thermal resistance. By looking at flex circuit offerings, embedded resistor laminates were found. They are available with or without polymer backing. They are flexible and the uniformity of resistor area is excellent. Adhesives used in flexible PCB industry also are available as thin as 13 microns.

6.13 Heater Design and Fabrication

The main suppliers of embedded resistor laminates are Dupont and Ohmega. All metal substrate is available from Ohmega. However, in that case the electrical insulation has to be ensured and additional processing steps would be required.

We used Pyralux® APR copper clad resistor laminate. Among the selections APR 02502518NC was selected as it had thinnest of dielectric and copper. The dielectric thickness is 25 micron. Although, primarily for having embedded resistor functionality for circuit boards, we found the laminate to be robust for heating use. Figure 6.10 shows the basic laminate structure.

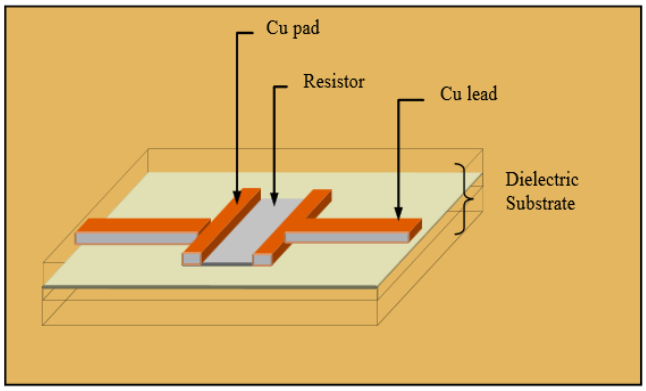


Figure 6.10 Laminate Structure

The heater was designed in Altium Designer. Top and Bottom Layers were used to specify NiCr and the top Copper layer. *Figure 6.11* shows the design of such heater. The side tracks (red) are copper and the blue area is NiCr. Since all area is filled with NiCr and due to controlled surface conductivity, the heating is extremely uniform. The inside tracks can be used to connect a sensor deposited over blue area. For example Platinum can be deposited after deposition of an insulating layer and hence the temperature can be measured.

6.14 Heater Fabrication Protocol

The heater fabrication protocol was developed after experimentation.

CAUTION: All processed should be done under UV-filtered light conditions to prevent poor quality products from incidental exposure that leads to unexpected polymerization of the photoresists.

Wafer from OmegaPly (Cu-Ni)

6.14.1 Part 1:

6.14.1.1 *Step 1*

Apply Photoresist Sheet on the front side of the Copper wafer

The front side of the wafer should be marked in advance.

Effective temperature in laminator: 120 ºC

To have a flat and even adhesion, hold the “top leaf” photoresist and the “bottom leaf” copper away from each other (picture) while feeding into the laminator.

Apply the photoresist on BOTH sides of the copper wafer.

Indication of success: The photoresist (light blue in color) adheres evenly to the copper surfaces.

6.14.1.2 *Step 2: Expose in UV with Mask 1*

Use negative photoresist mask. The pattern should be exposed to UV light.

The first mask’s patterns should expose the copper wires and the heater pad (electrical resistance region).

Expose the FRONT side of the wafer to UV for 25 seconds.

Indication of success: The exposed patterns should be darkened.

6.14.1.3 *Step 3: Develop the Photoresist*

Solution: 2.5 g Sodium Carbonate + 250 mL DI water
Temperature: Ambient
Dwell for 5 - 6 minutes.
Rinse in DI water for 2 - 3 minutes.
Indication of success: The red color of copper are visible at parts that were not exposed to UV light (light blue color) whereas the exposed patterns remain unchanged in dark blue color.
Step 4: Etch the Copper
Solution: 31 g Cupric Chloride + 65 g Ammonium Chloride + 250 mL DI water
Temperature: 50 ºC (the hot plate should be put at 90 – 100 ° C)
Dwell for 5 - 7 minutes
Rinse in DI water for 1 minute
Indication of success: The cupric red color should change to grey color of Nickel on uncovered photoresist.

6.14.1.4 *Step 5: Etch the Nickel*

Solution: 65 g Cupric Sulphate + 1 mL Sulfuric Acid 98% + 250 mL DI water

Temperature: 95 °C (the hot plate should be put at 180 -190°C)

Dwell for 5 minutes

Rinse in DI water for 2 minutes

Indication of success: The grey layer should be stripped off and the green polymer layer should be visible after blow dried.

Step 6: Strip off the Photoresist

Solution: 8 g Sodium Hydroxide + 250 mL Di water

Temperature: Ambient

Dwell for 2 - 3 minutes

Indication of success: The copper wires and copper pad should be COMPLETELY visible in red color.

6.14.1.5 *Step 7: Blow dry with nitrogen*

6.14.2 Part 2:

6.14.2.1 *Step 1: Apply Photoresist Sheet*

Same as Step 1 in Part 1 but only on the front side of the chip.

Step 2: Expose in UV with Mask 2

Same as Step 2 in Part 1 except Mask 2 should ONLY expose the copper wires but not the pad because we want to etch the copper pad.

6.14.2.2 *Step 3: Develop the Photoresist*

Same as Step 3 in Part 1.

6.14.2.3 *Step 4: Etch the Copper with special Ammoniacal Etchant*

Same as Step 4 in Part 1 except the DWELL time is 4 minutes.

BE CAREFUL not to over etch. When the copper is completely dissolved, and the grey layer is visible, it is good to take the chip out.

Note: NO nickel etching at this point. (No step 5 in part 1 repeated)

6.14.2.4 *Step 5: Strip off the Photoresist*

Same as Step 6 in Part 1.

6.14.2.5 *Step 6:*

Develop the back Photoresist

Peel off the resist cover sheet on the back of the chip.

Then repeat step 3 until the blue color of the photoresist disappears.

6.14.2.6 *Step 7 Blow dry with Nitrogen*

The chip is now ready for testing.

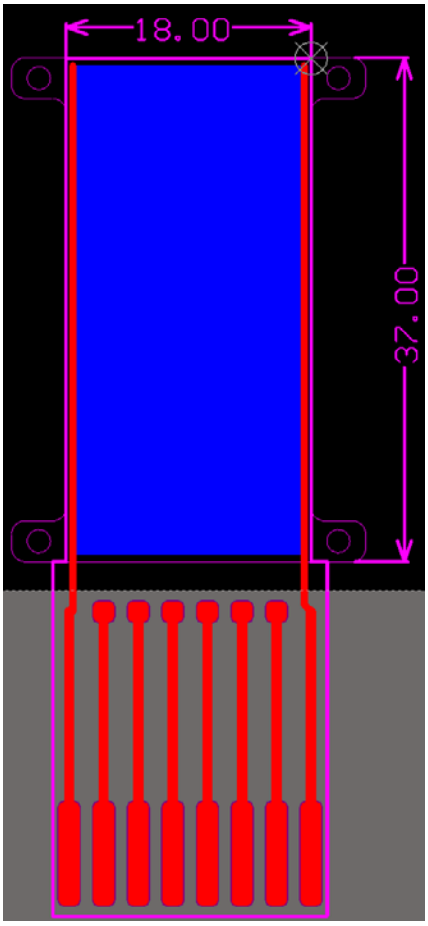


Figure 6.11 Heater Design

6.15 Implementation – experimental

To test the proof of concept the system was built on optical breadboard. Similar setup was used for this experiment with additions to the software. *Figure 6.12* shows the schematic of the setup and a picture of the setup is shown in *Figure 6.13*. SolidWorks design is shown in *Figure 6.14*.

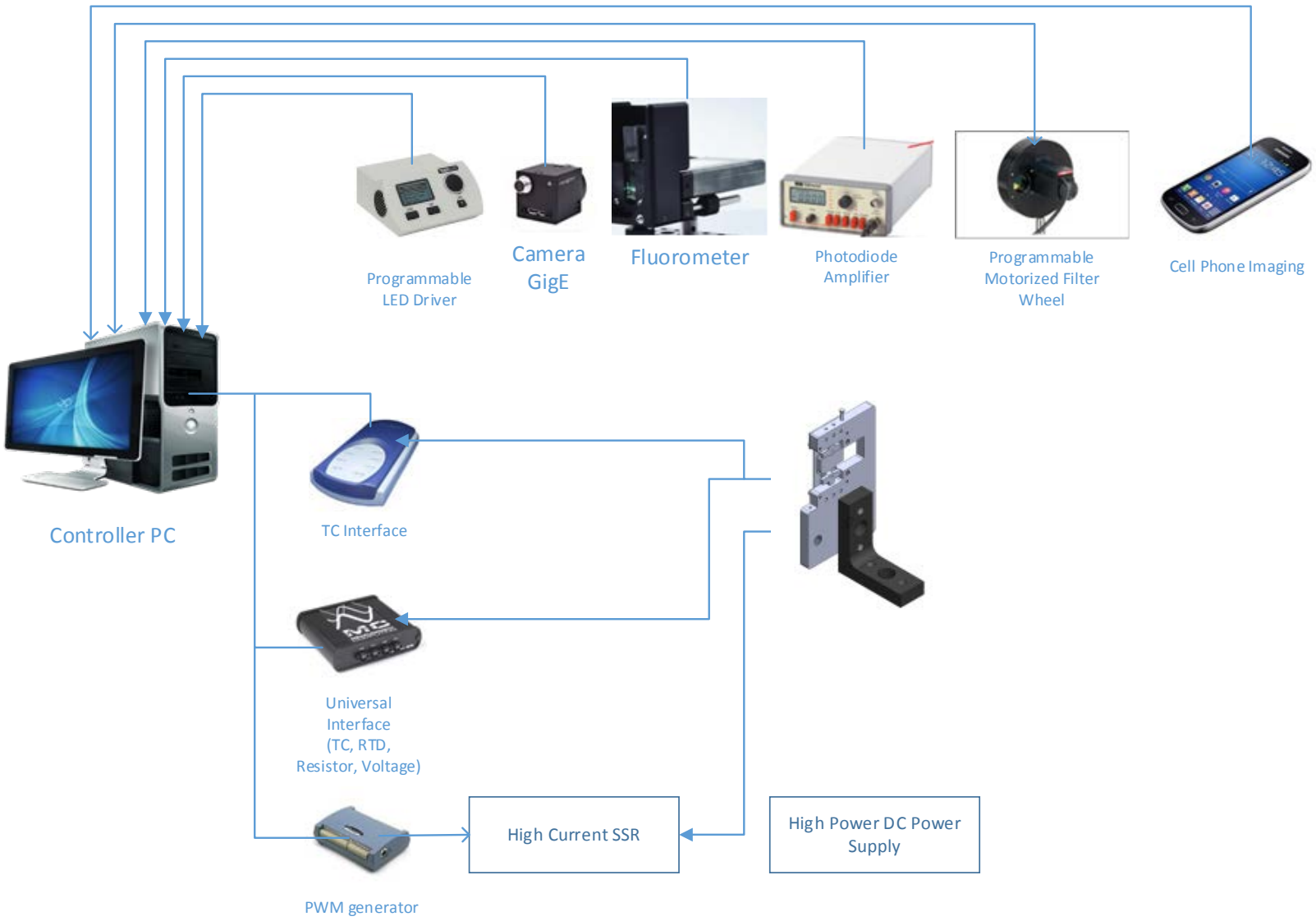


Figure 6.12 Test Setup Schematic

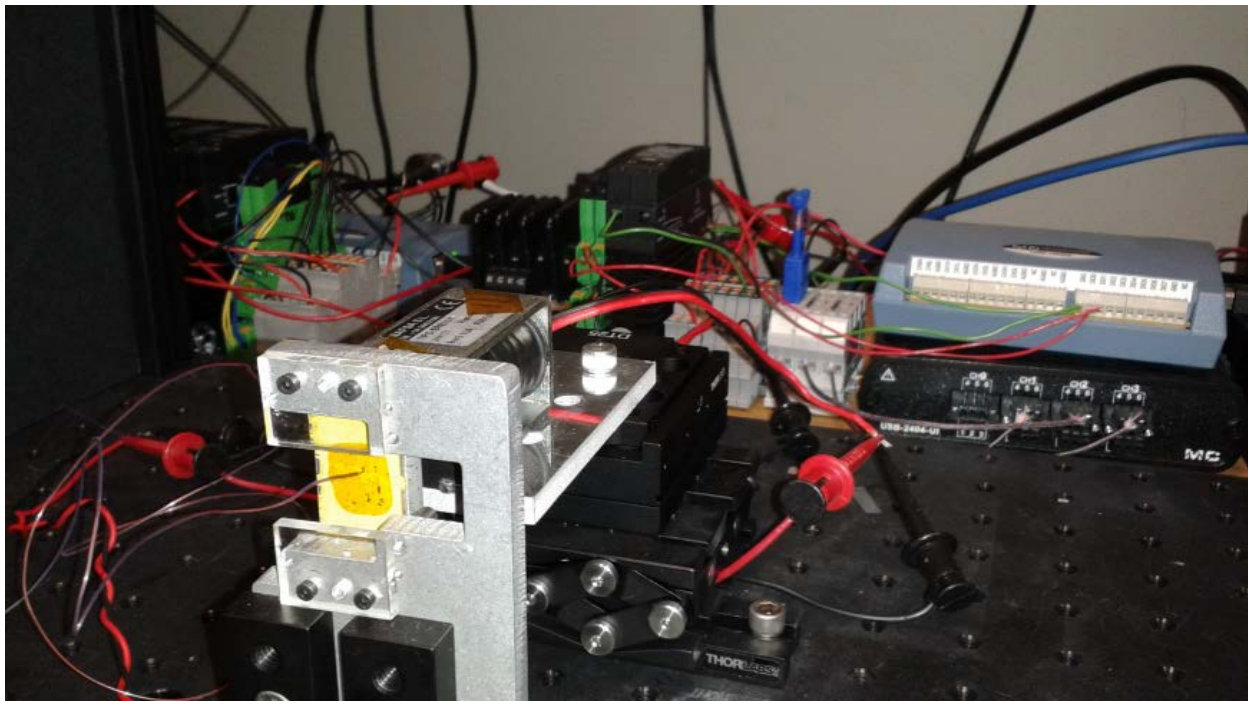


Figure 6.13 Picture of Test Setup

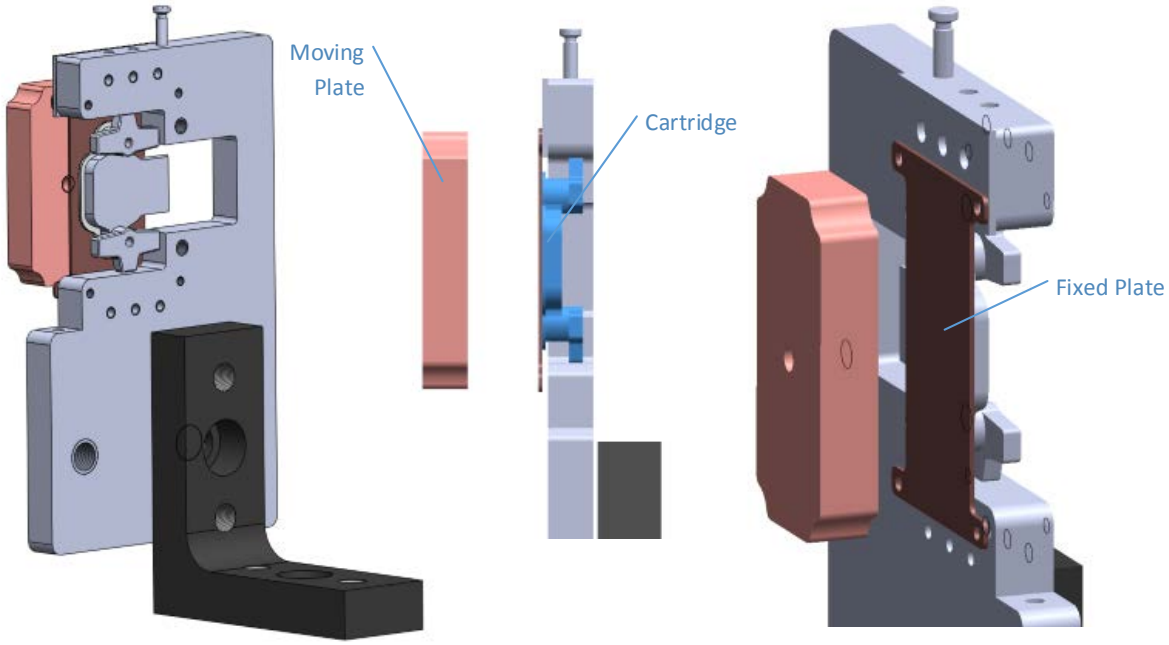


Figure 6.14 CAD Diagram of Contact Cooling Test Setup

6.16 Results

We could successfully cycle temperature while measuring at the fixed plate. This was a proof of concept demonstration. Various parameters can be improved including the heater design, quality of mechanics for contact and a secondary heat sink to disperse heat gathered during contact to a much bigger mass which can always be dissipating to the atmosphere. A ramp rate of about 7 degrees per second was achieved even with non-optimized system.

The temperature graph with contact cooling and resistive heating is shown in *Figure 6.15*.

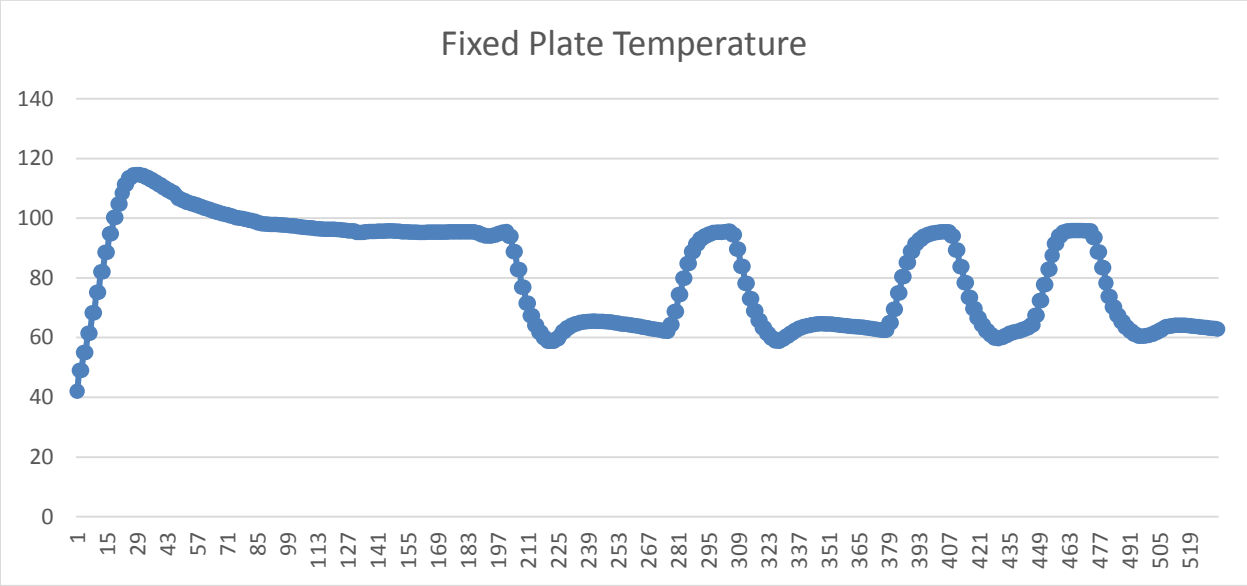


Figure 6.15 Temperature Control with Contact Cooling

6.17 Conclusion

We have suggested, designed, simulated and demonstrated a versatile approach to circumvent one of the major challenges for bring PCR at low resource settings. The method is high performance and provides many other benefits. Complete dust and water proof design are possible. Cost is low and the implementation can be exceptionally robust.

7 Low Cost, High Performance Optical Design for POC qPCR

7.1 Abstract

We report development of an optical technique which has the potential to solve long-awaited problem of high performance fluorescence detection at POC. Our design also works well with lab-on-chip devices. After thorough study of existing designs, we concluded that there were still shortcomings which does not allow their adaption to POC with desired features. We performed extensive design, analysis and simulations to optimize the designs. The performance is verified on bench top with real PCR reagents.

7.2 Requirements

The requirements for optical system for POC real time PCR based system are given below.

- Cost should be low
- Size should be small
- The life of the system should be long. The filters or optical elements should not degrade after use.
- System should not require precise or difficult alignment. This can make production and maintenance problematic.
- Good light collection efficiency. Should also allow required power to couple to PCR chamber for excitation.
- Should allow multi-color design for color based multiplexing.
- Integration or observation time should be low to allow fast readings.
- Should be robust to work in presence of bubbles.
- Preferably should work in presence of partial filling of cartridge.

7.3 Early Work and Problems

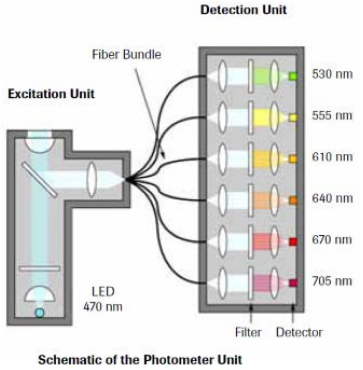
It is useful to review the designs used in commercial machines and research literature to find out the problems. It also helps us to look for opportunities, if any, in those designs. We want to see if existing designs can be modified to meet our requirements. It can also give us clues into merits and disadvantages of various approaches in implementing fluoresce detection in real time PCR.

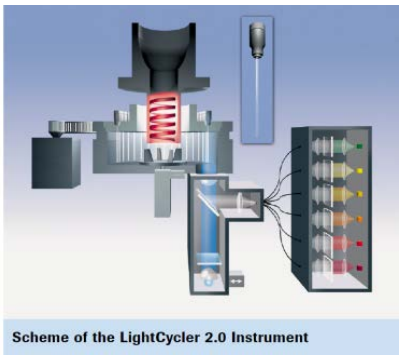
7.4 Commercial Machines

We first review the commercial PCR machines. Only few of these machines are for single cartridge based systems. Most of the machines are for multi-well plates. We have provided optical schematics for the optical designs of these machines in *Figure 7.1* . This is useful as the detailed technical specifications for the machines which can be useful in optical design are not publically available. However, by having the optical schematic a broad idea and approximations can be deduced.

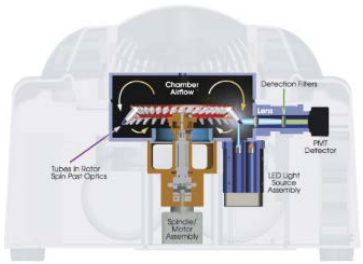
We can broadly divide these machines into two types depending on their collection optics. The first type 'images' all the reaction wells simultaneously. These typically used a camera (CCD is common). Roche Lightcycler, Illumina Eco, Thermo Scientific PikoReal, Matecycler and Bio-rad MiniOpticon fall in this category. This category is not attractive for POC applications where a single reaction well needs to be observed. In these machines, care has to be taken for optical leakage between wells and typically a reference dye (such as ROX) is used in all wells for calibration. Using a CCD also makes electronics much more complex than using individual photodiodes.

The second type detects fluorescence from each reaction individually and hence is time multiplexed. This is more compatible with POC systems. However, in order to observe multiple reaction vessels, more time is required. Also these machines require moving mechanical design. Roche Lightcycler, Qiagen RotorGene, Bio-rad CFX and Biometra TOptical are examples on machines in this category. Interestingly, all of these use dichroic mirrors or beam splitters in their designs except Rotor-Gene Q. All of the above move the reaction vessels except Bio-rad CFX and Biometra TOptical which move the optics over the PCR wells. All of these designs use lenses and various elements which require alignment. The cost and complexity of these assemblies seems high. The collection efficiency is also not very high since the sharp cutoff interference filters require normal angle incidence. To collimate the light from the reactions vessel, a certain distance needs to be maintained for using a lens.

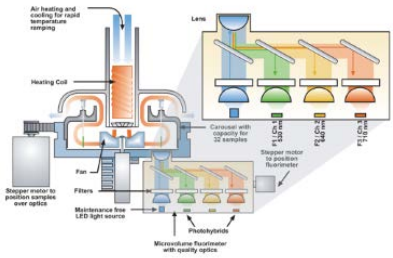




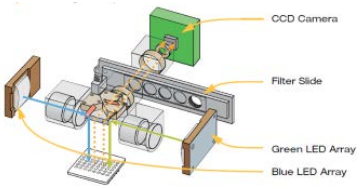
LightCycler



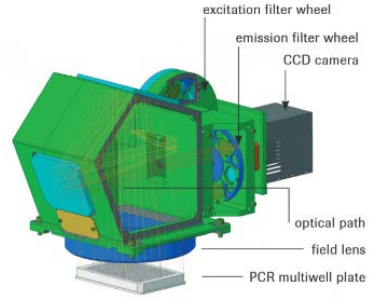
Rotor-Gene Q



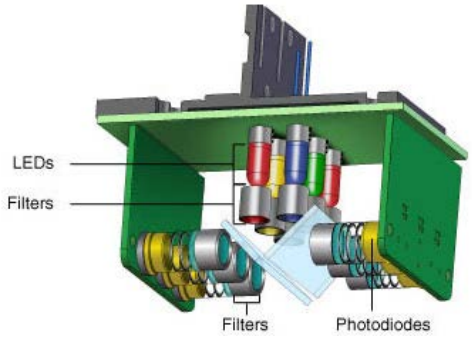
Idaho LightScanner 32



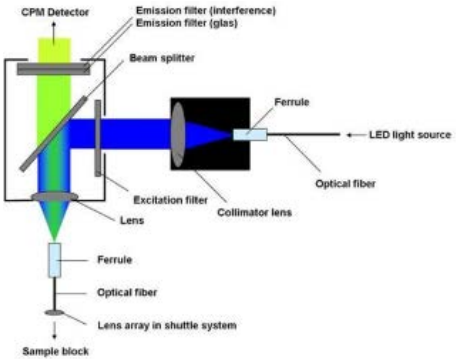
Illumina Eco



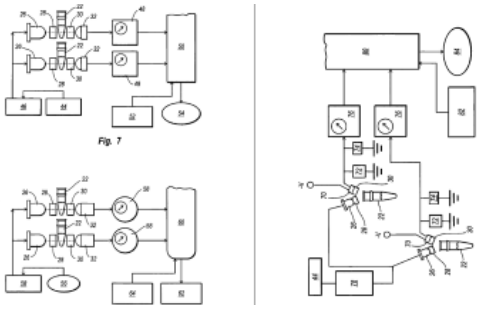
Roche LightCycler



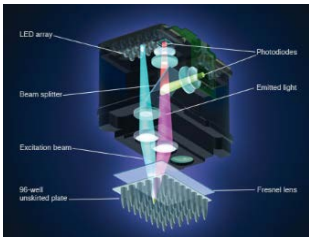
Bio-rad CFX 96



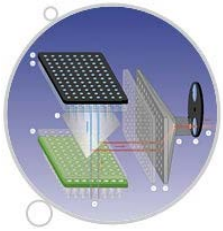
Biometra, TOptical



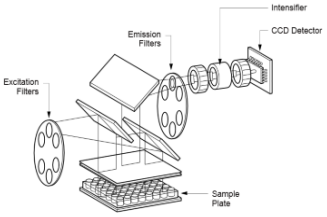
DxNA



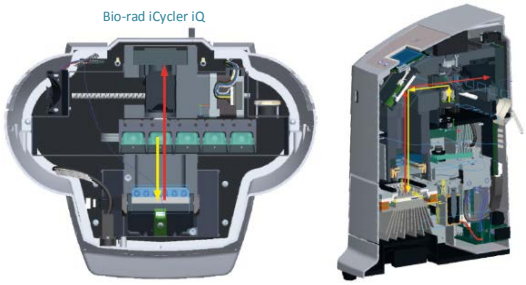
Bio-rad MiniOpticon



Mastercycler ep realplex



Bio-rad iCycler iQ



Thermo scientific PikoReal

Figure 7.1 Real Time PCR Machine Optical Design Schematics

An exception is design by DxNA which has LED and detector for each well. However, this design does not seems scalable for number of multiplex channels.

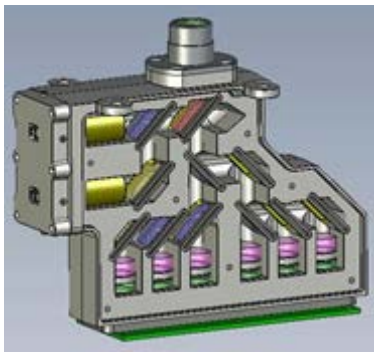
7.4.1 POC Instruments

There are not many available POC real time PCR or sample to answer machines including real time PCR detection. There are only few fully integrated systems which are available or are in development. The schematic and diagrams of their optical designs are shown in *Figure 7.2*. Cepheid has the largest installed base among all. Its optical system is a side based illumination and detection. The optics needs precise alignment. Also the shape is fixed. The collection efficiency seems to be low as the fluorescence from thin sides (about 1mm) is being observed. Most of the fluorescence will be directed in other directions due to shape of the chamber. By adding an optical read step, 6 seconds are added to the respective step. This is probably due to integration time required to collect emission signal. There are also multiple filters and lenses. Interesting, the detection channels use both dielectric and glass absorbing filters.

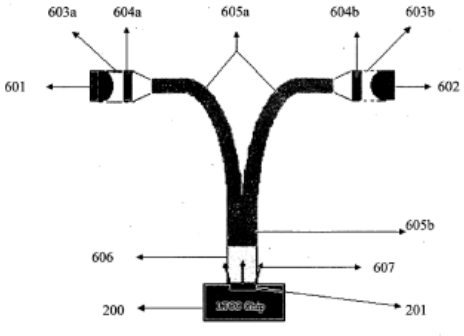
Enigma Optics is also complex and uses various dichroic elements and filters. Bigtec design used optical fibers. It is not clear how much of the reaction vessel's area is being optically interrogated. It also seems hard to multiplex as it can increase cost and complexity significantly.

The POC instruments in development including Quidel qNAT and Alere, which are funded by Gates Foundation are also interesting to look at. The Alere platform has a real time fluorescence imaging module. Although the details are not provided it's an epi-fluoresence setup and hence most probably would require dichroic mirrors and filters as in a conventional design. Alere platform uses propriety Competitive Report Monitored Amplification (CMA) technique[138]. It does not use classic real time PCR. A mechanical fluorescence background displacement unit is also used adding bulk and complexity. The cartridge has to be compressed reversibly mechanically for operation. This severely limits the technique and puts constraints on cartridge design. Image processing is required. A microarray has to be prepared. We believe this is a complex design and it would be hard to bring its cost and complexity down. Its advantage over multi-well qPCR based designs are also not clear.

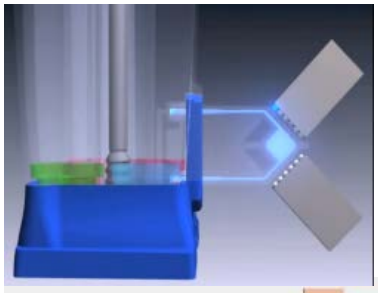
None of these designs seem to have a 'microfluorometer' or a system which can be compact and requires less optical alignment.

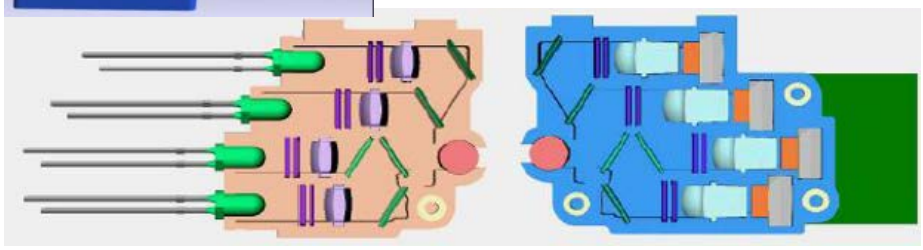


Enigma

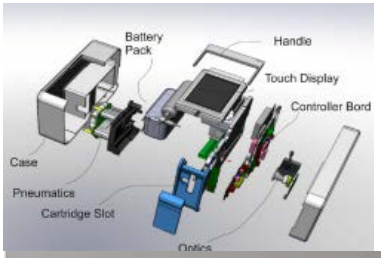


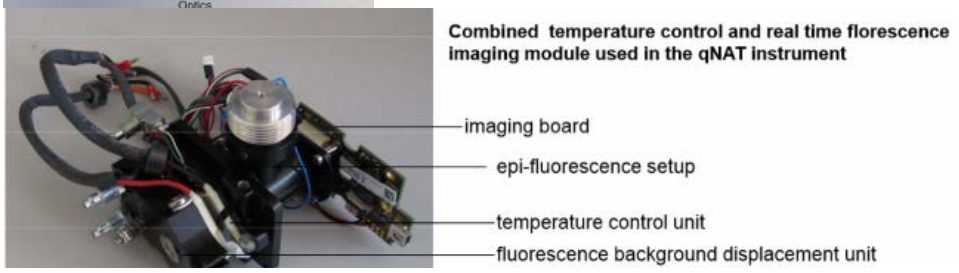
BigTech





Cepheid





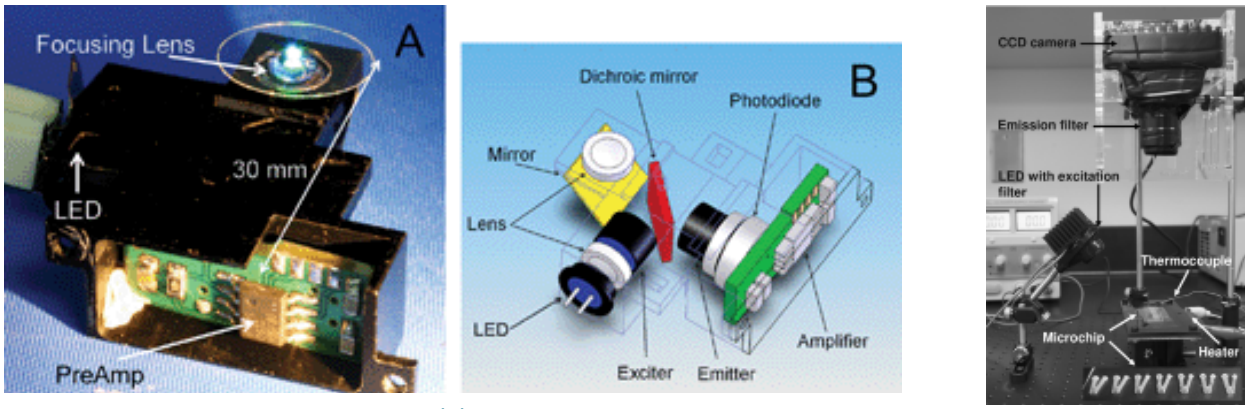
Alere

Figure 7.2 Optical Design Schematics for POC or single reaction vessel Instruments

7.4.2 Research Literature

We now turn attention to research literature. It was figured out that although there has been many lab-on-chip reactors [60], most of these use either commercial or bulky optical systems. Some of the researchers used optical microscopes. It seems that a coherent design of cartridge and the optical design in not done in most cases. One approach is to miniaturize the usual optical design [139]. However, this still remains expensive and the alignment and fabrication requirements of the conventional optical designs still remain. The benefit of this approach is size reduction but other parameters don't change. In fact it should be seen whether optical performance of these designs is comparable to commercial PCR machines.

Schematics using conventional approaches are shown in *Figure 7.3*. Usual design was miniaturized[139] and a camera was used on the bench[140].



(a) (b)

Figure 7.3 Example Fluorescence detection systems

There are few designs which use have excitation and emission in the same direction. The chip is typically between the source and detector. *Figure 7.4* shows main examples from literature. This approach is not attractive to real time PCR applications. For good thermal response, the height of the chamber should be low thus making the fluid flat and wide. At least one of the sides of the chip should be used for heating and cooling. Thus sandwich designs don't fit into this requirement. The advantage of this approach can be that whole area of the chip can be optically queried by large area detector. Thus this approach may work in some assay where temperature control is not required. It may be also used in case if some other form for thermal control is exercised. Another approach is to make transparent heaters. Non-directional filters are used[141] in some cases with polarization to reduce bleed though.

This technique does have the problem of putting additional requirements on filtration as a large amount of excitation can reach the detector.

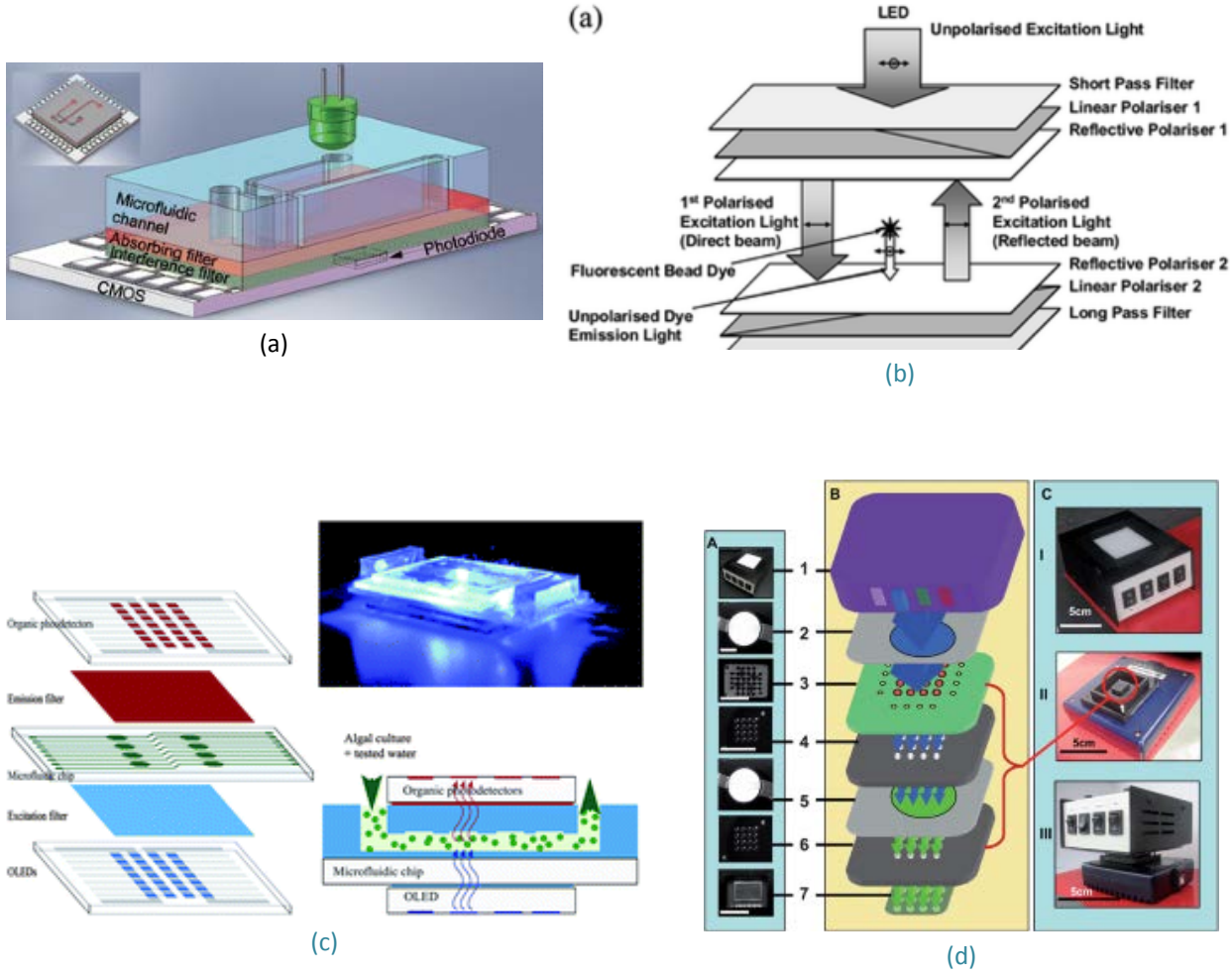


Figure 7.4 Designs with sandwich designs

The other approach has been to have excitation and emission in different directions. Many designs use orthogonal directions. Such designs typically don't require a beam splitter. Some key schematics are shown in Figure 7.5.

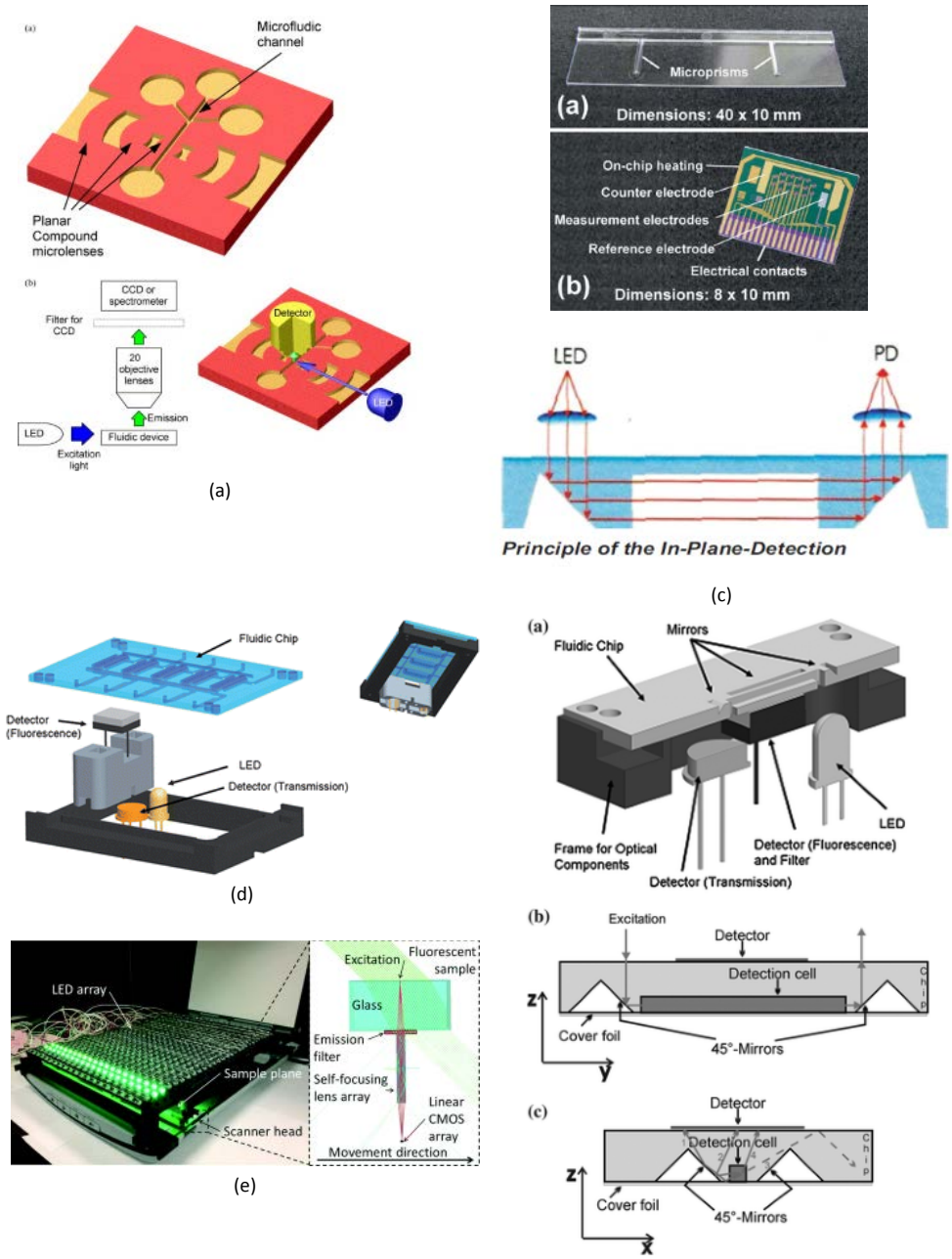


Figure 7.5 In Plane Excitation Designs

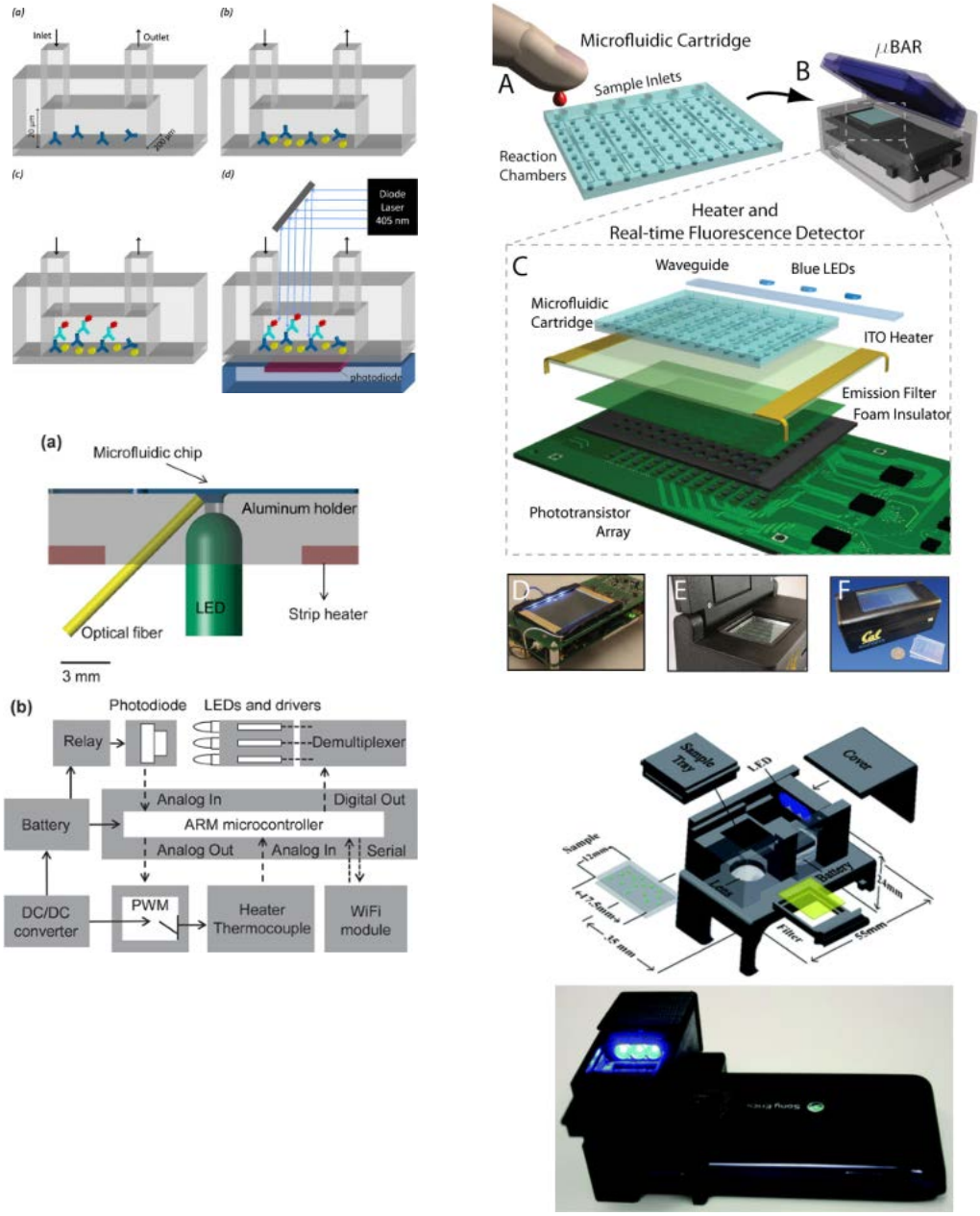


Figure 7.6 In plane and separate directions for excitation and emission based designs. (a). Uses planar micro lenses[6] (b). Microfluorimeter with integrated prisms [7]. (c) Gene-Z system [8]

Planar micro lenses which are a part of the chip are used[142]. In this design no excitation filter is used. However, the design was for a channel and not a large reservoir. Making lenses on chip also take chip area. An objective lens was used which is not good for large area observation. Microprisms were used to couple light at near critical angle[143]. This design was for evanescent fields and the region of interest was about 100nm only. A microfluorimeter was demonstrated with an integrated polymer chip [144]. The chip used integrated 45 degree mirrors to guide excitation and another set of mirrors to increase

fluorescence collection. The design is for a channel. The mirrors are on all 4 sides of the channel (chamber in our case). However, the fluid has to have an inlet on at least one side. The optical elements have to be under the chip and the size of the chip can increase. Hongying Zhu et al. used orthogonal excitation and a plastic emission filter for fluorescence imaging on cell phone[145]. This design is difficult to scale up for multiplex applications as it uses cell phone camera and putting multiple emission filters might not be trivial without causing significant interference. Also this is not a PCR chamber and no surface is being used for heating. The isothermal platform, 'Gene-Z', used a fiber to collect light. Such an approach is not attractive for POC use as it requires alignment and mechanical considerations [71]. Flatbed scanner was used [146] for gigapixel imaging. A waveguide was used to couple light from sides to the cartridge. ITO heater was used and one detector per reservoir was used. This design is not amenable to add more colors to the system.

The lens less imaging systems for POC are also reviewed[147].

7.5 Commentary

All the designs for side illumination miss an important point which becomes more relevant for wide and low height chambers - required for good thermal response in qPCR. The light from LED is not highly collimated. The first difficulty is to have light in a narrow slit like shape to be extracted from LED optics with appropriate efficiency. Any ray of light coming out of such narrow slit with little divergence will not illuminate the fluid once it reaches the polymer due to its higher refractive index. Thus if we use LED without a slit and very high collimation, most of the light will be trapped inside polymer and will not illuminate the chamber. Excitation light causes auto-fluorescence of the polymer. The path length is also not more than the length of the chamber for light which is well collimated.

7.6 Our Idea – side illumination

Our basic idea is coupled to design of our chip with a reflective bottom. However, the concept can be applied in a wide variety of modified applications. *Figure 7.7* shows a schematic of basic idea.

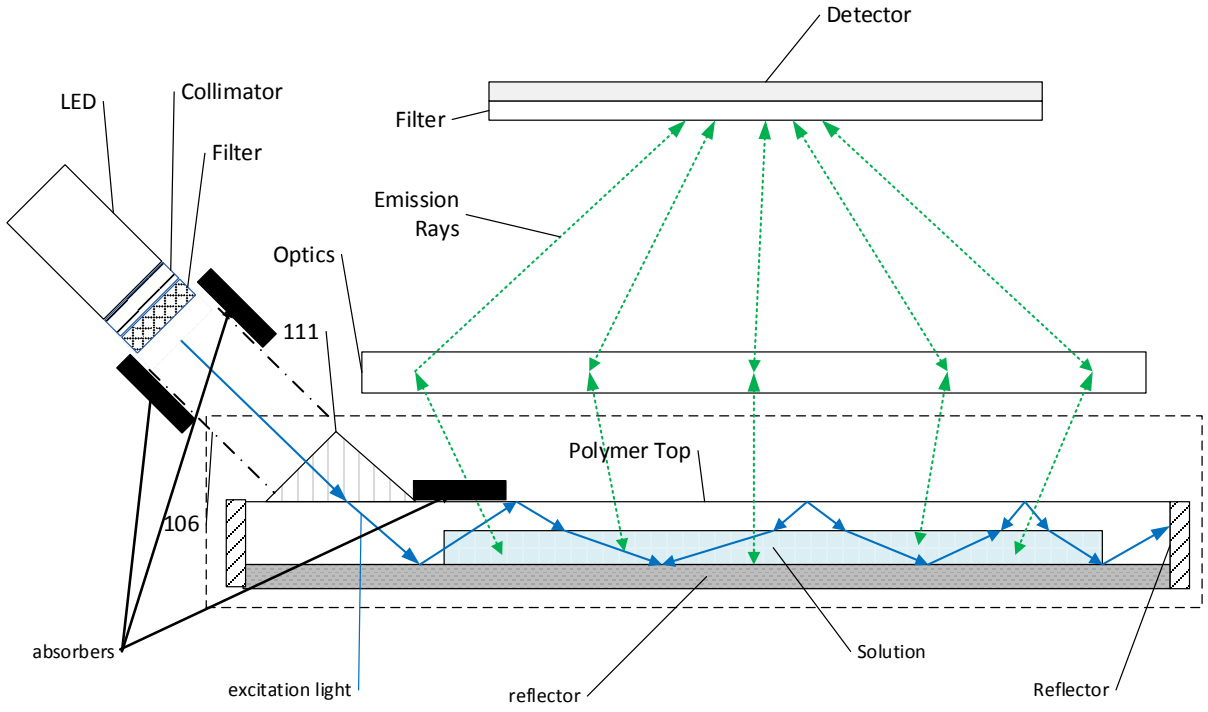


Figure 7.7 Optical Design Concept

There are various innovations in this design. The light is sent at an angle so that most of the light does illuminate the chamber instead of being trapped inside the polymer. The interaction length is also increased due to multiple reflections. Stray light is reduced due to TIR (total internal reflection) from the top polymer. In case of polymer bottom, the light can be totally internally reflected. However, we have shown that polymer can cause significant degradation to PCR speed. A reflective metal surface thus keeps the light inside the cartridge. It has the benefit that heater of any optical characteristics can be used. Fluorescence in the downward direction is reflected upwards. The prism can be molded as part of the chip. The prism can also be on sides thus keeping the top clear for optical interrogation.

A very useful feature is that a large area of the LED beam is coupled to fluid. More so this beam does not need to be super-collimated. We have already shown that top polymer can be thick without degrading the thermal response while increasing rigidity and allowing injection molding. Therefore excitation efficiency can be increased. Since the beam can have large area, various LEDs including high brightness LEDs and their low cost optics can be used to have very high excitation power levels.

One of the key benefits is that the alignment requirement are very loose. The LED beam can vary in angle and alignment without causing significant problems.

7.6.1 Extension to multiple chambers

The concept is useful for multiple chambers and even arrays of reservoirs as shown in *Figure 7.8*. Multiple separate detectors or a FPA (focal plane array) detector can be used. A moving detector can also be used. Multiple filters can also move or slide for multiplex operation. Multiple static filters is another option as well.

One of the advantages of this approach is that a single excitation system can be used to illuminate multiple chambers. A multi-well qPCR cartridge as a separate component or a part of an integrated cartridge can use this technique. This can also be used for microarrays. In this case coupler can be as simple as multiple holes in a plate to couple sensor's pixels with sites in the array.

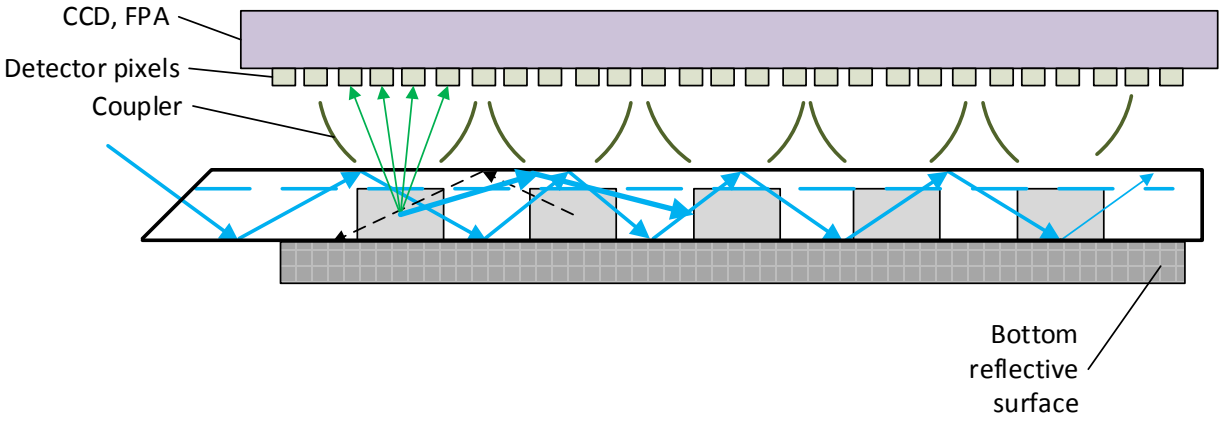


Figure 7.8 Extension for Multiple chambers

7.6.2 Use with Cell Phone

The basic idea can help realize a cell phone accessory which can do qPCR, isothermal amplification or fluorescence detection. The excitation can be separate or the LED of cell phone can be used. Camera of cell phone is used as a detector. *Figure 7.9* shows the schematic of the basic idea.

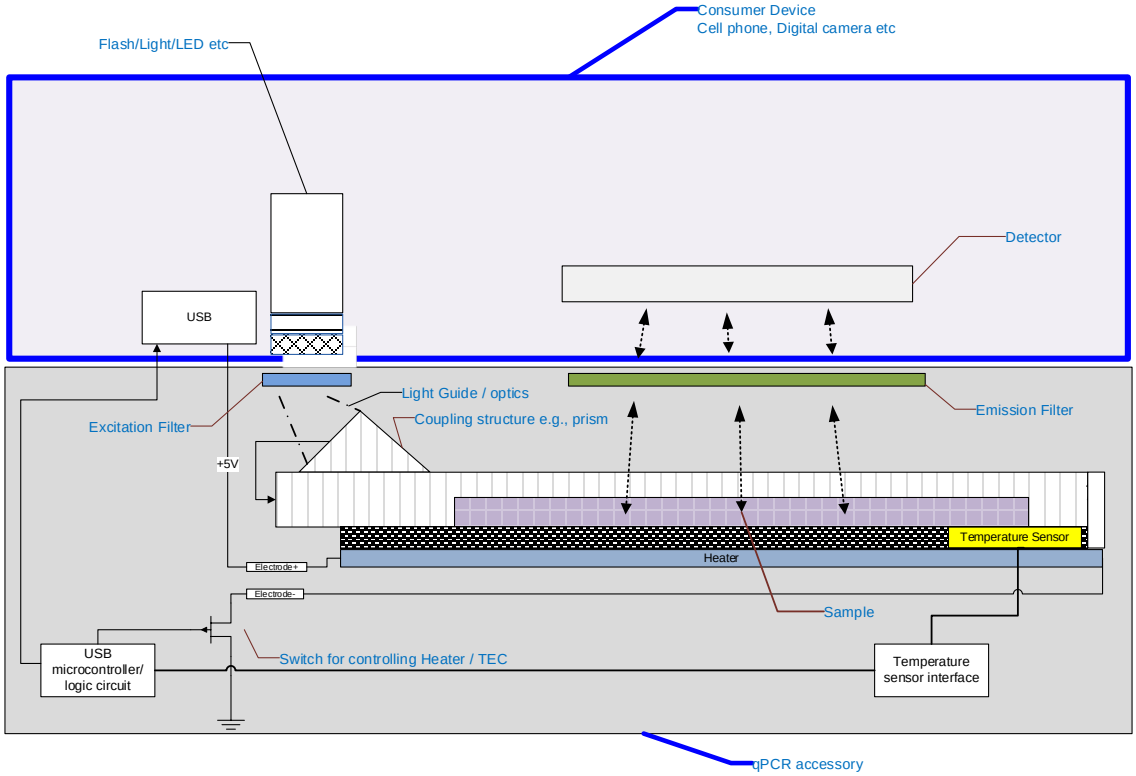


Figure 7.9 Cell Phone qPCR accessory

7.7 Detailed Design

Detailed design was performed to optimize the system and to know the parameters which are important.

7.7.1 Difficulty of design

The optimization of this design falls more under 'illumination engineering' which has much less literature than imaging optics. The process, although looking simple can be iterative, time-consuming and tedious. The merit functions are harder to design. There is a lack of formally trained people. There is also a lack of algorithms. The field is in its infancy[148]. As per the author

"So, at this time, the field of illumination design tends to be a rather hard discipline requiring the user to gain experience through trial and error, intuition, and the growing amount of literature in the field."

Figure 7.10 shows the design process from the book [148].

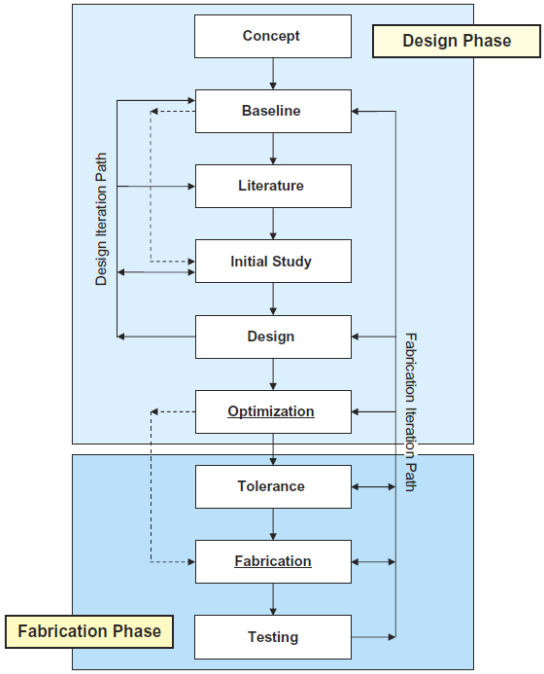


Figure 7.10 Design Process of Illumination System

7.7.2 Problems with purely side designs

It was considered that side illumination parallel to cartridge be used. In our case the chamber can be 100um-500um thick. A typical length will be 12 mm. Thus the ratio of length to height can be 120. The die size of chips used in HB (High Brightness) LEDs[149] is typically 2 mm. The surface of the die can be modelled as lambertian[150]. Ideally light should be collimated in the form of 12mm x 0.1mm beam with very narrow beam width. Even only considering length of the chamber and some side length for a total of 15mm and assuming a ray on top, the required collimation of beam is to be better than 0.5 degrees if the ray has to be inside the chamber for its entire length.

This fact is surprisingly not highlighted in most publications.

7.7.3 Light entry

The use of integrated prism is already mentioned. We also reiterate the advantage of light at an angle. The thickness of the cartridge top can be much more than the chamber height and even then the chamber can be well illuminated.

The conflicting requirement is the shorter the polymer height, the greater the light interaction can be due to multiple reflections.

To find out range of useful angles we do the following calculations.

Critical Angle for COP to air interface, $\theta_{c\_COP\_to\_air} = 40.81°$

Critical angle for COP to water interface, $\theta_{c\_COP\_to\_\text{water}} = 60.603°$

Angle in water after refraction, $\theta_{water} = \sin^{-1}\left(\frac{\eta_{COP}}{\eta_{water}}\sin(\theta_{COP})\right)$

Therefore, the useful range of angles is from around 40 degrees to 60 degrees. Hence a beam of 20 degrees can be coupled into the chamber.

Figure 7.1 shows the angle in water (with its surface vs the angle in the COP polymer).

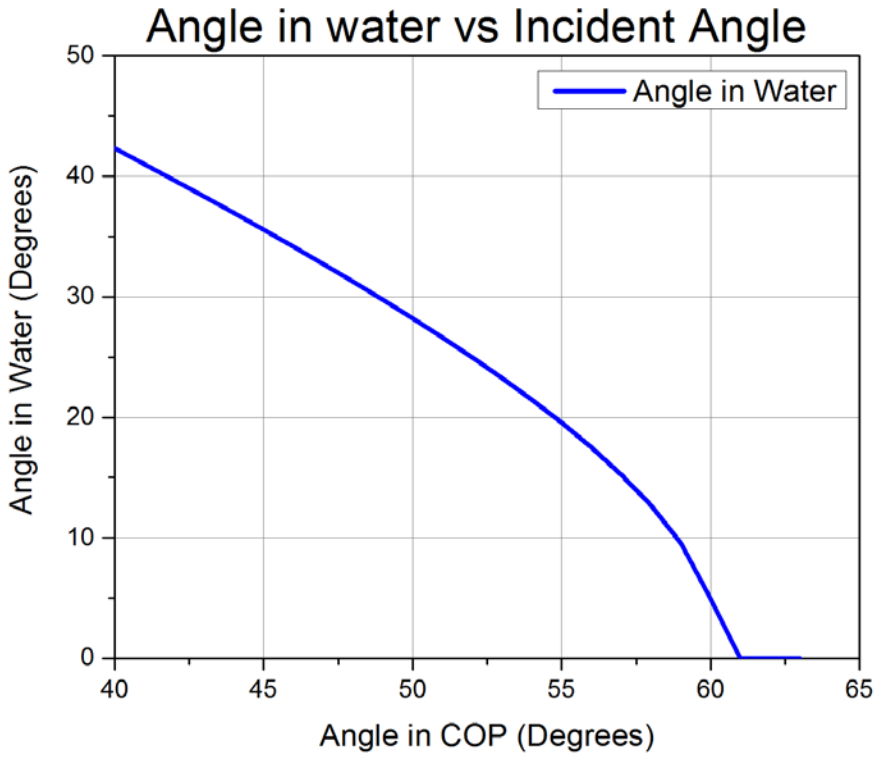


Figure 7.11 Angle of excitation rays in chamber (with surface) vs the angle in polymer (normal)

If we assume a uniform distribution of intensity vs angle in this 20 degree beam the distribution of the beam angles in water can be calculate and is shown in *Figure 7.12*. We note that more rays are at steeper angles and lesser at shallow angles with respect to the surface.

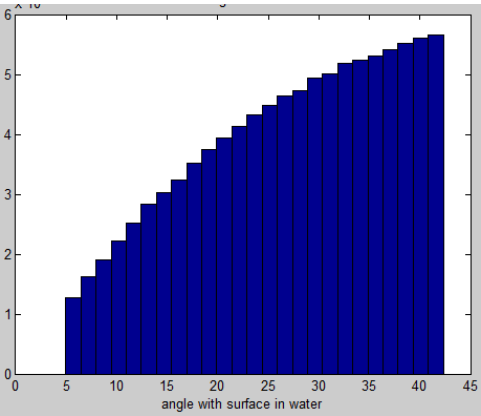


Figure 7.12 Distribution of Angles of Rays in water for a uniform input angular distribution

7.7.4 Coupling into Cartridge

The 20 degree angle is for inside the polymer. If light is introduced at a normal angle from air it will reduce in beam angle due to COP. The incident allowed beam angle is about 30 degrees.

7.7.5 Light guide design

The chamber is wide and narrow. To have efficient coupling to the cartridge, we should have a beam which can have 20 degree collimation in the narrow direction. The collimation in horizontal (or larger) direction is easier due to large size. LED lenses are available with various collimation characteristics. However, they are not designed to generate beams of these shapes. Light guides or light pipes are commonly used in LED based lighting. They are also used to relay light in displays which use LEDs as indicators. Light guide can provide homogenization and collimation as well. They are in various shapes [148, 151]. If we put an LED right next to the chip, lot of energy will be scattering due to typical wide beam widths of LEDs.

Another option is to put a slit next to a large collimating lens. However, this will bring a rather large object in close vicinity of the chip which can limit the observation area. A light guide can while improving the beam be a narrow structure, which can be used to couple light to the chip.

Another big advantage of light guide is that light guides designs can combine lights from various sources. This is very useful in multiple excitation sources as required in multiplex real time PCR. A light guide can bring light from various LED, lens and filter combinations to the chip. Putting multiples lenses near a small cartridge might not be feasible.

The light guide in our case is at least few millimeter in any dimension and the tolerances are also not critical. Mechanical problems and alignment issues encountered in fiber optics based light coupling is also avoided. The shape of the beam is well suited for light pipe based design.

There can be various designs of light guides. However a light guide which can be produced by laser cutting polymers sheets like PMMA are easy and quick to fabricate in our case. We used a simple rectangular design. This was also done since we used various commercial opto-mechanical components.

7.7.6 Effect of edge

One of our design merits is to reduce excitation light from reaching the detector. Using light at an angle which would excite the chamber causes an undesirable effect of having some of the light escape the polymer due to the effect of edge of the chamber.

The conceptual diagram is shown in *Figure 7.13*. Thus some rays do not stay inside the polymer due to the edge. Rays undergo refractions and reflections which can cause the angle at exit to fall below critical angle.

We define following quantities

$\theta_{exit,water}$ = Angle of ray after exit from water to polymer

θ_e = Angle of ray in polymer

θ_t = Angle of chamber edge with bottom

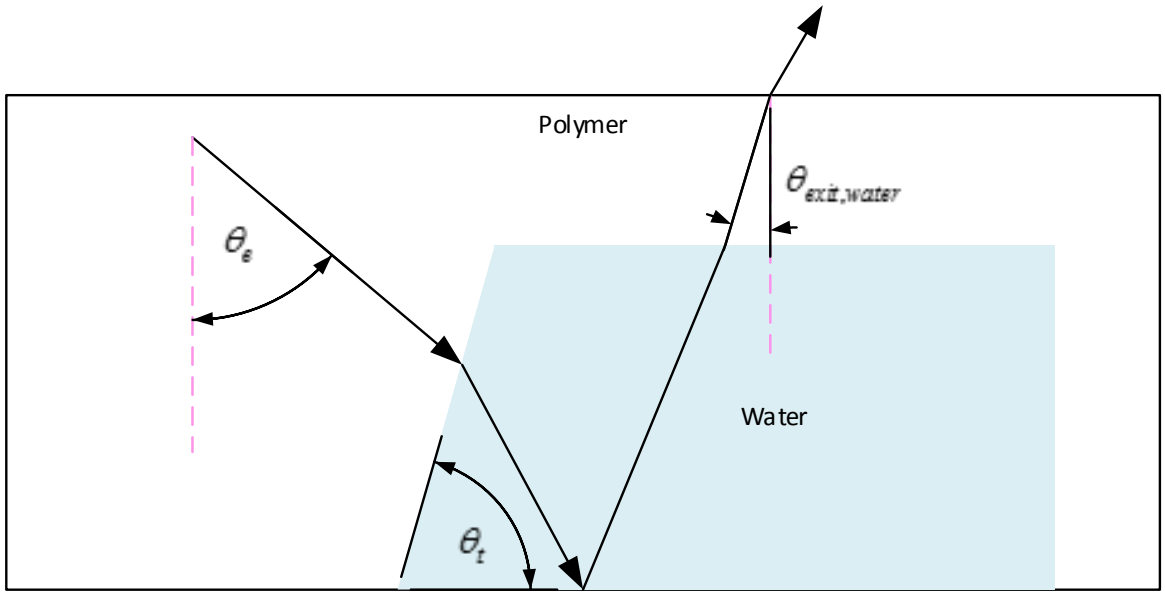


Figure 7.13 Light Escape due to Chamber Edge

After some manipulation the angle inside polymer after visiting the chamber through edge can be written as

$$\theta_{exit,water} = \sin^{-1}\left(\frac{\eta_{water}\sin\left(\theta_t + \sin^{-1}\left(\frac{\eta_{COP}\sin\left(\theta_e - \theta_t\right)}{\eta_{water}}\right)\right)}{\eta_{COP}}\right)$$

We can change the angle of chamber edge (θ_t) to see this effect. We already know the range of angle for excitation which are useful to illuminate chamber.

The plot for exit angle in polymer after going through chamber edge vs the incident angle in polymer for different chamber edge angles is show in **Error! Reference source not found.**. As can be seen the curve moves up as the chamber angle increseases. Thus lesser chamber angle causes a greater part of the beam to stary inside polymer. Chamber angles for *Figure 7.14* are between 30 degrees to 90 degrees.

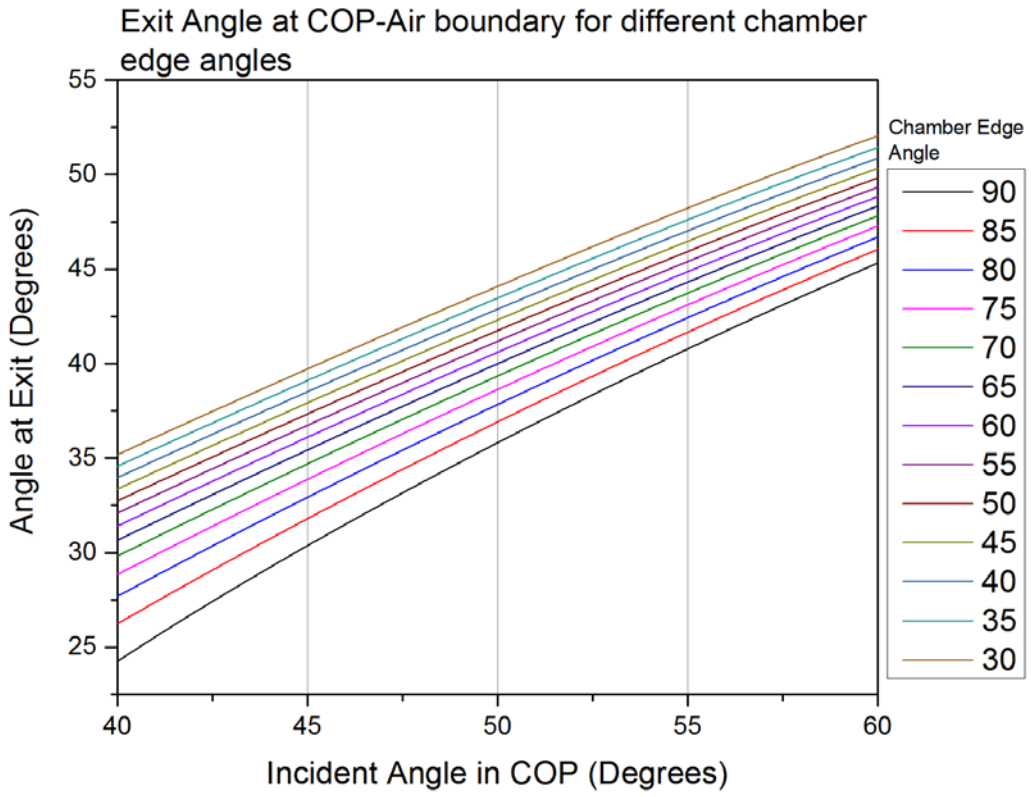


Figure 7.14 Exit Angle in Polymer after passing edge for different chamber edge angles.

The angle in the beam below which the rays will no longer stay inside cartridge is plotted in *Figure 7.15* as a function of beam incident angle.

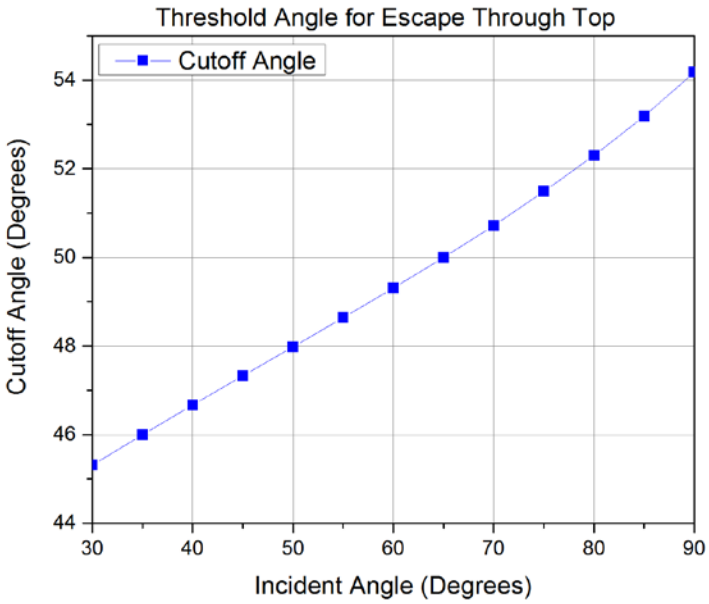


Figure 7.15 Cut-off Angle below which rays will escape

7.7.7 Light collection

The light collection efficiency is a very important parameter of optical design. Better light collection allows faster readings and better signal to noise ratio. It can also help in more flexibility in design. Intuitively, better collection efficiency can be obtained by recording signal over a larger area and be close to the cartridge. However, the excitation light can be a problem if the distance to cartridge top is too near in some cases.

7.7.7.1 *Effect of mirror*

The mirror finish in the chamber helps in increasing the collection efficiency by reflecting the downward directed fluorescence emission. Assuming that fluorescence is emitted Omni directionally and neglecting edge effect, about fifty two percent of fluorescence rays escape from the top of cartridge with this simple design. Rest of energy goes through TIR (total internal reflection). There are various ways to collect this energy as well. However, even with this simple design, a large portion of energy is already available at a convenient location – top of the cartridge.

7.8 Filter Choice and options

A large variety of filters can be used for design. We can broadly classify them as following

1. Plastic filters (used in architectural lighting)
2. Polymer filters
3. Absorbing glass filters
4. Camera Filters (various types)
5. Interference filters

The first four types are non-directional filters and do not need normal beam. Interference filters do need collimation to maintain the specification. However, they can be used at a tilt to change the pass band or to act as a dichroic mirror. To collect beam at all angles directly (without collimation or bending) non-directional filters are required. The glass absorbing filters also have better strong stop band and longer life. However, the pass band is not as steep as interference filters. Glass absorbing filters have much more variety in long pass applications than short pass or band pass response.

To find the appropriate filter combinations, we developed filter design software in-house. This is explained in the filter design software section.

7.8.1 Auto-fluorescence problem

The major disadvantage of glass filters in our case is their auto fluorescence. The autofluoroscence can be significant. Another problem is that the data for autofluoresence is not readily available. This makes it hard to model and design with such filters.

7.9 Modelling

To have an optimized design with all elements included, we decided to use high quality optical illumination software. We used Synopsys Lighttools and Zemax Optical Studio.

7.9.1 Why ray tracing

Ray tracing software is used in architectural and automotive lighting and many other applications. We did not find their much use in research publications for lab on chip. However, for a detailed design, these simulations are immensely valuable. The software programs have accurate models of sources like LEDs and many other elements.

These simulations give a range of useful data which is extremely difficult to get experimentally. More so ideas can be tried and results can be obtained without physically assembling and fabricating the optics. Many manufacturers provide models for their devices like lenses and LEDs which help in selection of components and gage the performance. These software couple with the 3D modeling software (SolidWorks in our case) to allow simulation on detailed models and take in to effect the full geometry for stray light and other calculations. Different parametric studies and optimizations are possible using these software.

However, they lack certain parameters for our case. Modelling of fluorescence is not direct and models for fluorescence phenomena are not integrated into these systems. Auto-fluorescence models are also not available for glass filters and other elements.

7.9.2 Simulations

In actual case, the excitation light excites the fluorophores in the solution and causes emission. However, a model of excitation vs emission is not available in these software. A full model should divide the chamber into small volumes and the fluorescence should be in accordance to the light intensity in that small volume. This can be very complex.

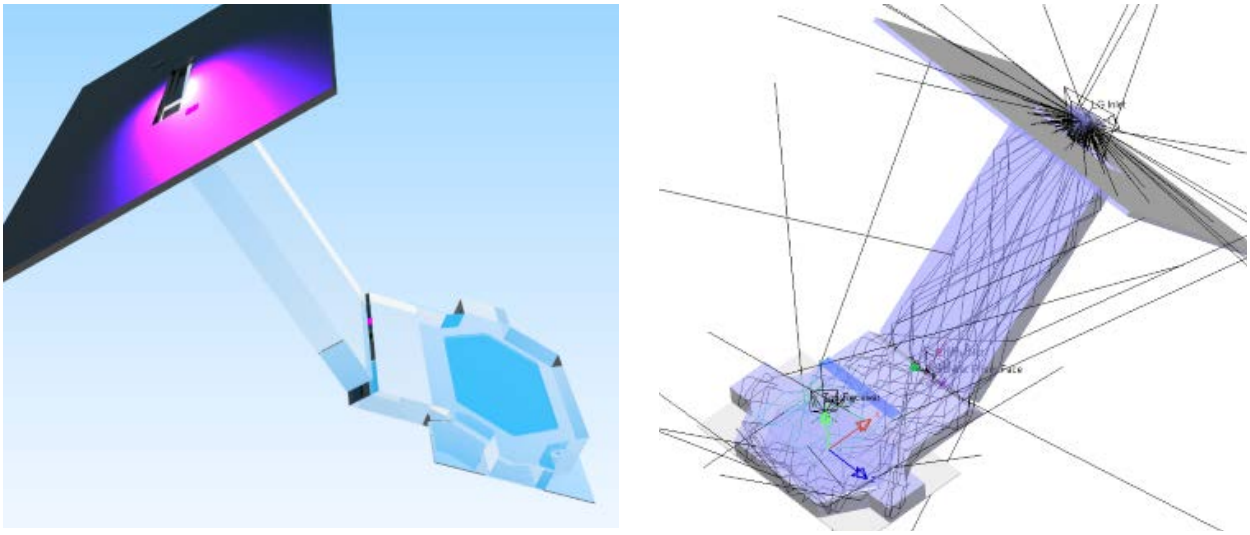
We therefore divided the excitation and emission simulations. We set our goals for each design. However, they both effect each other so the overall goals are also kept in mind.

7.9.3 Excitation Simulation

For excitation simulation, we have various elements to optimize. For a single color case, we have to use a LED, LED collimation optics, a light guide and the cartridge.

This simulation allows us to quantify stray light (excitation light escaping through the top). It also helps use to optimize geometry and to add additional elements like absorber to reduce the excitation light reaching detector. The simulation also shows us excitation efficiency or how much power from LED can be used to excite the liquid inside chamber. Again the analysis shows various directions the LED light can go so that we can put optical structures to absorb this unwanted light. Different phenomena like Fresnel effect can also be modelled.

There can be a wide variety of design for chamber illumination. We show analysis of a simple case. An Oslon SSL blue LED (Osram Optoelectronics) is used as excitation source. A simple rectangular light guide is used. The LED directly couples into the light guide. This can be the case if we use non-directional filter at excitation. Note that the diameter of integrated lens is about the same size as the height of LED. The setup is shown in *Figure 7.16*.



(a) (b)

Figure 7.16 Simulation Setup for Excitation. (a) Shows a rendered diagram. (b) Setup with preview rays

Simulation setup parameters and results are given below in *Table 7.1*.

Total LED Power Emitted (after filtration) = 1 Watt

Size of Top detector = 10mm x 10mm

Table 7.1 Excitation Simulation Results

Element	**Total Power Incident Watts**	**Average Irradiance** $\frac{W}{mm^2}$	**Max Irradiance** $\frac{W}{mm^2}$
Light Guide Inlet	0.65018	0.023572277	0.093534
Light Guide Outlet	0.65011	0.0235596277	0.024357
Prism Inlet	0.71520	0.0197075367	0.025804
Bottom of Chamber	3.55467	0.0283372685	0.13172
Top Detector (3mm from top)	0.016916	0.0001691513	0.000586
Top Detector (11mm from top)	0.001894	1.895564e-005	0.00015

There are various interesting things to note from simulation results.

More than 65% of LED light is coupled to this simple light guide. Almost all of this power makes it to other end of the light guide. The prism inlet has more incident power than the light guide outlet. This is not violating conservation principle but is the way the illumination design calculations are done. If a ray hits a surface multiple times it's counted in the same way. In this case this is likely due to some rays after entering the polymer make their way back to the prism inlet after undergoing reflections. The bottom of chamber has much more incident power. This is due to multiple reflections. As such it shows one of the very useful features of this design. Interaction length is increased due to multiple reflections. One can says that the cartridge sees the light from source 3.5 times. In this calculation, we has assumed that the rays are not attenuated by fluorophore excitation. To clarify ray traces are shown in *Figure 7.17*.

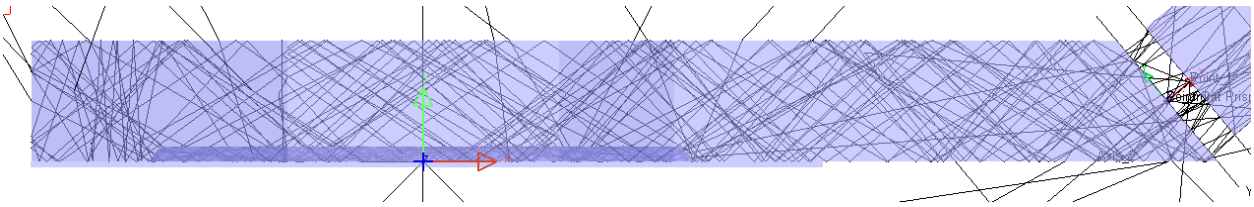
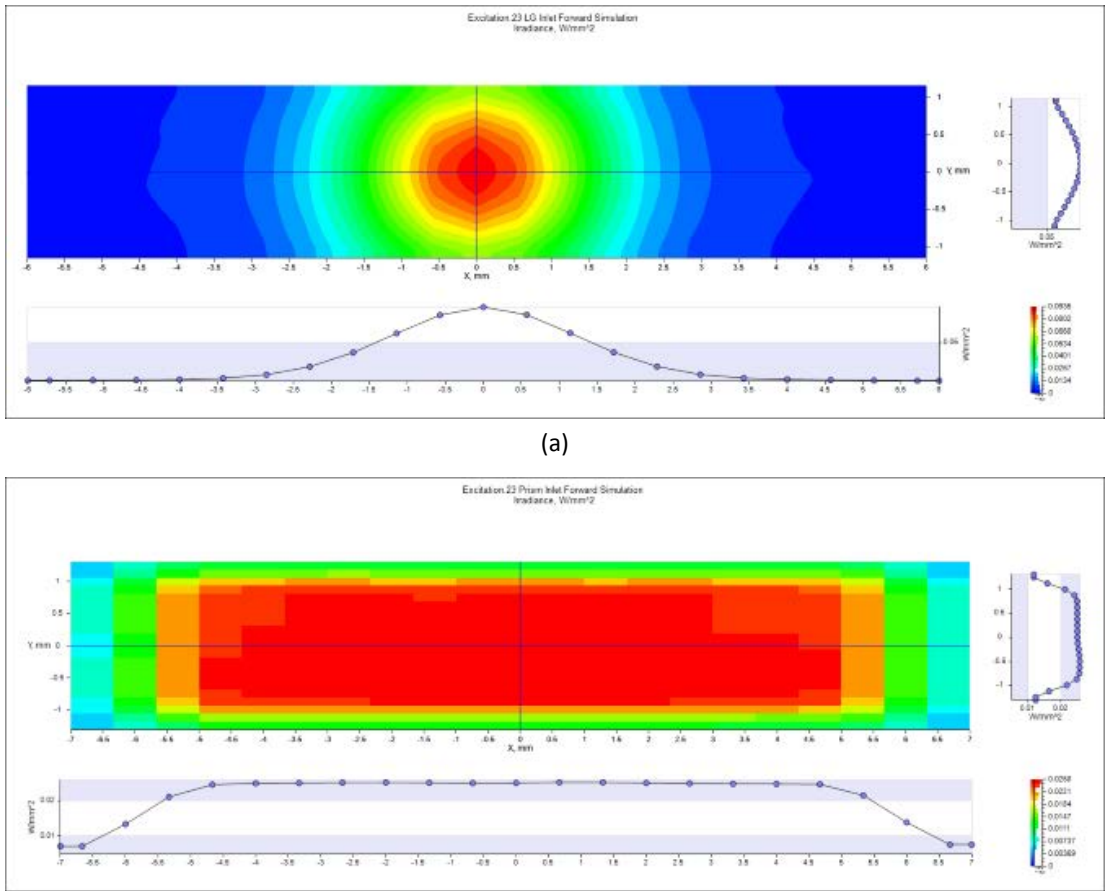


Figure 7.17 Excitation Ray Traces

Light guide also homogenizes the Light from LED before it enters the cartridge. The illuminance at inlet and outlet of light guide is shown in *Figure 7.18*. The pattern at output is much more uniform due to reflections inside the light guide.



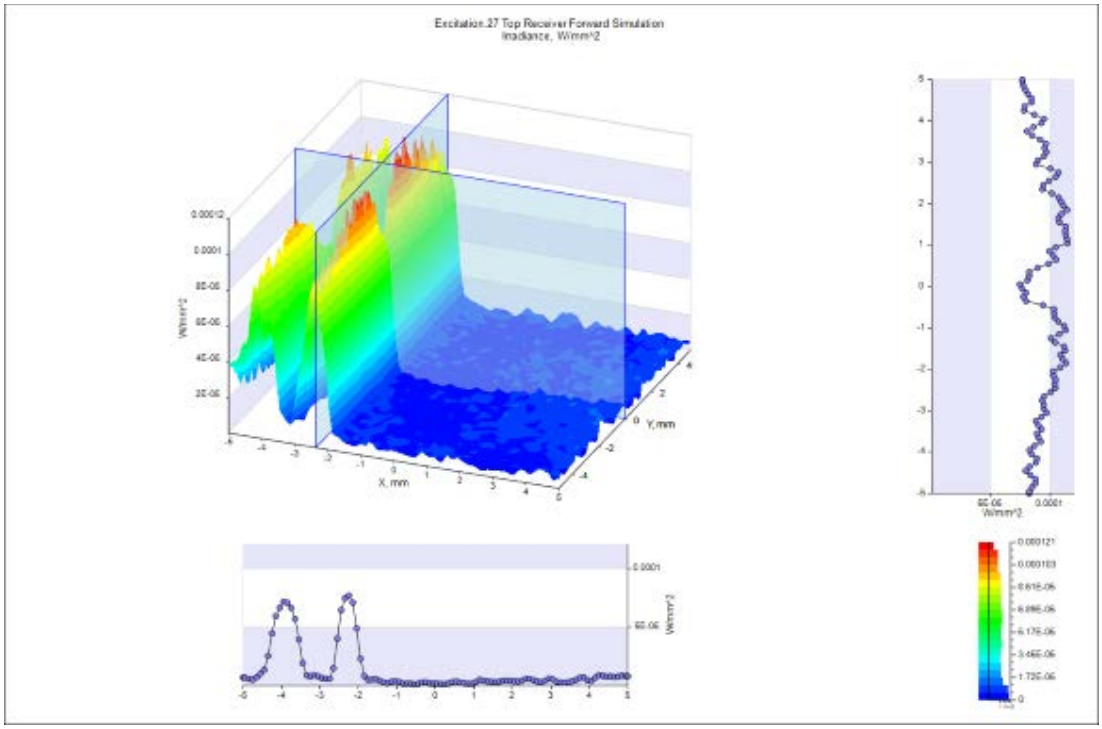
(a)

(b)

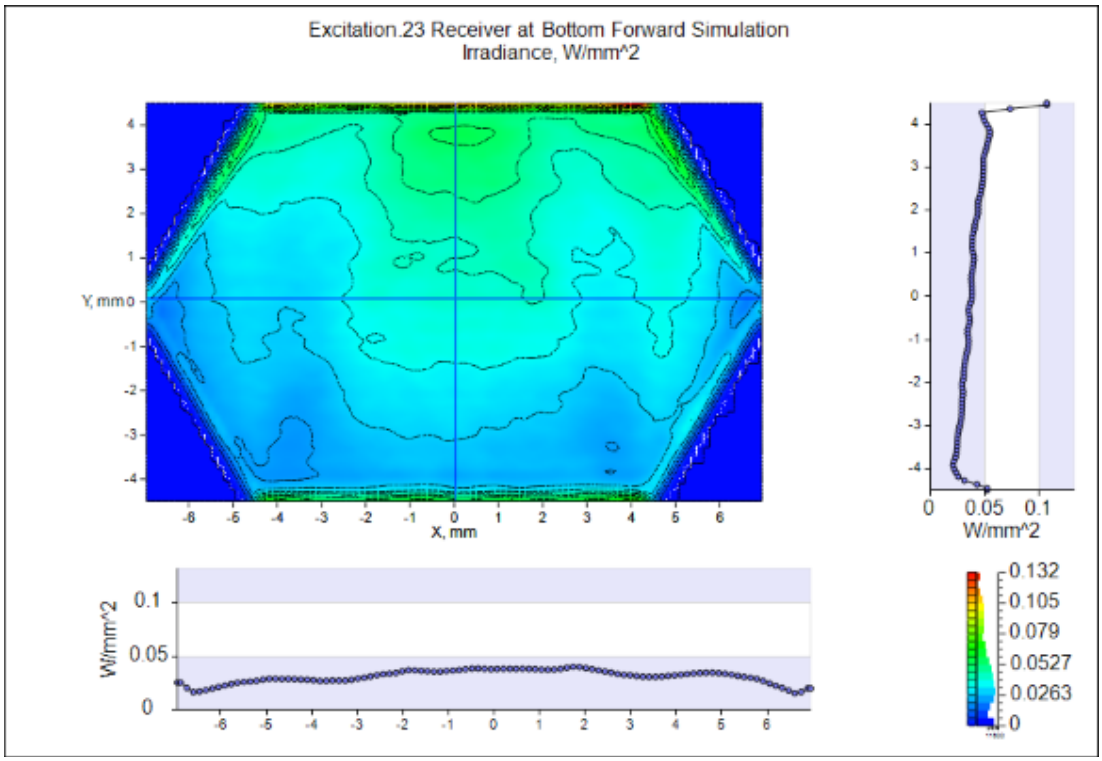
Figure 7.18 Illuminance Display for Light Guide. (a) Inlet Illuminance. (b). Outlet illuminance display

The Illuminance for the top detector and at the bottom of chamber is shown in *Figure 7.19*. The excitation light escaping and reaching to the top has a non-uniform pattern. Two lines of higher illuminance are noticeable. This can be used to position detector or filters appropriately. This effect is most likely due to chamber edge or the cartridge edge.

From the slice charts and the image, the illuminance is quite uniform in the direction orthogonal to excitation. The illuminance increases are little in the direction of excitation. This may be due to more chances of getting multiple reflections due to total internal reflection.



(a)



(b)

Figure 7.19 Illuminance Charts. (a) Illuminance at top detector. (b) Illuminance at bottom of chamber

It is interesting to see the change in escaped excitation light as we move away from the top of the cartridge. Shows graph of stray light as a function of distance from top of cartridge for a 10mm by 10mm detection area centered over cartridge.

We can reduce this effect by putting an absorber. The resulting graph is shown Figure *7.20*. The stray light power falls rapidly till the detector is about 6mm. This fact gives us guidance into the distance which might be useful for filters with high auto fluorescence.

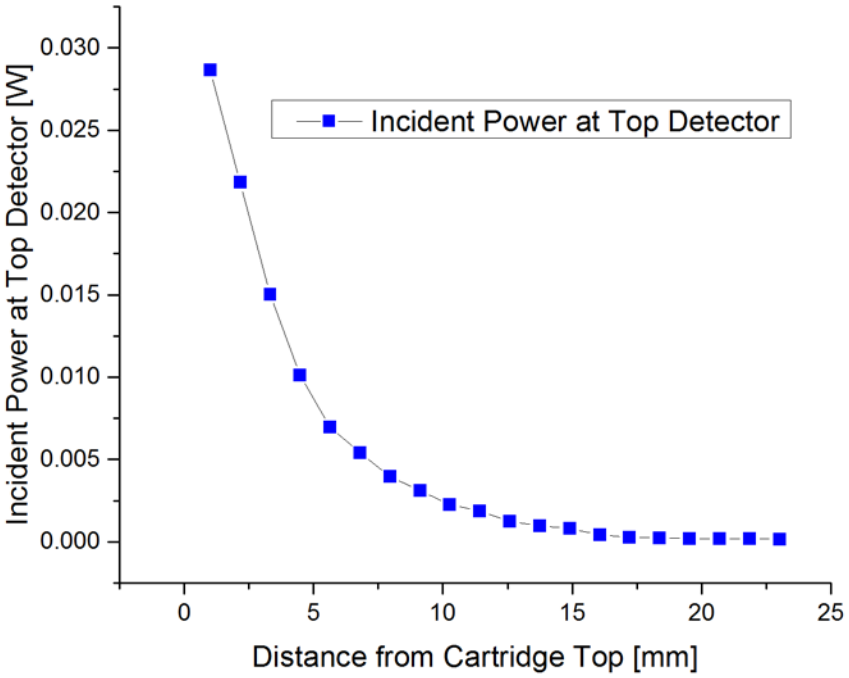


Figure 7.20 Stray light as a function of distance from Cartridge Top

7.9.4 Emission Simulation

The emission simulation is performed by assuming the chamber volume as a volume emitter. The simulation can be very complex if the excitation in chamber is not be uniform. In that case different sub-regions can emit with different power. We can try to design a system which excites the chamber with uniformity. However, we note that this is not an imaging system and the main purpose is to collect light on a single detector. The non-uniformity is not very important unless the light collection performance is compromised.

To simplify, we assume chamber as a uniform emitter. We have already shown the results of chamber illuminance in simulation for excitation light for a simple case.

Keeping in view the limitations of software, the bottom surface of chamber is assumed to be a surface emitter with total power of 1W. We again assumed a detection area of 10mm by 10mm.

Illuminance at detector is shown in *Figure 7.21*. The pattern is nearly symmetric in all quadrants. The pattern is also oval shape giving clues in to the design of optics required to guide the light to detector and the size of aperture which can be used for maximum light collection.

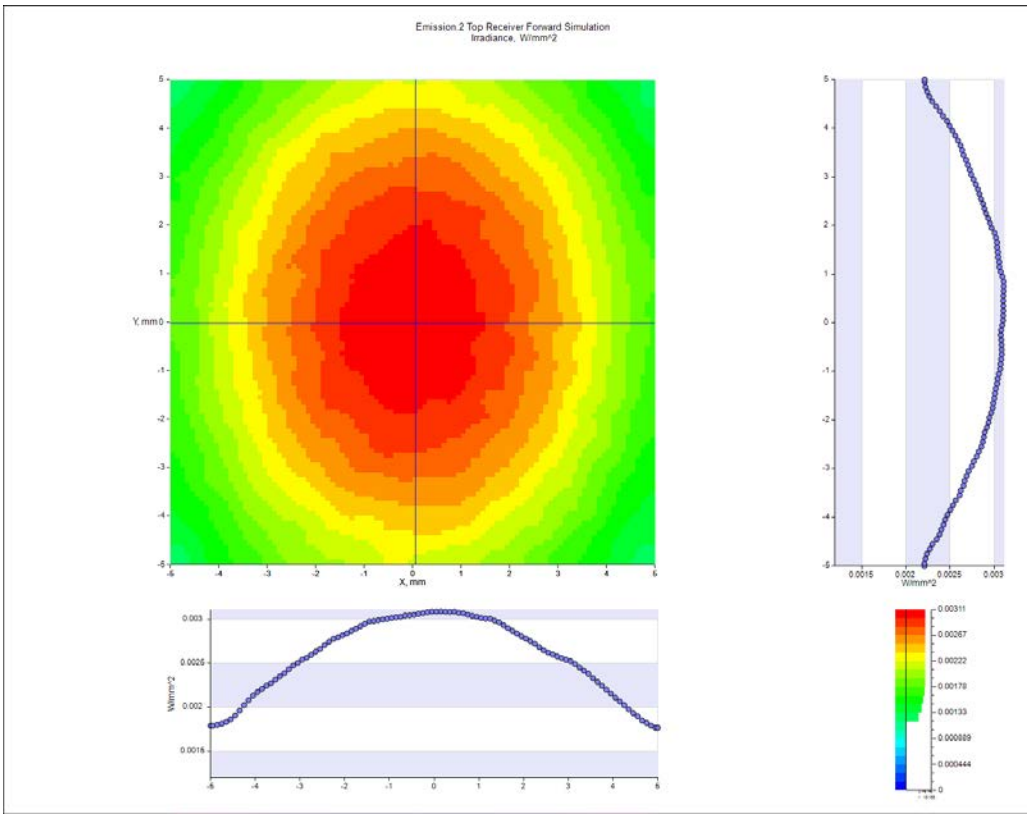


Figure 7.21 Illuminance at Detector (3mm from top of cartridge)

The graph of incident power on the detector as a function from the top of cartridge is shown in *Figure 7.22*.

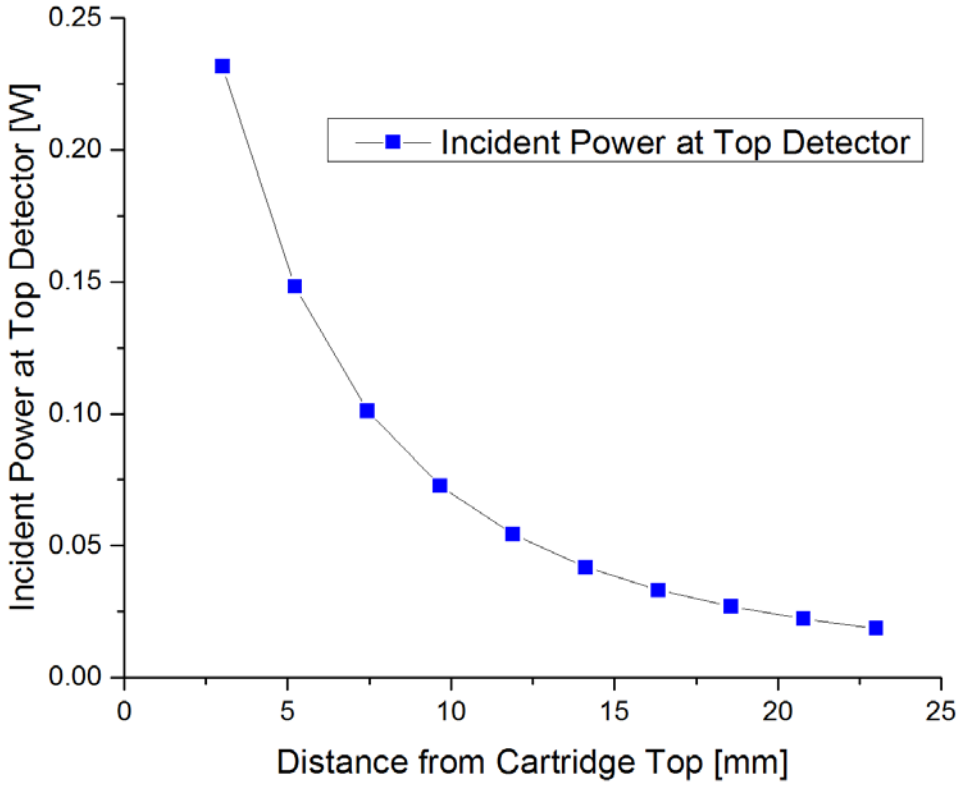


Figure 7.22 Total power incident at detector vs distance from top of cartridge

It is interesting to see the possibility of putting the detector on side. *Figure 7.23* shows two detector planes for both top and side observation. Interestingly there is a high illuminance line on the side as well which can be place for single or additional detectors.

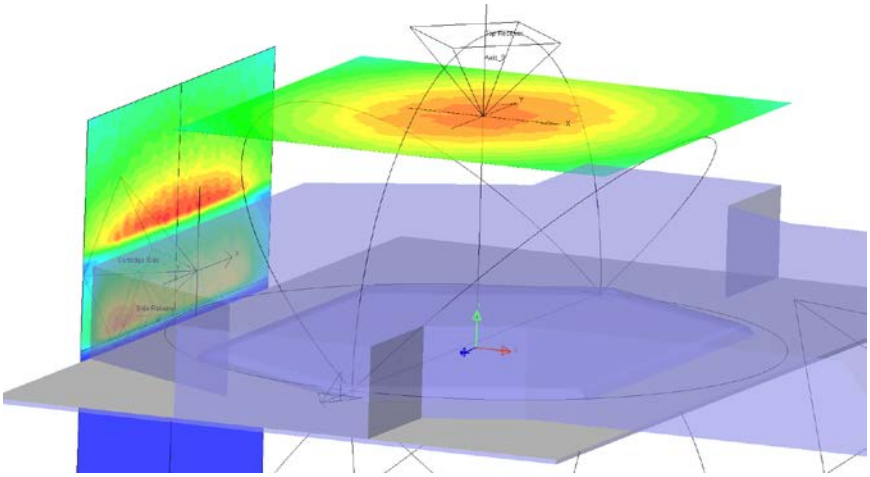


Figure 7.23 Detectors on Top and Side

7.10 Experimental and Test

The concept was tested on optical breadboard. The setup had to work in synergy with thermal control as well.

7.10.1 Setup

Since stray light is an important consideration the setup has to be carefully designed. We also wanted to measure the effect of misalignments and parameter changes. We selected Thorlabs lens tubes to house the optical components. Thorlabs has a unique cage system which allows attachment of various opto-mechanical subassemblies convenient. Various linear and rotational stages were used. *Figure 7.24* shows the setup on optical bench. The setup allows changing alignments and distances accurately. To test various filters we used cage compatible filter holders. We used laser cutting to fabricate the light guide from acrylic sheets. The light guide as held in the lens tube as well.

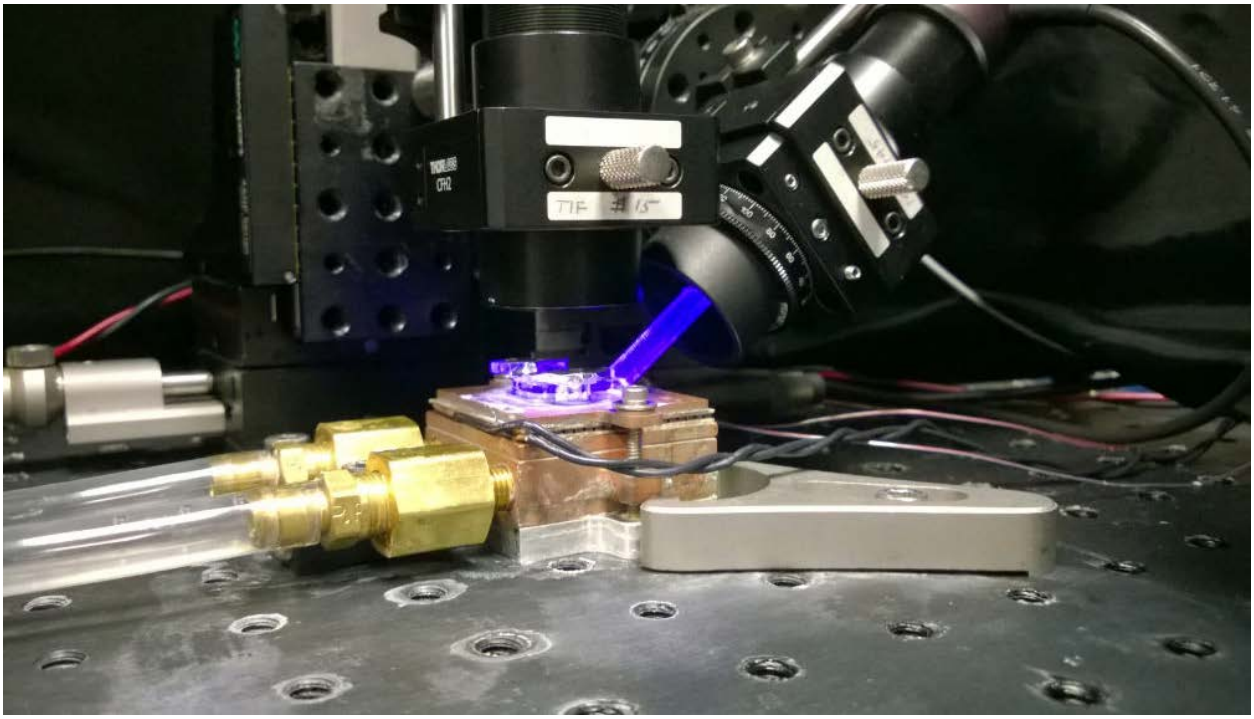


Figure 7.24 Optical Bench Setup

The instrumentation setup is as explained before. By developing extensive software, we could easily and quickly change various parameters for our assay. The optical detector was read though in-house developed electronics which is explained in its section. The electronics had automatic dynamic range matching. In contrast to various circuits given in literature with fixed dynamic range for the Analog to

Digital converter, we could tailor the dynamic range by changing the integration time dynamically allowing a very wide dynamic range.

We used the assays as described earlier and obtained the melt curve for a duplex assay. Melt curve is useful in measuring optical performance for intercalating dye based assays as we cover the whole range of expected signal levels. Melting curve has been suggested to be used for validation of real-time PCR instruments [152].

With an LED current of 100mA the integration time was less than 100ms for the entire range. That is very important since it shows that the measurements can be taken very fast and the reaction time does not need to be increased by much if we are taking measurements. In contrast, Cepheid SmartCycler, which has independent optics for each cartridge, adds 6 seconds to each cycle for real time observation. Our optics is updating faster than Qiagen ESE log as well. The measured data after processing is shown in *Figure 7.25*. The peaks are distinctively clear.

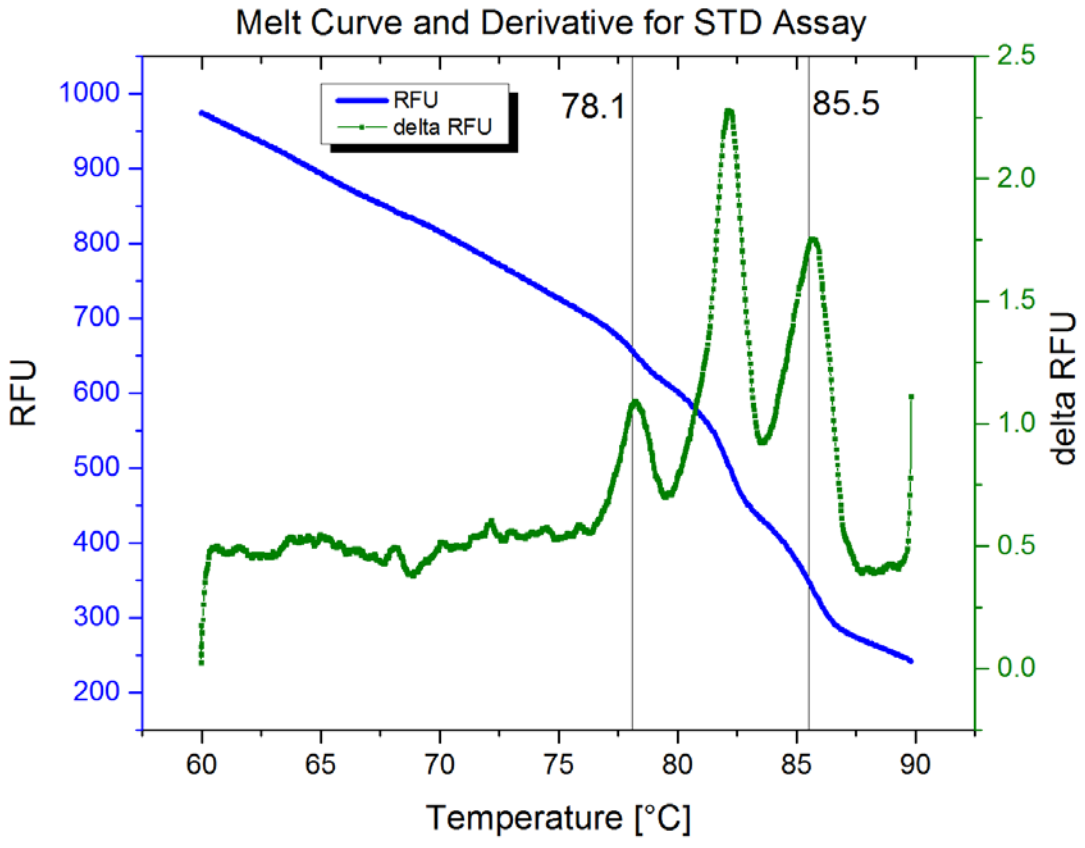


Figure 7.25 Melt Curve and Derivative for STD Assay

Amplification for Bio-rad reference assays is shown in *Figure 7.26*.

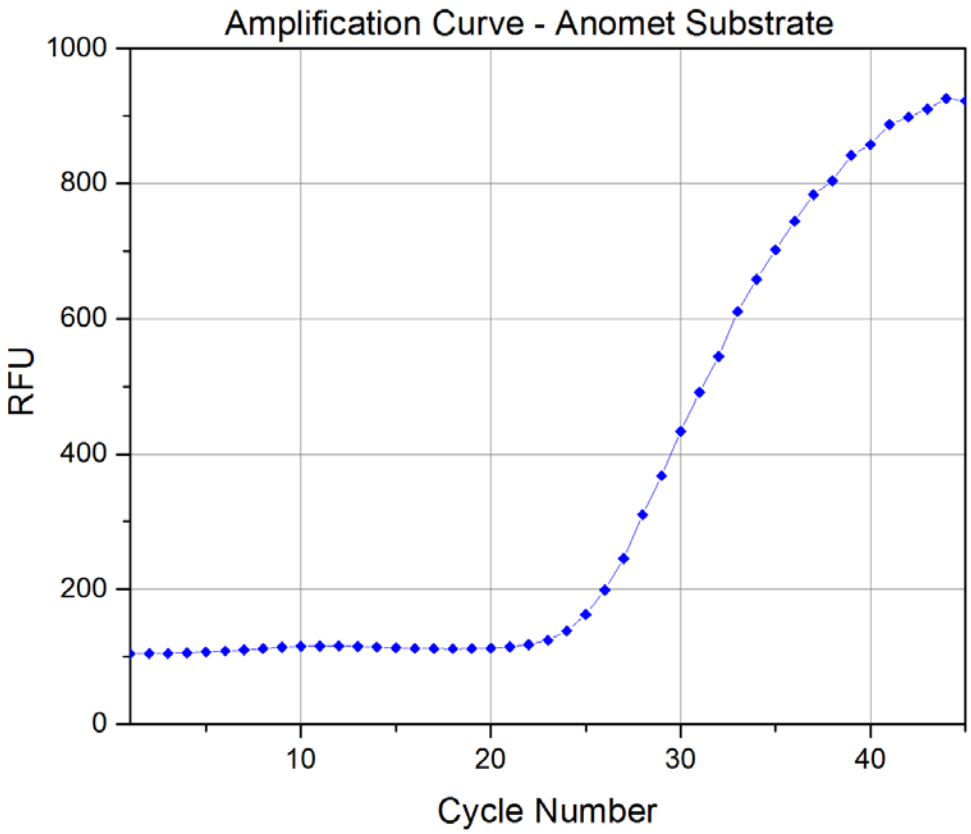


Figure 7.26 Amplification Curve in Cartridge

The fast reading also shows that a rotating filter wheel or a spatially separated design should work well for multiplexing. We integrated and synchronized the filter position of the filter wheel to do multiplex measurements. For this purpose we selected motorized filter wheel with very fast switch capability.

Also note that whole melt curve was obtained in 5 minutes with good resolution. Thus the cartridge thermal response works well to allow fast optical readings.

7.11 Advantages

There are major advantages of this design. Precise alignment is not required. This not only simplifies production but also allows equipment to remain operation with small dimensional change. High power can be coupled into the chamber with high efficiency. Due to high collection efficiency, the observation time is exceptionally fast. The design allows using different volumes of chamber without changing the optics. It also allows ways to have cartridges of different configuration use the same instrument. For example the optics can be designed so that cartridges with single or multiple wells can be used.

7.12 Conclusion

We have provided proof of concept implementation for a microfluorometer which meets many of the desired features. The design is applicable to many applications including multiplex real time PCR and other fluorescence bases assays like ELISA. The design in robust and well suited to POC instruments. We also have showed the modelling of the system. We hope that the design can be quickly modified and updated for changes in configuration using this approach.

8 Filter Selection Program

8.1 Abstract

Fluorescence detection optics can use from a large number of filters from various manufactures[153] [154] [155]. Filters are one of the most expensive components in typical optical designs. We wanted to reduce the cost of filters by choosing among various options to reduce cost. Due to the configuration of our optics, we also wanted to consider non-directional filters. We also considered plastic filters and camera filters which can be lower cost than the interference filters. Due to a large number of filters available from various manufacturers, it is difficult and time consuming to search and select the right filters. If the filter selection can be automated, then we can choose the filters which are most cost effective. In addition to filters, there are a large number of sources such as LEDs with different spectral characteristics. It's very helpful to shortlist the combinations which meet our requirements.

To perform numerical calculations we have to enter numerical data for the following.

1. Fluorophores
2. Excitation sources
3. Excitation filters
4. Emission filters
5. Detectors

8.2 Criteria / Challenges:

The filter program finds out certain information that can be a criteria to resolve the challenges of fluorescence detection.

We have loaded a big amount of data from various sources for the selection of components:

1. *Fluorophores/dye data*
    a. From [156]
    b. From [113]
    c. Alexa Flour dyes from [114]
2. *Excitation source:*

   a. LED data from Thorlabs [157]
3. *Filters:* we have a huge selection available, data sources are:
   a. Pubspectra [65]
   b. Lee Filters
   c. Hoya Filters [73]
   d. Thorlabs filters [88]

8.3 Notes

1. All the data is normalized for wavelengths from 200nm to 1200nm, with a step of 1nm.
2. Linear Interpolation is used wherever values are not available.

8.4 Specifications

The program can do analysis for component selection for both a singleplex and multiplex reaction

8.4.1 Singleplex reaction:

A singleplex reaction consist of a single source, dye, excitation filter, emission filter and detector. For this reaction following information needs to be calculated

8.4.2 *Excitation Efficiency*

This gives a measure of how much of the excitation from source is actually useful to excite the fluorophore.

Calculation: To find out excitation efficiency the program multiplies the spectra of source, excitation filter and excitation of fluorophore. It finds the area under the curve resulting from multiplication. Then this area is divided with the area under excitation source spectrum.

$$Ex(efficiency) = \frac{\sum_{200}^{1200}(source \times Ex\ Filter \times ExF)}{\sum_{200}^{1200} source}$$

Where ***ExF*** is excitation spectrum of fluorophore.

8.4.3 *Emission Efficiency*

This gives a measure of how efficiently the emission from Fluorophore is passed to the detector.

Calculation: To find it out the program multiplies emission spectrum of Fluorophore and the spectrum of emission filter, finds the area of under curve resulting from multiplication, and divides it by the area under emission spectrum of fluorophore.

$$Em(efficiency) = \frac{\sum_{200}^{1200} EmF\ x\ Em\ Filter}{\sum_{200}^{1200} EmF}$$

Where ***EmF*** is emission spectrum of fluorophore.

8.4.4 *Leakage*

It is the amount of excitation that makes its way into the detector.

Calculation: The program finds leakage by multiplying spectra of source, excitation filter, and emission filter. The area under the curve resulting from multiplication, represents the light that leaks from source to detector

$$Leakage = \sum_{200}^{1200} (source\ \times\ Ex\ filter\ \times Em\ Filter)$$

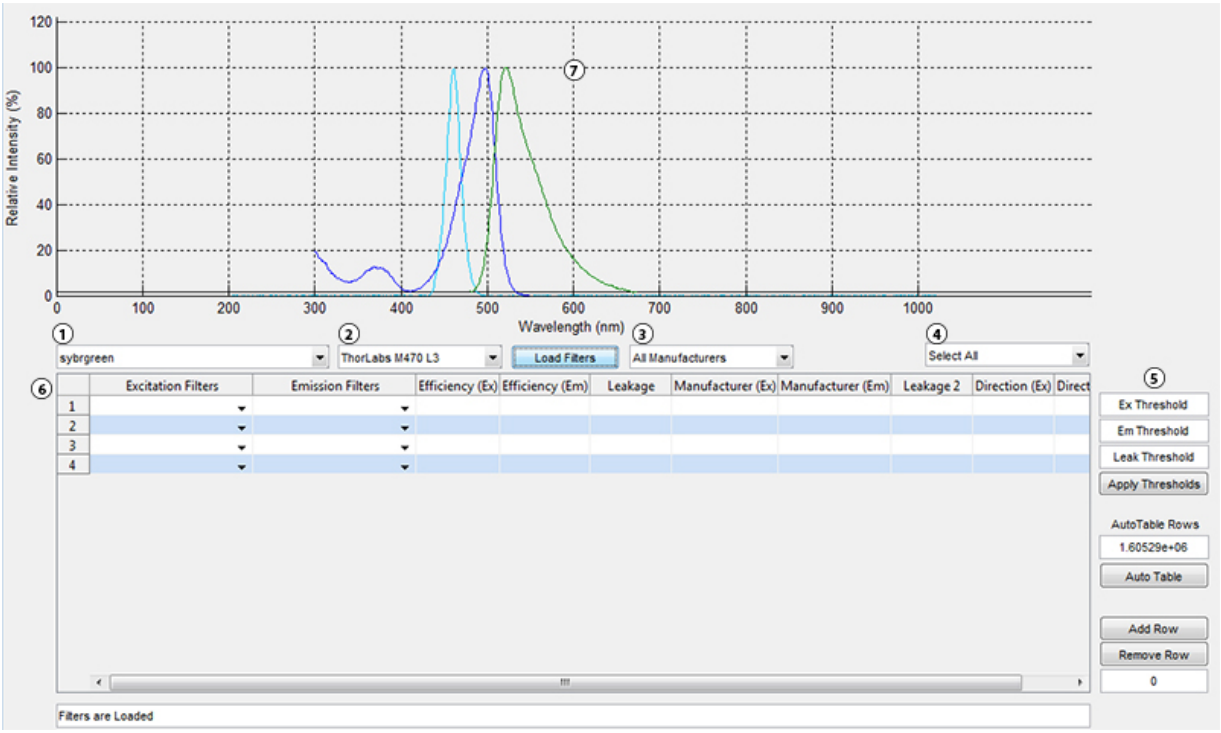
Goal: The goal is to minimize leakage as it can have significant effect on the output from detector, as the light from excitation source is a lot brighter than the light emitted from Fluorophore. Therefore we have to select filters such that leakage is minimal.

This information is used as a Figure of Merit (FOM) for selecting the filters.

On fixing the source and fluorophore the program allows to search for the best possible combinations of excitation and emission filters.

A threshold can be applied to all three FOMs, and a resulting list of combination of filters is presented, whose spectra are plotted so the user can further chose what is best.

8.5 User interface



1. Select fluorophore
2. Select source
3. Choose filters based on manufacturers for manual calculations
4. Choose filters based on manufacturers for automatic calculations
5. Filter out combinations based on threshold parameters (for automatic calculations)
6. Manual Calculations table
7. Combine graph

8.6 Multiplex reactions

A multiplex reaction consist of multiple sources, multiple combinations of excitation and emission filters, multiple fluorophores, and multiple detectors. Apart from the three FOMs used in singleplex reaction, there is one new FOM. Excitation efficiency is also different from singleplex reaction.

8.6.1 Excitation Efficiency

For simplicity the program considers that only one excitation filter is used for each source. The excitation efficiency is calculated for each source and now defines how much of spectrum of a source is useful for exciting the fluorophores used in a reaction.

Calculation: For each source the excitation efficiency is calculated by multiplying together source spectrum, excitation filter spectrum, and the paired fluorophore's excitation spectrum. The area under curve resulting from multiplication is then divided by area under source spectrum. So the Excitation efficiency per source is calculated by the fowling formula

$$\frac{\sum_{200}^{1200}(ExF \times source \times Ex\ Filter)}{\sum_{200}^{1200} source}$$

Where ***ExF(k)*** is excitation spectrum of fluorophore ***"k"*** and ***"n"*** is the number of fluorophores

8.6.2 Emission Efficiency

Emission Efficiency remains the same as in singleplex reaction, and is calculated for every fluorophore.

8.6.3 Leakage

In multiplex reaction leakage is similar to the one in singleplex reaction but needs to be calculated for each detector.

$$Leak(m) = \sum_{200}^{1200} (source\ (m) \times Ex\ filter\ (m) \times Em\ Filter(m))$$

8.6.4 Reciprocal Cross-talk

In multiplex reaction, fluorophores can have overlapping emission spectra, due to which there is a need that emission filters are selected such that the emission from a fluorophore only reaches its respective detector. However in practical systems even selecting the best emission filters combination still results in some cross-talk.

To explain this cross talk let's consider an example in which we have one source, one excitation filter, two emission filters and two fluorophores. Now the cross-talk phenomenon can be approximately quantified as,

$$\begin{bmatrix}\varphi_1\\ \varphi_2\end{bmatrix} = \begin{bmatrix}E_{11} & E_{12}\\ E_{21} & E_{22}\end{bmatrix}\begin{bmatrix}If_1\\ If_2\end{bmatrix} + \begin{bmatrix}b_1\\ b_2\end{bmatrix}$$

Where ***(b_1, b_2)*** is baseline caused by the background. [158]

Calculation:

In order to calculate this parameter which is an important FOM, first the elements in cross talk matrix are computed.

Each element of cross talk matrix $E_{mn}$is defined as the fluorescence reaching to detector "***m***" due to fluorophore "***n***". So $\mathbf{E_{mn}}$ can be calculated by multiplying the spectrum of ***mth*** emission filter with emission spectrum of ***nth*** fluorophore.

$$E_{mn} = \sum_{200}^{1200} (EmFi_m \times EmF_n)$$

Where $EmFi_m$ is **mth** emission filter's spectrum and EmF_n is emission spectrum of **nth** fluorophore.

Also each element in cross talk matrix is assigned a weight, which can be calculated by this formula

$$W_{mn} = \sum_{200}^{1200} (Source_n \times Ex\ Filter_n \times ExF_n) \times extinction\ coefficient \times source\ power$$

Where $ExF_n$is the excitation spectrum of nth fluorophore.

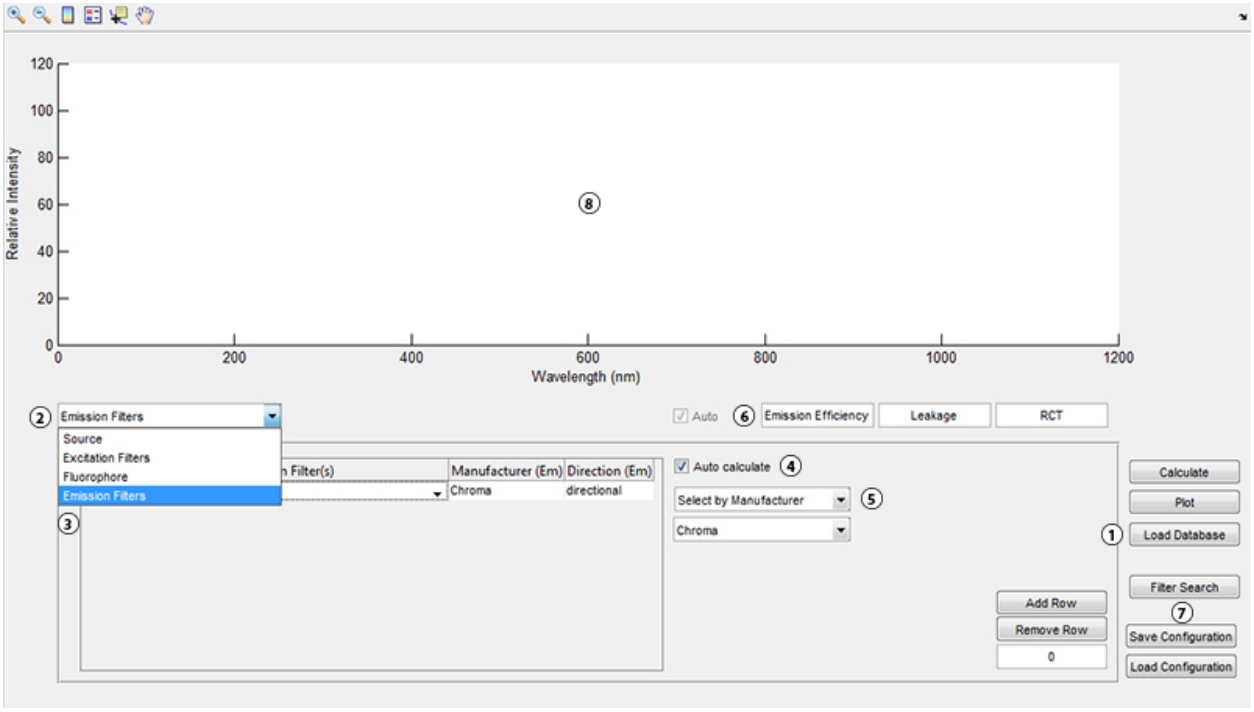
Now the reciprocal cross-talk for detector **"a"** is calculated as

$$RCT_a = \frac{F_{aa}}{\sum_{k=1}^{n} F_{ak}}$$

Where n is the number of fluorophores

And is basically a ratio showing the percentage of desired signal over total signal received by detector.

8.7 User Interface



1. Load all the database
2. Selection panel to select option table for either emission filters, source, fluorophore, emission filters
3. Table for selection/ calculations
4. Checkbox for automatic calculation
5. Filter out calculation based on either manufacturers or direction
6. Calculations thresholds
7. Save configuration which include selected source, excitation filters, fluorophores, emission filters
8. Combine graph

8.8 Filter Search

Filter search is an add-on feature of this application which allows a user to search filters based on different parameters. Filters can be find either matching with an ideal filter or a real filter from the database.

8.8.1 Ideal Filter based matching

In filter searching based on an ideal filter, each filter from the database is matched with an ideal filter of a known start and stop band. User can define the start and stop band of the ideal filter and thresholds i.e. percentage of area covered by filter in band, percentage of filter area out of band, total energy out of band.

8.8.1.1 *Percentage of area covered by filter in band*

It is defined as how much ideal filter's area is covered by each real filter from the database.

Calculation: First ideal filter's area is calculated by subtracting stop band value from the start band and multiplying it with 100.

$$Ideal\ Area = (Stop\ band - Start\ band) \times 100$$

Then overlapping area of each filter is calculated by adding all the values of real filter's transmittance curve starting from the start band value to stop band value. Finally the ratio of this overlapping area to the idea area is calculated which gives us the percentage of area covered by filter in band.

$$percentage\ of\ area\ covered\ by\ filter\ in\ band = \frac{overlapping\ area}{ideal\ area}\%$$

8.8.1.2 *Percentage of area out of band*

It is exactly opposite to the percentage of area covered by filter in band.

Calculation: First filter's area is calculated by adding all the values in the transmittance curve of that filter in the given region of comparison and then the ratio of overlapping area to this filter area is subtracted from one to get the ratio of area out of band.

$$\text{percentage of filter area out of band} = \left[\ 1\text{-}\ \frac{\text{overlapping area}}{\text{filter area}}\right]\%$$

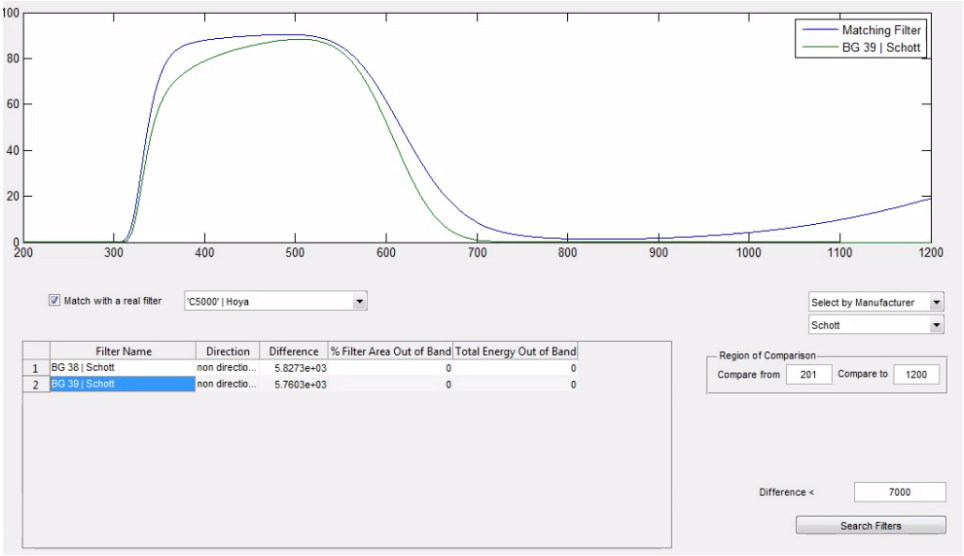
8.8.1.3 *Total energy out of band*

It is defined as the filter's transmittance outside the area of ideal filter.

Calculation: Subtracting overlapping area from the filter area gives total energy out of band.

$$total\ energy\ out\ of\ band = (filter\ area - overlapping\ area)\%$$

1.1.1. User interface



8.8.2 Real Filter based matching

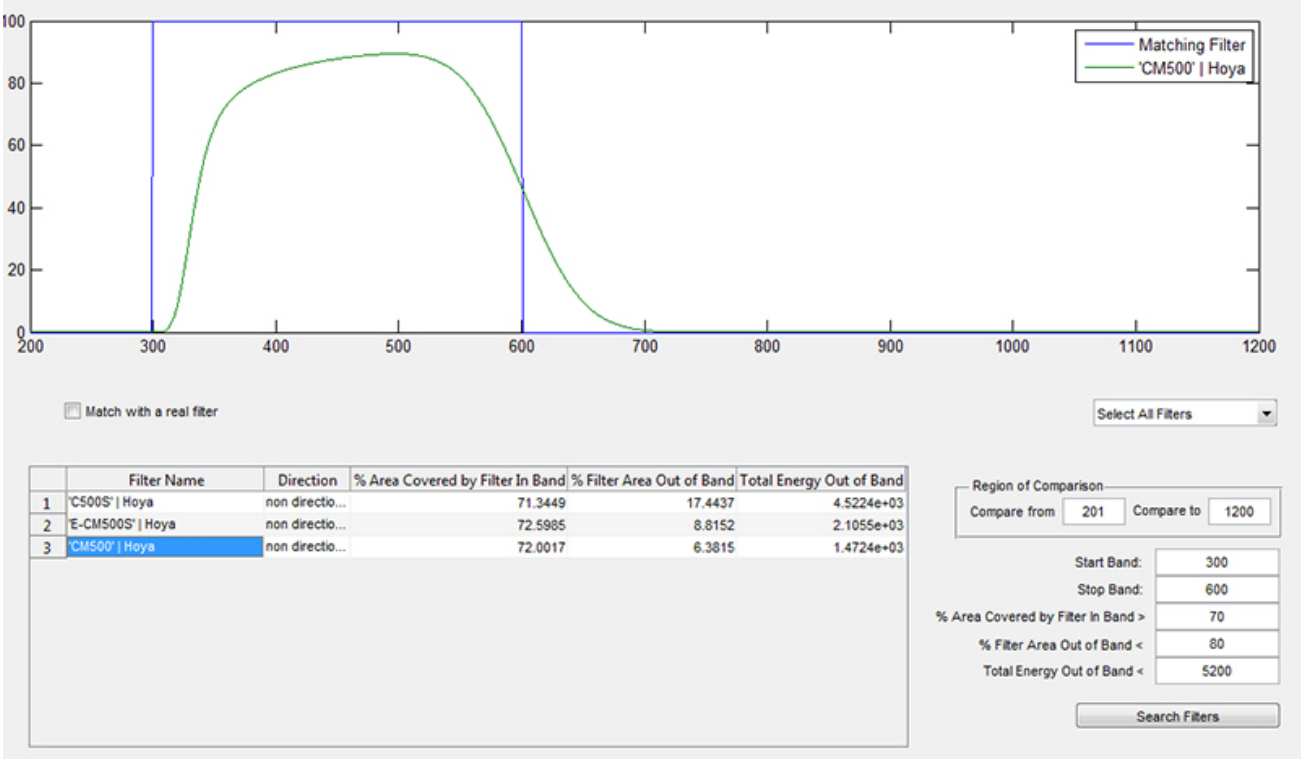
As the name suggest in this type of filter searching each filter from the database is matched with a real filter instead of an ideal filter. The only threshold is difference in this case.

8.8.2.1 *Difference*

It is defined as the measure of difference between each filter and the selected real filter.

$$difference = \sum(real\ filter - searching\ filter)$$

8.8.2.2 *User interface*



9 Robust and Flexible Sample Preparation Design for POC Diagnostics

9.1 Abstract

Sample preparation is one of the toughest challenges to bring PCR diagnostics to POC (Point of Care). We report design and testing of a novel design which can perform complex sample preparation for PCR at the POC (Point of Care). The design allows *different volumes* of samples and reagents for different protocols while not affecting the dead volume. This is extremely useful for different sample types and protocols. The design also allows different sample preparation steps in the same overall form factor of the disposable thus potentially allowing a *single universal instrument* to use a wide variety of disposables. *No discrete valves or on chip pumps* are needed. Vacuum or pressurized gas is also not required to operate the disposables. The design can be *injection molded* for low cost. A closed sample preparation system can built based on the design. The design has potential to include the PCR chamber to enable a full sample to answer cartridge at low cost. The design allows complex fluidic operations with a simple implementation. It also allows mix and match and on the fly reconfiguration and design thus resulting in truly *modular* and *customizable* design. The design is also useful for wide variety of applications including fluid manipulation, chemical and biological analysis, food safety, forensics, drug testing and many others.

9.2 Introduction

Sample preparation has been a challenge in design of integrated sample to answer systems[159]. Sample preparation for real time PCR is typically done in laboratory with bench top methods. Such methods require bulky and power hungry equipment like centrifuges and vacuum manifolds. There are various steps which have to be done manually. The robotic systems which automate many of these steps are large, expensive and not geared towards low cost and point of care (POC). There are

instruments which enable automation of various steps. However these are very expensive and require trained manpower and maintenance.

Over years there are many designs and products reported which try to take the sample preparation to POC. Various reviews for point of care testing are available [26-29, 95] [46, 91, 160] [91, 92] [161] [162] [163] [164]. Reviews focusing sample processing are also available [159, 165-171] [172] [173] [174]. However, sample preparation still remains a challenge[175]. The biggest remaining hurdles to bring MDx (molecular diagnostics) to POC are said to be reagent storage, sample prep, and integration with existing medical infrastructure and electronic record keeping.

Many of these try to tailor the design of chips or cartridges to well established chemistry while others try to have novel methods for sample preparation. Most of these designs have several constraints and need external elements which make it difficult to use them at POC in a robust fashion. Another limitation is that many of these designs are 'fixed' for sample type and the volume of sample thus making it less universal for a variety of assays. Partly due to limitations of 2d designs in chips, the complexity is high in many cases. The often overlooked thing in many of these works is the cost of production including assembly difficulty. Thus many of these designs are not easy to produce in large quantity. Another important element is the filling and sealing of reagents on the cartridge. Many designs are not amenable to automating this operation with flexibility.

9.3 Earlier Work

9.3.1 Commercial

There is no CLIA (Clinical Laboratory Improvement Amendments) waived sample to answer system for POC applications even in the US. No PCR tests are allowed at physician office. There are various companies which are working to get sample to answer systems including Cepheid,Luminex, Biocartis, iquum, enigma diagnostics, incubate, GenePOC, BioFire, Alere, Micronics, stat-diagnostica and Quidel. Of these Cepheid design requires a lot of power and its deployment in development world POC is debatable. Gates Foundation has given huge grants to both Alere and Quidel for development of their systems. Quidel is using technology from Northwestern University which developed this technology under a Gates Foundation's grant. Gates foundation has also funded other organizations for development of sample preparation component for POC Dx program.

Some companies have non-integrated offerings. Examples are Ustar, Bigtec, meridian and nanosphere. There are lab based sample prep only platforms like EZ1 Advanced and QIAcube from Qiagan, MagNA pure, COBAS Ampliprep, COBAS 4800 from Roche.

There are companies trying to develop systems to be deployed at POC. In first world market they are mostly targeted towards Physician clinics and pharmacies. After through deliberation Gates foundation is also inclined toward putting sample to answer systems at Clinical level. It seems that systems which are as portable as pregnancy test instruments are not a big focus.

9.3.2 Research Literature

Some selected examples of sample prep design can be looked in the references [176-189] [190] [191]. The designs have followed card like approaches which are closer to a 2D design or 3D approaches. Most of the efforts have used solid phase binding matrix for dna or rna extraction. We could not find a design which looked promising for our application. Also as the complexity increases, the resources needed to build the whole system can be out of capability of many research labs. 3D plastic based designs should be considered make by injection molding. Injection molding is a very common industrial there large scale production process. However, it's not common in research labs to have capability of =design and the production of injection molding parts. Injection molding also has lead times, mostly due to tooling which makes it expensive and slow. With the complexity of the chip, the cost of tooling can be a critical factor for a research effort. Search is typically trying to show a proof of concept and due to nature of plastic and polymers involved with complex designs, alternatives to molding are not trivial. Processes like machining can be very slow, and typically don't preserve the surface finish. Such machines also requires appropriate machines and expertise. Therefore, while evaluation designs from research, it should be considered if the process for fabrication and design is really useful for the mass production or is only good for the proof of concept. Another approach by some researchers is to involve industry to fabricate and help in design of the parts.

9.4 Requirements

We are determining requirements for a POC technology which consist of an instrument and a disposable. We want to use the same instrument for sample prep for a wide variety of assays.

The technical challenges for development of chip-compatible sample prep is reviewed[192]. Sample prep at the POC should eliminate manual steps to ideally allow an instrument to perform all steps after sample injection to recovery of real time PCR capable solution. To have an impact in the developing world the system should be low cost, rugged and reliable. The instrument should ideally allow *various sample types* to test for various diseases. It should allow *different volumes* of the reagents and samples for different panels. A *universal instrument* which allows testing for a wide variety of diseases by only changing low cost disposable cartridges can have a huge impact world over. To allow use of different chemistries (both current and future) the design should allow *adjustable steps or operations* without changing the hardware. For example blood and urine can go through different sequences of operations depending on the target one is looking for. For certain chemistry blood may need to be filtered to remove the cells while for others this may not be a requirement. Such flexibility makes design of sample preparation for POC very challenging with typical approaches.

An attractive feature is to be compatible with a large variety of assays already developed for various diseases. Thus qPCR is an attractive option for detection and quantification due to assay availability.

We can list the requirements for sample prep as follows.

i. Low Cost
ii. Easy to automate and optionally allows manual operation as well. Does not require instruments which are complex, expensive or power hungry. It is important to consider instrumentation with the design of the sample prep disposable.
iii. Allows porting of already developed assays.
iv. Freedom to operate – not blocked by patents
v. Allow different sample types including
    a. Blood
    b. Plasma
    c. Sputum
    d. Urine
    e. Stool
    f. Swabs
vi. Allow variable volumes of reagents and samples
vii. Allow variety of operations including
    a. Filtration
    b. Bind, Wash and Elute
    c. Homogenization, grinding, sonication etc.
viii. Allow fluid routing for different protocols
ix. Low dead volume
x. Easy to fill liquid reagents
xi. Dry Reagent storage should be easy
xii. Allow integration to qPCR based detection and possibly with other techniques like ELISA

xiii. Allow sample prep for both immunoassays and PCR
xiv. The cost of disposable should be low. Ideally the disposable in manufactured using injection molding.
xv. Can be used in a wide variety of conditions (like moving vehicles) and easily carried to low income POC conditions.
xvi. Allows easy transfer of prepared samples to tubes or cartridges.

9.5 Problems with Existing Approaches

Most of the designs do not fulfil the requirements. Some sample to answer systems are costly and complex. The commercial sample to answer systems required elaborate instrumentation which is not feasible for our purposed. For example the instrumentation for Cepheid is expensive and requires significant infrastructure.

Quidel instrument offers an innovative sample preparation solution. However, it remains to be seen that the method is efficient and can work in challenging conditions. Adding a new sample prep protocol also has to redo a large number of assays already available. The cartridge complexity and performance was not found appropriate for Micronics system. Although the cartridge is integrated, the complexity is high. Alere system does not use qPCR which brings patent protection problem. Also the design of cartridge does not seem to accommodate variable volumes easily. The instruments in all cases are expensive for its widespread use in low income places.

Most methods in Lab on chip applications using solid matrix to bind dna. The widely used bind-wash-elute protocol is used. New methods are Immiscible Filtration Assisted by Surface Tension (IFAST) [193] [194]and Biphase Flow[195] and immiscible phase purification[196].

9.6 Conceptual Design

We appreciated that in sample prep typically various kinds of fluid interact with some elements with a function. We call these elements as Functional Elements (FE).

We observed various injection molded fluidic manifolds as well. However, we could not find an appropriate item which can be directly used.

Our fundamental ideas was to move the elements instead of routing fluids though channels and other ways to stationary elements. In complex designs moving fluids requires valves and pumps which channels cause dead volumes.

The device consists of a rotating and or moveable structure (MS) which can have various functional elements (like DNA binding membrane, filters for bacteria or viruses, lysis structures etc.) integrated into it. The connections to various tubes, reservoirs, processing areas, reagent storage (CS) can be made simply by moving or rotating the MS.

The MS can replace a number of valves, channels and complex structures. In some cases only rotational motion is sufficient to operate the systems. This can be done conveniently using a low cost motor.

A large variety of operations can be performed by this design. Several embodiments are described below.

9.6.1 Concept

For an explanation of the concept, please refer Figure 9.1. Figure 9.1(a) translating MS moving structure while a rotary design is presented in Figure 9.1(b). The sliding motion can be along curved path as well. For purpose of explanation, both the implementations are very similar.

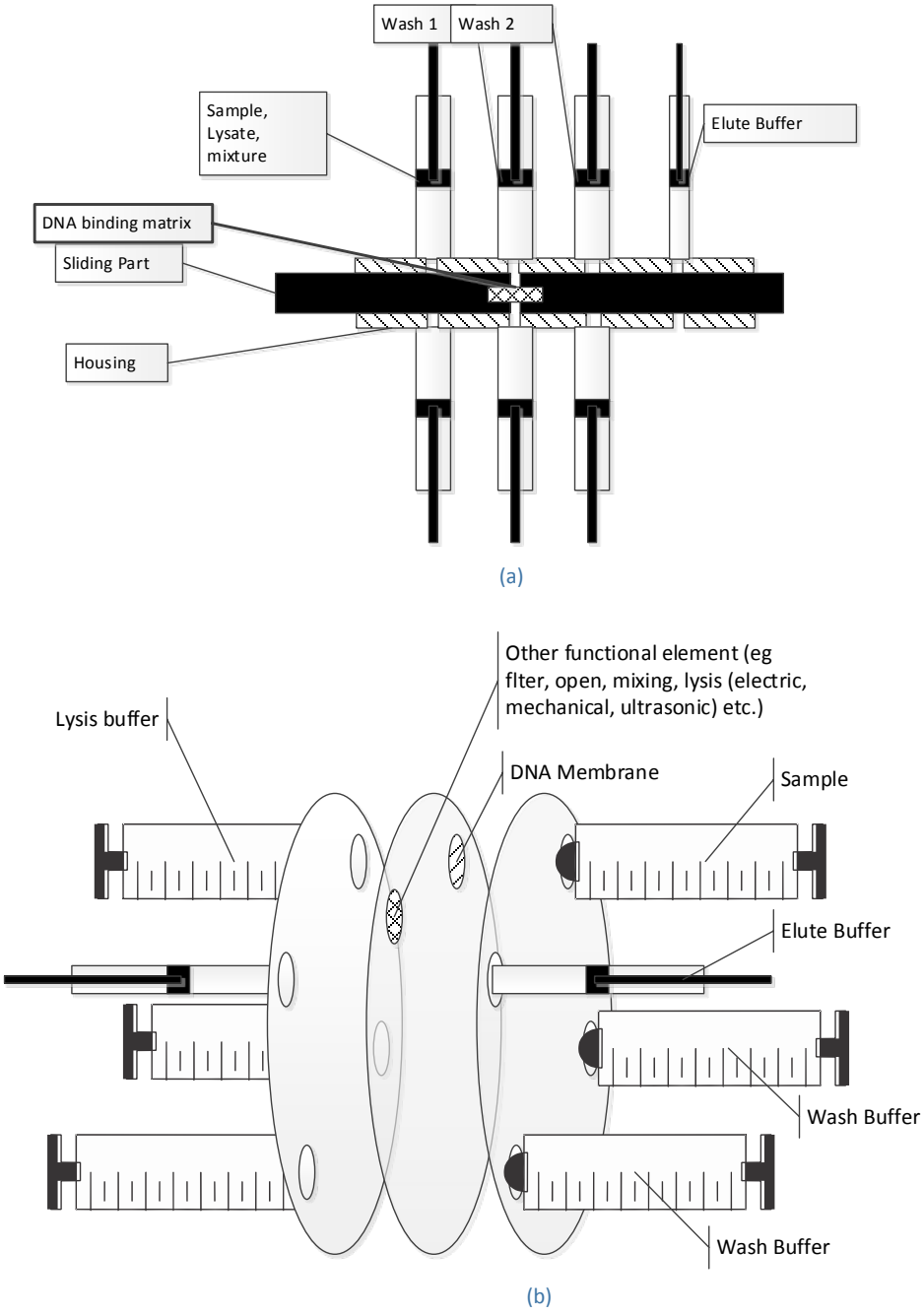


Figure 9.1 Sample Preparation Device Concept. (a) Translational Configuration. (b) Rotational Configuration.

In each case the MS can have straight (pass through) channels with functional elements or channel like structures. Both MS and CS can move in case of this design. Figure 9.2 shows a design with multiple functional elements in the MS.

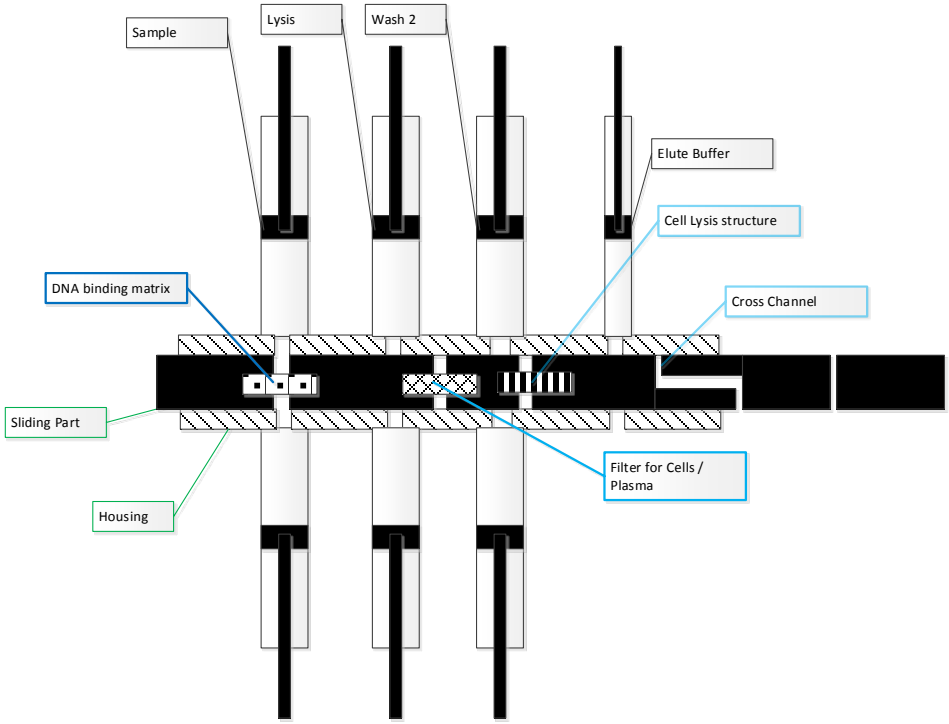


Figure 9.2 Multiple Functional Elements

The structure can be multilayered as well. In one embodiment the CS can also move as shown in fig 3. This will allow relative positioning of different CSs with respect to each other in addition to their relative positioning with respect to FEs in the MSs. Alternatively there can be multiple layers of MS as well. The distinction in MS and CS becomes blur in this case. Multiple thin layers can also assist in complex routing operations.

9.6.2 Functional Elements and Containing Structures

A wide variety of functional elements can be integrated into the systems. Some examples are Filtration, Mixing, Binding, Lysis, Cytometry, Analysis, De-bubbler, Di-electrophoresis, Impedance spectroscopy, Clear Channel and Capillary filling.

The CS can also be of various types. Some examples are

- Syringes with pistons as shown above.
- Custom syringe shapes with custom pistons
- Tubes with pinching mechanism to move fluid
- Planar reservoirs and channels with on chip or external pumps

- Pouches
- Blister packs
- Reservoirs with flexible membranes (which can be pressed to move the fluid)
- Pipettes
- Cartridges (to be filled)
- Tubes (to be filled e.g., PCR tubes)

To move fluids various kinds of MM can be utilized. Electrical motion via motors is a robust approach. Pneumatic pressure can also be utilized.

9.6.3 Sample preparation for PCR

Sample preparation for PCR is explained as follows. The sliding part has one channel with embedded DNA binding matrix in this case. The sliding part aligns itself to reservoirs/CS containing sample, wash and elute buffer in sequence. The fluid can move to and fro across the membrane rapidly thus allowing quick sample preparation and higher performance.

We consider low cost syringes acting as CS as an example. Thus sample obtained from a person using a syringe can be directly attached to the device. The syringe (or reservoir) attachments can be standard luer connections. Even in such design the dead volume is very low due to this novel design. It allows versatile sliding parts for a variety of operations. Also note that no valves are needed as the sliding structure is acting as a valve. The thickness of the sliding structure can vary.

It's possible to do both manual and automated operation. Since the pistons take the whole volume and can apply high pressures, this method is very robust and fast. The whole system can be ***closed*** to get rid of any contamination issues.

Fig 5 shows NA extraction operations in which the MS is rotating about an axis. It's still the sliding motion but arranged differently.

9.6.4 More complex and *universal* SP

Note that the sizes and relative distances of CS and MM structures do not affect the dead volume.

The design allows a truly universal design as we can have a variety of FE both in CS and MM. these can be used on demand and in a programmable fashion. For example some samples may not need filtration, in which case the filtration FE does not need to be moved in place. Also multiple lysis options can be integrated in MS and CS. For tough or gram positive bacteria, one CS can have beads. The sample in this case can be moved to lysis CS. Alternatively, there can be beads inside the MS.

The design also allows on the fly or field mix and match of modules for SP. For example there can be standard interfaces for CS's. Say, luer connectors are made standard. Then for a SP operation, the CS (like syringes) with suitable functionality can be attached. The MS can also be modular and assembled on spot.

9.6.5 ELISA

Separate ELISA or ELISA with PCR tests can be performed using this idea. If blood is being used as a body fluid, separation of plasma and cells can be performed using our inventions. The result can be read using fluorescence intensity.

Having one cartridge to perform both PCR and ELISA tests can simplify a lot of health related problems.

9.6.6 Cell Filtration

It is possible to separate blood serum, plasma and cells using this invention. Different filters can be moved in place to achieve the separation and further analysis downstream.

The design allows option to keep the cells or to keep the plasma. If cells need to be kept and plasma needs to be rejected, a FE in the MS can conveniently do this operation. If blood needs to be filtered, then a side CS can be used. The concept in later case is shown in Figure 9.3.

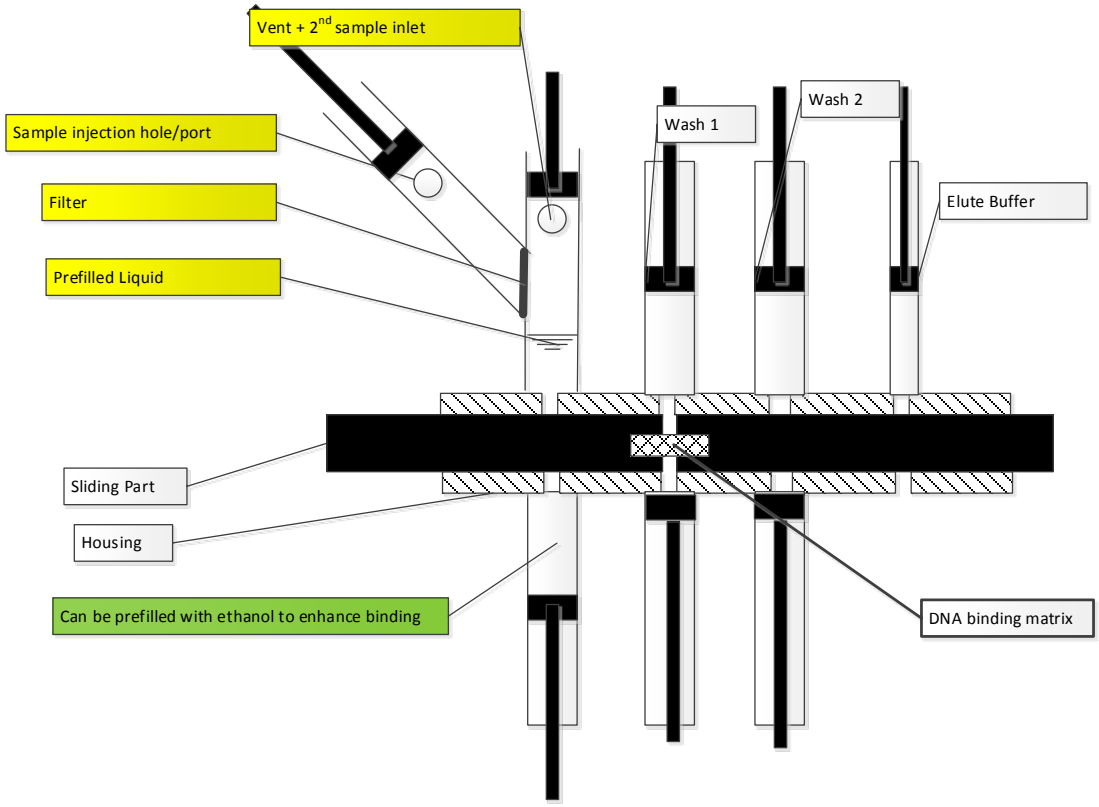


Figure 9.3 Filtration of Blood to Retain Plasma

9.7 Advantages

The design offers many advantages which are not typically available in existing product.

Adding an operation is adding another stage to the cartridge. The instrument does not need to change to allow such addition. Thus **very different protocols** can be ported to our design. The protocols can vary in number of steps and operations as well. However, in most of other designs, this is not easily done. Variable volumes are easy to manage and even same size cartridges can be used in some range. Due to syringe type structures and very low dead volumes, different volumes can be easily accommodated by just the position of respective syringe tip.

The instrument interface is very flexible and can be such that the cartridges can undergo many design iterations while the same instrument remains useful.

Clogging is another problem which happens even in Cepheid systems. Our design allows to and fro movement and positive displacement.

The DNA binding efficiency can increase by passing the sample multiple times though the DNA binding matrix. This is not done in most designs. However, our design let this to happen in a fast and easy way.

Design allows other diagnostic tests as well like ELISA and immune PCR.

The systems can work under vibration and changing attitude like in a vehicle, ship or aircraft. Venting is not required to move fluids as is the case with many other systems. The system is closed.

Multiple materials can be used in fabrication and integration of system with PCR cartridges or tubes is easy and well controlled. For movement of fluids across FE in the MS, both positive and negative pressure can be applied. There is no need to restrict buffers, for example, to be non-foaming.

9.8 Design and Fabrication

We designed the system in both linear and rotary configurations. We also varied the sizes of the devices. We first designed the systems for 3D printing. The surface quality of 3D printing is not appropriate for moving syringe tips as the surface has layers due to fabrication process. Nevertheless we had some success in using pipette tips with this approach. We designed the Stationary structure to be printed and used positive displacement pipette tips glued to the system as well.

We used a Teflon bar with a hole as the sliding part. We also used bars which were printed via 3D printing. Figure 9.4 shows and example of such a cartridge with 3D printed parts and a Teflon sliding bar. Design diagrams for top and bottom parts are also shown.

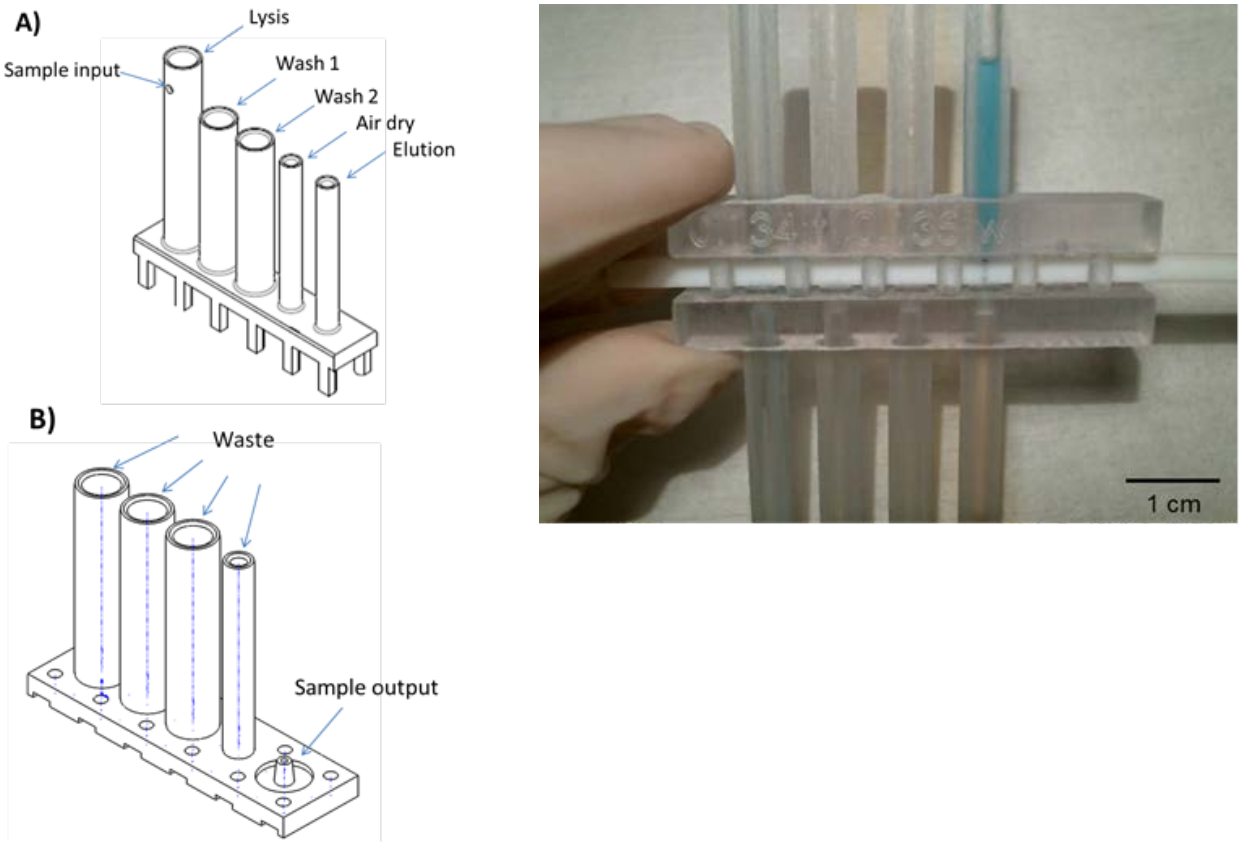


Figure 9.4 3D printed design implementation

It was kept in mind from early on that the designs should allow injection molding. To have more realistic systems, we changed the design to accept pipette tips from positive displacement pipettes and in other case to accept leur slip or luer lock syringes. This approach provides excellent and flexible storage of reagents and saves on 3D printing costs. The exposure of the fluid to 3D printed material was minimal. We found that Ethanol (used in wash steps) would attack ABS but the VeroClear material worked fine with it. The VeroClear material is also translucent and hence the movement of fluids or fluids with dyes is visible. Shows designs with pipette tips while shows design with luer syringes. Since the dead volume does not change by the spacing of various CS, the prototyping is flexible. We also made the stationary structure in metal.

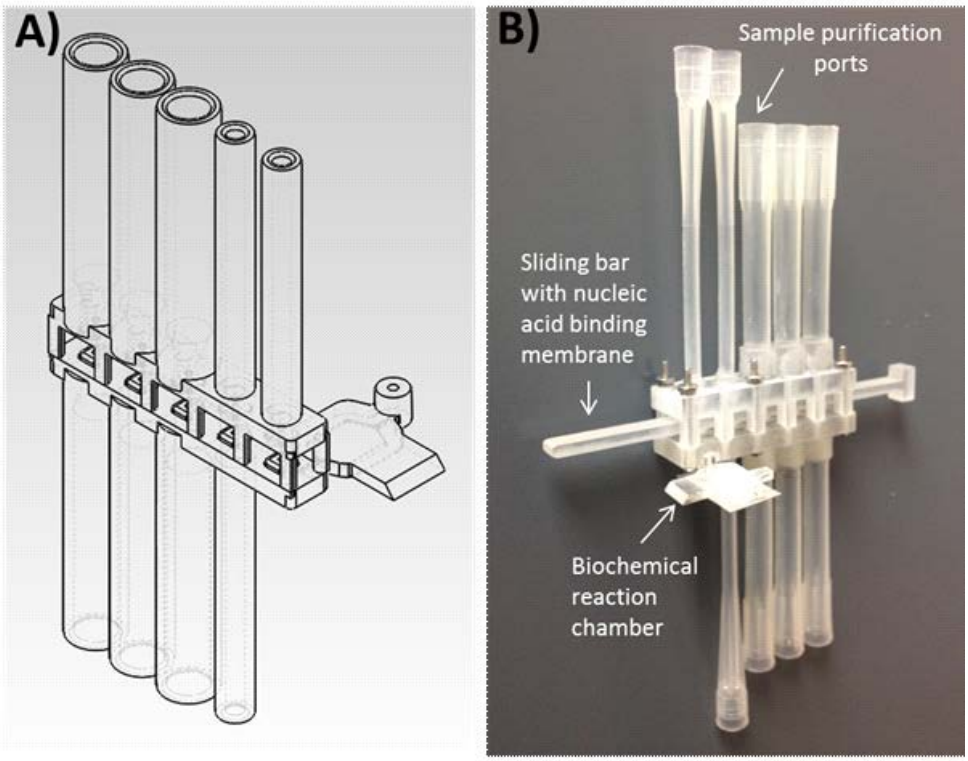


Figure 9.5 Design with Pipette tips

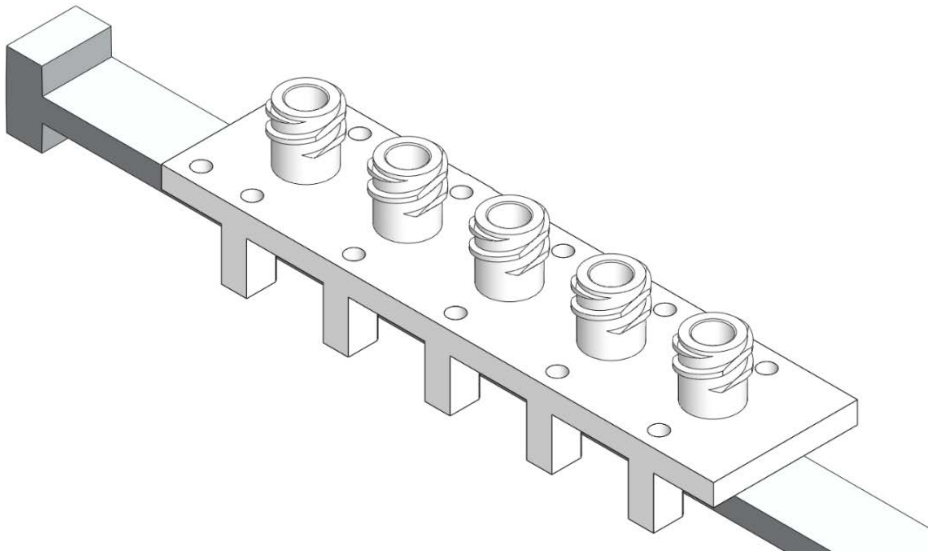


Figure 9.6 Luer Lock based connections

9.8.1 Injection Molding

The designs were also injection molded using COP 1420. Injection molding puts several constrains on design due to the process. After few iterations, the parts had little warpage. The residual stress in the injection molded parts can be taken care of by annealing. The injection molded parts are show. We used common syringe sizes to use the available syringe tips.

The design consisted to top and bottom parts with a rod between them. The top and bottom parts can be bonded using adhesives after the bar is placed in between.

Filling of liquid reagents can be done before bonding by filling from the side which contact the moving rod. A liquid handling robot can accelerate this process.

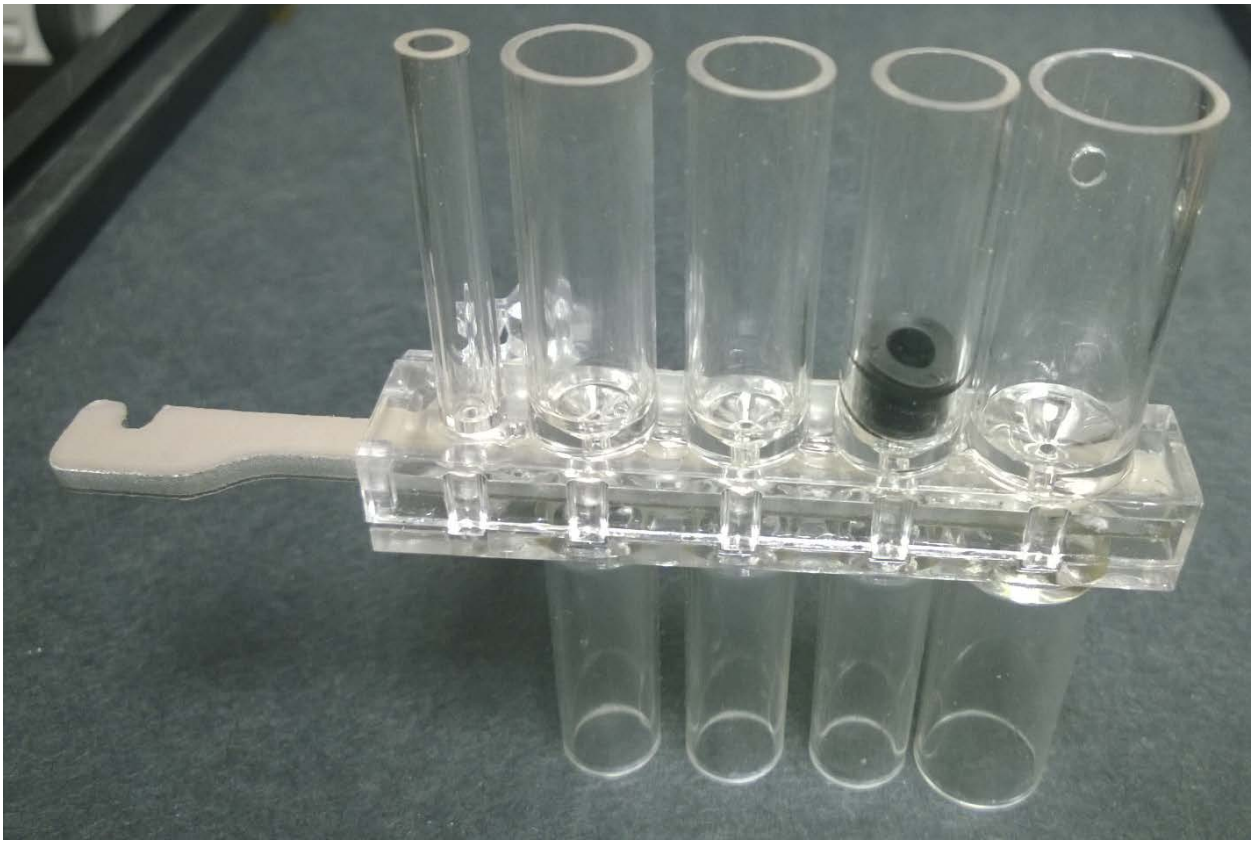


Figure 9.7 Injection Molded Cartridge

9.8.2 Seal Design

One of the key features of the design is the moving seal. As the moving bar of disc has to contact the fixed part at a number of places, friction should be kept low. At the same time there should be enough

pressure so that leaking of fluid is avoided. Seal can be achieved with flexible or elastomeric parts on either the fixed part or on the moving parts. The elastomer structures on fixed part has several advantages. It requires less area to be elastomeric. Also the moving bar or disc can be rigid which can make putting various kinds of dna binding matrices easy.

For prototyping we used backup rings as flexible material on the fixed parts. We also used protrusions on the fixed part. Figure 9.8 shows one such implementation.

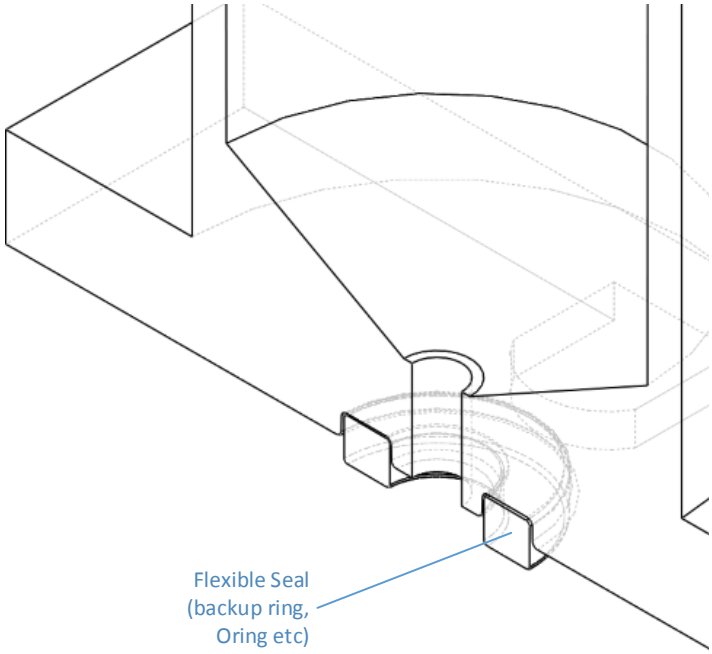


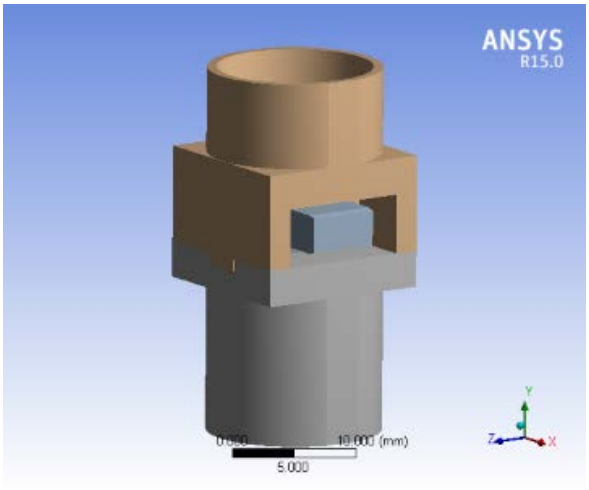
Figure 9.8 Seal using BackUp Ring

Seal design is rather difficult to control in prototyping due to exact dimensions required. It can also be a problem in injection molding since the tolerances of injection molded parts can vary due to shrinkage and warpage. Prototyping using metallic parts is an attractive way for seal design and is useful and quick method to arrive at the final design for the seal. CNC machining can provide good finish as opposed to 3D printing and the parts are robust and long lasting. However, it should be kept in mind that the final injection molded parts might bend under stress due to different elastic modulus.

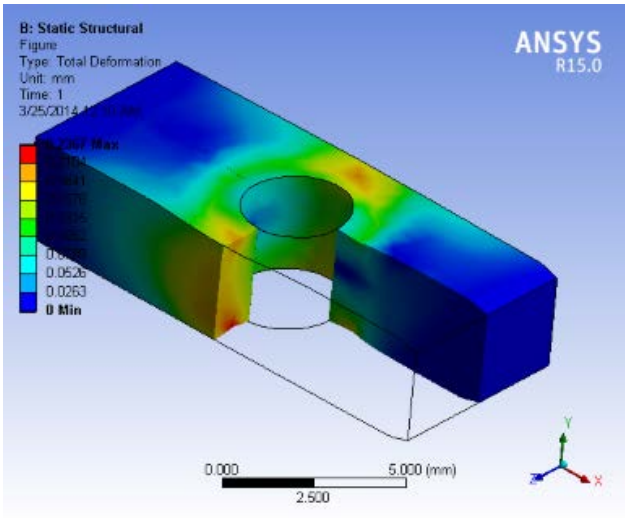
For a robust design several types of analysis have to be done. Both the part and seal design can use Finite element analysis. Such analysis provide various parameters. After this desing, modern injection molding software can be used. Injection molding software can export data to FE software for furtner analysis. As an example we show results from simulation for seal design. For evaluation of different

materials, data is needed which is not readily available. Ideally the coefficient of friction for different load conditions should be available for various parts. This is made complex since we can use various lubricants which will change this value. However, FE is very useful in seal design.

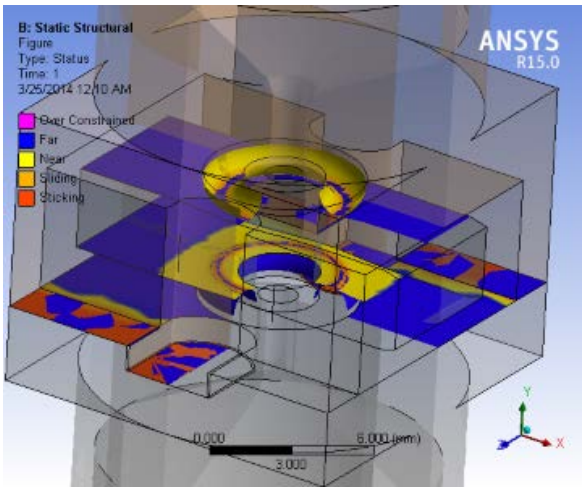
Shows results from a structural simulation. Such analysis shows the deformed shape, the contact status and the stress each material is undergoing.



(a)



(b)



(c)

Figure 9.9 Seal Design FEA. (a) Geometry (b). Moving Rod deformation. (c) Contact status

9.8.3 Instrument Interface

Automation of the devices can be done various ways. One is to use a motor with screw to drive the MS as in case of Figure 9.10. For rotary designs, a stepper motor can be used. Both manual and automated operations can be provided in one device.

In many cases no complex actuators are needed while still being able to precisely control fluid volumes and movement.

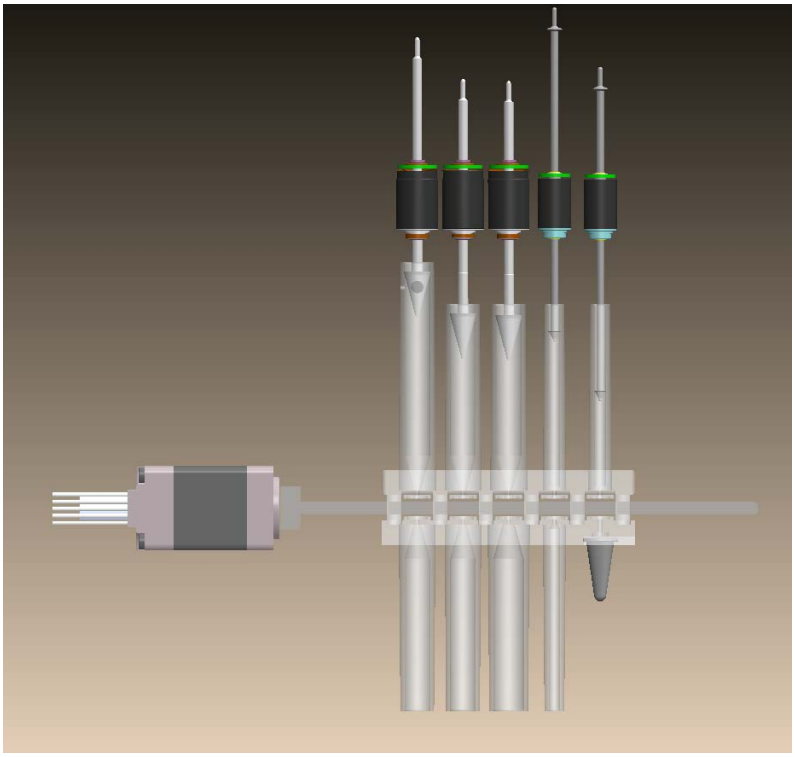


Figure 9.10 Automation Concept

9.8.4 Advantages of using syringe type CS

The use of syringes in movement of fluids have certain advantages over other techniques. The syringes are mass produced and are very reliable. They allow movement of all of the fluid inside the cavity. Thus some biomolecules are not left inside a cavity while movement like in other techniques. Also the amount dispensed can be very accurately controlled. The CS can be of different volume than the amount to be dispensed which allows operational and design flexibility using the same cartridge.

Syringes allow high pressure to be applied without failures. This feature allows rapid operation and many other options (like in lysis, filtration, mixing etc).

One option is EOP (Electro-osmotic pumping). This can be used to pump elute buffer with DNA in cartridge.

Other is to have a membrane which can press down on a reservoir.

9.8.5 Interface to qPCR and other reaction chambers

After sample processing, the required species can be introduced into one or multiple chambers. In case of qPCR, the last stage's actuator can push the elute buffer through the membrane.

The design is also useful for convective PCR. In that case the plunger can push and pull fluid back into the temperature zones. Air or an inert fluid in a narrow channel can be used. As the observation is being done at one temperature (e.g. annealing temperature), the optics has to observe only one location in case of one chamber.

Interface can be made to digital PCR as well. In that case the sample has to be distributed among multiple chambers. We have already shown that our optical design can work with multiple reservoirs or array style implementations.

9.9 Tests on Clinical Samples

The cartridges were tested on Clinical samples in Dartmouth. Even the cartridges were made using 3D printing, we obtained good results showing the promise of this design. Comparison was made with Qiagen EZ1 robot. For PCR we used Cepheid Smart Cycler Platform.

9.9.1 Trial 1 - A urine sample from a patient positive for *Trichomonas vaginalis* (TV)

9.9.1.1 *Protocol*

We used the following volumes and extraction protocol using Caltech's POC system. DNA was also extracted suing same urine volume with the EZ1 Qiagen Robotic system (the gold standard) for comparison.

	Extraction
Lysis and bind	650 µL urine+ 500 µL BE + 65 µL Pro. K (let stand 5 min). Add 500 µL ethanol (100%) & Mix
Wash 1	500 µL BW

Wash 2	600 µL B5
Air Dry	1 min
Elute	100 µL BE at 70° C

After extraction 25 µl reactions were run with primers specific for (TV) using Cepheid Smart Cycler platform.

After amplification, 4 µl of PCR product was run on gel.

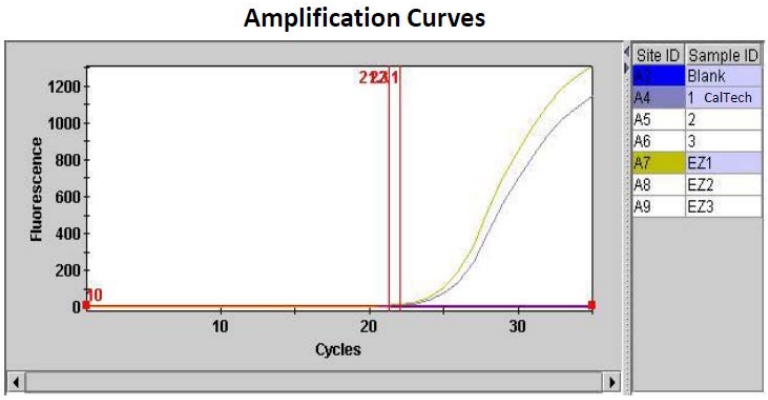
9.9.1.2 *PCR*

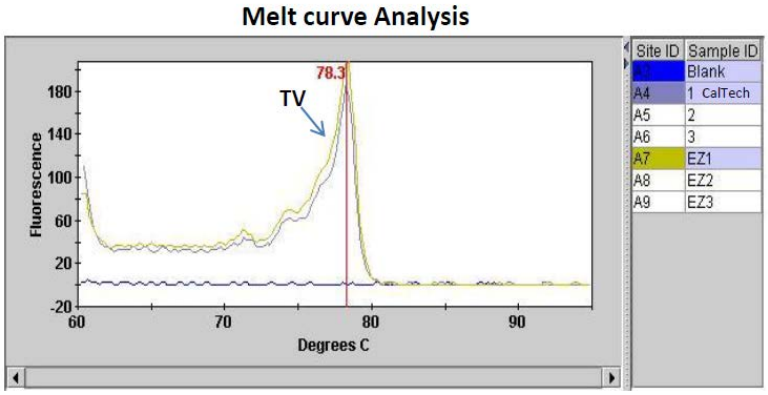
We tested the presence of *Trichomonas* by PCR using specifically designed primers and the Bio-Rad EvaGreen PCR master mix. We performed both melt curve analyses and gel electrophoresis to confirm PCR specificity. PCR conditions were as follows:

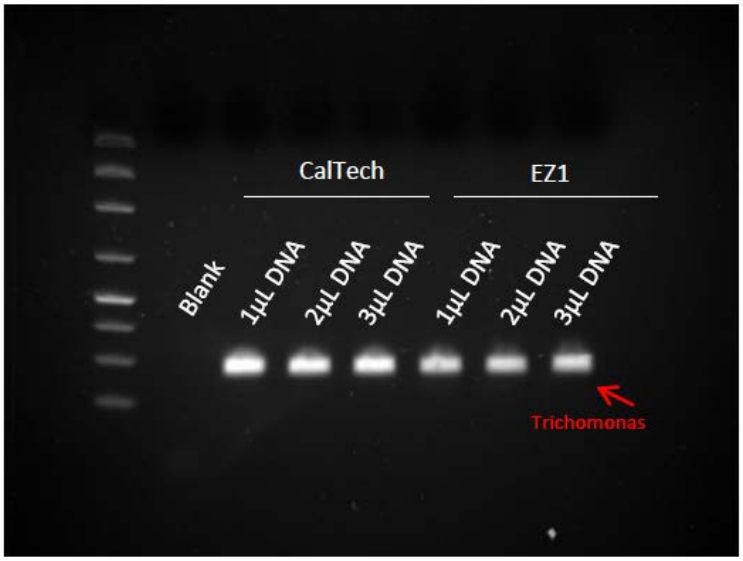
1) 95°C for 5 minutes
2) 35 cycles of :
    -95°C for 5 seconds
    -55°C for 30 seconds
3) Melt Curve: - 60-90°C, 0.2°C/second

-***We used 1, 2 or 3µl input DNA for PCR.***

-***4µl*** **amplified DNA was run on a gel**







9.9.1.3 *Observations*

Caltech POC system has similar Ct and brighter gel bands than the gold standard EZ1 robot. EZ1 is optimized for urine samples. Caltech's system extraction efficiency looks at least as good the EZ1 in this trial.

9.9.2 Trial 2 - A vaginal swab sample from a patient positive for *Trichomonas vaginalis* (TV)

9.9.2.1 *Protocol*

We used the following volumes and extraction protocol using Caltech's POC system. DNA was also extracted suing same urine volume with the EZ1 Qiagen Robotic system (the gold standard) for comparison.

	Extraction
Lysis and bind	650 µL swab + 500 µL BE + 65 µL Pro. K (let stand 5 min). Add 500 µL ethanol (100%) & Mix
Wash 1	500 µL BW
Wash 2	600 µL B5
Air Dry	1 min
Elute	100 µL BE at 70° C

After extraction 25 µl reactions were run with primers specific for (TV) using Cepheid Smart Cycler platform.

After amplification, 4 µl of PCR product was run on gel.

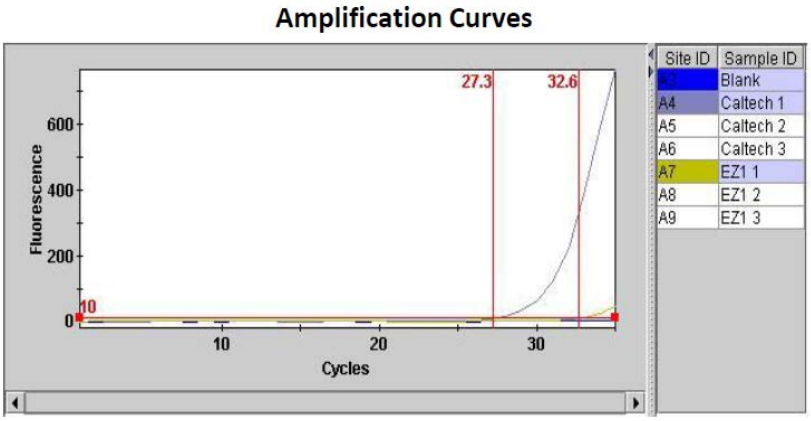
9.9.2.2 *PCR*

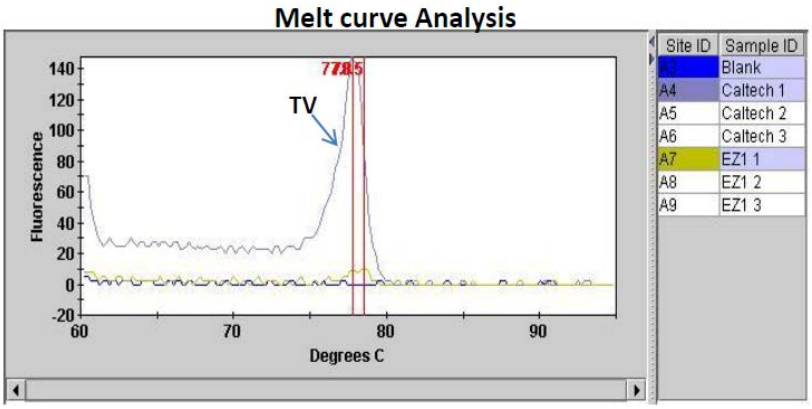
We tested the presence of *Trichomonas* by PCR using specifically designed primers and the Bio-Rad EvaGreen PCR master mix. We performed both melt curve analyses and gel electrophoresis to confirm PCR specificity. PCR conditions were as follows:

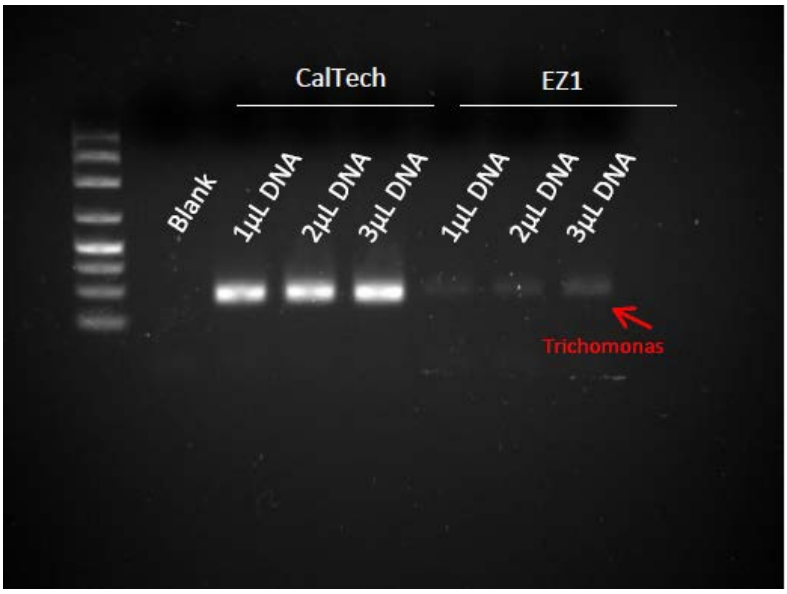
1) 95°C for 5 minutes
2) 35 cycles of:
    -95°C for 5 seconds
    -55°C for 30 seconds
3) Melt Curve: - 60-90°C, 0.2°C/second

-***We used 1, 2 or 3µl input DNA for PCR.***

-***4µl* amplified DNA was run on a gel**







9.9.2.3 *Observations*

Interestingly, only Caltech system could extract from this sample. This may be due to the design of Caltech's system. It is clear in this trial that Caltech's extraction efficiency is much better than EZ1 robot.

It is likely that the concentration of pathogen was much less in vaginal swab sample and Caltech's higher efficiency extraction outperformed the gold standard.

9.9.3 Trial 3 - A vaginal swab sample from a patient positive for *Chlamydia trachomatis* (CT)

9.9.3.1 *Protocol*

We used the following volumes and extraction protocol using Caltech's POC system.

	Extraction
Lysis and bind	650 µL swab + 500 µL BE + 65 µL Pro. K (let stand 5 min). Add 500 µL ethanol (100%) & Mix
Wash 1	500 µL BW
Wash 2	600 µL B5
Air Dry	1 min
Elute	100 µL BE at 70° C

After extraction 25 ul reactions were run with primers specific for (TV) using Cepheid Smart Cycler platform.

After amplification, 4 µl of PCR product was run on gel.

9.9.3.2 *PCR*

We tested the presence of *Chlamydia trachomatis* (CT) *by* PCR using specifically designed primers and the Bio-Rad EvaGreen PCR master mix. We performed both melt curve analyses and gel electrophoresis to confirm PCR specificity. PCR conditions were as follows:

1) 95°C for 5 minutes

2) 35 cycles of:

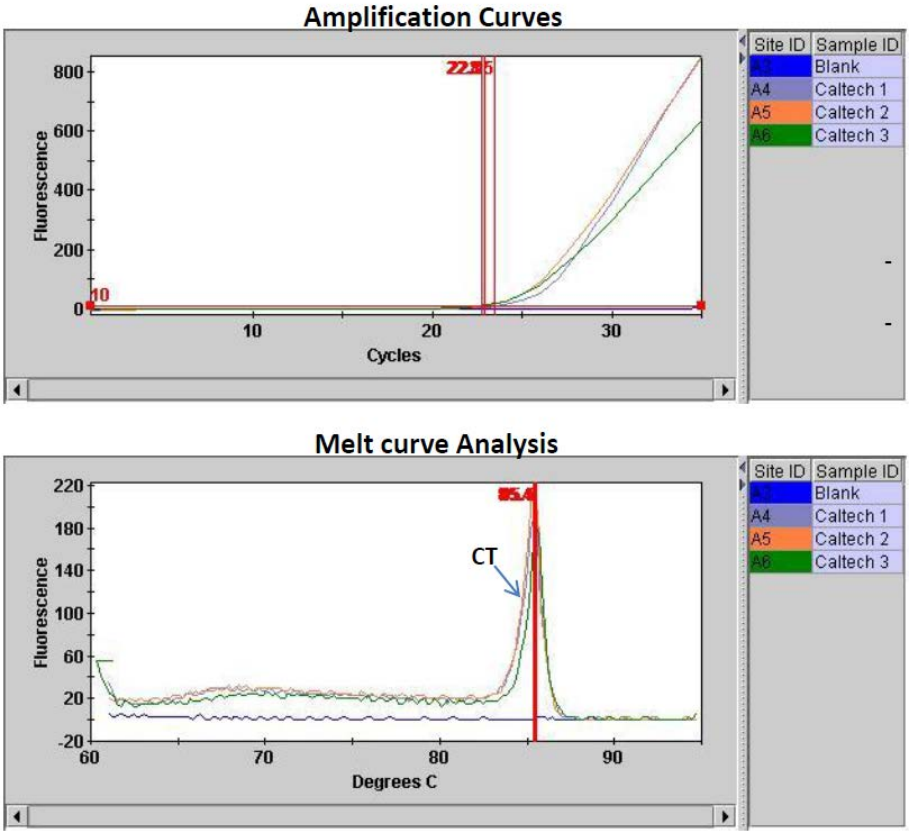
-95°C for 5 seconds

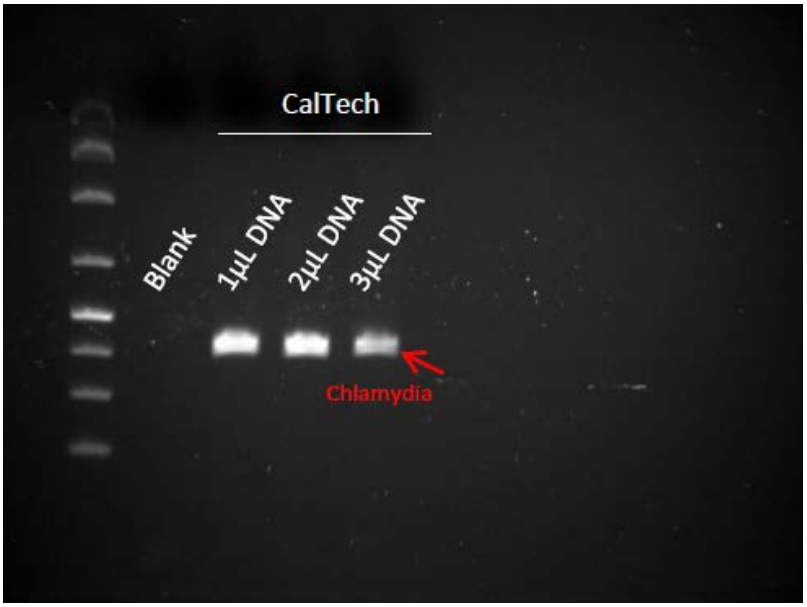
-55°C for 30 seconds

3) Melt Curve: - 60-90°C, 0.2°C/second

-***We used 1, 2 or 3µl input DNA for PCR.***

-***4µl*** **amplified DNA was run on a gel**





9.9.3.3 *Observations*

Caltech system successfully extracted *Chlamydia trachomatis* (CT) from vaginal swabs.

9.9.4 Trial 4 - A whole blood sample from a normal patient to evaluate genomic DNA extraction

9.9.4.1 *Protocol*

We used the following volumes and extraction protocol using Caltech's POC system.

	Extraction
Lysis and bind	650 µL Blood + 500 µL BE + 65 µL Pro. K (let stand 5 min). Add 500 µL ethanol (100%) & Mix
Wash 1	500 µL BW
Wash 2	600 µL B5
Air Dry	1 min
Elute	100 µL BE at 70° C

After extraction 25 µl reactions were run with primers specific for (TV) using Cepheid Smart Cycler platform.

After amplification, 4 µl of PCR product was run on gel.

9.9.4.2 *PCR*

We tested the presence of genomic DNA by PCR using primers targeting β-actin and the Bio-Rad EvaGreen PCR master mix. We performed both melt curve analyses and gel electrophoresis to confirm PCR specificity. PCR conditions were as follows:

1) 95°C for 5 minutes

2) 35 cycles of:

-95°C for 5 seconds

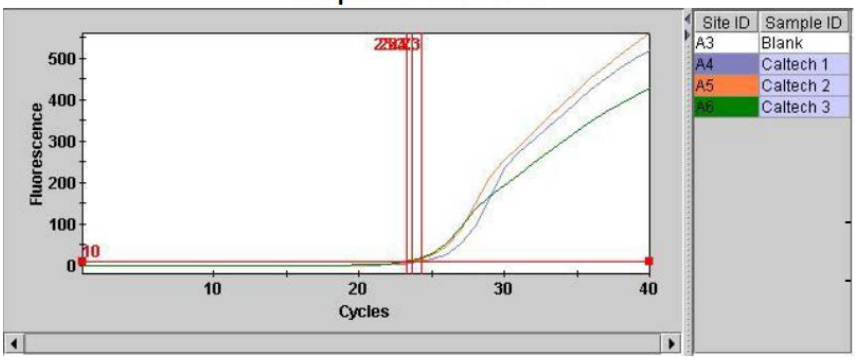
-55°C for 30 seconds

3) Melt Curve: - 60-90°C, 0.2°C/second

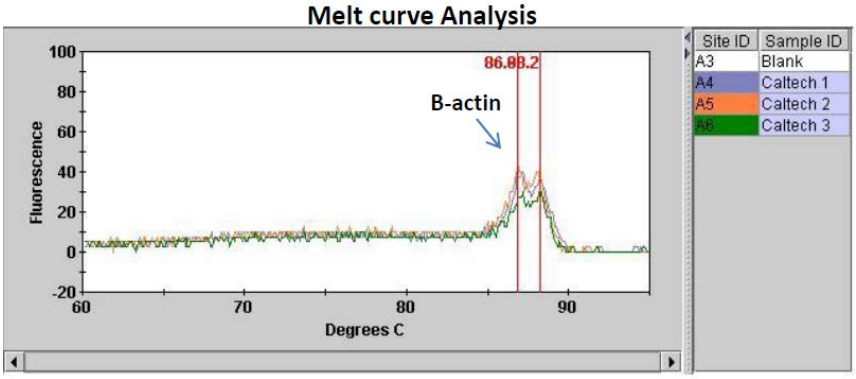
-***We used 1, 2 or 3µl input DNA for PCR.***

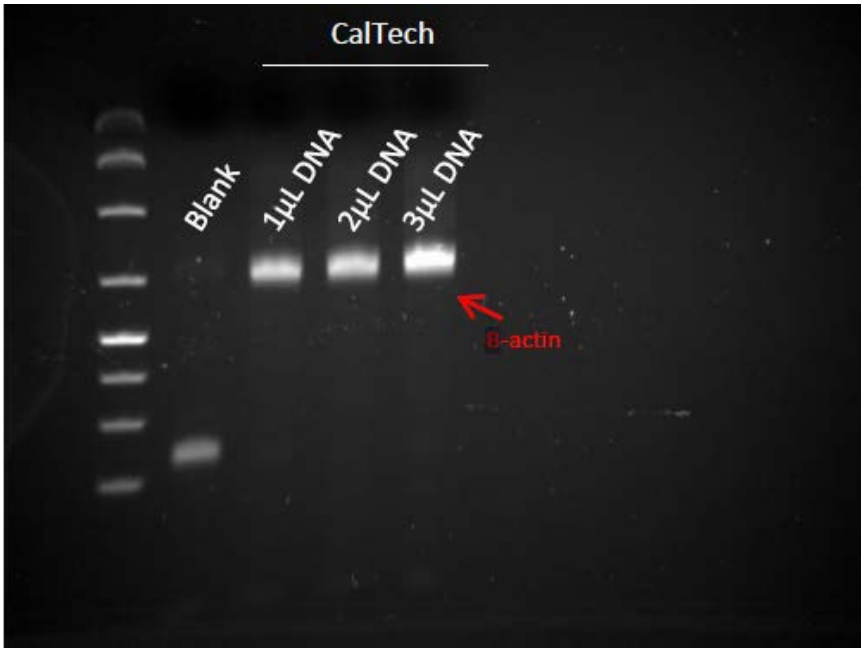
-***4µl*** **amplified DNA was run on a gel**

Amplification Curves



Melt curve Analysis





9.9.4.3 *Observations*

Caltech system successfully extracted *genomic DNA from whole blood. Extraction of both pathogenic and genomic DNA can be useful in personalized medicine, genetic disease testing and other applications.*

9.10 Conclusion

We have presented a new closed, robust, manufacturable, flexible and low cost design for sample preparation. Even with non-optimized system, we could extract dna from clinical samples with seemingly high efficiency. We hope that this design can outperform most of the current designs and can a significant contribution to point of care diagnostics worldwide.

10 Embedded Electronics

10.1 Introduction

POC instruments need embedded electronics for operation. Typically electronics is not developed in an academic setting and external instrument are used to prove the concept.

Among POC instruments, real time qPCR is one of the most intricate due to temperature control and optical detection for multiple channels. Various operations have to be synchronized and real time control, data collection and communications must occur. In some cases the optical signal can be weak and a high quality circuit design should be done.

The real proof of concept of a POC system should include the electronics as bench top electronics can have different designs and features which are not the optimum to implement in embedded electronics for POC.

Surprisingly we could not find an off the shelf module having desired features for our optical design even at the bench. Thus it cannot be always assumed that the embedded electronics and software will be easily developed for POC qPCR instruments. This is especially true if a highly flexible system is being developed to work with various signal levels and different cartridge designs.

There are only few point of care instruments with full embedded electronics reported for isothermal nucleic acid based tests [71] and we could not find full implementation for real time PCR based system in academic literature.

There are many advanced algorithms which can use the same elements in the instruments for better performance. For example modern control theory can make thermal performance better which could be worse if such algorithms are not employed. Modern estimation theory can also help in various measurements.

Smartphones are not always available or desirable at POC due to various reasons. In some settings they can be quickly stolen. A fully self-contained simple to use system is very important at POC. In the case of qPCR, the amplification and melt curves are not something which untrained people should use to decide clinical outcomes. In such cases the embedded electronics has to run various algorithms. It has to check

the validity of reactions and interpret the results. Electronics should be designed to be able to run these algorithms and have enough resources to allow upgrades for future assays and improvements in algorithms.

10.2 Overall Design

We designed the electronics to have a core system and various other elements depending on the use. The core system has processing and communication resources. Thus the same processor is used for all applications. In our case we considered following instrument configurations.

i. POC qPCR instrument
ii. POC sample prep instrument
iii. POC sample to answer instrument (this is also universal instrument)

We tried to have a single PCB (printed circuit board to have all or most of the electronics to save space and cost. It also helps in assembly.

10.3 Main Modules

The block diagram of the electronics is shown in Figure 10.1.

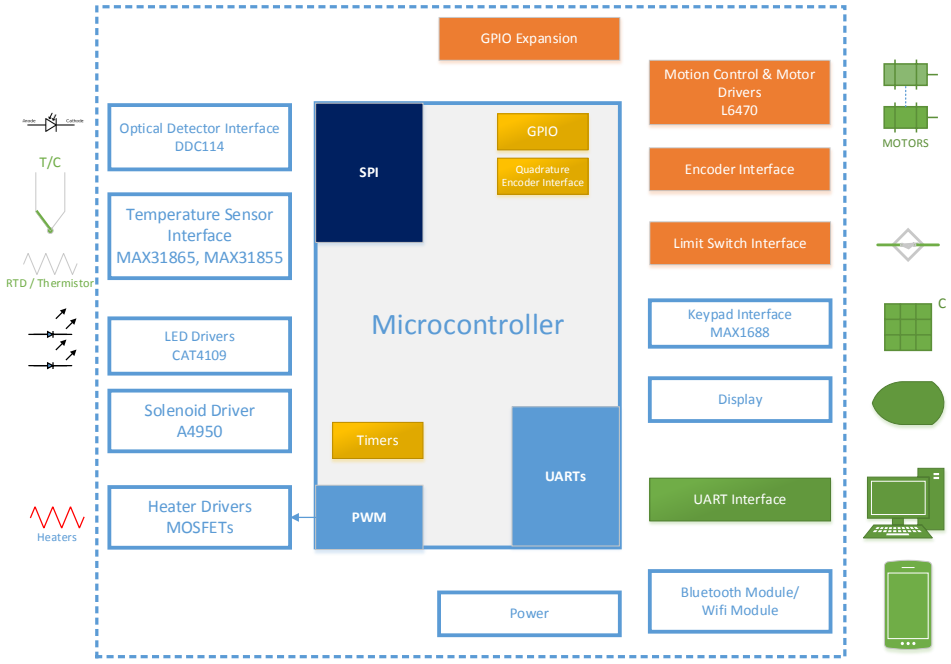


Figure 10.1 Block Diagram

10.3.1 Processor

We selected ARM core based processors due to their large penetration and low power consumption. They are one of the leading products for embedded systems. We selected 32-bit processing. This is done to allow running complex algorithms at a later stage. Another factor was the availability of tools for development. This is a very important factor. ARM microcontrollers are available from many manufacturers in various packages and configuration. We selected LPC1768 from NXP which is a 100 MHz 32 bit microcontroller with on-chip memory and a large number of peripherals.

10.3.2 Optical Interface

This is probably the most trick measurement in the system. Although simple circuits can be used for fixed configurations [139], these have limited dynamic range. The signal range in real time PCR is not very large if other parameters are fixed. However, if parameters changing, there can be issues with such designs. The parameters which can change are reaction volumes, chemistries and reactions types (for example ELISA vs qPCR). To have the design cover a large dynamic range and be sensitive, it should allow integration time control.

We selected DDC114 (current to digital converter) from Texas instruments. The book by Bob Pease helped in selection of this IC[197]. This is a unique IC which allows integration time for current to be programmed on the fly. Hence our system can have a very large dynamic range. At a fixed integration time, it has 20 bit resolution which is quite good.

10.3.3 Thermocouple and RTD interface

Lab on chip devices sometimes have integrated sensors. In other cases there can be non-conventional sensors in the instruments.

There can be non-standard RTDs. For thermocouple cold junction compensation is an issue for POC and was typically done by measuring temperature by an RTD or thermistor. Again owing to the book, we found AD7794 (analog front end). This IC has ability to interface both thermocouples and RTDs. The IC has to be programmed over SPI bus.

During the course of our work, two recent IC from Maxim Semiconductors became available. MAX3865 is an RTD interface where the RTD resistance can be changed by a resistor. MAX3866 is a thermocouple

to digital IC with on-chip cold junction compensation. Both of these are available on the electronics to allow different sensors to be used and tested.

10.3.4 Motion Control

We selected ST L6470 (Fully integrated micro stepping motor driver with motion engine and SPI). This is a powerful motion control IC for control of stepping motors and allows to run motion control algorithms.

10.3.5 LED Drivers

CAT4109 and CAT4101 were used as LED drivers. Both are from linear technology. CAT4109 can drive three separate LEDs with PWM control. CAT4101 can provide higher current drive. Both are linear devices. We selected them to make use of their low noise.

10.3.6 H-bridges

For bistable solenoids, we selected A4950 which is listed as ‘Full-Bridge DMOS PWM Motor Driver’. However we used it as an H-bridge.

10.3.7 Schematics

The schematics are attached as Appendix.

11 Developer Software

11.1 Introduction

The user interface for POC diagnostics especially in developing world should be very simple[36] [198] [39]. However, while developing the system we need detailed information about various elements. The software should allow to test various hardware options and set test parameters easily.

The software are specially designed in such a way that different instruments can be easily added or removed from the system. Two types of debugging software are developed for this system. One software is extended version of the end user applications and the other one is a data acquisition based software which allows to test and study crucial processes using evaluation boards.

11.2 Desktop Based

As mentioned earlier that desktop based debugging software is an extended version of the final application. Hence it not only contains all the critical methods to perform test but also provides different hardware controls which are necessary for the test engineers to test each hardware separately. In order to control hardware special serial protocol is developed which is communicated to the embedded device and then the embedded device decodes it and sends the necessary commands to each device.

11.2.1 Mode of communication

Embedded device and desktop debugging application can communicate with each other via UART using RS-232 protocol.

11.2.2 Features

11.2.2.1 *Heater Control*

Desktop debugging application allows a test engineer to separately control the connected heater. Test engineer can “on” or “off” the heater and thus a problem with heater can be easily detected during debug process.

11.2.2.2 *Cooler Control*

Similar to heater the cooler can also be controlled via desktop application and thus allows the engineer to predict whether there is a problem with cooler or not.

11.2.2.3 *LED Control*

LEDs connected to the embedded device can also be controlled via desktop application. Test engineer can on or off each LED connected to each channel. Beside this the value of current flowing through each LED can also be controlled. This help the engineer to test different current values and there effect on the fluorescence channels.

11.2.2.4 *DDC Control*

In the embedded device the fluorescence is read using photodiode connected to DDC114. This DDC114 are flexible in terms of its integration time and sensitivity. In order to get the optimum performance from the DDC114 different integration time and ranges can be selected depending upon the condition of the system. Thus to test these features and to get the optimum performance these parameters can be adjusted from the desktop application and thus test engineers can study the effect of changes in the parameters in real-time and thus developed the final algorithm for the embedded device.

11.2.2.5 *Temperature channel selection*

Options to select the desired temperature channel are also provided in the desktop application. Test engineer can select any channel in real-time.

11.2.2.6 *Temperature control parameters*

Temperature control parameters such as PID parameters and parameters for custom temperature control can be selected from the desktop application and thus the temperature controller can be tweaked in real-time.

11.2.2.7 *Define Assay*

Similar to the end user applications assay be defined using desktop application

11.2.2.8 *Load and Run assay*

Similar to end user applications assay can be loaded into the embedded device and test engineer can run it from the desktop application. Real-time graphs and results are shown in the desktop application.

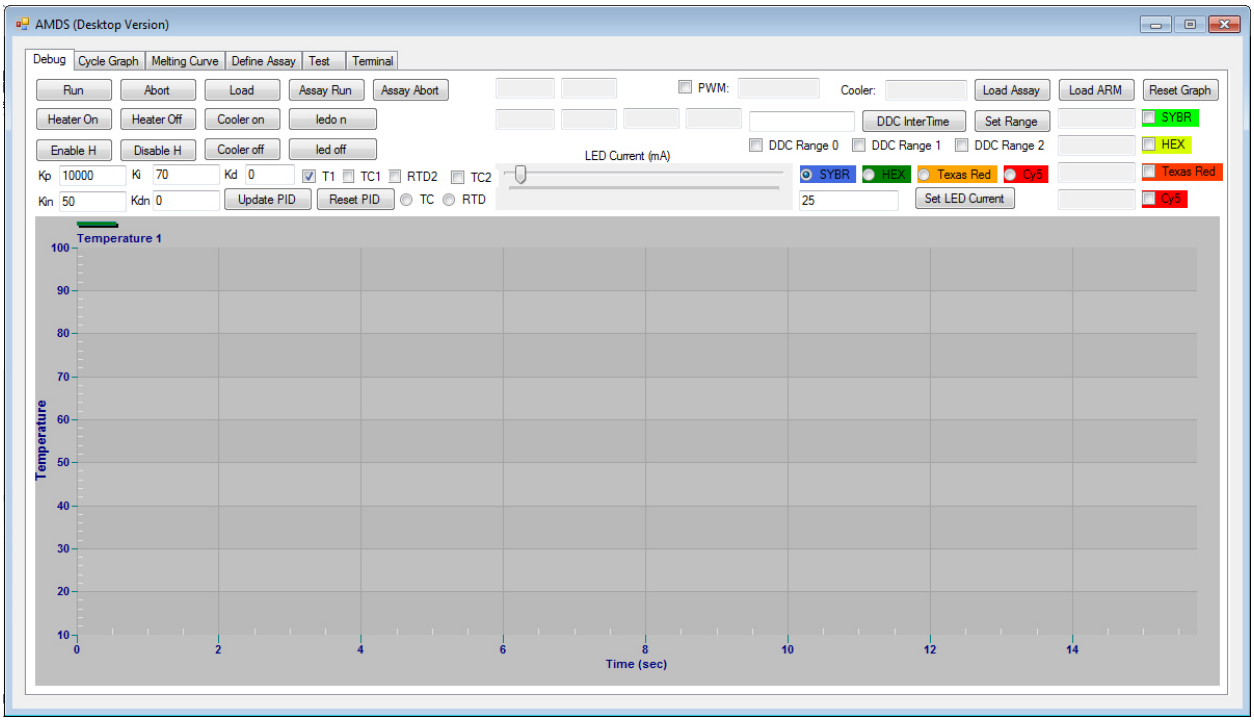
11.2.2.9 *Real-time graph*

A time base graph panel is shown on the main tab which shows the real-time graphs of all the connected hardware. This provide help to the test engineer in order to predict any malfunction in a particular hardware.

11.2.3 Tools Used

1. Visual Studio 2012, 2013
2. Gigasoft .Netcharts

11.2.4 User interface

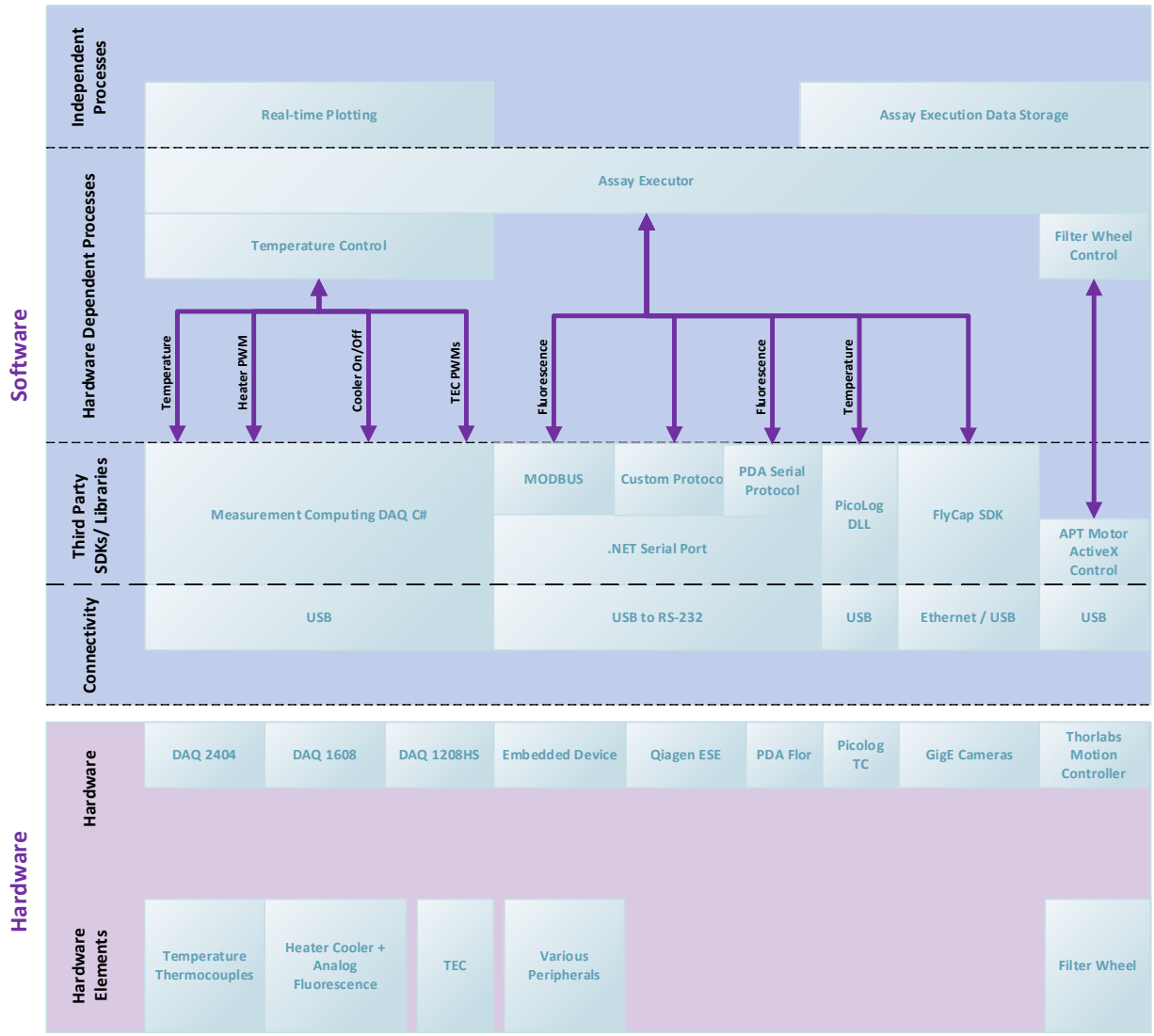


11.3 DAQ based

A DAQ based debugging application is made in C# using WinForms platform. The DAQ application allows executing QPCR assays with many other related features. The DAQ application allowed faster testing and development of algorithms such as Temperature control algorithms, dynamic range adjustments and others. The desktop application integrates a variety of commercial sensors and actuators. Tools such as Labview and Simulink have limited support for integration of commercial sensors and do not allow as much customization that can be achieved in developing a WinForms based application. A real-time

system is implemented synchronizing multiple connected devices including sensors, temperature control outputs, cameras and actuators. An interface to connect to our embedded platform is also included.

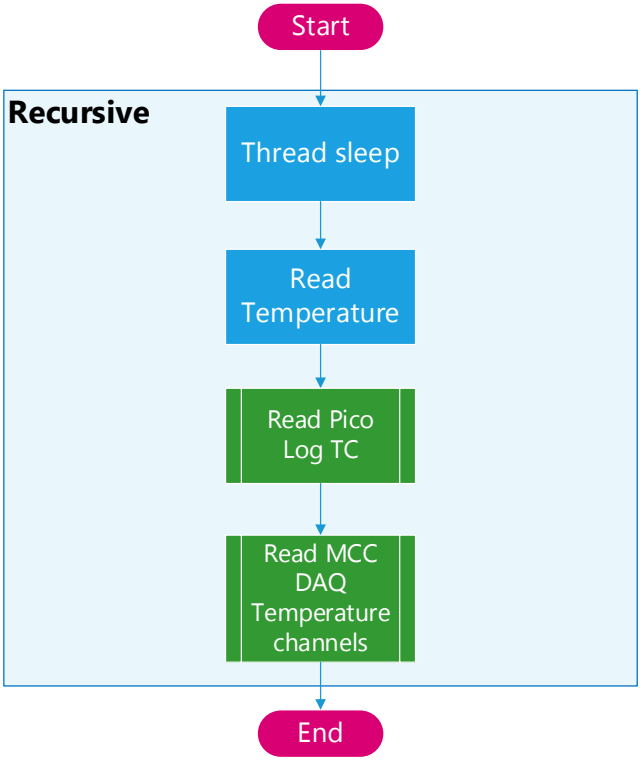
11.3.1 Architecture



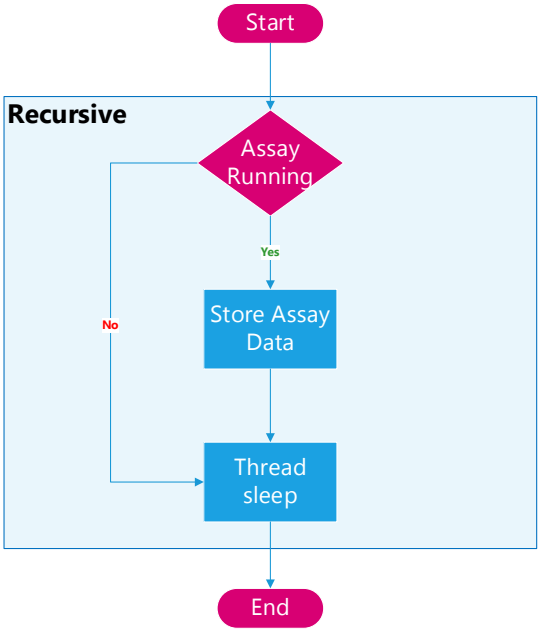
11.3.2 Parallel Programming

In order to achieve maximum performance, the program is developed using the concept of multi-threading. Most of the 3rd party libraries used execute in their own threads. Beside this some custom threads are also created. The purpose of doing multithreading is to speed up the program execution and to maintain synchronization between the critical methods.

11.3.2.1 *Temperature Read Thread*



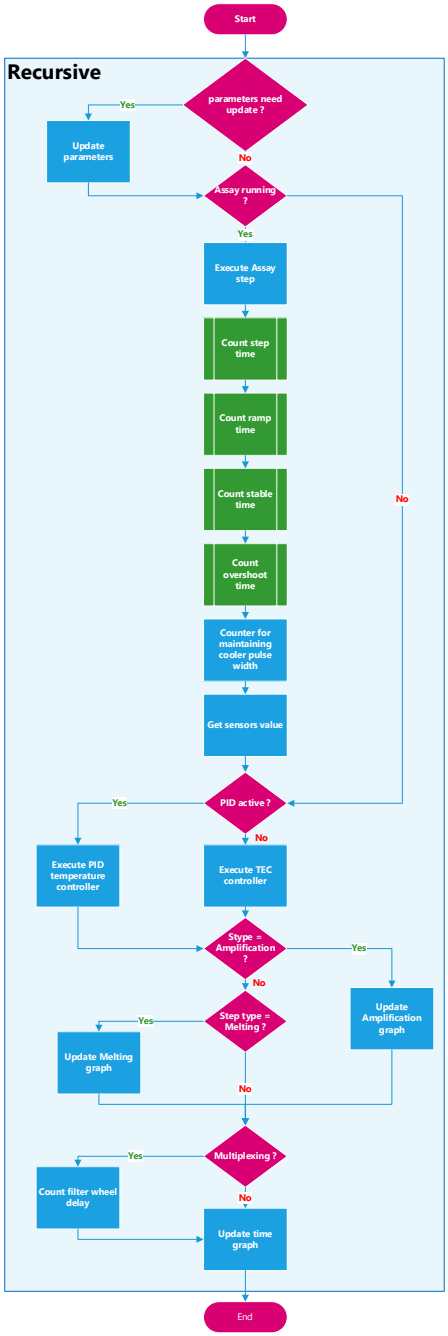
11.3.2.2 *Data Saving Thread*



11.3.2.3 *Main Thread*

Main feature of this thread is that along with the other execution code, it also execute all the time critical methods in a timer. The recursion interval of this timer is 0.2 seconds. It keep all the execution real-time

11.3.2.4 *Execution Timer*



11.3.3 Features

11.3.3.1 *Versatile Engine*

The application provides a versatile engine for complex real time automation applications. If offers a platform where upgrades and customizations can be made relatively quickly and easily compared to an embedded platform.

11.3.3.2 *Custom Assay*

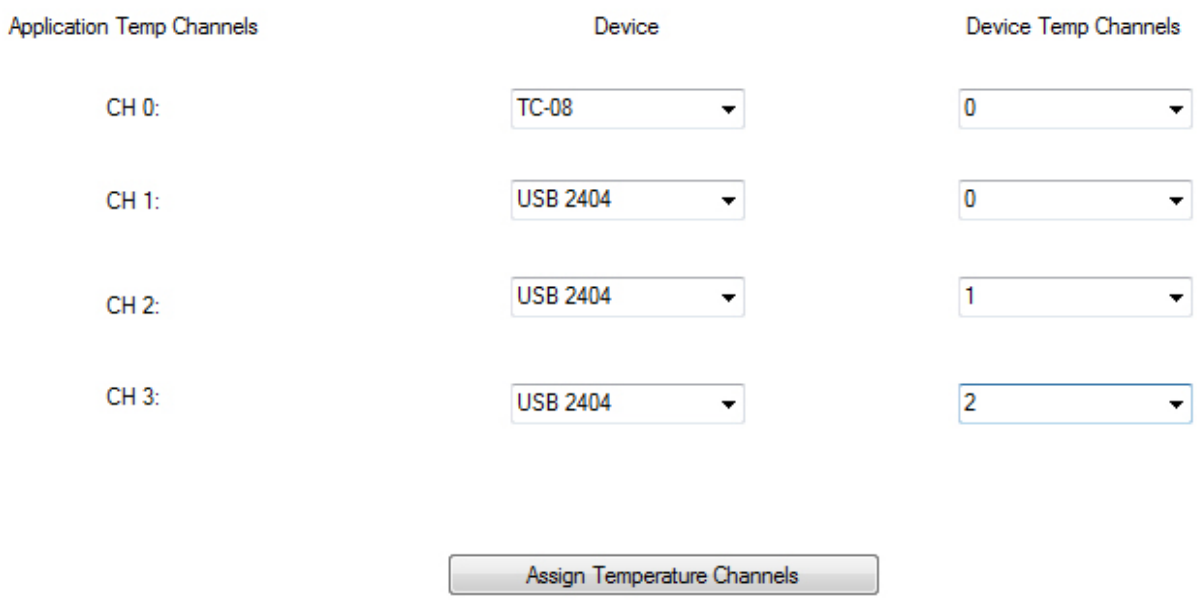
The application allows loading user-defined assays, which can be simply defined in any text editor or using the windows 8 application. The format of assay is same as in other applications, and assays defined in other applications are also compatible with the desktop application.

11.3.3.3 *Assay Execution*

The application supports executing both singleplex and multiplex assays. The assays are executed in similar fashion to as in the embedded device, but in desktop application various additional features are present.

11.3.3.4 *Temperature Channels Selection*

Before an assay is started a temperature channel needs to selected, on which the temperature will be controlled. As multiple sensors are connected to the desktop, data from multiple temperature channels is available. The two temperature sources are Pico Log Thermocouple and Measurement Computing Data Acquisition card. Both of these sources have multiple channels available, although data from only certain connected channels is useful. Therefore a feature is included to define temperature channels, where the sensor and its channel can be selected and assigned as temperature channel ‘n’ in the application.



11.3.4 Fluorescence Channels Selection

To measure the fluorescence again multiple sensors are connected. These include, Qiagen ESE, Tetrahertz Technologies PDA-750, and fluorescence can also be obtained from our embedded device.

11.3.4.1 *Fluorescence Sensors/ Channels*

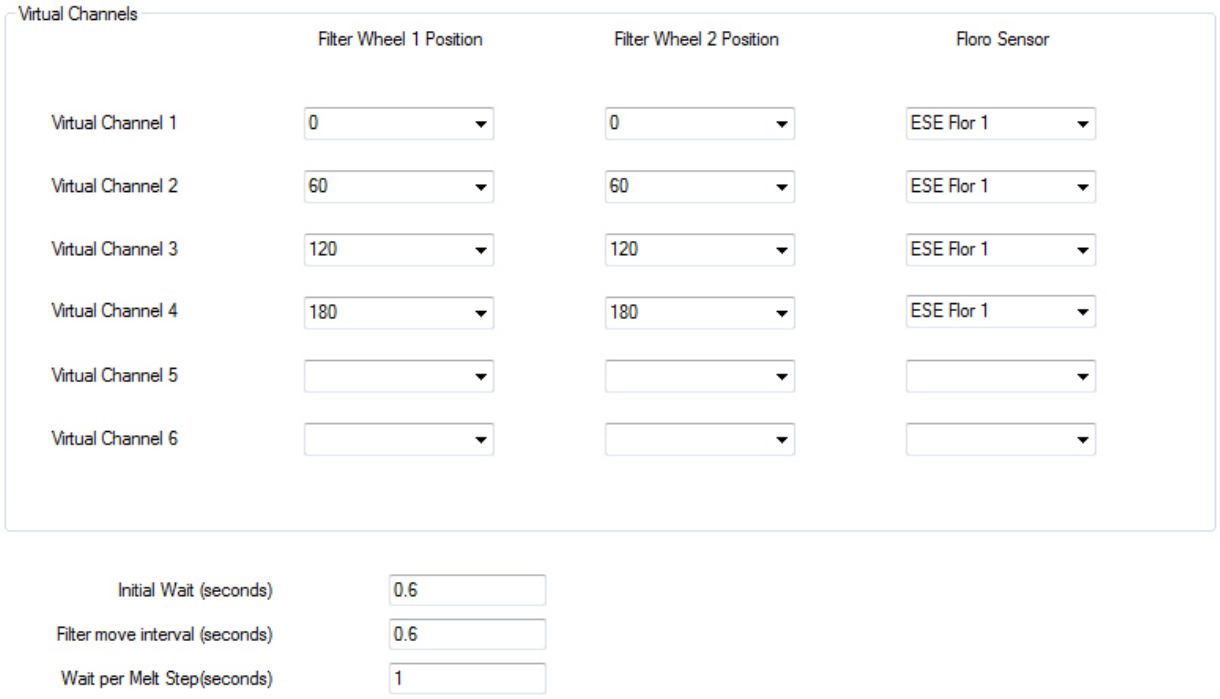
ESE has two detection channels and two excitation channels, and excitation channel for detection channel 1 can be set to either 1 or 2, providing a total of three combinations, and two active fluorescence channels. Our Embedded device has 4 fluorescence channels, whereas PDA has 1. Therefore resulting in a total of 7 fluorescence channels to choose from.

11.3.4.2 *Virtual Fluorescence Channels*

Now for a multiplex reaction two filter wheels are used, to make virtual fluorescence channels. To define a virtual channel, the positions of both filter wheels are selected and the sensor to be used is selected. During assay execution the filter wheels movement and reading sensors is synchronized. The synchronization uses three parameters.

1. *Initial Wait*, defines the time to wait at each optical step before fluorescence can be read, this is because fluorescence takes a little time to reach a stable value.
2. *Filter Move Interval*, defines the time to wait between each filter wheel move command, this delay accommodates the time it takes to move the filter and read value from sensor

3. *Wait per Melt step*, is used to provide extra time for temperature to get stable before virtual channels are read by moving filter wheels.



11.3.4.3 *Assay Speed Indicators*

Apart from real-time plotting of data from sensors, to get a measure of the temperature control performance and speed of assay execution current and previous steps' Ramp, overshoot and stable time are displayed. These indicators help in adjusting temperature control and assay execution parameters to optimize assay speed.

11.3.4.4 *Assay Data Export*

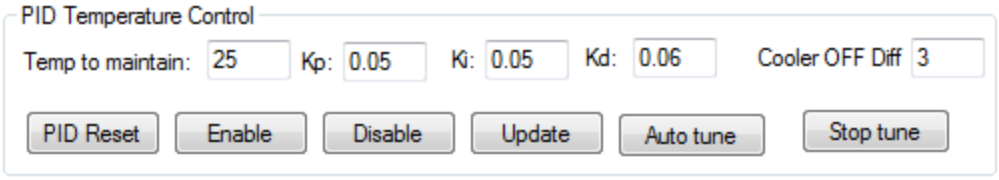
To have a record of assay data, which can be used for analysis later, all the assay data including raw data and graph plotting point is exported in a excel sheet. The assay execution data is saved and exported in excel sheet in background while assay executes, so that even in a case of a crash data is available for analysis which can also help in diagnosing the reason for crash. The assay data includes assay description and information such as total assay execution time which helps in determining assay execution performance.

11.3.4.5 *Temperature Control*

For temperature control two options are available.

11.3.4.5.1 Heater and Cooler

The heater is driven via PWM, while the cooler is controlled via a bi-stable solenoid. For temperature control via heater and cooler, two temperature control methods are available. These include a custom algorithm which uses a modified proportional control, and PID. PID auto tuning is also implemented which uses CC tune algorithm.



1. *Thermo Electric Cooling*

The thermo electric cooling uses a TEC from Thorlabs, it is controlled via two PWMs, one for each heating and cooling. The temperature control method used for it is again PID with a slightly different implementation.

11.3.5 Real-time Graphing

For real-time graphing of data from sensors and other information, plotting control Pro-Essentials from Gigasoft is used. There are three graphing controls in the application. The graph on main tab shows data from all enabled sensors, and PWM outputs to control temperature, with time on x-axis. Then there are two separate graph for cycling and melting plots. The subsets to be displayed in the graph are selected by the user according to requirement.

11.3.6 Camera Integration

GigE USB or Ethernet cameras support is also added to the application. These cameras take snaps at appropriate times during cycling. The snaps can be analyzed to get a measure of fluorescence at each cycling step. It also helps to identify any physical issues such as a cartridge leak.

11.3.7 Tools Used

The application is made in Microsoft Visual Studio 2012 using C# on .NET 4.5 Framework. Additional Third-party libraries used are:

11.3.7.1 *Measurement Computing DAQ Managed Library*

This library provides interface to DAQ cards. Three DAQ cards are used as shown in the diagram.

DAQ 2404: This DAQ provides analog channels and ports for thermocouples. Its used for temperature measurement via thermocouples and reading fluorescence from PDA via its analog interface.

DAQ 1608: This DAQ is used for providing pulse output to Cooler (solenoid) and PWM output to heater.

DAQ 1208: This DAQ provides two PWM outputs which are used to control TEC heating and cooling.

11.3.7.2 *Picolog Unmanaged Library*

This library is used to read thermocouples attached to PicoLog device, it has 8 temperature channels.

11.3.7.3 *Flycap SDK*

Flycap SDK allows controlling GigE cameras. These cameras can be connected via Ethernet or USB.

11.3.7.4 *APT Motor ActiveX Control*

This activeX control allows sending commands to APT Motor Controller from Thorlabs, in order to control the filter wheel. The control also provides a user interface showing the status of filter wheel.

11.3.7.5 *Gigasoft Pro-Essentials*

This control is used for real-time scientific plots

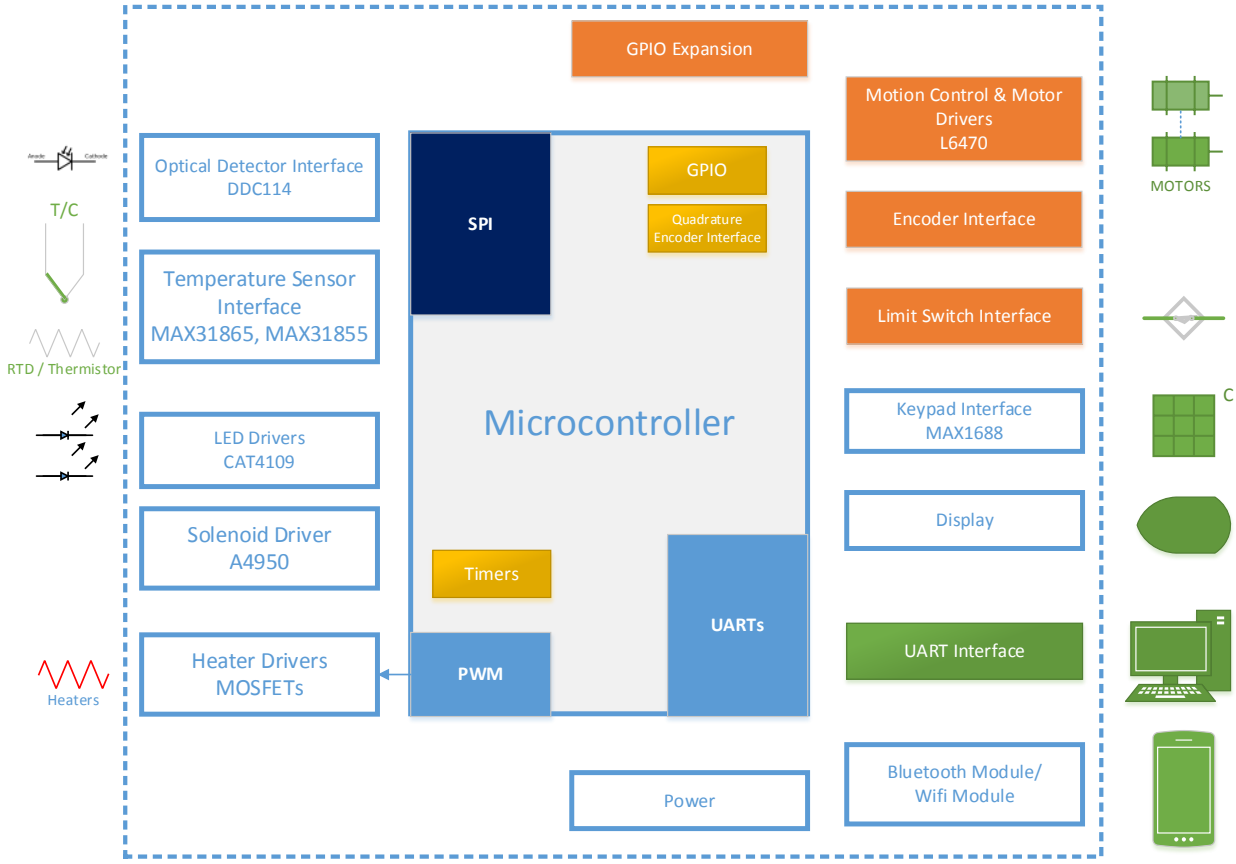
12 Embedded Software

12.1 Requirements

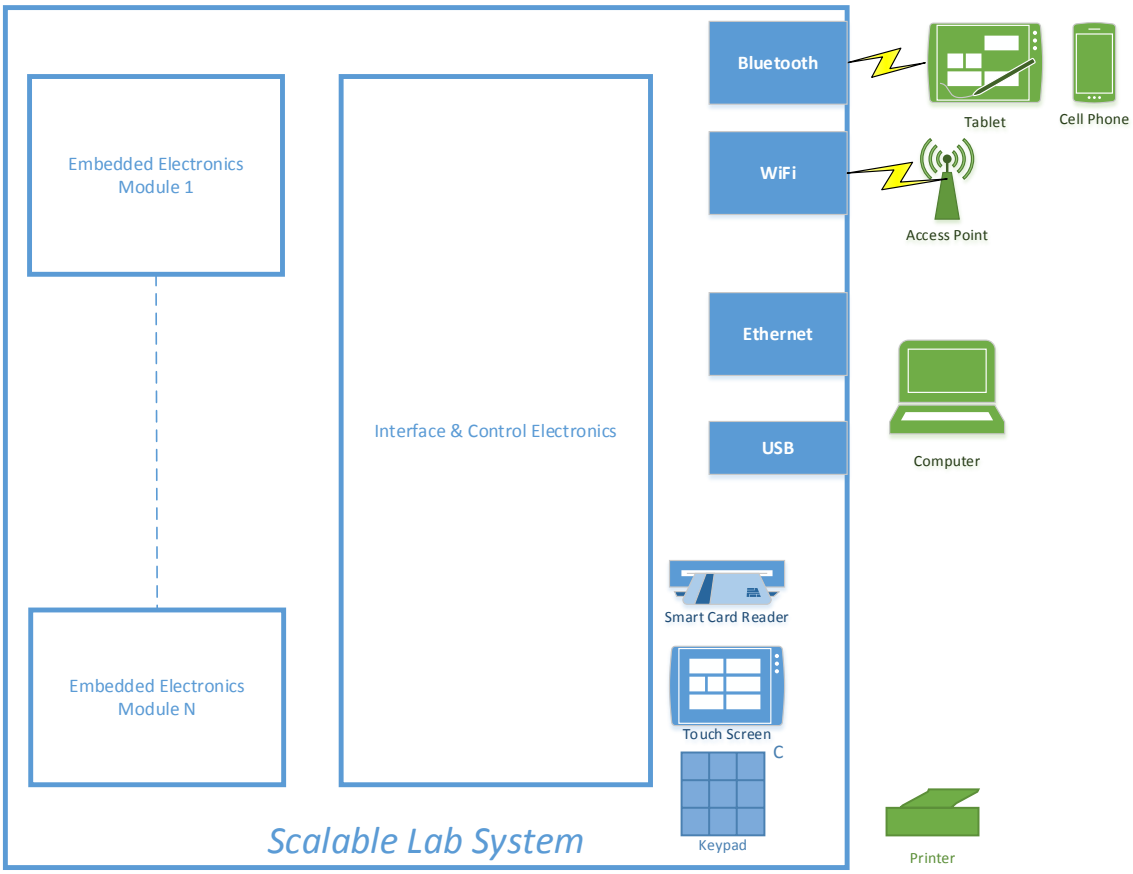
- Cost effective solution for running ADx
- Running dynamic assay
- Real-time sampling from sensors
- Fast PWM updates for precise temperature control

12.2 Architecture

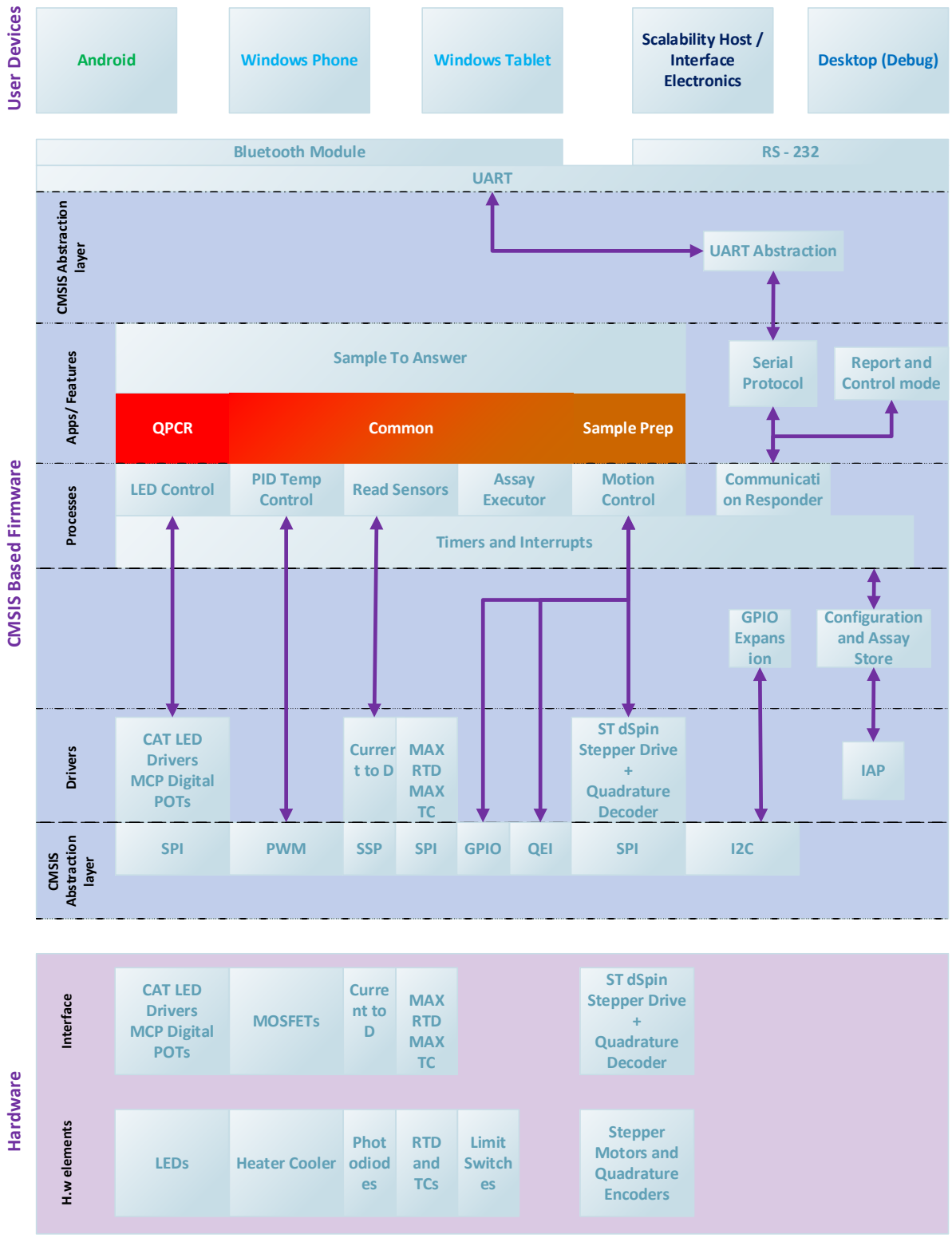
12.2.1 Hardware Architecture



12.2.2 Scalability



12.2.3 Software Architecture



12.3 User Devices

A single cartridge system can be connected simultaneously to multiple devices over Bluetooth and RS232. In addition to the scalability host connection discussed in previous chapter, the single cartridge system can be directly connected to a variety of user devices, without any scalability host.

User applications are made for different platforms including Android, Windows Phone 8, and Windows 8. This allows most smartphones and tablets to be connected to the single cartridge system over Bluetooth.

12.4 Firmware Overview

The microcontroller runs a firmware based on infinite loops design approach, and does not use any RTOS. The firmware uses Cortex Microcontroller Software Interface Standard library for providing a hardware abstraction for the microcontroller.

Some of the features of the microcontroller are not abstracted by CMSIS and custom drivers are used for them, such as In Application Programming, which is used to store assays and configuration parameters/

For external connected peripherals custom drivers are made to access and configure them.

- CMSIS benefits
- Easy portability among ARM Cortex M series
- Systematic, software design
- Easy to code
- Stable, well tested features

12.5 Features

The system is fully capable of running full sample to answer assays, and also provides the functionality to run QPCR or Sample Prep alone.

The assay execution is dynamic and can run user-defined assays. Some predefined assays are stored in the system, apart from those, the user can define a custom assay via a user-device such as Windows

Tablet and save it on the controller. These assays can be executed independently i.e. without the intervention of any host / user device.

The systems allows operation in two modes:

1. Normal mode
2. Report and Control Mode

12.6 Normal Mode

In normal mode the controller independently runs the selected assay, once it receives the command to start assay execution from a scalability host or a directly connected user device. All the peripherals are controlled by the embedded software in order to execute the assay. The assay progress and results are notified on all connected devices as the assay executes this provides the user devices with data to plot cycling and melt curves. As the assay execution is independent, in case of loss of communication between User Device and microcontroller, the last assay data is stored in the microcontroller, which can be retrieved by any connected user device.

The data stored during assay execution includes, cycling data points and melting curve data points. Although the melting curve data saved is sampled after every 1 second, whereas if user device is connected during assay, a smoother melting curve can be generated at user device as the data is sampled and provided every 100ms to the connected devices

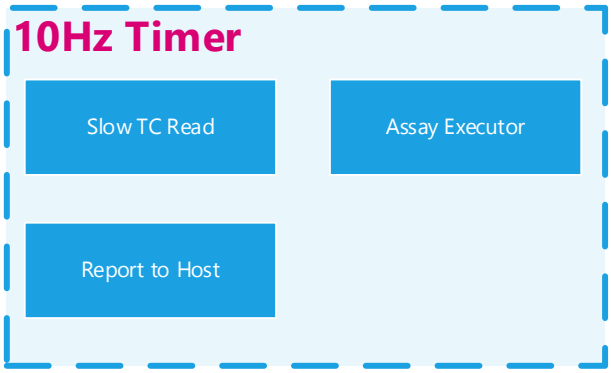
12.7 Report and Control Mode

Report and Control mode adds to the flexibility of system, in this mode the controller must be connected to a host (scalability host or user device). In this mode the host selects/enables the desired sensors connected to controller. Then the controller sends the most recent buffered values from those sensors to the host every 100ms. The host can then send commands to the controller in order to control the Heater PWM, Cooler state, LEDs state, and configure certain sensors parameters. This mode gives the host flexibility to execute an assay itself by controlling the peripherals connected to microcontroller. This feature is also useful for research and debugging purposes.

Certain error checking and reporting features are implemented in the system, so that if any sensor develops an error it is reported on the user device which helps in resolving any hardware issues that arise.

12.8 Processes

All the processes on the system are based on timers and interrupts.

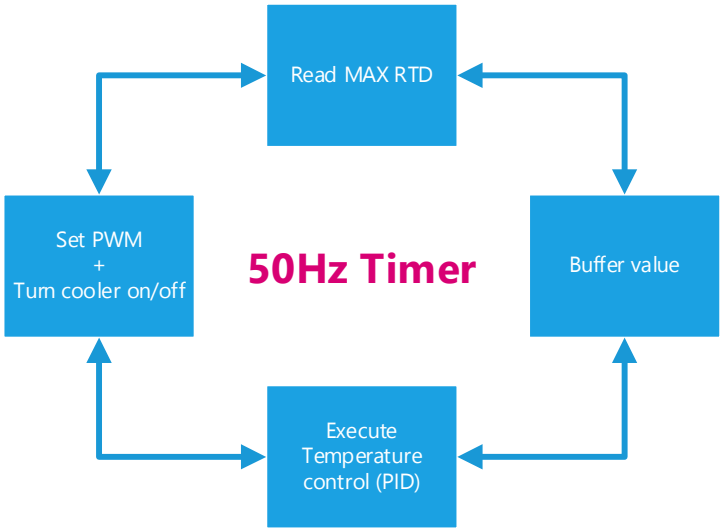


A 10Hz timer with pre-emptive execution is used for assay execution to count the step times, sampling temperature from Thermocouples and for reporting to host.

The data reported to host regularly after every 100ms includes:

- Melting graph plot points
- All sensors latest buffered values (In report and control mode)

Whereas the cycling graph plot points are sent right at the end of each cycling step.



MAX RTD supports faster sampling with maximum sampling time being 21ms, therefore a faster timer is used to sample the temperature from RTD, and this temperature value is buffered so it can be accessed by the 10Hz timer while reporting temperature data to host. Faster sampling and passing of process variable, that is temperature in this case, to the temperature control function allows improved control. The current temperature control method is a simple PID control with modifications for derivative kick and integral windup only. The output is of PID is bi-stable cooler, and PWM to control the heater which gets updated every 20ms.

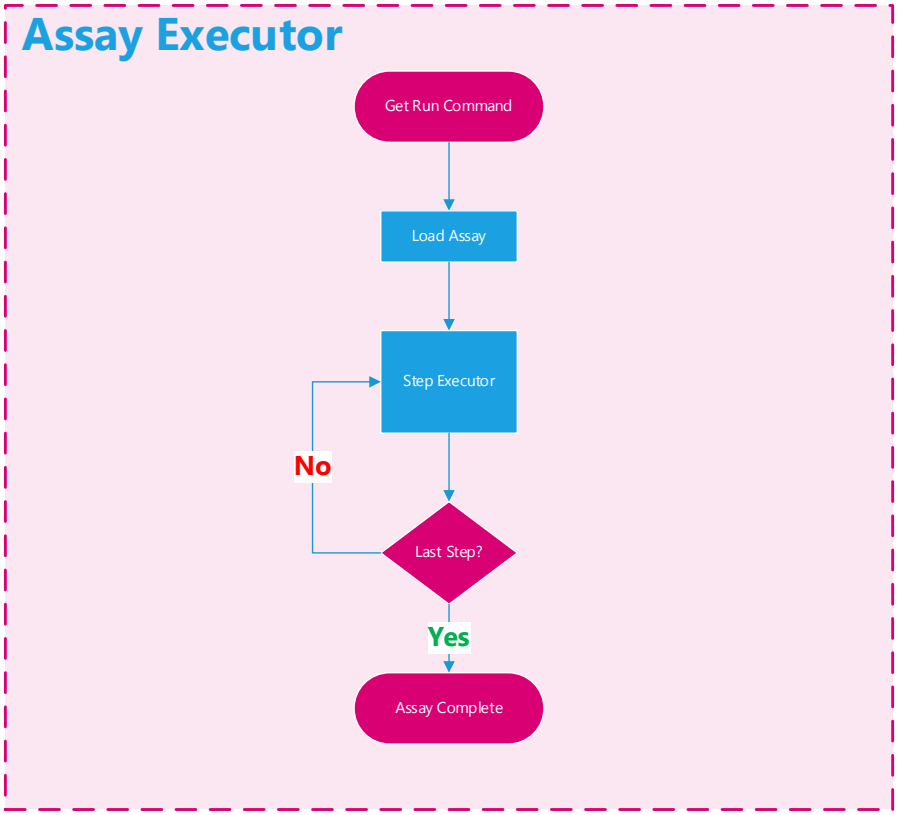
12.8.1 Assay Execution

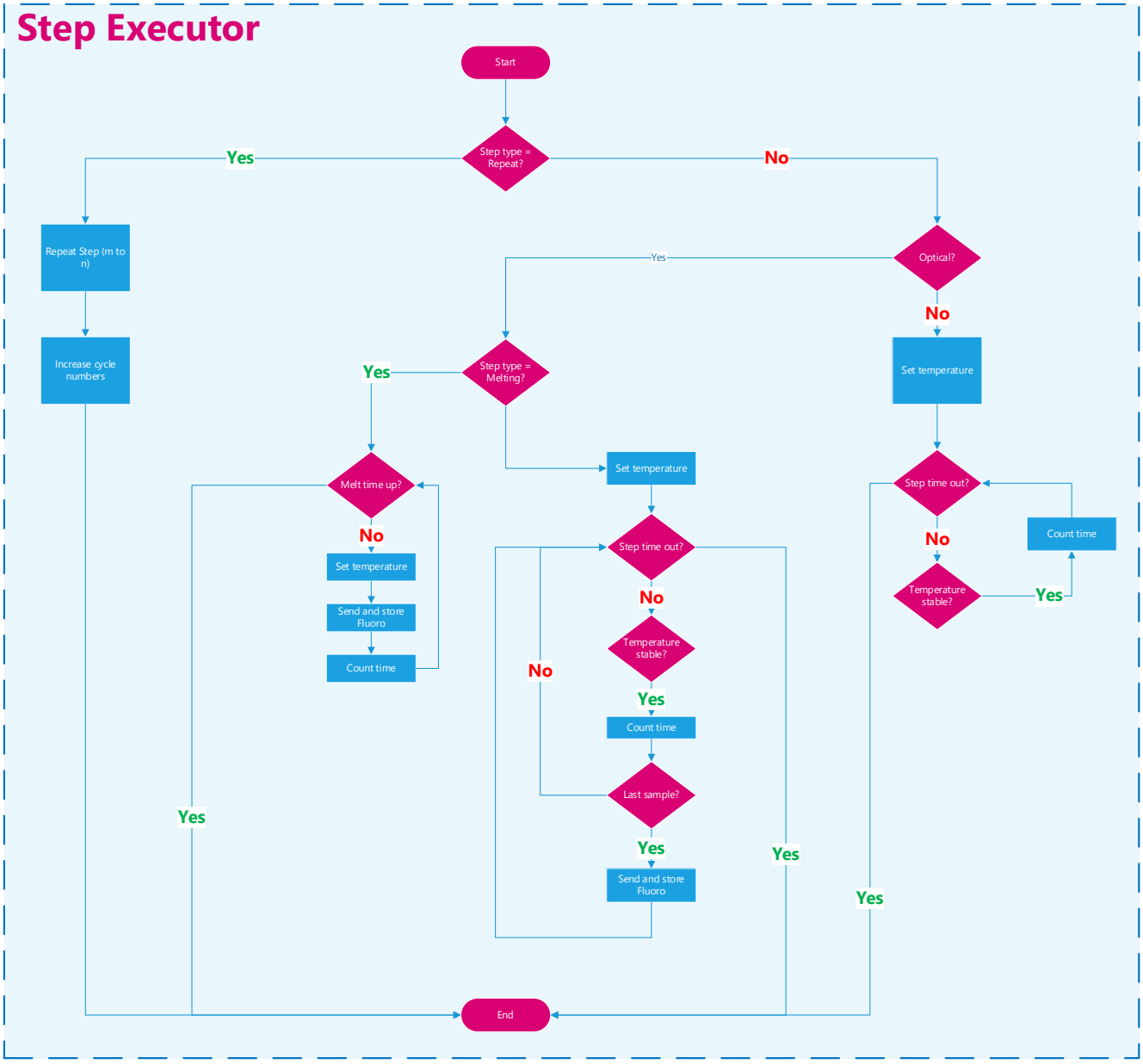
The assay executor starts running once a start assay command is sent from any connected host. The start assay command specifies the stored assay index. A custom assay can also be started by first loading the custom assay at specific index and then sending a command to start it.

A QPCR type assay has three types of steps:

- Set Temperature
- Set Temperature and read fluorescence
- Melting step

All steps have a fixed duration defined. Apart from these three steps, a repeat step can be defined, which repeats steps from one index to another. In this way completely customized assays can be executed.


Assay Executor
Get Run Command
Load Assay
Step Executor
No
Last Step?
Yes
Assay Complete



Once a start assay command is received, the assay to be executed is loaded in RAM via IAP. Each step is executed one by one until end of assay is reached. On a simple set temperature step, the Set Point of Temperature Control method is set. The time is counted once the temperature reaches close to the Set Point, and is considered to be stable. The maximum difference between Set Point and current temperature, before the temperature is considered stable, is defined by a configurable parameter “Temp Stable Range”. Which can be adjusted increase performance or to decrease overall assay execution time.

If a read fluorescence step, lies in a repeat cycle, then it's considered that cycling is in progress. In cycling the temperature is maintained similar to a normal step but right before the start of next step a the latest buffered fluorescence values for plotting cycling graph are stored in microcontrollers memory and also sent to the connected host

In a melting step the temperature set point is changed by either one of the policies:

- Temperature is changed every 100ms
- Temperature is changed in steps, according to the "melt step value" parameter defined in an assay

The fluorescence values are reported to the host every 100ms, also the values are stored every second in microcontrollers memory for retrieval if needed after assay completion.

12.8.2 Communication Handler/Responder

All communication with user devices is done via UART. CMSIS UART abstraction is used to implement interrupt based communication responder. The communication is done according to a custom Serial Protocol (see Appendix).

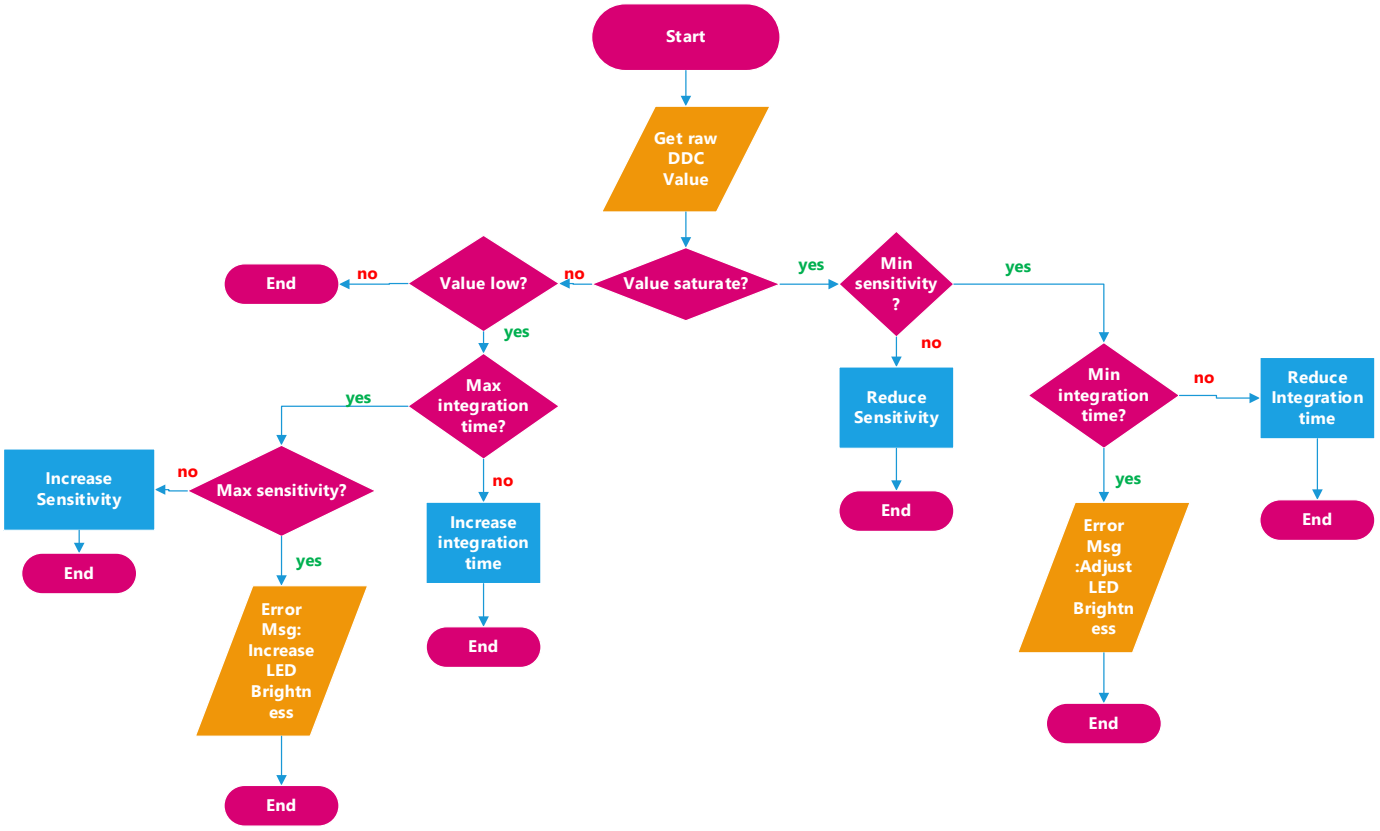
12.8.3 Fluorescence Reading and LED Control

Fluorescence is read by using a photodiode which is connected to DDC114 that a Current ADC. There are four channels available on the system which allows a multiplex reaction to be run. While measuring the value for once channel only the respective LED needs to be switched on. The DDC114 is used continuous reading mode and uses a swing buffer for the four channels.

A match timer with direct external out is used to toggle DDC_CONV input on DDC114, which switches current integration from channel 1 to 4. Respective channel LED is turned on and rest of the LEDs are kept off at every match timer interrupt. The duration of match timer, is the time that DDC integrates current on that channel.

DDC also allows its sensitivity to be configured, in order to increase range or sensitivity. Therefore to adjust the range, either sensitivity or integration time can be adjusted. These parameters can be saved in microcontroller.

For better performance, the integration time and sensitivity is dynamically adjusted at run-time. The following flowchart explains the dynamic range adjustment.



12.9 Drivers Description

12.9.1 IAP

A driver for In Application Programming is used to read and write on controller's flash memory while the system is running. The controller used has a 512kb Flash memory which is utilized, apart from storing code, for storing pre-defined and user-defined assays, and configuration parameters.

The configuration parameters data such as:

- LED Brightness settings for 4 channels
- DDC settings
- PID parameters

12.9.2 MCP4342

MCP4342 is the digital potentiometer used to set a resistance to control current provided to LEDs via CAT4109 LED driver. The driver uses CMSIS SPI abstraction to communicate with the digital potentiometer IC. The driver allows setting resistances, and moving the potentiometer wiper in single increment or decrement steps.

12.9.3 DDC114

DDC114 is a current ADC, which is used to measure the current from photodiodes. The driver uses CMSIS Synchronous serial port abstraction to communicate with the DDC. The driver configures and initializes the DDC, provides sensitivity adjustment and allows reading of values. The values are sampled from DDC on interrupt, and the sampling frequency depends on the integration time set.

12.9.4 MAX 31865 – RTD

The MAX31865 is an easy-to-use resistance-to-digital converter optimized for platinum resistance temperature detectors (RTDs). An external resistor sets the sensitivity for the RTD being used and a precision delta-sigma ADC converts the ratio of the RTD resistance to the reference resistance into digital form. The MAX31865's inputs are protected against overvoltage faults as large as Q50V. Programmable detection of RTD and cable open and short conditions is included.

12.9.4.1 *Converting RTD Data Register Values to Temperature*

The ratiometric ADC conversion results found in the RTD Data Registers can be converted to temperature with a few calculations. First, the Resistance of the RTD needs to be determined with the following equation:

$$R_{RTD} = (ADC\ Code \times R_{REF})/2^{15}$$

And then actual temperature is calculated by the following equation:

$$Temperature = \frac{\{Z_1 + \sqrt{Z_2} + (Z_3 + R_{RTD})\}}{Z_4}$$

Where,

Z_1 = 390830

Z_2 = 1758

Z_3 = 2

Z_4 = 115

The above formula is derived from Callendar-Van Dusen equation.

12.9.5 MAX 31866 - TC

MAX 31866 is the interface IC for connecting thermocouples. The driver uses CMSIS SPI abstraction to communicate with this IC. The driver configures the TC interface IC, provides fault identification, and reads the temperature values at the maximum available resolution that is 0.25 C.

13 End User Software Products

Cell phones have incredible penetration into even low income areas. Using technologies which leverage smartphone and cell phone networks can be transformative[199] [200].

End user products include software applications for different platforms such as Windows 8, Windows phone 8 and android. The overall requirements, architecture and major methods are common for all platforms to make management and future development less time consuming. Still there are few things that are tailored according to the platform specifications so that the applications can get maximum benefits from the native platform's API.

13.1 Requirements

1. Two-way communication with embedded software.
2. Standalone software (can be open or closed source)
3. Ability to run and abort assays of all types i.e. qPCR, S2A, SP.
4. Ability to create, edit, delete assays of all types.
5. Show real-time graphs specially fluorescence vs cycles and fluorescence vs temperature graphs. (software can also show temperature vs time graph but its optional)
6. Ability to show multiple fluorescence channels.
7. Ability to add/ load patients.
8. Management of patients and operators/ admin by either creating a custom EMR system or using an already developed one.
9. Saving, loading and analysis of each test's data, graphs and results.
10. Management of patient's history and symptoms and allow the operator to get suggestions based on geography and symptoms of the patient.
11. Ability to chat with the available doctor based on geography.
12. Real-time status of running assay i.e. running step type, cycle number in case of cycling.

13.2 Workflow

The workflow across all platforms is consistent.

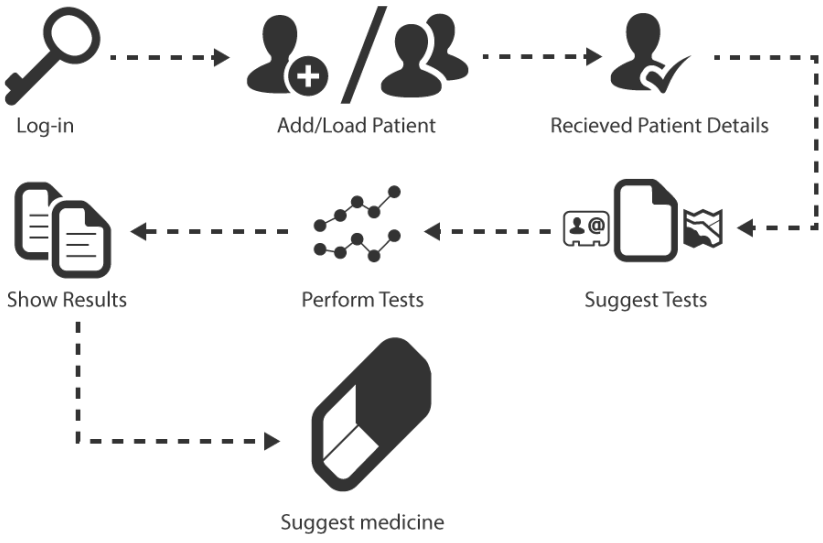
13.2.1 Summary

The basic steps to perform a test are given below:

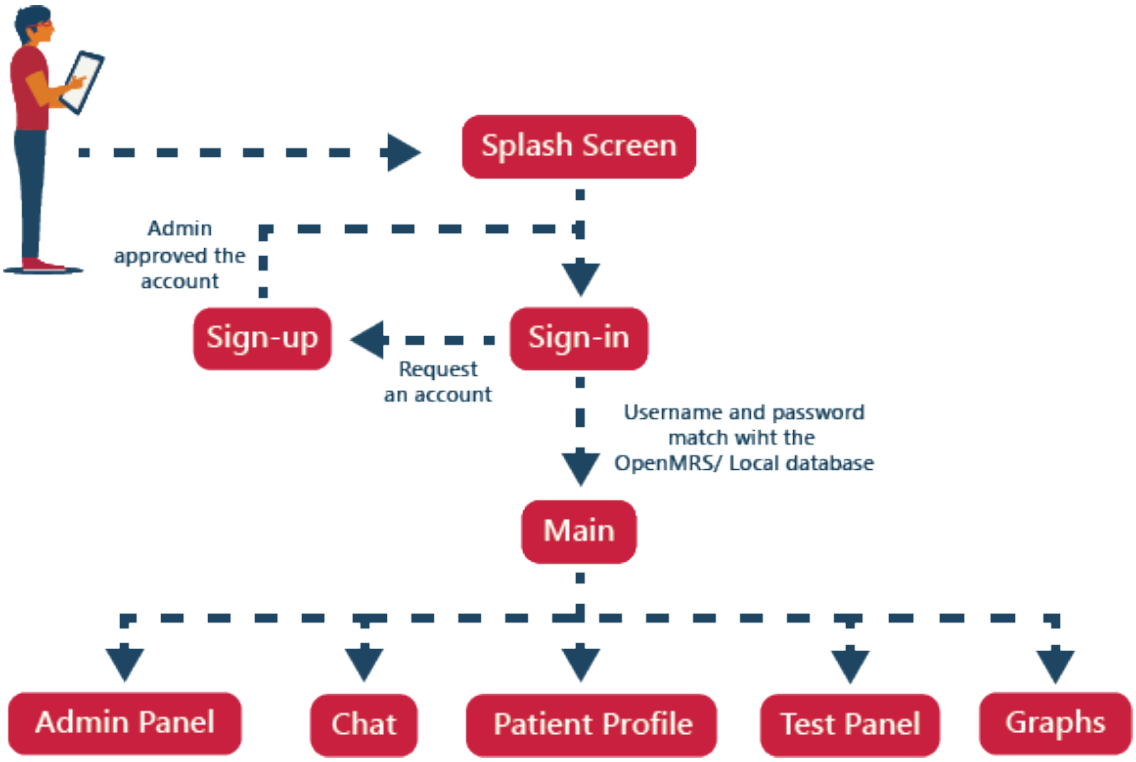
1. Login to the application.
2. Add patient or load patient's data (personal information, history and diagnosis).
3. Suggest a test based on patient's history, geography or as advised by the physician.
4. Find the hardware device containing embedded software.
5. Connect to the available device
6. Select test
7. Perform test

Show results and save them for future use

8. Take necessary actions on the basis of the result i.e. suggest a medicine, refer to a hospital or suggestion for more tests.



13.2.2 Detailed Workflow



13.2.2.1 *Sign-in*

At the start of each application the user will be prompt by a login screen. User will need to enter credentials such as username and password. The alternative way to log into the system is via smart card.

13.2.2.2 *Sign-up*

If the credentials do not match with the local database or the EMR system's database then the user will be asked to sign-up by filling a form. Once the user sign-up, the admin can activate account on behalf of each user.

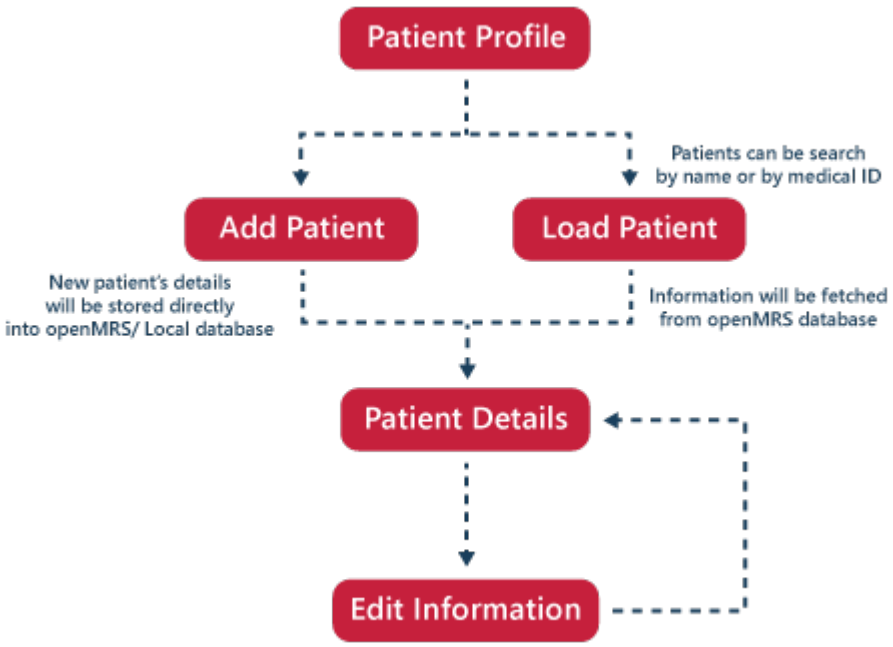
13.2.2.3 *Main*

Although the main screen is a little different depending on the platform of the application but the purpose of each main screen is same and that is to present different options to the user such as,

- Options for adding, editing, and loading a patient. (Patient's Profile)
- Options for performing a test including options for creating, editing and loading an assay. (Test Panel)

- Options for viewing different graphs and assay's status. (Graphs)
- Options for chatting with a doctor. (Chat)
- Options for Admin settings and records. (Admin Panel)

13.2.2.4 *Patient Profile*



Patients profile is further divided into number of options

13.2.2.4.1 Add Patient

User can add a patient into the database after filling a form. Form includes following fields.

1. Name
2. Sex
3. Medical ID
4. Country
5. Notes

User can automatically get patient's details via barcode or smart card.

13.2.2.4.2 Load Patient

An existing patient can be load into the application either searching by patient's name of medical ID.

13.2.2.4.3 Edit Patient

Patient's details can be edit at any time provided that the user has privilege to edit it.

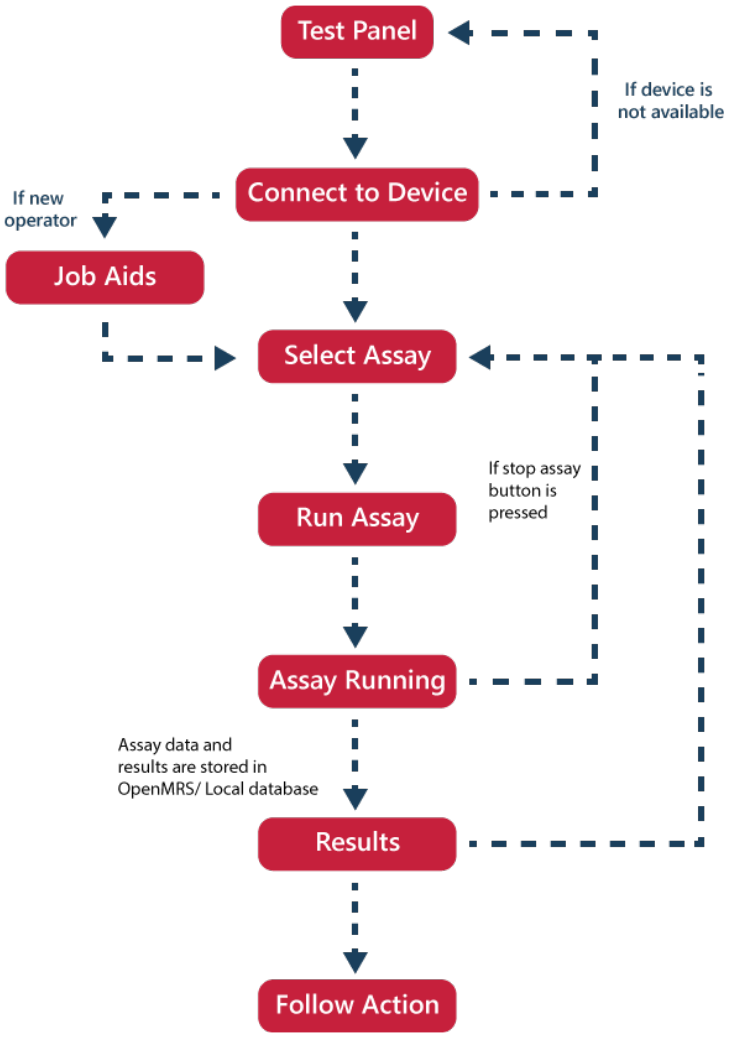
13.2.2.4.4 Patient's History

Application will automatically fetch patient's history if available. Patient's history include pas test records, symptoms, critical allergies etc.

13.2.2.4.5 Diagnosis

Application's diagnosis algorithm can suggest a test based on patient's history geography. Beside this a doctor can also suggest a test to perform.

13.2.2.5 *Test Panel*

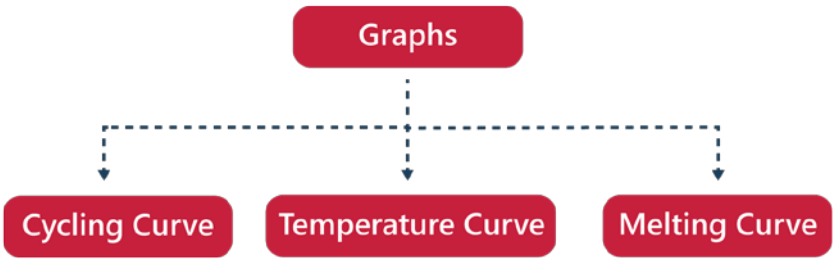


1. First it give options to find and connect to the device.
2. Once connected a job aid will be shown on the screen if the user is relatively new. User can either skip it or read all the steps.

Options for selecting a test will be shown on the screen, user can load a test of any type saved in the local memory or can select a default test to perform

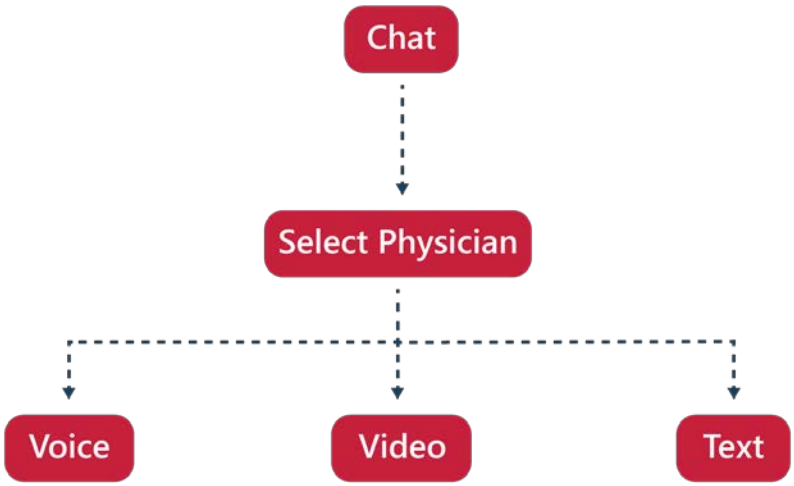
3. Once test is loaded into the system, button to perform the test will appear/ enable. User can click it to start performing the test.
4. User can abort a test midway if there is something wrong.
5. Once test is complete results will be displayed on the screen and will be stored into the database.
6. After that further actions can be taken accordingly.

13.2.2.6 *Graphs*



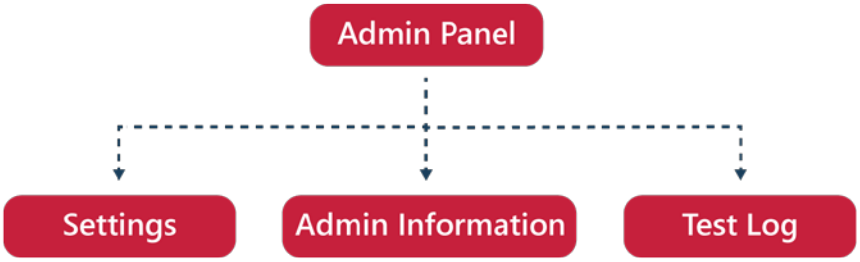
The graphs panel can show graphs of different types such as temperature curve, amplification curve and melting curve. User can view different fluorescence channels.

13.2.2.7 *Chat*



User can chat with a doctor based on the geography and availability. Chat can be either text, voice or video based again depends on the availability.

13.2.2.8 *Admin Panel*



The admin panel include options such as

13.2.2.8.1 Settings

It include different settings depending on the platform, some of the common settings are

- Access level
- Mode of operation

13.2.2.8.2 Admin Information

All the personal details of the user will be displayed under this tag. These details include

- Name
- Lab/ hospital name
- Contact number

It also include options to edit personal details and to change password.

13.2.2.8.3 Test Log

All the records about the test performed by the user will be shown under this tag. Every performed test according to the date will be shown in the form of a table and user can expand each row to view results and further details such as graphs etc.

13.3 Key Methods

13.3.1 Assay Parsing

Each assay is defined as a text which can be easily understand with very less knowledge about the system. This text base assay is parsed to get the details about each step.

13.3.2 Load to ARM

All the parse assay data is then converted into bytes according to the serial protocol given in Appendices. This bytes based data is then send to embedded device to take further action.

13.3.3 Serial State Machine

The data received from the embedded device is transferred to this method which decode it byte by byte so that the application can understand the received data. Received data is decoded according to the custom serial protocol, details about this serial protocol is given in Appendices.

13.4 Methods for Communication

13.4.1 Bluetooth

Applications communicate with the embedded device via Bluetooth. First the application find the device and then establish a connection to the embedded device. This is a two way communication in which the application continuously receive data from the embedded device while embedded device can continuously receive commands and data from the applications. Right now only a single device can

connect to an end-user application but the embedded device can simultaneously send and receive data from PC based debugging software.

13.4.2 Wi-Fi/ Ethernet

Wi-Fi/ Ethernet is use to communicate between the applications and EMR's data servers. These servers can be local or world-wide, depend on the complexity of the deployment. Again this is a two way communication. In case of non-availability of internet the data is stored in the application's local storage which can be sync with the main server when available.

13.4.3 Why not Wi-Fi for embedded device?

First the communication between Windows 8 application and embedded device was via Wi-Fi. Now with the additional communication between EMR servers and application via Wi-Fi, there was a need for an alternative mode of communication for embedded device since most of the tablets and smart phones can only connect to a single Wi-Fi hotspot. Thus the other most easily available method was Bluetooth which was finally used between end-user application and embedded device.

13.5 Windows 8/8.1 Application

Microsoft Windows has the biggest share in OS market and with the launch of Microsoft Windows 8 the line between a PC OS and OS for tablet is blurred which makes it even easier for the developers to develop one app that can work both on personal computers and tablets simultaneously. Some of the key benefits of Microsoft Windows 8 over other tablet OS are given below,

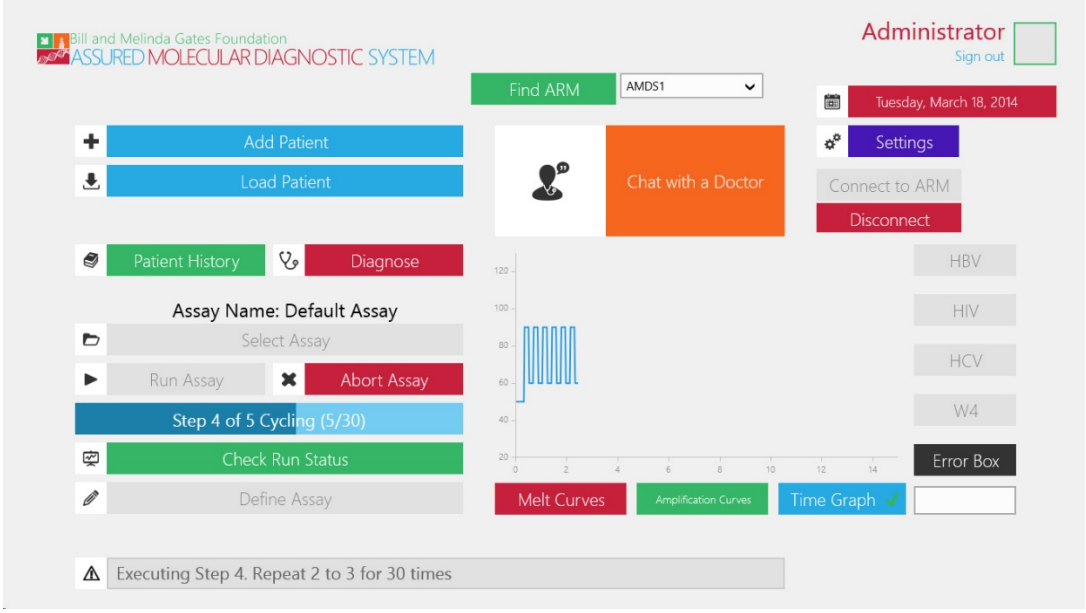
13.5.1 Benefits

1. *Single OS:* The biggest advantage of Windows 8 as mentioned earlier is that user can have a single OS on personal computers and tablets which makes it easier to develop, use and manage.
2. *Multiple users:* Tablets are the perfect form to pass around so others can share them. They are also very personal devices and sharing them properly requires a way to keep others out of your stuff. Windows has long supported multiple user accounts on a single PC and this can be leveraged on Windows 8 tablets.
3. *Use any peripherals:* The Windows ecosystem of third party peripherals is huge, and that extends onto the tablet. For example, it is easy to connect a 1TB portable hard drive for handling lots of large files on the go.

4. *Snap view:* Microsoft built the ability to run and display two apps side-by-side, a feature called snap view. This is a very useful way to work with two programs together. Some Samsung Galaxy Android tablets have this capability (called multiview) but it is more limited than the Windows 8 snap view.
5. *File encryption:* Security is important, especially for mobile devices that can be lost or stolen. A good way to keep data private no matter what is to encrypt everything so it can't be viewed without a security key.
6. *Run powerful software:* Windows 8 can run any legacy software which means even powerful programs like AutoCAD and Photoshop can be installed and used.

13.5.2 User Interface

Windows 8/8.1 application is completely designed keeping in mind guidelines of metro UI style established in Windows 8 onwards. Major benefit of this UI style is that keeps design overheads as less as possible and most of the UI styles can be easily generated via code. Metro UI also use less space in the memory make it ideal for portable devices such as tablets. Another benefit of metro UI is that it's developed especially for touch systems which enhance user experience and allow to get maximum efficiency from the PC and tablet's touch hardware.



13.5.3 Tools Used

1. Microsoft Visual Studio 2012, 2013
2. Telerik controls for Windows 8/8.1

13.6 Applications for Mobile Platforms

13.6.1 Comparison between Android, WP8 and iOS

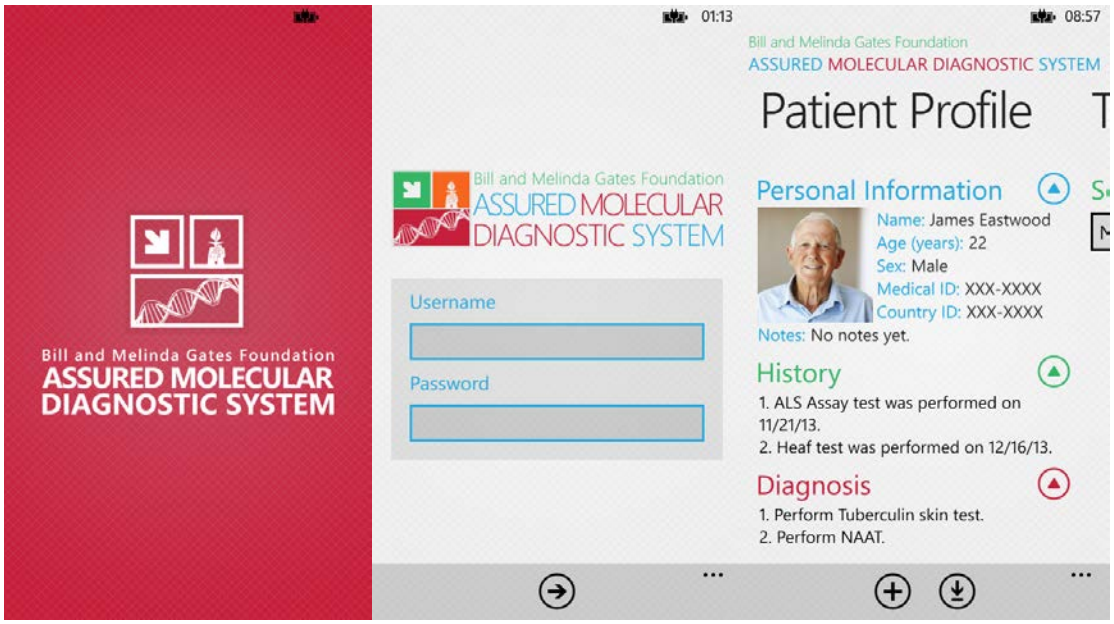
	iOS	Android	Windows Phone 8
Market share	14.2%	79%	3.3%
Unit Price (average)	$635	$268	287
API	Closed source	Open source	Closed source
Developer Community (Size)	Big	Biggest	Medium
Annual growth rate	10.9%	9.5%	46.2%
Native development language	Objective-C	java	C#

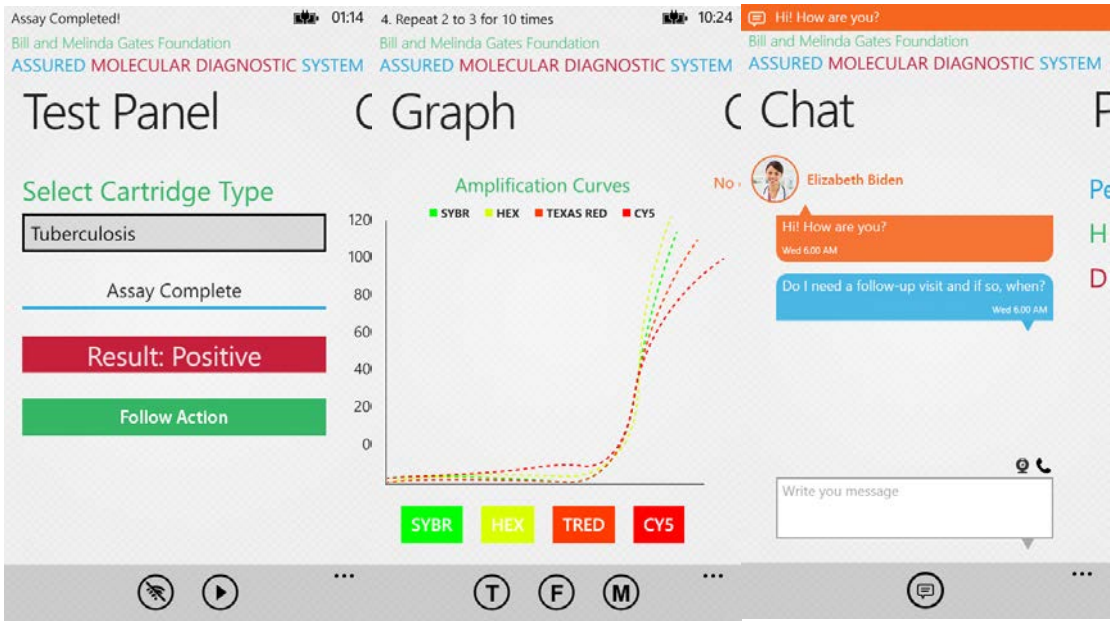
13.6.2 Windows Phone 8 Application

Windows phone 8 was chosen as the first mobile platform for our mobile application since it was easy to port windows 8 application to windows phone 8 considering both follows same development language and same UI style.

13.6.2.1 *User Interface*

Similar to Windows 8, for windows phone 8 same metro UI style and guidelines are followed. To make it easier for the user similar program hierarchy is used in the windows phone 8 app as in Windows 8. But one thing is kept in mind that the mobile app should be mobile friendly and gives a user a close feeling to native windows phone 8 application.





13.6.2.2 *Tools*

1. Microsoft Visual Studio 2012, 2013
2. Telerik controls for Windows Phone 8

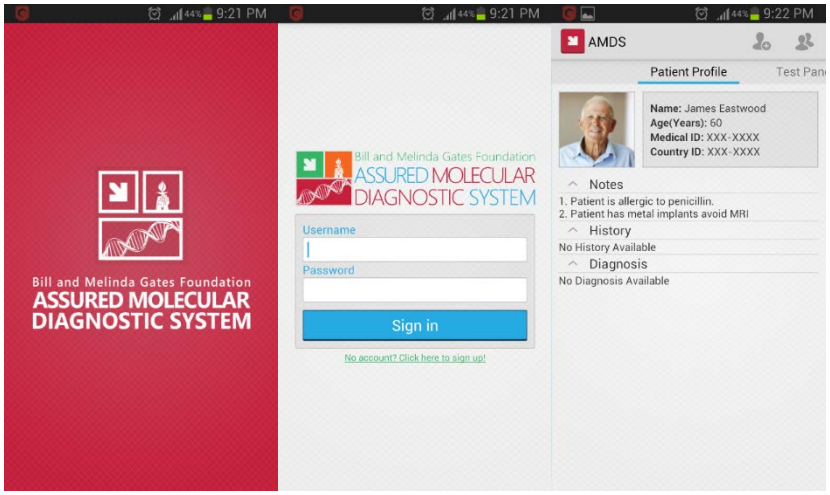
13.6.3 Android Application

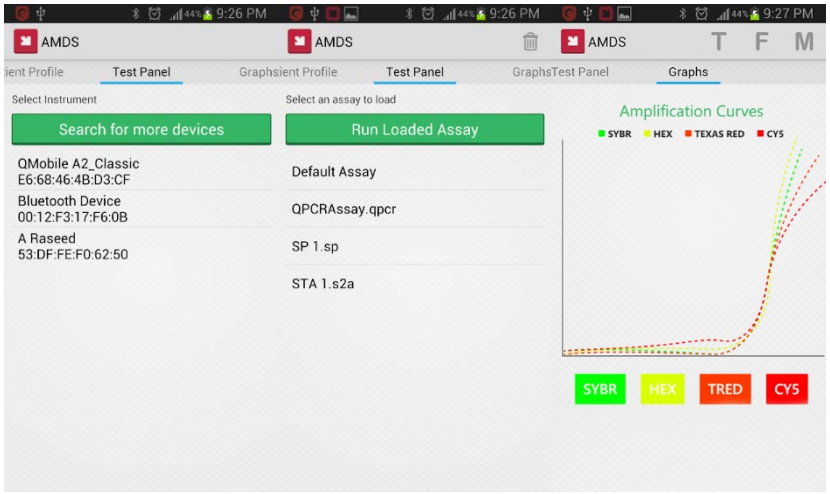
Since android is the most used mobile platform with market share of 79%, it was compulsory to develop an android application for our system so that more users can get benefits from this system. In order to

decrease porting time from previous C# based applications (W8 and WP8) to a java (Android) based application we used xamarin and hence we wrote the android application in the same C# environment. This not only helped us to decrease porting time but also allowed us to maintain critical methods once and thus our applications is less prone to logical errors.

13.6.3.1 *User Interface*

UI design and development for the android application was little tricky since we need to keep a balance in style so that the user must feel the android application closer to the applications of other platforms but at the same time user must feel it as a native android application. Hence same Windows phone 8 color combination and program hierarchy is followed but the UI elements are placed according to android UI guidelines.





13.6.3.2 *Tools*

1. Microsoft Visual Studio 2013
2. Xamarin
3. TeeChart for .NET (Xamarin) Chart for Android

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