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Automation of Tangential Flow Filtration for Purification of Biosynthetic Spider Silk

Candace N. Clark
Utah State University

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AUTOMATION OF TANGENTIAL FLOW FILTRATION FOR PURIFICATION OF BIOSYNTHETIC SPIDER SILK

by

**Candace N Clark
Eric Anderson, Andrea Olson, Cole Peterson**

**Thesis submitted in partial fulfillment
of the requirements for the degree**

of

**HONORS IN UNIVERSITY STUDIES
WITH DEPARTMENTAL HONORS**

in

**Biological Engineering
in the Department of Biological Engineering**

Approved:

Thesis/Project Advisor
Dr. Randolph V. Lewis

Departmental Honors Advisor
Dr. Wynn Walker

Director of Honors Program
Dr. Christie Fox

**UTAH STATE UNIVERSITY
Logan, UT**

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Abstract:

The objective of this project is to create a user-friendly, automated flow regulation system for a tangential flow filtration (TFF) process. These filtration units are crucial for the initial stages of extracting the spider silk proteins from transgenic goat milk. Automated flow regulation systems do exist, but not at an appropriate scale for this application. This system will allow bench-top studies to be conducted with automated efficiency. Spider silk is an amazing material and if commercially available, it could be used in hundreds of applications. Currently, cost effective production is the most significant issue stopping large-scale production and use of spider silk. An increased availability of spider silk would allow for exploration into new applications. The TFF system already produces valuable spider silk protein and automating the process will result in increased quantities of spider silk proteins. With larger amounts of protein available, researchers are better able to explore the many exciting applications of spider silk proteins.

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1 Project Summary

The aim of this project is to design an automated tangential flow filtration (TFF) system in order to purify spider silk proteins from transgenic goat milk (Figure 1). The outcome of this design project will be a mid-scale filtration system suitable for providing a working stock of spider silk proteins.

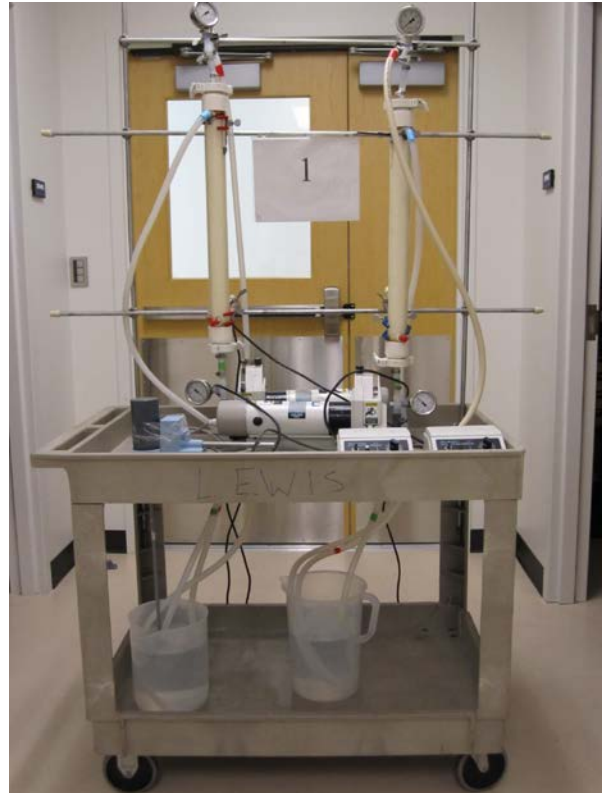


Figure 1: Image of Current TFF system in storage

2 Aims

The objective of this project is to create a user-friendly, automated flow regulation system for a tangential flow filtration process currently in use. These filtration units are crucial for the initial stages of extracting the spider silk proteins from transgenic goat milk. This process is going to be used for multiple years, so establishing an automated system will save countless hours and large amounts of valuable product over the course of its use. Some aims and criteria include:

- Increased operational efficiency
- Wide applicability – can be used in different systems
- Controls – easy and accessible
- Computer Regulation - computer program to control the flow
- Flow Regulation-This is the main design concern. It will cut down user input and reduce overflow concerns.
- Quality Control

This system design is possible and very practical. Automated flow regulation systems do exist, but not at an appropriate scale for this application. Our system will allow bench-top studies to be conducted with automated efficiency.

3 Background

3.1 Spider Silk

Spider silk is an extremely impressive material with not only high amounts of tensile strength, but also a large range of elasticity. Commercializing the production of spider silk is a goal of Dr. Randolph Lewis that could be very beneficial for multiple industries. Producing spider silk on a large scale has been a challenge in the past, as spider farming is impractical. The spiders are territorial, cannibalistic and simply do not produce quantities of silk large enough to be utilized in industry.

Several innovative methods have been developed to produce spider silk fibers at larger levels. One method involves using transgenic goats. Each goat contains one of the two genes (1) that encode the spider silk protein that is produced in their milk. This milk is collected and processed through a TFF system. The spider silk proteins are separated from large fats and proteins. This concentrated protein is collected from the TFF and further processed. The final result is purified, synthetic spider silk. Though not as strong as natural spider silk, synthetic silk is still a strong competitor against materials such as steel and Kevlar (5). As of now, 6-8L of milk can be processed through a filtration system to obtain roughly 1000 meters of silk. One of the challenges now is to find ways to modify the synthetic fibers to make them stronger. This is done through trial and error, using a variety of experiments. However, to conduct any of these tests, purified silk protein must be available.

Currently, the most effective way to acquire more spider silk proteins is to increase the operational efficiency of the TFF system. This is the major reason for designing an automated control system. After working with the TFF process for 5 months, the need for an overflow failsafe is evident. It is frustrating to have an entire milk run compromised due to overflow or a hose failure. It also takes a long time to clean up and to clean out the filters once the system has become imbalanced. Many laboratories are manned by undergraduates who cannot afford to be at the laboratories all day. This means that there is even more risk in letting the system alone, since there is very little time dedicated to balancing the flows. Having a flexible system that can be scaled up easily will also be beneficial when larger amounts of milk become available to process.

3.2 Tangential Flow Filtration

There are a variety of tangential flow filtration systems with automated features on the market, so what is the value in designing a new one? While there are several systems available, they are extremely limited in their size options. The current TFF system has expanded beyond the bench top but is far from being industrial scale. The system has a current working volume of 6-8L, and could possibly be increased to 14-15L in the future. There are mini-scale TFF systems designed

for bench top use, but they can only handle total feed volumes of 0.5L (2). Other small systems are also available, but have a maximum operating volume of 1L. Other systems available with feed automation and flow control are large scale, designed for volumes of 300L or more (3). Another concern is cost. While the larger systems could be implemented, they can cost upwards of \$200,000 and require a larger feed stream than currently available. Building our own system with flow regulation in place is estimated at under \$20,000 and it will fit the purpose of the lab much better. While industrial scale is likely a future possibility, arrival at that point demands a solution to the current volumetric requirements.

3.3 Current Extraction Method

The first step in processing the milk is to de-fat it so it can be applied to the TFF units (Figures 1- 2). This filtration system houses 2 columns with differing retention sizes. The first column contains a 750kiloDalton (kDa) filter. The milk is pumped into this column where smallfats and other contents are filtered out of the flow stream. The whey (product from column 1) is pumped to the second column. This column contains a 50 kDa filter. This filter traps the spider silk proteins and collects them into a second container known as concentrated, clarified whey (CCW). This permeate is then recycled back into the 750 kDa TFF column. The filtration must be repeated continuously because of the affinity for spider silk proteins to bind to small fat globules retained in the 750 kDa column. This process takes approximately 24 hours to reach a minimum purity standard.

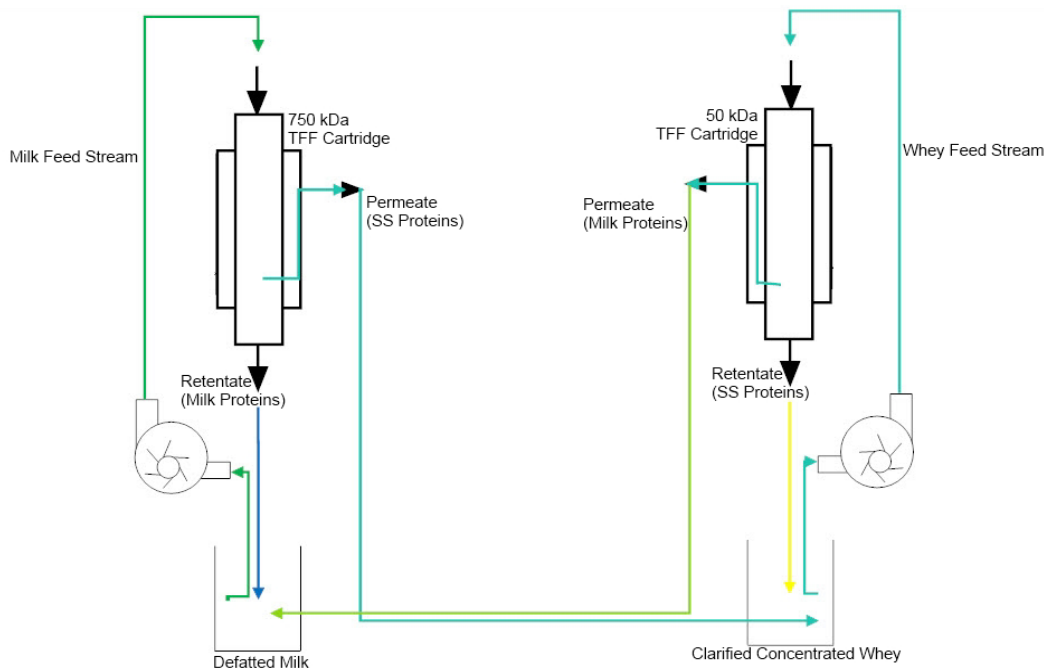


Figure 2: Overall Process Diagram. Defatted milk enters 750kiloDalton (kDa) TFF cartridge. Spider silk (SS) proteins are separated from the milk and are carried through the permeate tube to the concentrated, clarified whey container. The whey is then pumped through the 50 kDa TFF cartridge. The 50 kDa further purifies the spider silk, leaving spider silk proteins in the retentate stream and sending other proteins through the permeate tube to the 750 kDa TFF cartridge. This cycle continues for up to 24 hours to reach minimum quality standard.

All of the liquid streams are fed through the process using a system of peristaltic pumps. Both pumps run in tandem, creating a dual feedback loop. This feedback loop becomes unbalanced over time as the viscosity of the two liquids changes. This makes it necessary to comparatively maintain the liquid levels in the uptake containers. The current process requires lab personnel to manually balance the 750 kDa feed stream with the 50 kDa output stream at frequent intervals to ensure neither of the reservoirs overflow. It often takes upwards of six hours to successfully balance the two pumps. While manual balancing is effective, it is time intensive and is prone to failure. There is no way to predict the speeds of the flows for future runs, as each batch of milk varies in fat and protein content.

This process must run for 24 hours and it is unreasonable to have personnel constantly monitoring the process, often into the night. Overflow results in loss of the concentrated product in addition to tedious cleanup procedures. The filtration columns can also be damaged if allowed to run dry.

3.4 Available Tangential Flow Systems

Tangential flow filtration systems are widely available today, but only in very limited volume options. There are small scale TFF systems with automation, but they are designed for volumes up to 1L. Other large systems are available, but they operate at large volumes compared to our system, upwards of 300L (4). These systems can cost upwards of \$200,000 and would be too large to be practical for our requirements.

The entire process of extracting spider silk protein using this filter system is new, and as such, a filtration system specifically designed for this application does not exist. This is a novel attempt of scale up to commercialize biosynthetic spider silk production. Though filtration systems are available, they do not exist for the scale of this application. Designing this system will facilitate the overall production of silk proteins by simplifying the filtration process. This simplification will result in decreased personnel hours, thus more research can be accomplished.

4 Significance and Innovation

Though this system is only a part of the overall spider silk production process, it is critical to provide the necessary spider silk protein to conduct studies. Future applications of research findings may be important to textile and medical industries. It is possible that the need for mid-scale automated TFF systems will be realized by other researchers ready to scale their research up beyond bench-top application. It may also be a marketing opportunity to aid other labs in need of flow regulation for their systems. Since it is simple and adaptable, it can be converted for other systems.

This approach is innovative, as nothing exists commercially for flow regulation on TFF systems of this size. There are several automated systems available for small and large scales, but nothing for mid-scale applications. It is a simple design that will make the lab efficient and will be easily understood by new users.

5 Approach

The objective of this project is to create a user-friendly, automated flow regulation system for a TFF process currently in use.

We have opted to maintain the current filter and reservoir system because it is functional and does not contribute to the current flow problem (Figure 2). After a system of sensors has been optimized for the procedure, larger volume containers or filters of higher-flow potential may be considered. To address the most pressing problem of over/under filling, we propose to use sensors to determine the fill level of either container. The feedback from these sensors will be applied to a Visual Basic program, which will subsequently switch the appropriate pump on or off to prevent overflow or running dry. Once fill levels are within normal limits, the pump action will be reversed to allow the process to continue. This is effectively a mechanization of the current manual method, in which the operator observes the level of the whey container and turns the pump on or off. Future methods could be based on process parameters other than fill level, such as system pressure or flow rate.

5.1 Timetable

Completed

- Design: General design considerations will be worked out with the entire team, first with individual designs. Team collaboration will identify the most feasible and workable aspects of individual designs into a general, first design by Sept 20.
- Modeling: Numerical and visual modeling will be spearheaded by Eric and Andrea. Debugging of computer control programs will represent the greatest time requirement and may take two to three weeks. Modeling should be complete by November.
- Materials: Materials acquisition, headed by Candace, will be minimal. Three weeks' time will be allotted before beginning construction of a prototype. This can be done concurrently with modeling considerations, meaning modeling will be complete by November 1.
- Interim report filed by December 7.
- Construction: Construction phase will begin Spring semester with Cole taking lead.
- Testing: Testing will run as long as necessary, but a working model will be produced confidently by early February.
- Further revision: Remaining time until April 1 will be spent refining the apparatus.
- Report: Final report will be complete by April 27.
- Presentation: First week of May is when the team will report final results

See Appendix 1

6 Design Considerations

The following criteria were considered:

- Liquid level sensing: Non-contact sensors to reduce contamination and protein fouling. Allows for easy cleaning.

- Control pump setting: Turn pumps on/off depending on sensor readings. Pumps controlled by a relay.
- Simple user interface and operation: Easy set up and operation. Little training required to operate sufficiently. User guide provided to aid operation.
- Operate at 4°C: System mounted on a cart for easy transportation. Non-cold room compatible equipment made portable.

7 Design Process

Three main design areas were considered: sensor type, programming method, and hardware mounting.

7.1 Sensors

Multiple types of sensors were considered for detecting and monitoring the volume levels of the whey product (Table 1). Conductivity and mechanical floats were initially considered. Conductivity sensors proved to be unfavorable because multiple sensors were needed. Additionally, they required an analog-digital converter, operated in contact with the solution, and were expensive (\$75-100 per sensor). Mechanical floats also proved undesirable. While inexpensive (\$30 per sensor) and simple, they had large surface areas and were thus prone to contamination. Piezo-resonant sensors were also considered, but were eventually ruled out due to high cost (\$140 per sensor) and unpredictability. Electro-optical sensors were then considered and met the desirable criteria. The sensors chosen use infrared (IR) wavelength. They provide a measure of volume in the reservoir using a single sensor without making contact with the solution. They were also the cheapest sensor (\$13.60 per sensor). This sensor was specifically designed and optimized to communicate data to a computers I/O board.

Table 1: Sensor Decision Matrix

LIQUID LEVEL MONITORING AND CONTROLS				
Supplier	Part Name	Part Number	Type	Est. \$
Sensorex	CS150 K = 0.1	CS150	Conductivity	-
Sensorex	CS150 (TC) K = 1.0	CS150	Conductivity	-
Sensorex	Loop Powered Blind Contacting Transmitter	CT1000	Transmitter	-
Gems	Multi Point Level Switch	LS-3000	Float	\$150.00
Gems	Single Point Level Switch	LS-3000	Float	\$30.00
Warrick	Series 3R-3T Probes		Conductivity	\$80.00
Warrick	Series 3W Probes		Conductivity	\$20.00
Gems	Electro-Optical	ELS-1100	Electro-Optical	\$100.00
Gems	ExOsense Piezo-Resonance Sensor		Resonance	\$137.00
Phidgets	Sharp Distance Sensor 2D120X (4-30cm)	3520-0	Electro-Optical	\$13.60
Phidgets	IR Distance Adapter	1101-0	Adapter	\$10.70
Phidgets	Interface Kit	1018-2	I/O Board	\$77.60

7.2 Programming

There were many programming options available, three candidates were considered. C++ was free and flexible, but the team had no prior experience. LabVIEW was also considered. It was deemed unsuitable due to its expense and needless complexity. Visual Basic was chosen due to previous team experience and its cost (free). Visual Basic was preferred over Visual Basic for Applications (VBA), as Excel did not lend itself to our application. Visual Basic is a widely used language and vast amounts of information are available online. (See Appendix 2 for code).

7.3 Mounting Schemes & Hardware

Mounting of the sensing system was the final step to finish the volume control system (Figures 3-4). The infrared sensor had variable sensitivity at different distances. The movement of hoses and the bucket affected the sensitivity of the sensor. The first prototype was an over-bucket design. This proved to be unstable and not easily adapted to different buckets. Additionally, a propensity for contamination existed. A second design incorporated a side clamp-on method. This was easily removable and had no contact with the whey. Hoses interfered with the sensors, giving false readings. Foaming of the whey product was another problem and also contributed to false readings. The final design maintained a clamp on design and incorporated a pipe to isolate the sensor and stabilize it (Figure 5).



Figure 3: An over bucket mounting scheme. This design was ruled out due to needless complexity, instability, and potential for contamination.

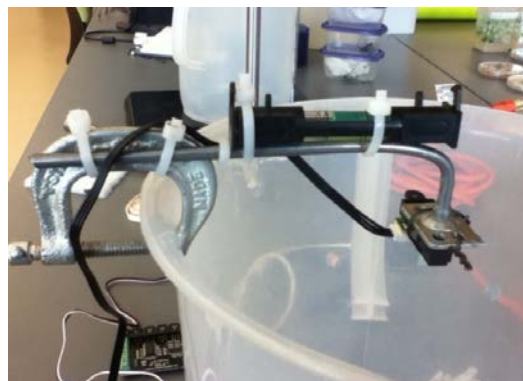


Figure 4: Clamp on design. This was chosen for its adaptability to different buckets, and simplicity



Figure 5: Final prototype. Note the addition of a PVC chamber for the sensor, this was added to prevent hose and foam interference with the sensor.

8 Final Design

The TFF columns were mounted on a wheeled cart with the hoses and buckets underneath the columns. The sensor, attached to a pipe, was placed in the product bucket, where it reads the liquid level. The sensor sends the value to an I/O board and then to the computer via USB. The VB program then compares the sensor reading to control parameters and sends an output signal to a relay. The relay is wired in line with the pumps' power supplies, and determines whether the pump is left on or shut off. This then regulates the levels of the fluid and prevents overflow over the course of the process.

9 Results

This automation system has been successfully tested in 8 runs. The system was maintained within acceptable parameters and no failure occurred. A Western blot was done to assure quality control standards in the automated runs (Figure 6). All standards were met and the automated TFF system was demonstrated to produce a similar protein quality to the unautomated process.

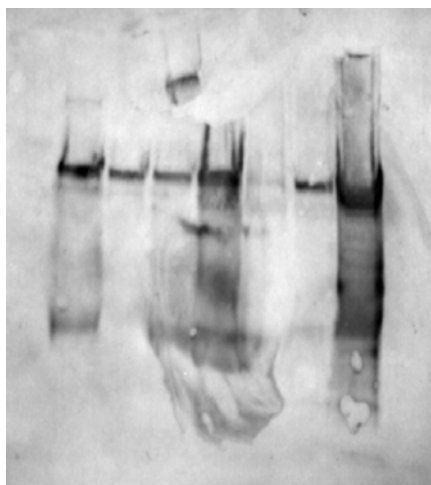


Figure 6: Western Blot demonstrating that a minimum quality control standard is maintained through use of the automated TFF system.

9.1 Cost Analysis

The project was initially estimated to cost around \$1000. After completing the final version, the total was \$796.15 (Table 2). This places the project 21% under budget. This cost estimate also includes parts that may need replacement over the lifetime of this system. Our cost analysis is only analyzing the volume control system and does not include the cost or lifespan of the elements of the TFF (pumps, columns, etc.) This new design saves time, estimated at being 75% cheaper per run based on reduced labor costs. After completing 8 runs, the system has saved \$480. At the present rate, the savings will exceed the cost of the system by May 30, 2012.

Table 2: Final Cost Analysis

Items	Cost
Computer	\$450
Interface Kit	\$80
Sensors and accessories	\$150
Mounting System and cart	\$100
Books	\$16.15
Total	\$796.15

10 Future Work

Future work will include the addition of a pressure monitoring device. This will serve to shut down the system in the event of catastrophic failure to preserve membrane integrity. Addition of a variable rheostat to control flow rates will also be considered. This would replace the hard pump shut off of the current system and allow for continuous pump operation. While this will considerably increase the complexity of the VB code, it may increase the efficiency of the process. A wireless interface will also be added to eliminate hazardous wiring from the cold room to the control computer outside. A data logging system, coupled with a remote failure notification system (i.e. SMS message/email notification of failure) will be implemented.

A Failure Modes Effect Analysis (FMEA) was developed to aid in future work (Appendix 3).

11 Conclusion

An automation system for the TFF was successfully developed. This system has replaced manual observation and control of the beginning purification step. With increasing need for silk in the future, a non-automated process would require a full-time position to continually control flows. Automation would be much preferred. In order to have industrial application for this process, automation would also be necessary. The risk of using a manually operated process would be costly and unacceptable. Spider silk is an amazing material and if commercially available, it could be used in hundreds of applications. Right now, cost effective production is the most significant issue stopping large-scale production and use of spider silk. An increased availability of spider silk would allow for exploration into new applications.

The TFF system already produces valuable spider silk protein and automating the process will result in increased quantities of spider silk proteins. With larger amounts of protein available, researchers are better able to explore the many exciting applications of spider silk proteins.

Description of Personnel

Team

- Candace Clark – Designer: Responsible for weekly progress reports. Prior experience with TFF, also other lab techniques. Experienced in instrumentation programming.
- Eric Anderson – Designer: Responsible for tasks not otherwise designated.
- Andrea Olson – Designer: Responsible for modeling, Prior experience with TFF.
- Cole Peterson – Designer: Responsible for construction and instrumentation selection. Extensive laboratory experience, knowledge base.

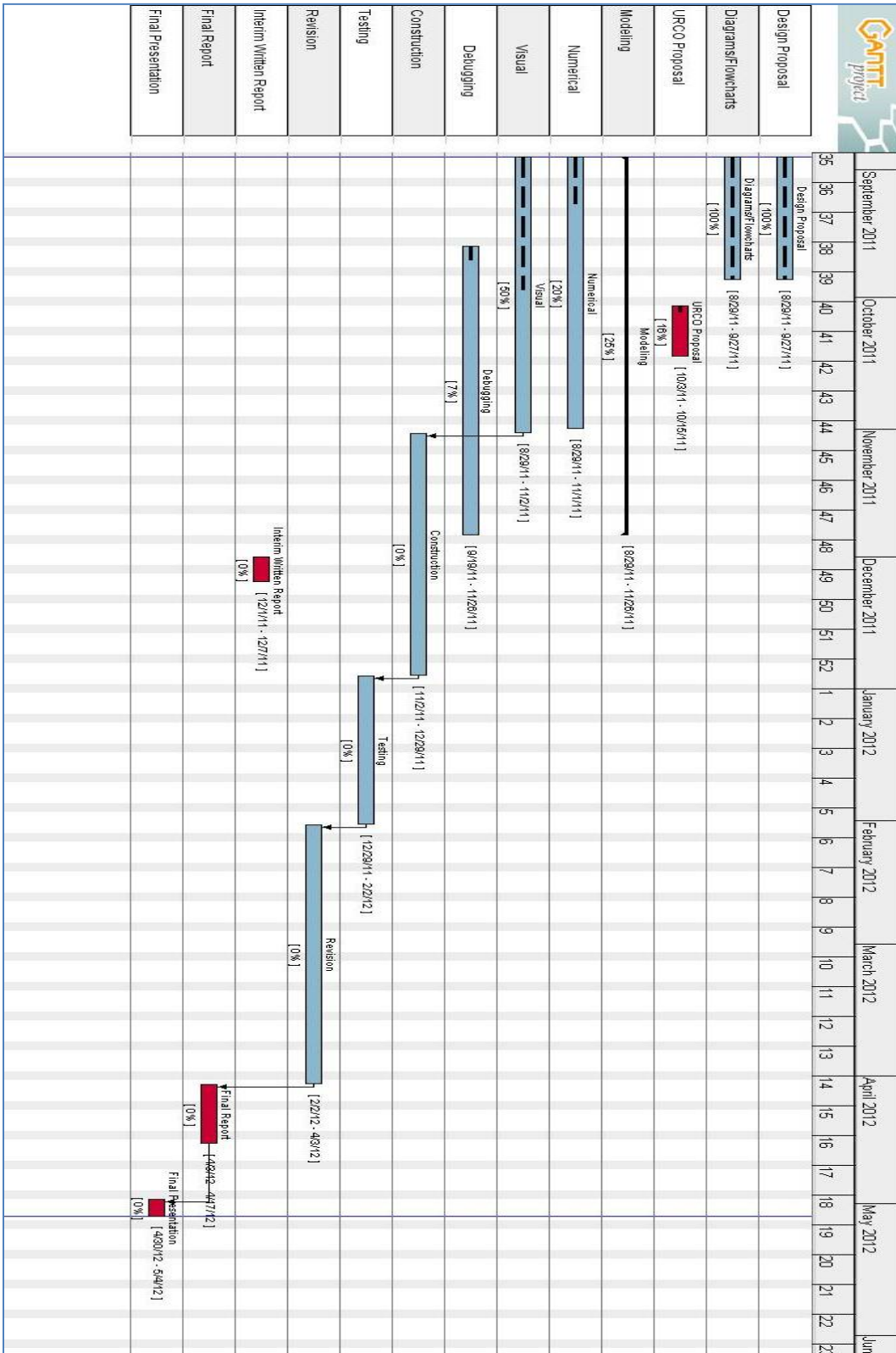
Support

- Dr. Randy Lewis –Primary investigator on spider silk research.
- Justin Jones, M.S. –Purchasing, project oversight and TFF training.
- Dr. Tim Taylor – Faculty Mentor: Advising, class instruction.
- Brianne Bell – Laboratory assistance

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Appendix 1: Timetable



Appendix 2: VB Code

```
PublicClassTFF_Control
DimWithEventsphidget_input, phidget_outputAsPhidgets.InterfaceKit
PublicStart_button, timer_conditionAsBoolean
Dim Alert AsNewList(OfString)
Dim low, high AsInteger
Dim val1 AsDouble
Dim distance, boxwidthAsDouble
Dimhightripped, lowtrippedAsBoolean

PrivateSub Form1_Load(ByVal sender AsSystem.Object, ByVal e AsSystem.EventArgs)
Handles MyBase.Load
MsgBox("Press OK to activate pumps. Balance pumps and press Start to begin management
program.")

Sens_track.Value = 0
Sens_track.Enabled = False
sensitivity_text.Text = ""

phidget_input = NewPhidgets.InterfaceKit
phidget_output = NewPhidgets.InterfaceKit
'To reduce code complexity we assume that there is one PhidgetInterfaceKit
'attached to the PC before the program is run.
Try
phidget_input.open(176314)
phidget_output.open(179521)
Catch ex AsException
MessageBox.Show(ex.ToString())
EndTry

EndSub

PrivateSubphidget_input_attach(ByVal sender AsSystem.Object, ByVal e
AsPhidgets.Events.AttachEventArgs) Handlesphidget_input.Attach
level_1.BackColor = Color.White

Ifphidget_input.sensors.Count> 0 Then
Sens_track.Enabled = True
Sens_track.SetRange(0, 1000)
Sens_track.Value = phidget_input.sensors(0).Sensitivity
sensitivity_text.Text = Sens_track.Value.ToString()

phidget_input.ratiometric = False
EndIf
EndSub

PrivateSubphidget_output_attach(ByVal sender AsSystem.Object, ByVal e
AsPhidgets.Events.AttachEventArgs) Handlesphidget_output.Attach
```

```
phidget_output.outputs(3) = False
phidget_output.outputs(0) = False
```

```
EndSub
```

```
PrivateSubphidget_input_detach(ByVal sender AsSystem.Object, ByVal e
AsPhidgets.Events.DetachEventArgs) Handlesphidget_input.Detach
    level_1.BackColor = Color.Tan
    Sens_track.Value = 0
    Sens_track.Enabled = False
    sensitivity_text.Text = ""
EndSub
```

```
PrivateSubphidget_output_detach(ByVal sender AsSystem.Object, ByVal e
AsPhidgets.Events.DetachEventArgs) Handlesphidget_output.Detach
    Start.BackColor = Color.Tomato
```

```
EndSub
```

```
PrivateSubphidget_input_Error(ByVal sender AsObject, ByVal e
AsPhidgets.Events.ErrorEventArgs) Handlesphidget_input.Error
    MessageBox.Show(e.Description)
    phidget_output.outputs(3) = True
    phidget_output.outputs(0) = True
```

```
EndSub
```

```
PrivateSubphidget_input_SensorChange(ByVal sender AsObject, ByVal e
AsPhidgets.Events.SensorChangeEventArgs) Handlesphidget_input.SensorChange
```

```
'Pump 1 is 50 pump: is output 0, orange cord.
'Pume 2 is 750 pump: is output 3, beige cord.
```

```
'Timer2.Stop()
Ifphidget_output.outputs(3) = FalseThen'Pump 2
    pump2.BackColor = Color.LawnGreen
```

```
Else
    pump2.BackColor = Color.Red
```

```
EndIf
Ifphidget_output.outputs(0) = FalseThen
    pump1.BackColor = Color.LawnGreen
```

```
Else
    pump1.BackColor = Color.Red
```

```
EndIf
```

```
distance = 2076 / (e.Value - 1)
boxwidth = 75
    Panel3.Size = NewSize(boxwidth, distance * 3)
'Level_vis2.Size = New Size(width, distance2 * 2)
```

```
    level_1.Text = distance.ToString("f1")
'level_2.Text = distance2.ToString("f1")
```

```
IfStart_button = TrueThen
Iftimer_condition = FalseThen
```

```

'VOLUME CONTROL IR DISTANCE SENSORS
'Have some sort of reset
'distance Numbers are being displayed as distance values (cm) from top
'<<All pumps are backwards...False condition = on, TRUE condition = off>>

Ifhightripped = TrueThen
' After level gets too high, signals this case and waits until level subsides
' to about mid-way before resuming the other level-monitoring code below.

If distance >= 10 Then
hightripped = False

EndIf
ElseIflowtripped = TrueThen
' After level gets too high, signals this case and waits until level subsides
' to about mid-way before resuming the other level-monitoring code below.
If distance <= 10 Then
lowtripped = False
EndIf
Else
If distance <= 9 Then
'Level in bucket is too high, need to lower level

phidget_output.outputs(3) = True'pump 2 is off
phidget_output.outputs(0) = False'pump 1 is on

Alert.Add(DateAndTime.Now.ToString&"      High level")

high = high + 1

                Timer1.Interval = 10000
Timer1.Start()
timer_condition = True
hightripped = True
ElseIf distance >= 12 Then
' Level in bucket is too low, need to raise level

phidget_output.outputs(3) = False'pump 2 is on
phidget_output.outputs(0) = True'pump 1 is off

Alert.Add(DateAndTime.Now.ToString&"      Low level")

low = low + 1

                Timer1.Interval = 10000
Timer1.Start()
timer_condition = True
lowtripped = True
Else' Both Pumps Running

phidget_output.outputs(3) = False'pump 2 is on
phidget_output.outputs(0) = False'pump 1 is on

EndIf

```

```

EndIf
EndIf
EndIf
'Timer2.Interval = 10000
'Timer2.Start()
'val1 = e.Value
EndSub

PublicSubStart_Click(ByVal sender AsSystem.Object, ByVal e AsSystem.EventArgs)
HandlesStart.Click
IfStart_button = TrueThen
Start_button = False
Start.Text = "Start"
phidget_output.outputs(3) = False
phidget_input.outputs(0) = False
DimhighstringAsString = high.ToString, lowstringAsString = low.ToString
ForEach _string AsStringIn Alert
Me.AlertBox1.Text += _string
Me.AlertBox1.Text += Environment.NewLine
Next
phidget_output.outputs(3) = True
phidget_output.outputs(0) = True

MsgBox("High trips: "&highstring&Chr(10) &"Low trips: "&lowstring)

Else
Start_button = True
Start.Text = "Stop"
EndIf
EndSub

PrivateSubphidget_input_InputChange(ByVal sender AsObject, ByVal e
AsPhidgets.Events.InputChangeEventArgs) Handlesphidget_input.InputChange
EndSub
PrivateSubphidget_input_OutputChange(ByVal sender AsObject, ByVal e
AsPhidgets.Events.OutputChangeEventArgs) Handlesphidget_input.OutputChange

EndSub
PrivateSubphidget_output_Error(ByVal sender AsObject, ByVal e
AsPhidgets.Events.ErrorEventArgs) Handlesphidget_output.Error
MessageBox.Show(e.Description)
phidget_output.outputs(3) = True
phidget_output.outputs(0) = True

EndSub

'Private Sub phidget_output_OutputChange(ByVal sender As Object, ByVal e As
Phidgets.Events.OutputChangeEventArgs) Handles phidget_output.OutputChange
'    If Start_button = True Then
'        phidget_output.outputs(3) = True
'        phidget_output.outputs(0) = True
'        Alert.Add(DateAndTime.Now.ToString& "        Relay Error!")
'        MsgBox("Error with output: please check lines to pumps.")

```

```

' End If

'End Sub
PrivateSub Form1_FormClosing(ByVal sender AsObject, ByVal e
AsSystem.Windows.Forms.FormClosingEventArgs) HandlesMe.FormClosing
phidget_output.outputs(3) = True
phidget_output.outputs(0) = True
RemoveHandlerphidget_input.Attach, AddressOfphidget_input_attach
RemoveHandlerphidget_input.Detach, AddressOfphidget_input_detach
RemoveHandlerphidget_input.Error, AddressOfphidget_input_Error
RemoveHandlerphidget_input.InputChange, AddressOfphidget_input_InputChange'(greened this
one)
RemoveHandlerphidget_input.OutputChange, AddressOfphidget_input_OutputChange
RemoveHandlerphidget_input.SensorChange, AddressOfphidget_input_SensorChange
RemoveHandlerphidget_output.Attach, AddressOfphidget_output_attach
RemoveHandlerphidget_output.Detach, AddressOfphidget_output_detach
RemoveHandlerphidget_output.Error, AddressOfphidget_output_Error
'RemoveHandlerphidget_output.InputChange, AddressOfphidget_output_InputChange
'RemoveHandlerphidget_output.OutputChange, AddressOfphidget_output_OutputChange '(greened
this one)
'RemoveHandlerphidget_output.SensorChange, AddressOfphidget_output_SensorChange

Application.DoEvents()

phidget_input.close()
phidget_output.close()

EndSub

PrivateSubinputTrk_Scroll(ByVal sender AsObject, ByVal e AsSystem.EventArgs)
HandlesSens_track.Scroll
Try
DimiAsInteger
Fori = 0 To phidget_input.sensors.Count - 1
phidget_input.sensors(i).Sensitivity = Sens_track.Value
Next
sensitivity_text.Text = Sens_track.Value.ToString()
Catch ex AsException
MessageBox.Show(ex.Message.ToString())
EndTry
EndSub

PrivateSub Timer1_Tick(ByVal sender AsSystem.Object, ByVal e AsSystem.EventArgs) Handles
Timer1.Tick
timer_condition = False

EndSub

'Private Sub Timer2_Tick(ByVal sender As System.Object, ByVal e As System.EventArgs)
Handles Timer2.Tick

' If level_1.Text = 0 Then
'     If Start_button = True Then
'         phidget_output.outputs(3) = True
'         phidget_output.outputs(0) = True

```

```
'            Alert.Add(DateAndTime.Now.ToString& "            Sensor Error!")
'            MsgBox("Error with sensor")
'        End If
'    End If
'End Sub
```

```
PrivateSub Panel13_Paint(ByVal sender AsSystem.Object, ByVal e
AsSystem.Windows.Forms.PaintEventArgs) Handles Panel13.Paint
EndSub
EndClass
```


Appendix 3: Failure Mode Effects Analysis

Function	Potential Failure Mode	Potential Effect(s) of Failure	S	Potential Cause(s) of Failure	O	Current Process Controls	D	RPN	CRIT	Recommended Action(s)		
Sense fluid level	Sensor becomes disconnected	Process continues unregulated	8	User error: cord problems	4	User observation	10	320	32	Wireless capability		
				Mechanical vibrations	2							
	Sensor reads inaccurate values	Process continues unregulated	8	Foaming	4	User observation	10	320	32			
				Frequent pump cycling	4	Errors due to environment	4					
						Sensor is immersed	2					
Pump control	Program malfunctions	Process continues unregulated	8	Change in operating conditions	4	Error alerts	3	96	32	2 nd string programming		
				Frequent pump cycling	4	Program errors	3	Log of pump-state decisions	2			
				Relay becomes disconnected from PC	8	User error: cord problems	4	Shut-off	1		32	32
	Pumps become disconnected from relay	Process becomes unbalanced	8	User error: cord problems	2	User observation	10	160	16			
				Electrical hazard	8	Mechanical vibrations	1					

Author's Biography:

Candace Clark will graduate with a B.S. in Biological Engineering. As an undergraduate research fellow, she has been involved in a variety of research projects. Candace researched optimizing algae growth for biofuel production, which resulted in the upcoming publication of the manuscript, "*Effects of Light Environment on the Volumetric Productivity of the Green Algae, Neochlorisoleoabundans.*" Candace was selected for a Department of Energy Science Undergraduate Laboratory Internship at the Idaho National Laboratory, where she researched metabolic engineering of thermoacidphilic bacteria. Candace's current research involves optimizing and automating synthetic spider silk protein purification under the direction of Dr. Randy Lewis, supported by an Engineering Undergraduate Research Program award. As a participant of the USU honors program, Candace will graduate with honors in departmental and university studies.

Candace is involved with the Engineering Council and Biological Engineering Club, where she helps plan and carry out activities. She is also active in the recruitment of new engineering students participating in outreach as a College of Engineering Ambassador. Candace grew up in Rigby, Idaho. She is the daughter of Michael and Diana Clark and is very proud to be a fourth generation Utah State Aggie engineer. Candace plans for full time employment after graduation.