Optimization of Multi-Centrifuge Steps in Biotechnology Automation

Aura-Maria Cardona
Department of Electrical Engineering
Florida Atlantic University
777 Glades Road
Boca Raton, FL 33431
acardon5@fau.edu

Chingping Han
Department of Computer Science and Engineering
Florida Atlantic University
777 Glades Road
Boca Raton, FL 33431
han@fau.edu

Zvi Roth
Department of Electrical Engineering
Florida Atlantic University
777 Glades Road
Boca Raton, FL 33431
rothz@fau.edu

ABSTRACT

Design of an automation line is a multi-objective optimization problem involving throughput, yield, floor space and cost constraints. This paper examines the feasibility of computer-aided automation design for biotechnology applications using Arena™ software. A generic case study chosen for this paper involves a sequence of steps in a preparation process of RNA from tissue-cultured cells. These steps involve repetitive usage of centrifuging operations to perform separation of biochemical substances. A centrifuge typically operates on multiple sample tubes that are loaded symmetrically within the centrifuge. The tubes must be loaded onto the centrifuge by a pick-and-place device (typically a robot manipulator). Consecutive centrifuging steps may involve multiple centrifuges as well as robots, or some measure of equipment sharing. Automation design necessarily involves a tradeoff among conflicting objectives. Ultimately all objectives (throughput, yield, space utilization and process cost) may be cast in a unified cost structure to allow an optimal design decision. The paper demonstrates such a design process.

Keywords

Biotechnology, Automation, System Modeling, Arena™ software, Throughput, Cost

1. INTRODUCTION

Biotechnology processes, as other industrial processes, require specialized equipment to be able to handle the specific samples intrinsic to the field, their quantities, and obviously the actual operations that take place in the processes. When assembling these processes into production lines various challenges and constraints have to be encountered. Following the completion of genome sequencing of many organisms, and in order to decipher the function of genes and proteins, demand for automated experimentation and protocols became large.

In [1] the importance of and need for automated solutions for biotechnology processes is illustrated by means of a specific process for preparation of RNA from tissue-cultured cells. Some constraints that biotechnology equipment has were highlighted. Biotechnology operations have to be very accurate as there is no possibility of automatically monitoring every single step in a process. In order to assure that the output is within specifications, the essentially open-loop operations require precision. Another feature of many biotechnology processes is the relative high cost of processed materials. The samples and reagents involved in such processes tend to be expensive and sometimes very scarce and therefore waste must be kept to a minimum. Many biotechnology process steps may require stringent environmental conditions and tight timings constraints.

A centrifuge is used to separate solids suspended in liquid. The apparatus rotates at very high speed and the centrifugal forces separate particles that are suspended in a substance according to their relative mass. This process varies in time depending on the substance that is being separated. It could take as few as 15 seconds, but there are substances that require higher speeds and longer times.

The need to use a centrifuge within an automated biotechnology process imposes a need to employ pick-and-place devices, typically robotic arms to be able to load the centrifuges. Centrifuges available nowadays for biotechnology applications do not allow any other means of integration.

This paper discusses protocols that use multiple centrifuge steps. Common configurations of repeating centrifuge steps are analyzed and generalized. Section 2 describes the specific biotechnology process, which is the subject of this study; the complete process is briefly outlined along with a more detailed description of the process subset that requires computer-aided optimization of automation design. Section 3 describes the models used in the Arena™ simulations. Section 4 describes the throughput, cost and footprint considerations when selecting an automation configuration. Section 5 focuses on high-throughput implementation. Section 6 explores open issues and future research directions.

2. PROCESS DESCRIPTION

As in [1] this paper involves a generic case study of a procedure for the isolation of RNA from Drosophila Schneider 2 cells that were transfected with a plasmid expressing dact mRNA (RE37047) [2]. The overall goal of the procedure is the preparation of RNA from tissue-cultured cells. This process when done manually consists of 20 steps:

1. Retrieval of one tube full of frozen cells.
2. Adding of 350 µl of RLT buffer followed by gentle vortexing until cells are lyzed.

3. Transferring of the lysate into a QIAshredder spin column placed in a 2ml collection tube.
4. Centrifuging of symmetrically arranged tubes for 2 minutes at 14,000 rpm in a 4°C incubator.
5. Adding of 350 µl of 70% ethanol to the lysate in the collection tube.
6. Transferring of the combined 700µl, including any precipitates that may have formed, to an RNeasy mini column in a 2ml collection tube.
7. Centrifuging of tubes for 15 seconds at 14,000 rpm.
8. Discarding of the flow-through and reattaching the collection tube to the mini column.
9. Addition of 700µl of buffer RW1
10. Centrifuging of tubes for 15 seconds at 14,000 rpm.
11. Discarding of the flow-through and the collection tube.
12. Transferring of the RNeasy mini column to a new 2ml collection tube.
13. Adding of 500µl of the wash buffer RPE into the column.
14. Centrifuging of tubes for 15 seconds at 14,000 rpm.
15. Discarding of the follow-through and reattaching the collection tube to the mini column.
16. Addition of another 500µl of the wash buffer RPE into the column.
17. Centrifuging of tubes for 2 minutes at 14,000 rpm.
18. Adding of 50µl of RNase free water and attaching a new collection tube.
19. Elution of the RNA from the column by centrifugation for 2 minutes at 14,000 rpm.
20. Storage of the collecting tube in a freezer at -20°C.

These 20 steps can be grouped into 17 consecutive automation stations when setting a conceptual manual-labor production line [1]. Such manual-labor production line is shown in Figure 1.

The subset of operations chosen for computer-aided design optimization demonstrated in this paper (Stations 14 through 17) represents a set of operations present in the isolation of RNA as well as in many other biotechnology protocols that require pick-and-place manipulation. This group of process steps is also interesting from an optimization point of view due to the possibilities for shared equipment. This subset of operations is included in biotechnology protocols whenever washing or eluting a sample, or simply if more than one reagent is added and a subsequent separation operation is needed. The set of operations consists of five stations: the first station is where the sample arrives into the system. It could be coming from storage, or from a previous step in the protocol. The second station is a centrifuge station. In this station the sample is centrifuged for a certain amount of time (two minutes in our case study). The third station is a dispensing station. In this case study a wash buffer is added to the sample. The fourth station is a second centrifuge station. Finally, the fifth station is where the sample exits the system, possibly to the next station or, like in this case, to a storage unit because the output of this station is the overall final product. A block diagram of the subset of operations is shown in Figure 2.

Four possible configurations for batch production of RNA are explored. In order to have a consistent optimization study, the same equipment and lab ware is used for all configurations simulations. This ensures that the conclusions drawn are due to the difference in automation configuration and not due to the difference in equipment and/or lab ware. Evaluating different types of equipment to realize specific process steps is a future research direction that will not be discussed in this paper. The idea is to evaluate these four possible configurations comparing throughput, floor space and cost: the cost of producing the samples and the value that these samples represent. The set-up with the best return on investment for a given product pricing strategy might be considered the best.

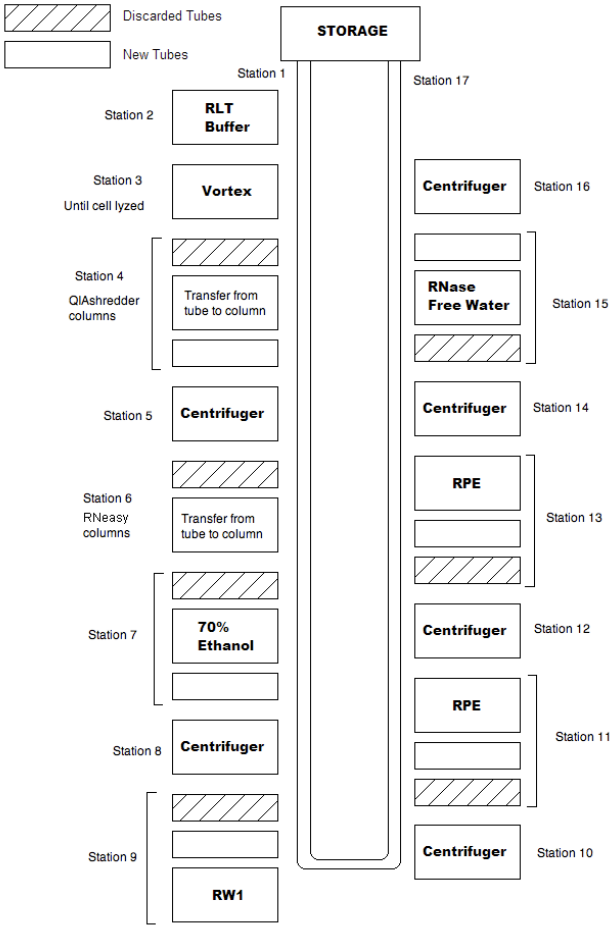

Figure 1. Conceptual design for a production line for the preparation of RNA from tissue cultured cells

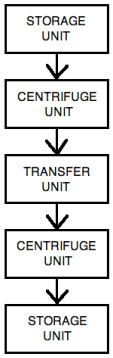

Figure 2. Subset of operations studied.

The above batch production automation layout candidates all represent a "low-throughput" solution, whereas a high-throughput solution is explored in a later section. It is important to keep in

mind that any high-throughput automation strategy necessitates customized equipment. The investment in customized tooling makes sense if the actual increase in potential revenue is significant due to the considerable increase in throughput.

2.1 Candidate Configurations for Automation Design

Configuration 1: Dual robots and centrifuges arranged linearly

The first configuration involves a robot for each centrifuge. Each of the robots loads and/or unloads just one centrifuge as it transfers the lab ware to and from the centrifuge station. Below is a detailed description of each of the stations for Configuration 1:

Station 1: Represents the previous station where the sample enters the system. In this specific case and for the purpose of modeling and simulation the sample is assumed to be in storage prior to entering the system.

Station 2: In the Centrifuge Station the sample is centrifuged for 2 minutes. In the simulations a standard centrifuge is used that has a rotor that can fit up to six 1.5ml collecting tubes.

Station 3: The Dispensing Station consists of a commercial dispensing unit where a reagent is added to the column and the collecting tube.

Station 4: A second centrifuge station is identical to Station 2.

Station 5: A storage unit is where the sample exits the system. The samples are kept in a storage unit for future use.

Since centrifuges can only be loaded and unloaded from the top, and tubes need to be put symmetrically into slots, a pick-and-place device is needed to perform such an operation. The best solution is to use a robotic arm capable of loading and unloading the equipment, and help with the manipulation of lab ware among stations. The layout for Configuration 1 is shown in Figure 3.

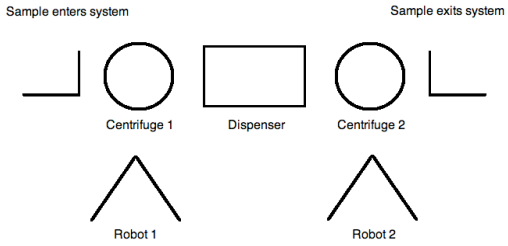

Figure 3. Block Diagram for *Configuration 1*

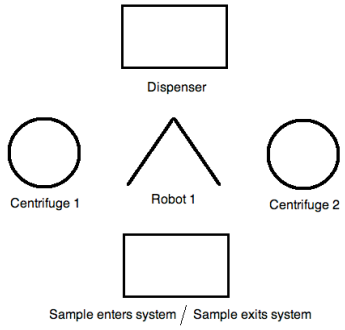

Figure 4. Layout Diagram for *Configuration 2*

Configuration 2: Single robot serving two centrifuges in a circular geometry.

Relatively speaking robots are among the more expensive hardware in the automation line, if not the most expensive. The second configuration is a setup aimed at cost reduction. The two centrifuge stations share one robot that loads and unloads tubes to and from both centrifuges. A circular geometry enables all stations to lie within the workspace of the robot. The circular geometry enables also the share of storage units, and this is the main difference stations-wise with the previous configuration. Configuration 2 layout diagram is shown in Figure 4.

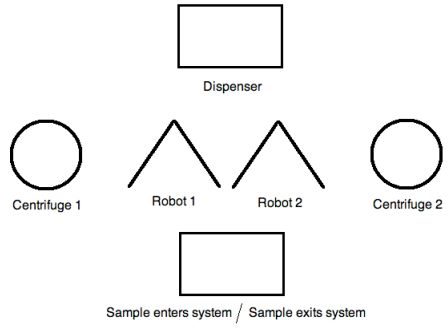

Figure 5. Layout Diagram for *Configuration 3*

Configuration 3: Dual robots and two centrifuges arranged in a circular geometry

Configuration 3 conceptually retains the throughput obtained whenever a centrifuge utilizes a dedicated robot for each centrifuge. The only caveat is to prevent the robots from having intersecting workspaces. Configuration 3 layout diagram is shown in Figure 5. Configuration 3 has the same stations and structure as Configuration 2.

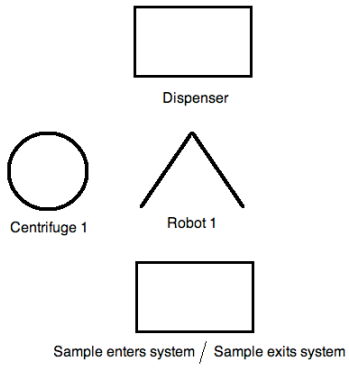

Figure 6. Layout Diagram for *Configuration 4*

Configuration 4: Single Robot and single centrifuge strategy in a circular geometry

The fourth configuration aims at reducing both footprint and equipment cost, at the expense of lower throughput. Reachable robot workspace constraints dictate a circular geometry. The same storage unit is used to store "samples" – initial product, and RNA the "final product". The layout diagram of Configuration 4 is shown in figure 6. The single centrifuge station performs both centrifuging process steps.

2.2 Equipment used in the Numerical Example

In order to allow quantitative assessment typical present costs of commercially available equipment (obtained from web resources or by direct price quotes) are listed below:

Robot (certified for biological applications):
Fanuc M-430iA [3] (Figure 7)
Price: $54,900
Footprint: 320 x 320 mm

Figure 7. Fanuc M-430iA [4]

Centrifuge:
Kizker MZ011 [4] (Figure 8)
Price: $3933
Footprint: 275 x 347 mm
Rotor:
Kizker MZ011_2424
Price: $305

Figure 8. Centrifuge Kizker MZ011 and Rotor Kizker MZ011_2424

Standard Equipment:

Dispenser: 600 x 400 mm

Storage Unit: 1100 x 600 mm

Labware:

Columns & Reagent:

QIAGEN – Rneasy Mini Kit [5] (Figure 9)

Price: $227.00 per 50 units

Centrifuge tubes:

Kizker G013 True-Lock 1.5ml Tubes (Figure 9)

Price: $36.50 per 1000 units [4]

Figure 9. Shows the lab-ware used. Centrifuge [4] tubes and Columns [5]

3. SIMULATION WITH ARENA™

Arena™ software by Rockwell Automation has the capability of executing detailed analysis of complex manufacturing processes. Arena™ calculates all timings, queues, and time usage information for the various resources in the model, all costs associated with the entities, and it can also associate statistical attributes to each element of the model (that might include realistic sub-system failure probabilities accounting for less than perfect yields) [6].

The four candidate configurations (discussed in Section 2) were modeled in Arena™ to obtain throughput and cost information. The total footprint information was calculated based primarily on the robots workspaces. The Arena™ block diagram for the first three configurations is shown in Figure 10.

The difference between the first three evaluated solutions has to do with the way lab ware is handled and manipulated. Configurations 1 and 3 use two robotic arms, whereas Configuration 2 uses only one. Advance transfer library templates available in Arena™ describe the robots and their motion. Robots are modeled using the Transport template, allowing the specification of path type, the distance covered, and manipulator velocity, initial position, and initial status. The robots actions are described using Arena™ "request" "move", and "free" blocks. This allows each modeled robot to be requested, loaded, be moved from one station to the next, be unloaded, and finally be freed up to be able to tend another station that may request its services.

It is important not to get misguided by the Arena™ block names. For Transport operations the names are: "Station", "Request Pickup", "Load Robot", "Move to Station", "Unload Robot", and "Free Robot". The block numbers enumerate the different blocks that perform the same operation, but it does not mean (watching Figure 10) that there are five robots to be unloaded in the system. It just means that five robot operations are performed within the system. Arena™ cannot work with replicate names and that is why the blocks have to be numbered. Which robot is attending which block is not explicitly shown in the diagram. This type of information is included in the blocks setup and is described for each configuration below.

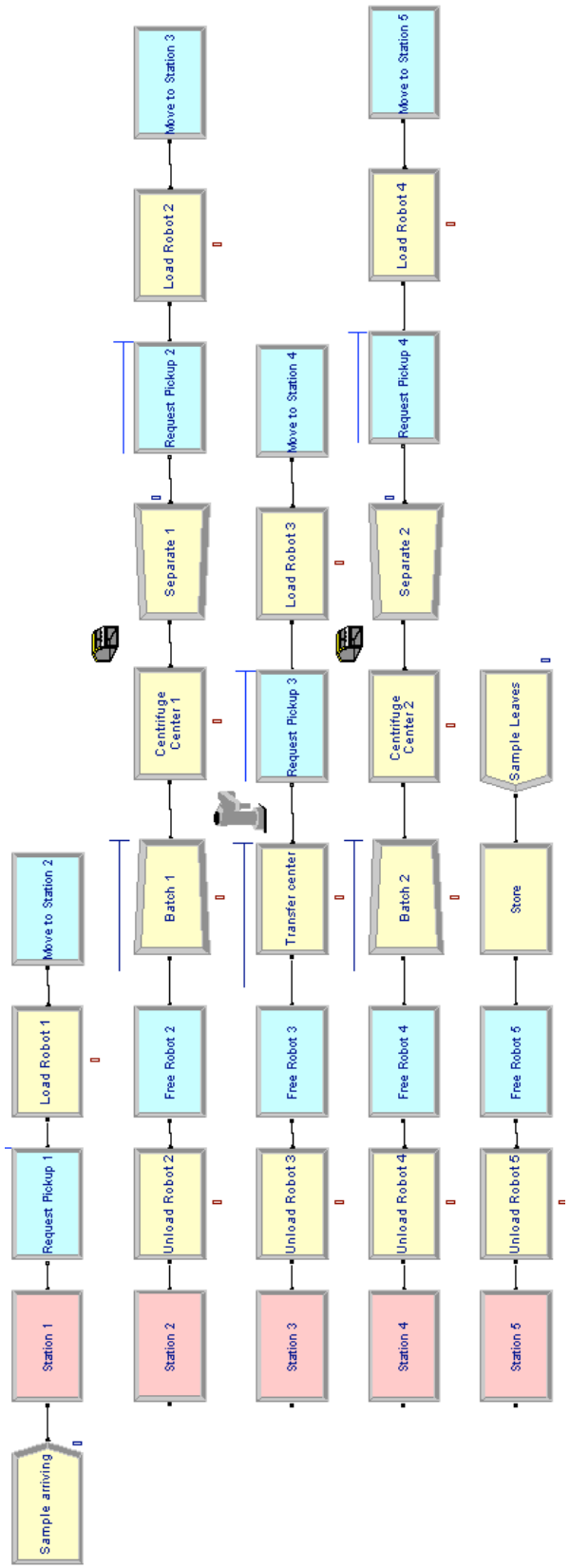

Figure 10. Arena™ model for *Configuration 1, Configuration 2 and Configuration3*

Configuration 1: Dual robots and centrifuges arranged linearly

The required Arena™ modeling steps for any sample to go through the system are as follows:

1. The sample enters the system to Station 1
2. Station 1 requests the pick-up of the sample by Robot 1
3. Robot 1 Picks-up the sample
4. The sample is moved to Station 2
5. The sample is dropped in Station 2, Robot 1 is freed, and the sample waits for another five samples, which come in the same way, so that Centrifuge 1 can start running.
6. Once six samples are ready inside Centrifuge 1, Centrifuge 1 runs for 2 minutes.
7. The samples are unloaded one at a time and the request for Robot 1 to pick-up the sample is issued.
8. Robot 1 picks-up the sample
9. The sample is moved to station 3
10. The sample is dropped in station 3, the Robot is freed, and the sample is left in the Transfer Center queue.
11. The Transfer Center dispenses 50ul of RNase free water into the column with the sample.
12. Station 3 requests the pick-up of the sample by Robot 2
13. Robot 2 picks-up the sample
14. The sample is moved to Station 4
15. The sample is dropped in Station 4, Robot 2 is freed, and the sample waits for another five samples, which come in the same way, so that Centrifuge 2 can start running.
16. Once six samples are ready inside Centrifuge 2, Centrifuge 2 runs for 2 minutes.
17. The samples are unloaded one at a time and the request for Robot 2 to pick the sample is issued.
18. Robot 2 picks-up the sample
19. The sample is moved to station 5
20. The sample is dropped in Station 5, Robot 2 is freed and the sample is stored leaving the system.

Configuration 2: Single robot serving two centrifuges in a circular geometry.

In this configuration, every station requests Robot 1, and Robot is in charge of manipulation every piece of lab ware throughout the equipment.

Configuration 3: Dual robots and two centrifuges arranged in a circular geometry

The required steps for the samples to move through the system are the same as in Configuration 1.

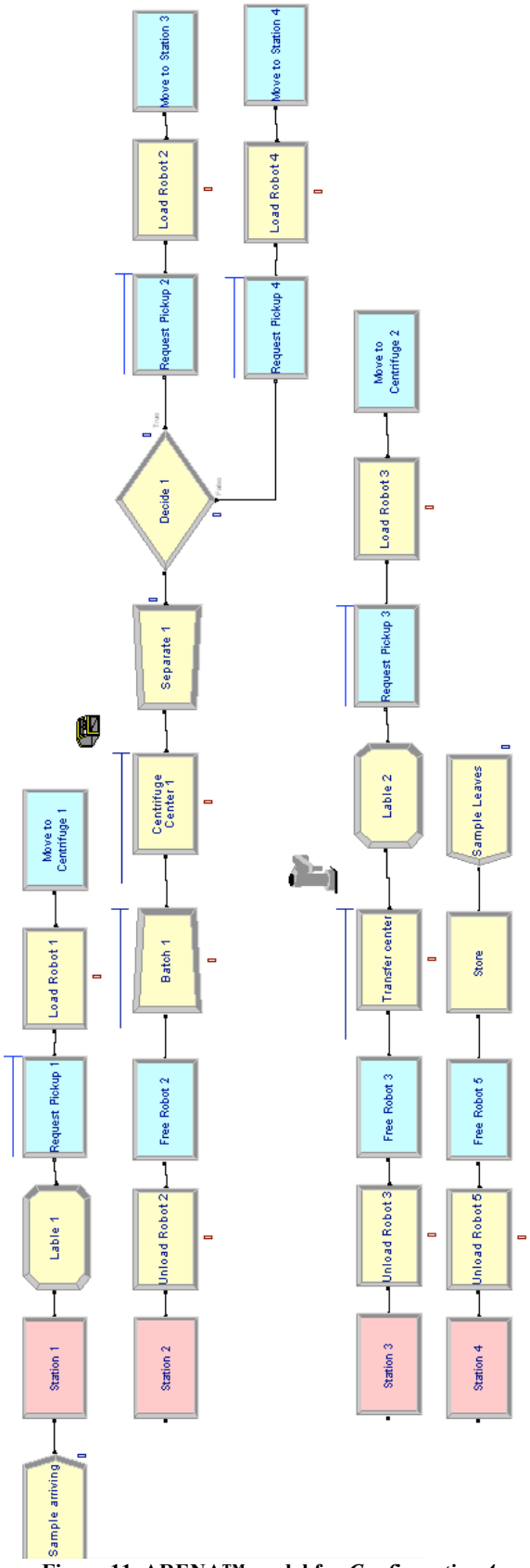

Figure 11. ARENA™ model for *Configuration 4*

The Arena™ model used to simulate Configuration 4 is considerably different than the ones discussed above. Figure 11 shows this model.

Configuration 4 maximizes equipment sharing. It uses a single robotic arm performing the task of two and similarly for the centrifuge.

Configuration 4: Single Robot and single centrifuge strategy in a circular geometry

Configuration 4 has one Robot that moves the samples and lab ware from one station to the next, and also it has only one centrifuge station that is used for both centrifuge runs.

Simulations assumed a single eight hours shift a day. The entities (modeling the tubes) were assumed to be entering the system at a normally distributed period, having a mean of 60 seconds and a standard deviation of 10 seconds.

4. AUTOMATION CONFIGURATION SELECTION

The candidate automation configurations are evaluated through a comparison of throughput, cost and footprint.

Production line cost is calculated by adding the cost of the various equipment modules, and the "cost of the layout footprint". There is also a cost associated with the lab ware involved depending on how many samples are processed. An approximated model of the production line cost is:

$$C_{PL} = N_R * C_R + N_c * C_c + N_S * C_S + N_D * C_D + FP * C_{FP} \quad (1)$$

Where each C denotes a "cost per unit" and each N denotes the "number of units" for the production line, robot, centrifuge, storage unit and dispenser, respectively. FP is the total floor space area (needed based on the system footprint), and C_{FP} is the cost per unit area.

The most common method of integrating the multiple objectives is via a return on investment constraint. That is, by stipulating a specific return on investment time period.

To set the price for the product, one needs to ensure that after the investment is returned profit exceeds the line operating costs. These variable operating costs include the cost of the lab ware and the cost of the "input samples". The variable daily cost C_V is calculated using:

$$C_V = C_{VU} * N_U \quad (2)$$

Where C_{VU} is the variable cost per unit, and N_U is the number of units produced per day.

The daily income I is:

$$I = P * N_U \quad (3)$$

Where-P is the price per unit sold and N_U are the number of units sold. It is assumed that every unit made is sold.

The daily revenue R after the investment is returned is:

$$R = I - C_V = P * N_U - C_{VU} * N_U \quad (4)$$

Note that P and C_{VU} are independent of the automation configuration choice.

The revenue earned per day divides the cost of the production line to give T the number of days to return the investment.

$$T = \frac{C_{PL}}{R} \quad \textbf{(5)}$$

Assuming that the only costs considered is the hardware cost. It has to be clear that this is a highly simplified model and this is why this figure is used instead the classical return on investment value. Many cost items are not taken into consideration such as: the set up of equipment, the design of the line, the monthly utilities, and transportation cost among many others. It is safe to assume that the real time that it takes to return the investment is several times longer than the one calculated above.

Some interesting observations can be drawn from the above cost model. The return on investment is of course inversely related to the set price. In many biotechnology applications the demand for samples is relatively inelastic with respect to product unit price. That is, changes in product price has little affect on the quantity demanded, thus increasing the price increases the total revenue.

Whenever comparing different automation implementation configurations, what dictates which configuration is better, is the ratio between the two respective values of the times to return the investment *T* denoted as SR (the Selection Ratio criterion).

$$SR = \frac{N_U}{C_{PL}} \quad \textbf{(6)}$$

The configuration to be selected is the one maximizing SR. Choosing the best configuration does not depend on the price set to the product. Finding the best configuration depends on the cost of the equipment used and the throughput of the configuration.

Numerical example

Table 1 provides simulations throughput results for an 8-hour shift. Throughput is defined as the total number of units prepared per single shift. Time per sample is the average time to produce one sample.

Table 1. Simulation Throughput Results

Configuration	Throughput (number of samples)	Time per sample
1	645	45 sec
2	523	55 sec
3	645	45 sec
4	534	54 sec

If the only objective of automation is to maximize throughput, the configuration that uses two robotic arms, one to handle each centrifuge station, will have a greater throughput. In one 8-hour shift a two-robots solution will produce 122 samples more than a single robot implementation. Another way to quantify the optimality of the two-robot solution is to say that each sample is produced 10 seconds faster, compared with the single robot implementation. Throughput for two-robot solutions is not two times larger than for one-robot solutions. What dominates the throughput in this numerical example is the time that the centrifuges need to run which is independent of the configuration selection. The queues to each centrifuge do vary with the configurations but their impact on the total throughput is secondary. The cost of the product line in this example is dominated by the unit cost of the robot arm. Doubling the number of robots almost doubles the production line cost, however the throughput does not increase by the same rate.

Using (1) – (5) the cost of the production lines, income, revenue, and return of investment can all be calculated.

Table 2. Equipment Prices

Equipment	Price ($)
Robot	54,600
Centrifuge + Rotor	4,238
Dispenser	2,500
Storage Unit	1,200

Typical equipment prices are found in Table 2. Since Arena™ cannot automatically layout the equipment, computation of the floor space has to be done manually. In order to calculate the footprint the configurations are sketched, and the total square footage needed to setup the equipment is found. The total area is then multiplied by 1.5 to have space to accommodate other equipment not included in the model like storage for the lab ware, and additional space for operators to work. For the sake of example the capital cost per square foot is assumed to be $40.00. An alternate way to include the floor space cost is to add it to the line operating cost (e.g. $0.5 per square foot per one day shift for commercial rented space). When laying out the equipment, the first consideration made is that the robot should be able to reach the different units that it serves. The robot used in this paper's example can reach any point within 90cm from its base center. The equipment modules were spaced 20cm apart. As a method of providing safety margin as well as a simple way of estimating the production line area, a rectangle containing the robots circular reachable space is created. Total area is then multiplied by a factor of 1.5 as explained earlier. Figure 12 to 15 shows the layout and total area for the different configurations. The resulting peripheral margin, area and FP value for each configuration appear in Table 3. In this example the cost associated with floor space plays a negligible role. This may be the situation for most biotechnology applications. In other words, tight floor space constraints may be used just to screen out candidate automation configurations, but play only a minor role in the selection process of best configurations among those that do meet the floor space constraint.

Let us now compute the variable cost per unit. Since the initial sample in this case is cultured-cells its cost is neglected in this example: the cells are samples taken from patients and grown to get the desired quantities. This is not always the case though. Biological raw samples may sometimes be expensive. The price for an RNeasy mini kit is $227.00 and it contains 50 units. The variable cost for each unit is $4.54.

Table 3. Layout Results

Configuration	Peripheral Margin (cm)	Area (sq-ft)	FP value (sq-ft)
1	1080	69.75	104.63
2	720	34.87	52.30
3	760	38.75	58.12
4	720	24.87	52.30

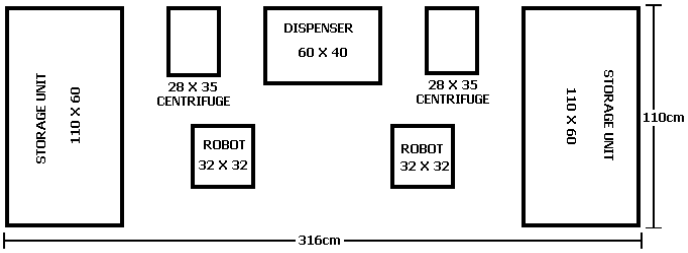

Figure 12. *Configuration 1* layout

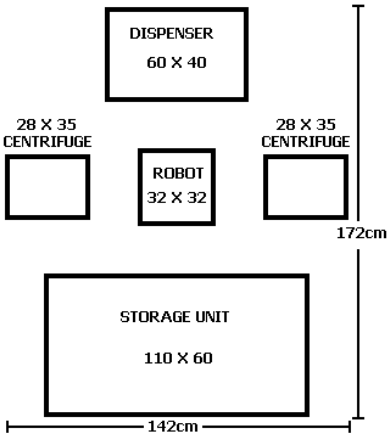

Figure 13. *Configuration 2* layout

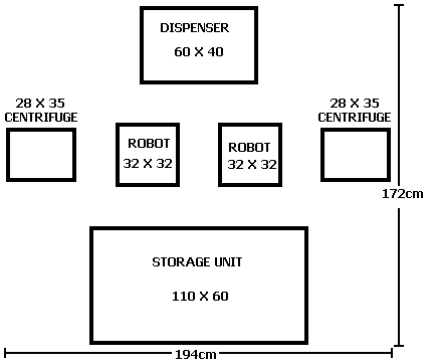

Figure 14. *Configuration 3* layout

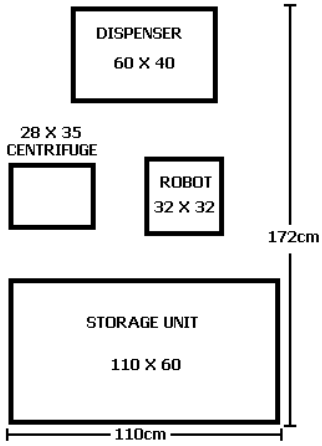

Figure 15. *Configuration 4* layout

The final factor missing is the price set per unit produced. For the purpose of calculating numerical values to compare the performance of the configurations a P = \$6.00 is assumed. Recall that P does not affect the configuration selection process.

By (1)-(5) production line cost, variable cost, revenue and the time needed to return the investment can be calculated for the sake of comparing the configurations. Table 4 shows the cost results for each of the configurations.

Table 4. Cost Results

Configuration	C_{PL} (\$)	C_V (\$)	R (\$)	T (days)
1	126,761.20	2,928.30	941.79	135
2	68,866.00	2,374.42	765.58	90
3	123,700.80	2,928.30	941.79	132
4	64,630.00	2,424.36	779.64	83

Obviously $C_{PL4} < C_{PL2} < C_{PL3} < C_{PL1}$. If the only objective were to minimize cost then the best answer would be *Configuration 4*.

Configurations 2 or 4 with only one robot return the investment twice as fast as *Configurations 1 or 3* that use two robotic arms. This is as the robots are by far the most expensive pieces of equipment used, constituting about 80% of the cost of the production line. Further analysis of the simulation results reveals that the utilizations of the robots in a two-robot configuration are much lower than that of the utilizations in the one-robot configurations.

For a severe cost constraint scenario Configuration 4 appears to be optimal.

The results obtained when using (6) to compare all the configurations among them appear in Table 5.

Table 5. Comparison Results

Configuration	SR
1	0.0051
2	0.0076
3	0.00.52
4	0.0082

Configuration 4 maximizes the SR selection measure.

In cases of severe floor space constraint, *Configuration 3* might be the best solution since it has the largest throughput with the smaller footprint.

If hypothetically money and footprint are not an issue, and the constraint is that the solution has to have two robotic arms (possibly dictated by other parts of the entire automation line) the best solution may be neither Configuration 1 nor 3. Instead the best solution in such a case might be to build two Configuration 4 setups in parallel. The footprint of a double-configuration-4 is only one tenth larger; the cost of the production line increases by only 1%, however the throughput increases by 40%.

5. SIMULATION RESULTS OF HIGH-THROUGHPUT IMPLEMENTATION

The above configurations are plausible candidates for batch production systems. To explore a High-Throughput (HT) strategy solution to the same configurations some design adjustments have to be made. Most HT systems require customized tooling and other equipment. A common format of the arriving samples is that

of a 96-well plate containing 96 samples (Figure 16); the centrifuge needs to be capable of running with these plates, and the dispenser should use a different tip capable of dispensing 96 wells at a time. This tooling and equipment changes necessitate a significant increase in cost (see Figure 17).

Figure 16. 96-well plate [5]

There are four HT configurations that can be analyzed using the previous Arena™ simulation setups. The main change with respect to the low-throughput simulations is that the centrifuge is ready to run with two entities (arranged symmetrically 180° apart), and each entity passing through the system represents a 96-well plate.

Figure 17. 96-plate rotor for High-Throughput centrifuge

Table 6 shows the throughput for an 8-hour shift for the various converted-to-HT configurations, and Table 7 shows typical prices of HT equipment.

Table 6. HT Simulation results

Configuration	Number of wells in 8-hours	Number of samples	Time per well
1-HT	966	92,736	30 sec
2-HT	764	73,344	38 sec
3-HT	966	92,736	38 sec
4-HT	762	73,152	54 sec

Table 7. High Throughput equipment costs

Equipment	Price ($)
Robot + Gripper	57,600
Centrifuge + Rotor	13,106
Dispenser	10,500
Storage Unit	1,200

Tables 1 and 6 reveal that entities throughput for HT implementation increases by almost 50%. This is due to the difference in the total queuing time. For low-throughput solutions, entities need to wait inside the centrifuge until all six slots are filled. In the HT case on the other hand since the rotor can only accommodate two entities, the total queuing time becomes significantly shorter.

The price for a QIAquick96 kit is $632.00 and it contains 4 plates. The variable cost for each plate is $158.00 or $1.65 per sample. The variable cost for an 8-hour shift (per day) for each of the configurations is calculated using (2). The variable cost per day for Configuration 1-HT is $C_{V1\text{-}HT}$ = $157,368.00, for Configuration 2 is C_{V2} = $120,712.00, for Configuration 3 is C_{V3} = $157,368.00, and for Configuration 4 is C_{V4} = $120,396.00.

Modified cost of the production line and revenue for each of the HT configurations is shown in Table 8.

Table 8. High-Throughput Cost Results

Configuration	$C_{PL\text{-}HT}$ ($)	R_{HT} ($) (per day)
1-HT	158,356.40	399,048
2-HT	86,983.60	319,352
3-HT	158,227.20	399,048
4-HT	86,627.60	318,516

In this example we observe that the revenue does not significantly change from one configuration to another; the cost of a two robot implementation is double that of a single robot implementation. In a high-throughput solution it appears best to always try to minimize the number of pieces of HT equipment.

The paper explores only five steps from a process that has 20. Cost analysis of HT implementation of the complete RNA production process is deferred to a later study.

6. OPEN ISSUES AND FUTURE WORK

This paper discussed a specific application example and so far it is not clear how generic the example is in terms of equipment cost and layout of equipment. In this example the robotic arm is the most expensive piece of equipment and it represents a high percentage of the total cost of the production line. This could not always be the case. There are other robots used in biotechnology, pharmaceutical and food applications. There are also pick and place devices available which could perform just the loading and unloading of the centrifuge. The manipulation of the lab ware has in such a case to be done by other means like conveyors. Each choice has its advantages and disadvantages. The evaluation and comparison between an architecture that uses robotic arms, and one that uses pick and place devices and conveyors is an open issue.

The paper results are tied to a specific RNA production protocol and one may speculate how this same setup and configurations work for a different protocol. In biology and chemistry not all samples behave the same way. It has to be taken into consideration that when dealing with live samples there is only an expected tendency, but that does not mean that every single cell reacts exactly as the rest, not even mentioning that different samples are expected to behave differently; the samples might need different timing, and environmental conditions among others. If for other type of samples different centrifuge times are needed, the results might change drastically.

This paper was a first approach to understand the different cost and protocol factors that have to be taken into account.

There are additional questions that have not been answered. One deals with the potential of Arena™ as a tool to perform

automation design optimization. Arena™ has a feature called OptQuest that has to be further explored, which allows searching for optimal solutions within the simulation model. Automation design is a typical example of a decision making problem, where the goal is to find the best set of values for a group of available variables. OptQuest for Arena™ might be able to address that.

A second issue is related to the yield of the different protocols. Not all protocols produce the same yield. It is very important to figure out the best way to add yield considerations to the optimality criterion. That would represent a more robust decision method.

To help address the core issues of multi-centrifuge lab setup we did not include the statistical variations into our models with the only exception of arrival intervals. The variations in yield, centrifuge operation time, load-unload operation, and breakdowns were not simulated. However those issues can be easily addressed by modifying our basic model. Blockages, as well as the storage space were not concerns in such conditions.

Future works may involve the study of different types of equipment for the same configurations explored in this study. It may also involve the integration of the different configurations in a complete biotechnology procedure

The cost method used to evaluate the different automation configurations should represent more realistically some variables that were simplified and others that for the purpose of this study were neglected. Variables such as utilities, design and construction costs along with maintenance cost should be taken into consideration and a weighted cost modeling method should be developed.

7. REFERENCES

[1] A.M. Cardona, Z. Roth. *Design Considerations in High-Throughput Automation for Biotechnology*. 2008 Florida Conference on Recent Advances in Robotics, FCRAR 2008

[2] *Biotechnology Laboratory I Manual*. Biotechnology Laboratory I, Biological Science, Florida Atlantic University, Fall 2006.

[3] FANUC Robotics America, Inc. M-430*i*A/2F™ www.fanuc.co.jp

[4] Gerlinde Kizker – Products for Biotechnology www.kisker-biotech.com

[5] QIAGEN – Sample and Assay Technologies www.quigen.com

[6] Kelton, W., Sadowski, R., Sadowski, D. *Simulation with Arena*. McGraw Hill, New York, NY, 2002.

[7] Groover, M. *Fundamentals of Modern Manufacturing Materials, Processes, and Systems*. Prentice Hall, Upper Saddle River, NJ, 1996.

[8] Singh N. *Systems Approach to Computer-Integrated Design and Manufacturing*. John Wiley & Sons, Inc., New York, NY, 1996.

[9] Hardin, C. Edwards, J., Riell, A. Presutti, D., Miller, W., and Robertson, D. Cloning. *Gene Expression, and Protein Purification Experimental Procedures and Process Rationale*. Oxford University Press, New York, NY, 2000.