



Direct observation of individual cell behavior within a biofilm
by Andrew Ramsey Rice

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering

Montana State University

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Abstract:

The ability of microorganisms to form biofilms has been well characterized. Bacterial cells can transition from a planktonic state to a sessile state, replicate and subsequently populate a surface. Organisms which are the first to colonize a “clean” surface are referred to as “primary” biofilm cells. The progeny of the first generation of sessile cells are known as “secondary” biofilm cells. This study examined the growth of both primary and secondary biofilm cells.

The organism of interest in this study was *Pseudomonas aeruginosa* PA01. A parent strain, deficient of the ability to synthesize green fluorescent proteins (GFP) and a construct strain which constitutively produced GFP were used. This organism was grown in planktonic and biofilm environments. A parallel plate flow cell reactor was used with a glass substratum for the biofilm experiments. A population of only GFP+ cells was studied for planktonic and primary biofilm experiments, whereas a mix of GFP+ and GFP- cells were used to examine the growth of secondary biofilm cells.

It was found that primary cells apparently undergo a change in phenotype from planktonic to biofilm form and also experience a lag in their growth. This is referred to as a surface associate lag time. Planktonic and secondary biofilm cells both grew at a faster rate than the primary biofilm cells.

The growth of secondary biofilm cells is characterized by replication, emigration, and movement. Each of these behaviors was directly observed for individual cells. A dilute mix of GFP+ cells in a predominantly GFP- biofilm allowed was used. It was discovered that cells which are destined to emigrate are more active in terms of movement than their remaining counterparts. Also, cells emigrate at a median time 1/3 of that of the median time to replication. Finally, at least 1/3 of all cells emigrate which is consistent with the “spreading” mode of biofilm growth of *Pseudomonas aeruginosa*.

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MONTANA STATE UNIVERSITY-BOZEMAN
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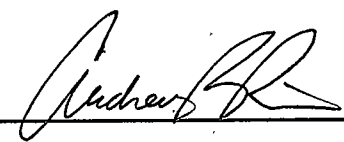
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ABSTRACT

The ability of microorganisms to form biofilms has been well characterized. Bacterial cells can transition from a planktonic state to a sessile state, replicate and subsequently populate a surface. Organisms which are the first to colonize a "clean" surface are referred to as "primary" biofilm cells. The progeny of the first generation of sessile cells are known as "secondary" biofilm cells. This study examined the growth of both primary and secondary biofilm cells.

The organism of interest in this study was *Pseudomonas aeruginosa* PA01. A parent strain, deficient of the ability to synthesize green fluorescent proteins (GFP) and a construct strain which constitutively produced GFP were used. This organism was grown in planktonic and biofilm environments. A parallel plate flow cell reactor was used with a glass substratum for the biofilm experiments. A population of only GFP+ cells was studied for planktonic and primary biofilm experiments, whereas a mix of GFP+ and GFP- cells were used to examine the growth of secondary biofilm cells.

It was found that primary cells apparently undergo a change in phenotype from planktonic to biofilm form and also experience a lag in their growth. This is referred to as a surface associate lag time. Planktonic and secondary biofilm cells both grew at a faster rate than the primary biofilm cells.

The growth of secondary biofilm cells is characterized by replication, emigration, and movement. Each of these behaviors was directly observed for individual cells. A dilute mix of GFP+ cells in a predominantly GFP- biofilm allowed was used. It was discovered that cells which are destined to emigrate are more active in terms of movement than their remaining counterparts. Also, cells emigrate at a median time 1/3 of that of the median time to replication. Finally, at least 1/3 of all cells emigrate which is consistent with the "spreading" mode of biofilm growth of *Pseudomonas aeruginosa*.

CHAPTER 1

INTRODUCTION

It is important to understand how microbial biofilms form because of the complexity, prevalence, and impact of these communities. During the initial events of biofilm formation, the cells which originally colonize a particular surface become the parent organisms of the ensuing biofilm. The behavior exhibited by these cells appears to vary according to species (Lawrence and Caldwell, 1987a). Parent cells can form dense microcolonies and then disperse in a matter of minutes (O'Toole and Kolter, 1998a). Upon replication, daughter cells can pack tightly near the parent cell location, spread out upon the surface, be cast back into the bulk fluid, form long chains of cells, or roll along the substratum (Lawrence and Caldwell, 1987a; Korber *et al.*, 1989; Power and Marshall, 1988; Shapiro and Hsu, 1989; Dalton *et al.*, 1994).

Purpose

The general purpose of this research project was to examine the importance of initial events; or, to determine if the initial colonization pattern of cells had any

bearing upon the biofilm that resulted. This research involved studying the processes which occur after initial events to understand how a biofilm develops from a population of parent cells.

The practice of extrapolating the behavior of individual cells to that of a general population has been suggested by (Gualtieri *et al.*, 1985) in the study of the motion of microorganisms. The consequence of this approach yielded two related areas of results: a quantitative description of individual cell behavior within an emerging biofilm matrix and a more qualitative account of an effect of the phenotypic transition of planktonic cells to sessile cells. When taken together, it is evident that the process of biofilm development is quite dynamic. Cells may have different growth rates depending upon their point of origin. They also are constantly repositioning themselves within the biofilm matrix; some move relatively short distances while others completely relocate over distances of hundreds of microns.

Much of the work responsible for the present scope of knowledge of biofilms was gained through direct observation approaches rather than efforts that were "hypothesis driven" (Costerton, 1999). It has been postulated that further gains in this field will stem from "very basic, extremely cross-disciplinary and sometimes esoteric research" (Palmer and White, 1997). The results of this study follow in this theme and are a product of direct, in situ observation of biofilm development utilizing flow cell and Confocal Scanning Laser Microscopy technology.

Technology

Microscopy and Image Analysis

The microscope is a key instrument for the investigation of bacterial behavior. Various means of employing the microscope in conjunction with the study of sessile organisms have been reviewed (Madigan *et al.*, 1997). Transparent and/or opaque substrata coupled with automatic image analysis is one of the most prevalent combinations currently used in this field of research. The use of various fluorochromes in biofilm research is also becoming a method of choice due to the ability of specific organisms and various physiological parameters to be visualized with differing degrees of sample disturbance (McFeters *et al.*, 1995). This research and others (Kuehn *et al.*, 1999; Davies *et al.*, 1998) are exploiting a new and indispensable technology which utilizes the insertion of genes into target bacteria which code for the production of a green fluorescent protein (GFP). Cells which possess the ability to produce this GFP are in essence, self-labeling. The protein provides a valuable way to observe individual bacteria in a biofilm with minimal to no sample preparation or disturbance.

Computer technology, image analysis techniques and video capture capability have enabled many researchers to conduct experiments that utilize time lapse microscopy to illuminate sequential events in biofilm processes (Mueller, 1996; Power and Marshall, 1988; Shapiro and Hsu, 1989; Lawrence and Caldwell,

1987a; Habash *et al.*, 1997; Dalton *et al.*, 1994). This technique has enabled various modes of "recolonization" to be visualized (Lawrence and Caldwell, 1987a), the discrimination between and quantification of cell growth and attachment in the rate of surface accumulation (Mueller *et al.*, 1992) and the rates of locomotion of bacteria on surfaces (Power and Marshall, 1988).

Confocal scanning laser microscopy (CSLM) has become the premier tool for studying biofilms in three dimensions. The major advantage of this microscope system is the ability to eliminate out of focus haze and produce very thin optical slices. When coupled with automated stages that can step in increments of sub-microns, very accurate three-dimensional images of biofilms can be created (Lawrence *et al.*, 1991). CSLM has been used to determine fractal dimensions of biofilms (Hermanowicz *et al.*, 1996). Three-dimensional imaging of morphological changes in biofilms has been used to illustrate the findings of Dalton *et al.* (1994). Moller *et al.* (1996) used fluorescent probes and CSLM to map the location of certain cells within a biofilm matrix. Further applications of CSLM and fluorescent probes have been reviewed by Costerton *et al.* (1995b).

Parallel Plate Flow Cell

The parallel plate flow cell has also become an important piece of equipment for the study of biofilms. The type that is used most commonly was first described by Sjollema *et al.* (1989) and modifications of which have been utilized by researchers at the Center for Biofilm Engineering (Camper *et al.*, 1994; Hamilton *et*

al., 1995; Davies *et al.*, 1993; Mueller, 1990; Scheuerman, 1996; Escher, 1986). The primary advantage of such a reactor system is that it allows the researcher to obtain images from biofilms with minimal physical disturbance. Real time studies of biofilm formation can also be carried out in flow cell reactors and time lapse images can be obtained. Many studies which have examined the initial attachment, detachment, and growth of bacterial cells on glass surfaces along with measurements of rates and types of movements have been conducted with a parallel plate flow cell reactor in conjunction with time-lapse microscopy (Korber *et al.*, 1994; Lawrence *et al.*, 1987b; Lawrence and Caldwell, 1987a; Lawrence *et al.*, 1989; Caldwell and Lawrence, 1986; Korber *et al.*, 1989; Lawrence *et al.*, 1991). The research presented in the following chapters was carried out using similar methods.

Organization

The following terms will be used in this report and need to be clarified in advance:

1. Primary biofilm cells: Those cells which are the first to colonize a surface. In essence they are the first generation biofilm cells and have transitioned from a planktonic environment to a sessile state.

2. Secondary biofilm cells: Any generation of cells which arise from primary biofilm cells. These cells do not undergo a change in phenotype for they originate in a sessile state.
3. Emigrate: The action of a cell moving from its previous location. Cells which emigrate do not necessarily detach and partition into the bulk fluid. On the contrary, they usually remain intimately associated with the biofilm, but travel to a new location in what is termed a recolonization event (Lawrence *et al.*, 1987b).

This report is organized in two main sections; each describing a portion of the total research project. Both parts are a contribution to the understanding of biofilm development. Chapter 2 presents the more qualitative exposition of the effects of a change in phenotype upon primary biofilm cells and Chapter 3 is a quantitative description of the behavior of secondary biofilm cells. Two strains of the same organism, *Pseudomonas aeruginosa* PA01, were used in these experiments and are described below in more detail. The experiments that dealt with planktonic and primary biofilm cells used only the GFP+ strain, whereas the secondary biofilm experiments utilized a mix of GFP+ and GFP- strains; with 1% of the cells being GFP+. The content of each of these sections will be condensed and submitted as manuscripts to be published at a later date.

CHAPTER 2

APPARENT SURFACE ASSOCIATED LAG TIME OF PRIMARY BIOFILM CELLS

Background

Biofilms are complex communities of microorganisms pervasive in the natural environment. The current conceptual model of a biofilm portrays an ingeniously complicated multi-species entity where ecological microniches are created and occupied by specific organisms (Costerton *et al.*, 1995b; Costerton, 1995). Bacteria are known to attach to both abiotic materials and other organisms in all manner of aqueous environments. Following attachment of the primary colonizing bacteria, growth of the sessile population and induction of new organisms into the community occurs. The resulting biofilm is an intricate assortment of micro- and macroorganisms (Fletcher, 1996) which can be responsible for many types of human infections, the corrosion of metals, the reduced efficiency of industrial heat dissipation equipment, and the degradation of waste materials (Costerton *et al.*, 1995b).

Because of these pertinent biofilm effects, researchers are interested in knowing how these communities form. The term used to describe the first stages

of biofilm development is "initial events" and has been thoroughly studied but is not well understood. Surface topography can affect the manner in which bacterial cells colonize a substratum (Camper *et al.*, 1994; Scheuerman *et al.*, 1998). Cells which first colonize a particular surface become the parent organisms of the ensuing biofilm. The behavior exhibited by these cells is thought to vary according to species (Lawrence and Caldwell, 1987a). Parent cells can form dense microcolonies and then disperse in a matter of minutes (O'Toole and Kolter, 1998a). Upon replication, daughter cells can pack tightly near the parent cell location, spread out upon the surface, be cast back into the bulk fluid, form long chains of cells, or roll along the substratum (Lawrence and Caldwell, 1987a; Korber *et al.*, 1989; Power and Marshall, 1988; Shapiro and Hsu, 1989; Dalton *et al.*, 1994).

Prior Research

Initial Events Description. One of the most useful results from research regarding initial events in biofilm formation is a conceptual model of biofilm accumulation. The framework for this model was first established by Escher in 1986. When a "clean" substratum first comes in contact with an aqueous environment, a layer of organic molecules is almost instantaneously formed on the surface. This layer is known as a conditioning film and may have a substantial

influence on the subsequent biofilm as many surface bound nutrients can be present in this film (Chamberlain, 1992; Marshall, 1996).

Bacterial cells are then transported from the bulk solution to the substratum. This can also be a rapid process with measurable attachment within as little as 30 seconds (Vanhaecke *et al.*, 1990). Several factors, including flow regime, motility and relative concentration of cells in the bulk fluid influence the rate at which cells are transported to the solid-liquid interface. Once organisms have come into contact with a substratum, there is a two-stage adsorption process: reversible adsorption followed by irreversible adsorption. Reversible adsorption is a weak bonding of the cell to the surface which is facilitated by one or a combination of the following: van der Waals forces, electrostatic and steric interactions, and polymer bridging. The time span of reversible adsorption is on the order of minutes. During this time cells may desorb from the surface and partition back into the bulk solution (Escher and Charaklis, 1990). Desorption of cells may also be a fairly rapid process. In an experiment that measured attachment and detachment during the first 120 minutes of biofilm formation, 60-80% of detaching cells had a surface residence time of less than 10 minutes (Wiencek and Fletcher, 1999).

Irreversible adsorption is a more permanent association of a cell with the substratum and is accomplished by the production of extracellular polymers. Net accumulation of a biofilm involves growth of the population of irreversibly attached cells, and the detachment of surface bound organisms either via erosion, sloughing or abrasion (Charaklis, 1990). Detachment events have usually been

thought of as mechanical failures of the biofilm matrix due to an applied shear force, but recent research has shown that these events may be initiated by enzymatic changes brought about by certain segments of the biofilm population as a response to environmental conditions (Davies *et al.*, 1998)

The processes involved in the initial events of biofilm formation, described above, have been succinctly summarized by (Mueller *et al.*, 1992):

1. Conditioning of the substratum by organic molecules
2. Transport of cells from the bulk water to the solid liquid interface
3. Adsorption of cells on the substratum
4. Transformation of irreversibly adsorbed cells to reversibly adsorbed cells
5. Desorption of reversibly adsorbed cells from the substratum into the bulk water
6. Growth of irreversibly adsorbed cells
7. Erosion of cells from adsorbed colonies into the bulk water.

Attachment. As described previously, the creation of a conditioning layer on a "clean" substratum has been thoroughly investigated and reported (Marshall, 1996). The initial attachment of cells to a substratum is influenced by the nature of the surface to which organisms are adhering. On a topographically homogeneous, stainless steel substratum, *Pseudomonas aeruginosa* displays a completely random spatial pattern of attachment. However, for a slightly rougher substratum, this organism exhibits a degree of aggregation, thereby proving that the topography of the substratum does have an influence on the initial attachment of this organism (Camper *et al.*, 1994).

Further examination of the influence of topography on bacterial adhesion was performed by monitoring the attachment patterns of bacteria to a smooth silicon substratum in a flow cell reactor. The surface was marked by defined grooves creating a well-characterized structural heterogeneity. Results again showed that substratum topography has a bearing upon the bacterial attachment pattern. Cells were shown to preferentially attach to regions of the surface just downstream of the grooves whereas control experiments run with bacterial sized beads did not show the same pattern (Scheuerman *et al.*, 1998).

Mueller (1996) has reported on a combination of processes in initial events of biofilm formation. Attachment, detachment and growth were observed using time-lapse microscopy and a parallel plate flow cell. Substrata of stainless steel, copper and silicon were exposed to a flowing bulk solution of *Pseudomonas aeruginosa* and nutrient media until a certain surface density was reached to monitor attachment and detachment rates. Subsequently, flow of sterile, cell free, nutrient media was initiated and growth and erosion of the attached cells was monitored. Silicon was the smoothest of the three materials and had the lowest rate of adsorption. Copper was the roughest material and showed the highest rate of irreversible attachment. However, no growth of attached cells on this surface was evident which is a result of the toxic effects of copper. Growth rates on silicon and stainless steel were found to be 0.28 hr^{-1} and 0.33 hr^{-1} respectively. This research showed that the net accumulation of cells on a surface was a result of attachment,

detachment and growth with growth being insignificant in the very early stages of biofilm development (Mueller, 1996)

Direct observation of attachment has been accomplished (Lawrence *et al.*, 1987b). In this report, the characteristic behavior of *Pseudomonas fluorescens* was described. The planktonic cells approach the surface and attach. Rotational behavior was seen for up to half an hour on the surface with rotation gradually slowing and ceasing resulting in a longitudinal irreversible attachment. Rotational behavior was also seen with detaching or emigrating cells prior to moving to a nearby location where the sequence of rotation and attachment was repeated. Following longitudinal attachment, cell division occurred resulting in two daughter cells with a clear space of 0.2 to 0.3 μm . The standard colonization and growth pattern for *P. fluorescens* is adsorption to a surface in single cells, development of 8-16 cell microcolonies, and emigration of the daughter cells to form 8-16 cell microcolonies elsewhere.

Observed differences between planktonic and biofilm cells. There is a wide range of research topics which address various aspects of attachment and subsequent growth of sessile cells. It is known that planktonic organisms are much more susceptible to antimicrobial agents than those in a biofilm (Brown and Gilbert, 1993). Organisms which partition from near starvation conditions in the bulk fluid can flourish on a surface. This behavior is explained by the greater

amount of surface associated nutrients available to sessile cells (Habash *et al.*, 1997; Kjelleberg *et al.*, 1982). These studies illustrate advantages for cells to adhere to surfaces rather than remain in the bulk environment.

One of the first efforts to address differences, or lack thereof, between sessile and planktonic cells was reported by Bakke in 1984. In this work, it was shown that *Pseudomonas aeruginosa* does not behave differently in planktonic cultures than biofilm cultures where diffusional resistance is neglectable. This conclusion allowed the kinetic and stoichiometric parameters for *P. aeruginosa* derived from planktonic cultures to describe steady-state biofilm processes (Bakke *et al.*, 1994). A contradictory finding by (Moller *et al.*, 1995) showed that the relationship between substrate concentration and growth rate for planktonic cells was not observed for sessile cells. A detailed review of surface and planktonic growth has examined several other contradictory findings where growth of a sessile population deviated from that of a planktonic population. The conclusion was that the lack of consistency is due to a variety of experimental conditions and analytical methods. More correlateable research is needed in this area (van Loosdrecht *et al.*, 1990).

Another difference between biofilm and planktonic environments is seen in the composition of a dual species biofilm. Under identical planktonic conditions, *Klebsiella pneumoniae* has a specific growth rate double that of *Pseudomonas aeruginosa* and therefore controls the population distribution in that environment. However, when the two organisms are present in a biofilm, *K. pneumoniae* can not

displace *P. aeruginosa* due to the manner in which each behaves in a sessile environment (Stewart *et al.*, 1997).

Phenotypic differences between planktonic and biofilm cells. It is now a widely accepted premise that bacteria undergo phenotypic changes when a transition from a planktonic state to a sessile state occurs. In essence, the switch from a planktonic type organism to a sessile one is mediated by a large number of regulated genes which are either expressed or down regulated depending upon the environment. Adhesion to a surface appears to rapidly initiate the phenotypic expression of a range of genes not used by planktonic organisms (Costerton *et al.*, 1995b; Costerton, 1995; Korber *et al.*, 1995; Costerton and Lappin-Scott, 1995a).

Evidence of this type of behavior was first presented by Dagostino *et al.* in 1991: Two types of marine bacteria were shown to express unknown genes upon attachment to surfaces via the use of a *lacZ* reporter system. The same organisms did not express these genes while in a planktonic environment (Dagostino *et al.*, 1991). In many cases, genes which are involved in EPS production are up regulated after an attachment event (Fletcher, 1996). Supporting research has shown that new protein synthesis is required for *Pseudomonas fluorescens* to form biofilms on abiotic surfaces. The production of these unknown proteins is part of a speculated common genetic pathway used to form biofilms on a range of abiotic surfaces (O'Toole and Kolter, 1998b).

Specific research in this area has identified a portion of the regulated genes that are being expressed. An example is the *algC* gene which is a key component in the alginate production pathway in *Pseudomonas aeruginosa*. Within the span of minutes after a planktonic cell adheres to a surface, this gene is expressed and eventually results in the abundance of alginate which is a primary constituent of the EPS produced by biofilms of this organism (Davies *et al.*, 1993).

The fact that biofilm cells are markedly different than planktonic cells is not trivial as this may help explain the increased resistance to antibiotics (Costerton *et al.*, 1995b) and other morphological changes (Dalton *et al.*, 1994).

Purpose

To initiate the development of a biofilm, bacterial cells in suspension are transported to a solid/liquid interface and adhere. Once these cells are in a sessile environment, they replicate and cover the surface to form a biofilm. The process of becoming a sessile organism may involve a sweeping number of genetic changes which causes the sessile cell to become phenotypically distinct from its planktonic counterpart (Costerton *et al.*, 1995b). In the experiments described here, *Pseudomonas aeruginosa* PA01 cells underwent this transition and there appeared to be a temporary halt in their growth and division cycle. This lag time may be a result of viable cells directing metabolic energy into other products, such as extracellular materials, rather than into replication (Fletcher, 1996).

The planktonic cells used for the experiments were cultivated in a chemostat reactor and then transferred to a parallel plate flow cell reactor for observation with CSLM. Also, inocula were placed in a batch reactor as a control. Under the conditions of these experiments, the results show that growth of primary biofilm cells is negligible during the first 12 hours following attachment, while no difference in growth is seen in planktonic cells which never became sessile. The growth of secondary biofilm cells was slower than planktonic cells but significantly faster than primary biofilm cells. Nutrient conditions for all three types of cells were held constant.

This finding is a result of direct, in situ observation of biofilm cells in a parallel plate flow cell visualized by confocal laser scanning microscopy. The purpose was to qualitatively compare the growth characteristics of cells in planktonic, primary biofilm and secondary biofilm environments.

Materials and Methods

Overview

The growth of bacteria in sessile and planktonic states was examined in this research project. The original bacterial cells which colonize a clean surface are referred to as "primary" biofilm cells and the subsequent progeny cells are termed "secondary". Both primary and secondary biofilm cells were observed using a parallel plate flow cell reactor and planktonic cells were studied in a standard batch culture. Batch culture experiments were conducted for periods of 6 and 12 hours while flow cell experiments lasted 8.5 and 12 hours.

Organism

The organism used in this study was *Pseudomonas aeruginosa* PA01. *Pseudomonas aeruginosa* is a motile, aerobic, Gram negative rod. This particular strain contained a plasmid that conveyed a resistance to 150 mg/l of carbenicillin and encoded the production of a green fluorescent protein (GFP). The GFP was constitutively expressed, thus enabling each cell to be "self labeling". The excitation wavelength of the GFP was 488-nm and an emission maxima was at 512-nm. This organism was obtained from Dr. Mike Franklin, at Montana State University.

Stock Culture

Stock cultures of the organism were prepared by streaking for isolated colonies on R2A agar (Difco) which contained 150 mg/l of carbenicillin (Sigma). Isolated colonies were then transferred to fresh R2A/carbenicillin agar and incubated until a confluent lawn was present. The culture was harvested, placed in a 2% peptone - 20% glycerol solution and stored in 2.0-ml vials at -70°C . For each experiment, approximately 1.0 ml of frozen stock culture was used as an initial inoculum.

Equipment Preparation

The equipment used in this study included media and waste containers, assorted tubing, a chemostat reactor, a parallel plate flow cell reactor, an inoculation vessel, a bubble trap, and a batch reactor. These components are illustrated in Figures 2.1 through 2.4 and described in following paragraphs.

The nutrient media for each experiment was prepared and sterilized separately from the remainder of the items as explained in the following section. The various vessels and tubing were cleaned by washing with soap and water and sterilized by autoclaving dry for 20 minutes at 121°C and 20 psi. The tubing upstream from each of the reactors was replaced for each experiment.

Media

The same nutrient media was used in the chemostat, flow cell, and batch reactor. The constituents are listed below in Table 2.1:

Table 2.1. Media Composition

Constituent	Amount (grams per liter)
K_2HPO_4	0.70
KH_2HPO_4	0.30
$(NH_4)_2SO_4$	0.10
$MgSO_4 \cdot 7H_2O$	0.01
Glucose	0.40

The potassium salts and ammonium sulfate (Fisher) were added to glass carboys, an appropriate amount of reverse osmosis water was added and the containers were sealed and autoclaved for 15 minutes per liter. The solution was cooled to room temperature before the remaining constituents were added. Proper amounts of glucose (Fisher) and magnesium sulfate (Aldrich) were dissolved in approximately 25 ml of reverse osmosis water and added to solution via syringe and a 0.2-micron syringe filter.

Chemostat Operation

A chemostat was used to grow inocula for both the flow cell and batch culture experiments. The reactor was operated at room temperature with a volume of 500

ml and a 5-hour residence time. The culture in the reactor was well mixed by a magnetic stir bar and plate and aerated by the laboratory pressurized air system through bacterial air vents (Gelman Sciences). A schematic diagram of the system is shown in Figure 2.1. After sterilizing all reactor components the system was assembled. The chemostat was filled with sterile nutrient media, inoculated with 1.0 ml of frozen stock culture and operated in batch mode for 24 hours. The pump was then turned on to supply media to the reactor and the effluent was pumped to a waste container. The bacteria were grown in a chemostat environment for 48 hours (approximately 10 residence times).

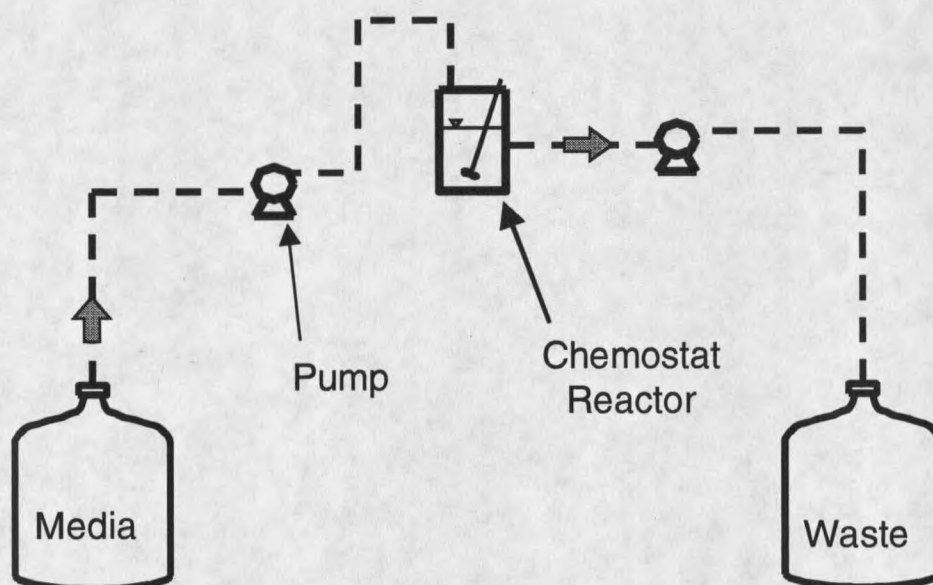


Figure 2.1. Schematic of one chemostat reactor system.

Batch Culture

A batch reactor was utilized with the media described above. 1.0 L of media was sterilized in a 2.0 L Erlenmeyer flask. An inoculum was withdrawn from a 48-hour chemostat culture and placed in the vessel. The batch culture was incubated at room temperature and well mixed using a magnetic stir bar and plate. Population measurements were made at the beginning, middle and end of a 12-hour period.

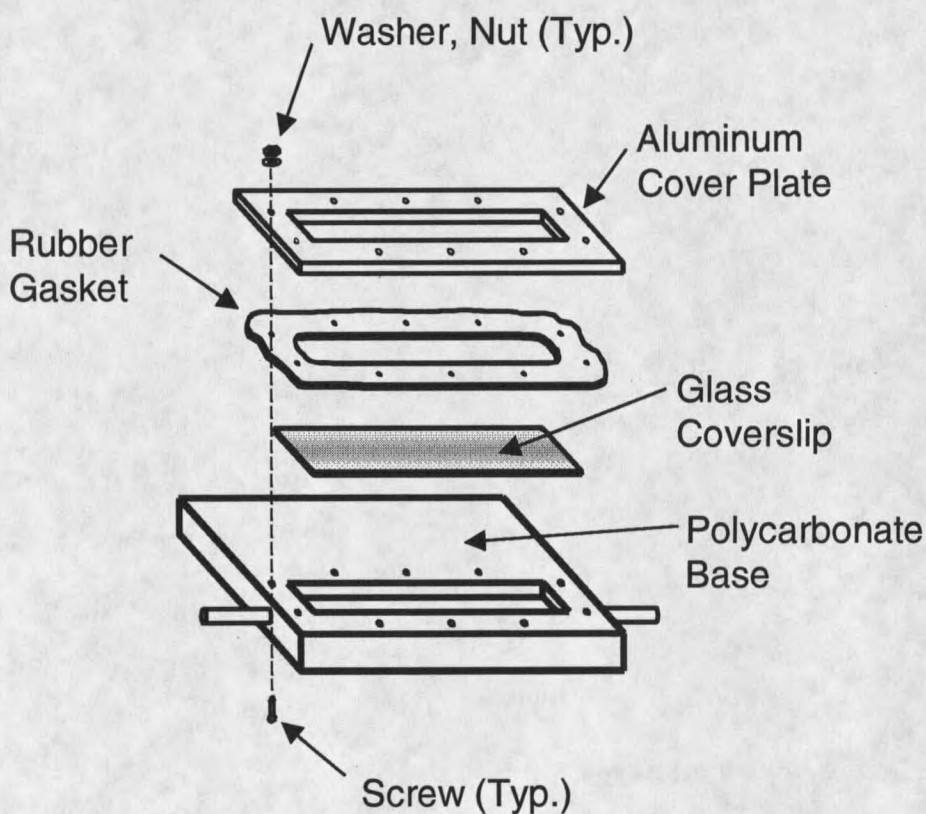


Figure 2.2. Parallel plate flow cell reactor.

Flow Cell Cleaning

Cells growing in an attached or sessile state were observed on the "wet side" of the coverglass in a parallel plate flow cell reactor (Figure 2.2). Cleaning of the flow cell prior to each experiment was carried out as follows:

The polycarbonate base was submersed in 95% ethanol for 30 minutes and then subjected to UV light for 30 minutes. Each experiment utilized a new glass coverslip that was washed twice with antibacterial soap and rinsed twice with 95% ethanol. The coverslip was then autoclaved for 20 minutes and dried at 115 C for 24 hours. Other assorted materials (tubing, coverplate, gasket, screw and nuts) were autoclaved for 20 minutes. After all parts of flow cell were sterilized or cleaned, the flow cell was assembled inside a biological hood.

Flow Cell Operation-Primary Cell Experiments

The entire flow cell reactor system (Figure 2.3) was set up on a laboratory cart to facilitate transportation to and from the microscope lab where the experiments were completed.

Prior to inoculation, the flow cell and all upstream tubing were filled with sterile media to purge air from the system. Approximately 15 ml of inoculum were withdrawn from a 48 hour chemostat culture and placed into the inoculation vessel, upstream of the flow cell. The inoculum was rapidly mixed using a magnetic stir bar and plate. A portion of the stirred inoculum was pumped through the flow cell

for approximately 1 minute. After cells had been introduced into the flow cell reactor, the inoculum flow was terminated and flow of sterile, cell free media commenced. The flow rate was set at 2.0 ml/minute which is laminar flow ($Re \sim 5.5$) with flow channel dimensions being 12 mm x 1.5 mm in cross section and 37 mm in length.

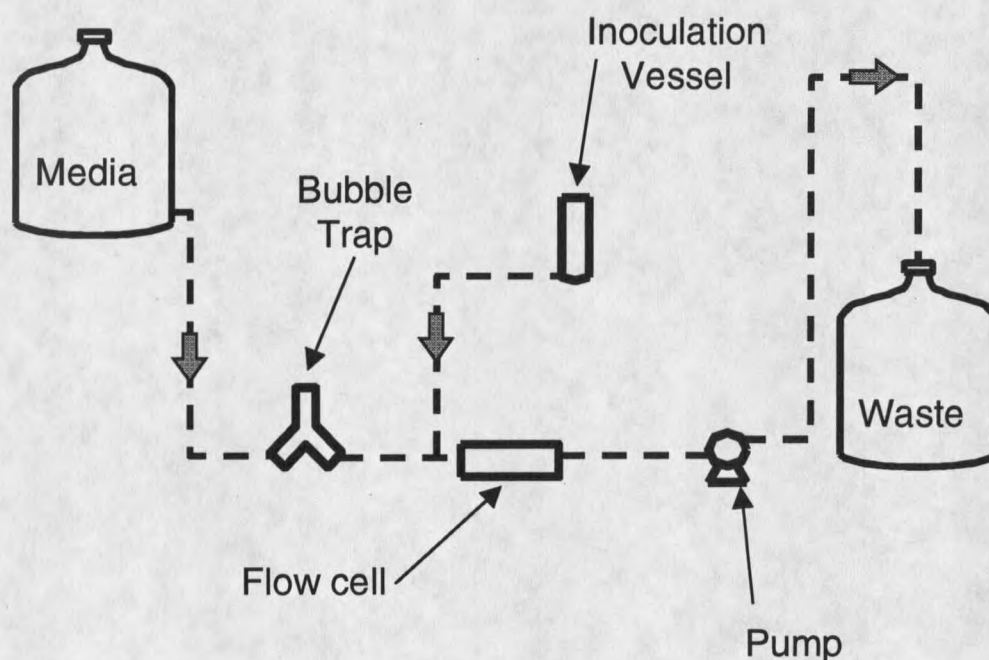


Figure 2.3. Schematic of flow cell reactor system

In order to prevent bubbles from collecting in the flow cell, the media container was elevated so that the upper surface of the media was approximately 0.5 meter higher than the flow cell with the media effluent port at the bottom of the container. The effluent line from the flow cell was at an elevation approximately level with the reactor. By aligning the system in this manner, the elevation of the media reservoir

served to slightly pressurize the reactor, preventing the formation of bubbles in the flow cell. A bubble trap (Figure 2.4) was also used downstream of the media reservoir to remove any large bubbles that may have entered the line during the initial start up or media container exchange. The pump is located downstream from the flow cell to minimize the amount of upstream tubing subject to initial cell inoculation. In this arrangement, the pump serves to hold flow back at the proper rate; without the pump in place, the media container would drain completely by gravity in a matter of minutes.

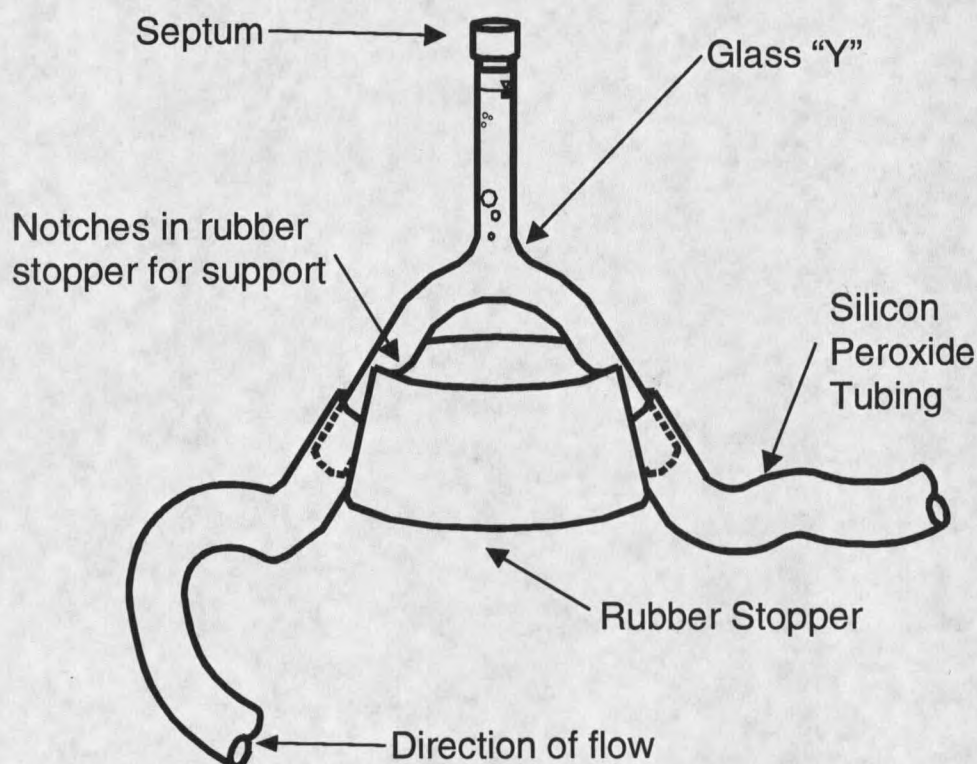


Figure 2.4. Bubble trap.

After inoculation of the flow cell reactor, the adhered cells were subjected to uninterrupted, cell free nutrient flow for 10 minutes. Subsequently, a random field of view was chosen to be observed. The flow cell reactor was left in place on the microscope stage and not moved for the duration of the experiment. The cells in this field of view were then observed using a confocal laser scanning microscope every 15 minutes for a period of 12 hours to determine population growth. At each interval, the desired field of view was first checked for focus by eye with transmitted white light. Subsequently, the image was captured using the laser light source. This method greatly reduced the amount of time the sample was subjected to bleaching and heating from exposure to the laser beam. Nutrient flow was halted temporarily for each image to prevent pulsing of the coverglass.

Flow Cell Operation – Secondary Cell Experiments

The flow cell was prepared and inoculated in the much the same fashion as detailed above and is described in the corresponding section in Chapter 3 of this report. Secondary biofilm cell experiments utilized a dilute mix of GFP+ cells in predominantly GFP- environment. This allowed for the certain identification of cells and their progeny. After inoculation, the flow cell reactor remained in operation, but not observed for a period of 24 hours. During this period of continuous sterile media flow, the biofilm grew to a depth of several cells (3-10 μm). The cell growth observed in this type of experiment was strictly growth of the secondary population.

Data Acquisition and Analysis

Image Capture and Analysis

Various TIF format images from both batch culture (cells captured on filter) and flow cell experiments were produced using a Leica TCS-NT Confocal Microscope System. The fluorescence emitted from the GFP produced by the bacteria was used to locate individual cells. The excitation of the protein was achieved with the 488-nm laser light source. The fluorescent emission, maxima at 512 nm, was registered after passing through a BF filter block (500 to 550 nm, cutoff).

The TIF image files were imported into an image analysis software package, Image-Pro Plus, Version 3.00.00 (Media Cybernetics). The software was calibrated based on the actual dimensions (100 μm x 100 μm for images captured using 100X, 1.4 N.A., oil immersion objective) of each image. The auto-count feature was set to identify all objects (cells) within a specified area range, usually 0.5 to 2.5 μm^2 . The software automatically assigned each cell an object number, and x and y coordinates, in μm , with the origin being the upper left corner of the image. Occasionally, several cells were overlapping and had to be manually tagged. In this event, the software still took record of the x,y coordinates of the object, however it was the researcher's task to identify and tag the locations of each of the overlapping cells.

Planktonic Cells

To determine population growth, direct cell counts were performed at the beginning and end of 6 and 12-hour periods using only GFP+ cultures. This was accomplished by removing 10 ml of the culture, vortexing to break up cell clusters, and filtering onto the surface of a 25 mm diameter, 0.2 micron pore size polycarbonate filter (Osmonics).

The filters were then placed on microscope slides and the fluorescent cells were examined using the same microscope, light source and filter settings described previously. From each filter, 12 to 15 separate fields of view were counted to determine the approximate number of cells per filter from which the number of cells per ml in the batch culture were extrapolated.

The average beginning and end cell concentrations and corresponding standard errors of the mean were calculated for each sample. Population increase was determined by dividing the change in cell concentration by the beginning concentration.

Biofilm Cells

Each of the cells in the initial field of view, or image, was identified and assigned an object number using image analysis software, as described previously. The original colonizing cells, their fate, and progeny were tracked over

the course of the experiment using the images and the object numbers. The following quantities were counted in each experiment:

N_0	Initial number of cells on the surface
N_{0E}	Number of original cells which emigrate
N_{0D}	Number of original cells which divide
N_{0R}	Number of original cells which remain but not divide/emigrate
n_P	Number of progeny cells arising from dividing cells
n_{PE}	Number of progeny cells which emigrate

From this data, various counts and percentages were determined as follows:

Table 2.2. Descriptive Quantities and Formulas

Value	Formula
Total number of cells, n_T	$N_0 + n_P$
Base number of cells, N_B	$N_0 - N_{0E}$
% of original cells that emigrate	$\{ N_{0E} / N_0 \} \%$
% of original cells that divide	$\{ N_{0D} / N_0 \} \%$
% of original cells that do not divide or detach	$\{ (N_0 - [N_{0E} + N_{0D}]) / N_0 \} \%$
% Surface accumulation increase	$\{ n_P / N_0 \} \%$
% Population increase	$\{ n_P / N_B \} \%$
% Population detach	$\{ (n_{PE} + N_{0E}) / n_T \} \%$

Results

Summary

Three types of experiments were performed which dealt with the growth of planktonic, primary biofilm and secondary biofilm cells. In each case, the purpose was to quantify the growth of each population to determine any differences between primary biofilm cells, their progeny, and their planktonic counterparts.

As was described previously, planktonic cell counts were obtained from GFP+ batch cultures of various ages. The growth of two 6-hour cultures and four 12-hour cultures were measured and percent population increase was calculated.

Four flow cell reactor experiments were conducted for primary and secondary biofilm cells. Primary cell experiments were run with only GFP+ cells and secondary cell experiments used a mix of GFP+ and GFP- cells. The length of these experiments varied from 8 to 12.5 hours. Each time, a specific field of view was observed for the duration of the experiment and individual biofilm cells were tracked. Cells growing in the attached state were quite active. Many cells moved around during the experiment, the total distance moved being in the tens of microns. Also, a number of the cells detached or emigrated to leave the field of view. Without tracking individual cells on a 15-minute interval, it is difficult to follow those cells and make any accurate judgment concerning their growth. Due to this

behavior, it was necessary to maintain the same field of view. The result is detailed information on a limited number of cells.

Planktonic Population Increase

The cell concentrations of the batch cultures were measured at the beginning and end of each experiment. The average increase after 12 hours was 981%. The results are listed below in Table 2.3. The percent population increase was determined by:

$$\%Increase = \frac{\text{Change_in_concentration}}{\text{Initial_concentration}} * 100$$

Table 2.3. Planktonic Growth Results

Culture ID	Time (hr)	Initial Concentration (cells/ml)	SEM*	Final Concentration (cells/ml)	SEM*	% Population Increase	Average% Population Increase
mar30-1	12	1.40E+04	1.08E+03	1.08E+05	4.78E+03	671.43	~ 12 hr 981 %
mar30-2	12	no count	N/A	1.44E+05	6.32E+03	N/A	
mar30-3	12	1.31E+04	5.79E+02	1.72E+05	7.23E+03	1212.98	
average		1.36E+04	8.30E+02	1.41E+05	6.11E+03		
jan29-1	12.17	1.91E+05	7.95E+03	3.13E+06	1.10E+05	1538.74	
jan29-2	12.17	2.25E+05	7.07E+03	no count	N/A	N/A	
jan29-3	12.17	2.30E+05	4.95E+03	1.38E+06	4.47E+04	500.00	
average		2.15E+05	6.66E+03	2.26E+06	7.74E+04		
jan21	5.95	5.88E+04	1.84E+04	2.08E+05	8.63E+03	253.74	~ 6 hr 194 %
jan18	5.45	9.94E+04	8.60E+03	2.32E+05	1.24E+04	133.40	

* SEM – Standard error of the mean

Primary and Secondary Biofilm Populations

A direct measurement of population growth was not possible due to the movement and emigration of biofilm cells. Because of this complication, an initial field of view was chosen and the cells identified. These original cells were then observed for the duration of the experiment and their fate was recorded. The description of the data and the calculations have been listed previously; the results are presented below in summary in Table 2.4 and in detail in Table 2.5.

The two key calculations made to illustrate the growth of biofilm cells are percent population change and percent surface coverage change. Invariably, there were cells present in the original field of view which emigrated before the end of the experiment. It is unknown whether these emigrating cells divided during the remainder of the observation period. The measurement of population increase is determined for only cells whose fate is known. Surface accumulation increase is a measure of the change in total numbers of cells attached in a field of view and is affected by cell division and emigration. There was no reattachment of cells in any field of view during these experiments of GFP+ cells. This is not surprising however, due to the very small percentage of GFP+ (therefore, visible) cells within the reactor.

The four experiments evaluating the behavior of primary biofilm cells were each carried out in separate reactors. The four sets of data resulting from the secondary biofilm experiments were taken from two reactors. The data from

experiment "nov21" had the cells arranged in such a way that three areas of interest (AOI) were used, and thus three sets of data were created.

Table 2.4. Summary Biofilm Cell Growth Results

Experiment	Time (hr)	% Surface Accumulation Increase	% Population Increase
Primary Biofilm Cells			
jan21	12.57	26.1	28.6
feb02	12	0	0
mar29	12.42	26.5	31
average	12.33	17.53	19.87
Secondary Biofilm Cells			
oct21	11	58.3	107.7
nov21-aoi#1	10	250.0	250.0
nov21-aoi#2	10	141.7	170.0
nov21-aoi#3	10	100.0	200.0
average	10.25	137.5	181.9

The planktonic population observed in the current project exhibits an average of nearly a 1000% increase over a 12-hour period and roughly a 200% increase in nearly 6 hours. The planktonic cells that were used to inoculate the batch reactors originated in a chemostat culture with a residence time of 5 hours, which corresponds to a doubling time of 3.5 hours (Madigan *et al.*, 1997). If the cells were to continue to replicate at that rate, these population increases are exactly what would be expected. From this information, it is evident that the cells which were transferred from the planktonic state in the chemostat to the planktonic state of a batch culture incurred no significant lag time and continued to replicate without any hindrance at a specific growth rate of 0.2 hr^{-1} .

This was not the case with cells that made the transition from a planktonic state to a sessile state. In these experiments, the primary cells that originally colonize the surface are the first, or parent, generation of biofilm cells. The primary cells, as a group, only achieved an 18% increase in surface coverage, and a 20% actual population increase over 12 hours. Also, only 7% of the primary cells divided during the first 12 hours after initial colonization. Due to such a great proportion of cells exhibiting no growth, it was not possible to determine a representative mean time to division needed to estimate growth rate.

Once a primary cell replicates, the two daughter cells are known as secondary biofilm cells. These cells are different in one significant way from their parent cells. These bacteria did not undergo a change in environment; they originated in an attached state and did not partition onto the surface from the bulk, or planktonic phase. This group of cells did not grow as fast as their planktonic counterparts, but did grow at a greater rate than the primary cells. In approximately a 10-hour period, there was a 138% increase in surface accumulation and a 182% population increase. The mean specific growth rate was 0.1 hr^{-1} .

Comparing the patterns shown by primary and secondary biofilm cells, it is evident that the secondary cells are replicating at a significantly faster rate. In general, the secondary biofilm cells are more active than the primary cells; 76% of primary cells neither divided nor emigrated compared with only 6% of secondary cells. Also, only 7% of primary cells replicated during the 12-hour observation period compared to 65% of secondary cells.

Table 2.5. Detailed Biofilm Growth Results

Primary BIOFILM CULTURE EXPERIMENTS															
Experiment:	Time(hr)	begin # cells (N _b)	# of orig. emigrate cells (N _{ec})	# of orig. cells divide (N _{eo})	# of orig. cells not div/emgt (N _{en})	# of cells from divison (N _r)	# of daughter emigrate cells (N _{ec})	total end # of cells (n)	base # of cells (N _b)	% original emigrate	% original divide	% original not div/emgt	% Surface Accum.	% Pop. Increase	% Pop. Detach
jan15	8.37	13	3	1	9	1	0	14	10	23	8	69	7.7	10.0	21.4
jan21	12.57	46	4	7	35	12	7	58	42	9	15	76	26.1	28.6	19.0
feb02	12	95	19	0	76	0	0	95	76	20	0	80	0.0	0.0	20.0
mar29	12.42	34	5	2	27	9	5	43	29	15	6	79	26.5	31.0	23.3
Total		188	31	10	147	22	12	210	157	16	5	78	11.7	14.0	20.5
								Avg. %							
								16.6							
								7.2							
								76.2							
								15.1							
								17.4							
								20.9							

Secondary BIOFILM CULTURE EXPERIMENTS															
Experiment:	Time(hr)	begin # cells (N _b)	# of orig. emigrate cells (N _{ec})	# of orig. cells divide (N _{eo})	# of orig. cells not div/emgt (N _{en})	# of cells from divison (N _r)	# of daughter emigrate cells (N _{ec})	total end # of cells (n)	base # of cells (N _b)	% original emigrate	% original divide	% original not div/emgt	% Surface Accum.	% Pop. Increase	% Pop. Detach
oct21	11	24	11	13	0	14	14	38	13	46	54	0	58.3	107.7	65.8
nov21-aoi#1	10	4	0	3	1	10	3	14	4	0	75	25	250.0	250.0	21.4
nov21-aoi#2	10	12	2	10	0	17	10	29	10	17	83	0	141.7	170.0	41.4
nov21-aoi#3	10	8	4	4	0	8	4	16	4	50	50	0	100.0	200.0	50.0
Total		48	17	30	1	49	31	97	31	35	63	2	102.1	158.1	49.5
								Avg. %							
								28.1							
								65.6							
								6.3							
								137.5							
								181.9							
								44.6							

Discussion

The purpose of this research was to contrast the growth of bacteria in three different settings: Planktonic, primary biofilm and secondary biofilm. Due to the length of the lag time exhibited by primary biofilm cells, it was not possible to determine a specific growth rate for that population. However, it was possible to estimate the specific growth rate of the planktonic and secondary biofilm population. The reason for comparing growth in these environments is to show that cells which transition from a planktonic state to a biofilm state endure a lag time or a period of no replication. The primary biofilm cells do not replicate at the same frequency as do their planktonic counterparts or their progeny.

In the past, the strict definition of the term planktonic has referred to cells which are suspended in solution and not attached to a surface or neighboring cells. A sessile organism has been defined as an organism that is attached to a surface and not suspended in a liquid culture. In recent years, the distinction between the two terms has come to imply not only environmental differences, but phenotypic differences as well (Costerton *et al.*, 1995b; Costerton, 1995; Korber *et al.*, 1995; Costerton and Lappin-Scott, 1995a; Fletcher, 1996). As an underlying tenet of this current research, it is believed that a change in phenotype does not occur instantaneously. Accordingly, a detached biofilm cell which is suddenly resuspended in the bulk fluid is an organism in a planktonic environment, but is expressing a biofilm phenotype. Research currently in progress is investigating

the time frame over which such changes in phenotype occur (Pulcini, 1999). For the scope of this discussion, the terms planktonic and secondary biofilm refer explicitly to phenotype, while the term primary biofilm describes a cell in transition.

Bakke *et al.* (1994) proposed that growth rates of *Pseudomonas aeruginosa* biofilm cells and planktonic cells are the same provided the microenvironment of the cells is the same. Therefore, the kinetic parameters determined for a planktonic culture could be used to predict biofilm behavior while the biofilm is not experiencing any diffusion limitation (Mueller, 1990). The biofilms studied in this research are only a few microns thick and do not have any diffusion limitations; however, there are differing growth rates depending on the source of the cell. This result shows that the conclusions drawn by Bakke *et al.* do not apply for very early stage biofilms.

Differences in the growth of planktonic and biofilm cells have been described previously (Moller *et al.*, 1995; Kjelleberg *et al.*, 1982; Habash *et al.*, 1997; Moller *et al.*, 1996; Dalton *et al.*, 1994; van Loosdrecht *et al.*, 1990; Xu *et al.*, 1998; Korber *et al.*, 1994; Bakke *et al.*, 1994). The results of these studies vary, indicating that planktonic cells grow faster, slower and the same as biofilm cells. The purpose of this current research, again, is not to refute or support any of these findings. Rather, the aim is to show that cells that undergo a change in phenotype from planktonic to biofilm, without a change in nutrient conditions, also endure a lag time in their rate of replication.

The population increase of primary cells is virtually nonexistent in contrast to the growth experienced in the first 12 hours of a planktonic culture. From these results, it is apparent that the cells which transition from a planktonic phenotype to a biofilm phenotype endure a lag phase in their growth before significant biofilm accumulation can occur.

Quantifying the growth of a population of biofilm cells has been accomplished by others (Korber *et al.*, 1994; Lawrence *et al.*, 1987b; Lawrence and Caldwell, 1987a; Lawrence *et al.*, 1989; Caldwell and Lawrence, 1986; Korber *et al.*, 1989). In each case, the specific growth rate for various species of adhered cells was determined in a parallel plate flow cell via time lapse microscopy. Their results suggest that the organisms in each case did not exhibit any lag type behavior but rather were replicating from the moment of attachment. This would appear to contradict the findings of this research. However, their inocula consisted of biofilm phenotype cells rather than cells with a purely planktonic phenotype. Therefore, no lag time would be expected due to no change in phenotype occurring.

Mueller also has studied the behavior of sessile cells on various surfaces (Mueller *et al.*, 1992; Mueller, 1996; Mueller, 1990). The flow cell reactor system used in these studies is operated in a similar manner to the one described herein and the organism is the same species but a different strain: *Pseudomonas aeruginosa* ERC. Mueller does not specifically discuss the aspect of a lag time but the data presented indicate the occurrence of such an event. The inocula for these experiments were drawn from chemostat culture where the cells would truly

be expressing a planktonic phenotype. Therefore, any adherence and subsequent growth on a surface would necessitate a change to a biofilm phenotype.

These results show that when *Pseudomonas aeruginosa* PA01 cells undergo transition from a planktonic environment to a sessile one, there is a lag time in growth associated with this change. This conclusion fits well with the idea that these cells have changed phenotypically as well. If the number of genes being up or down regulated is as large as has been postulated (Costerton *et al.*, 1995b) then this would constitute a major change. Also, if several of these genes are involved in the production of extracellular materials (Davies *et al.*, 1993), then it would seem that a portion of metabolic energy is now being required for this task at the expense of creating new cells. Overall, the results of this research fit very well with the current conceptual model of biofilm formation.

CHAPTER 3

MOVEMENT, REPLICATION, AND EMIGRATION OF SECONDARY BIOFILM CELLS

Background

Biofilms are communities of microorganisms that can be found in virtually every type of aqueous environment. A biofilm is an ingeniously organized multi-species entity where ecological microniches are occupied by specific organisms (Costerton *et al.*, 1995b; Costerton, 1995). Bacteria can attach to both abiotic and biotic materials as is evidenced by many types of human infections, the corrosion of metals, the reduced efficiency of industrial heat dissipation equipment, and the degradation of waste materials (Costerton *et al.*, 1995b).

Once bacteria have colonized a surface, growth of the attached population and induction of new organisms into the community occurs. The resulting biofilm is an intricate assortment of micro- and macroorganisms (Fletcher, 1996). Because of the amazing complexity, prevalence, and impact of microbial biofilms, it is important to understand how these communities form. The first stages of biofilm development, "initial events", have been studied in detail but are poorly understood. It is known that topography affects the manner in which bacterial cells

colonize a surface (Camper *et al.*, 1994; Scheuerman *et al.*, 1998). Cells which originally colonize a particular surface are the primary biofilm cells, or parent organisms of the ensuing biofilm. It has been reported that a certain species of primary cells forms dense microcolonies and then disperses in a matter of minutes (O'Toole and Kolter, 1998a). After replication of biofilm cells, daughter cells can spread out upon the surface, pack tightly near the parent cell location, be shed into the bulk fluid, roll along the substratum, or form long chains of cells (Lawrence and Caldwell, 1987a; Korber *et al.*, 1989; Power and Marshall, 1988; Shapiro and Hsu, 1989; Dalton *et al.*, 1994). It has been postulated that the various types of behavior exhibited by these cells is species dependent (Lawrence and Caldwell, 1987a).

Prior Research

Initial Events Description. One of the most valuable results from initial events research is a conceptual model of biofilm development. The processes involved in the initial events of biofilm formation have been summarized by (Mueller *et al.*, 1992):

1. Conditioning of the substratum by organic molecules
2. Transport of cells from the bulk water to the solid liquid interface
3. Adsorption of cells on the substratum
4. Transformation of irreversibly adsorbed cells to reversibly adsorbed cells
5. Desorption of reversibly adsorbed cells from the substratum into the bulk water
6. Growth of irreversibly adsorbed cells
7. Erosion of cells from adsorbed colonies into the bulk water.

Attachment. The first step in biofilm formation is the creation of a conditioning layer on a "clean" substratum and has been thoroughly investigated and reported (Marshall, 1996). The second step, the initial attachment of cells to a substratum, is influenced by the nature of the surface to which organisms are adhering. Given a topographically homogeneous substratum of stainless steel, *Pseudomonas aeruginosa* displays a completely random spatial pattern of attachment. However, for a slightly rougher substratum, this organism exhibits a degree of aggregation, thereby indicating that the topography of the substratum does have an influence on initial attachment (Camper *et al.*, 1994; Hamilton *et al.*, 1995).

A further examination of the influence of topography on bacterial adhesion has been carried out (Scheuerman *et al.*, 1998). This was accomplished by observing the attachment patterns of bacteria to a smooth silicon coupon marked by distinct grooves creating a well-characterized structural heterogeneity. Results again proved that substratum topography has an impact upon the bacterial attachment pattern. Cells preferentially attached to regions of the substratum surrounding the grooves whereas control experiments with bacterial sized beads did not show any specificity in terms of adsorption location.

Growth. Growth of sessile cells occurs after attachment. Three types of measurements of growth rates of sessile bacteria have been presented which involve the time required for cell fission, the specific increase in cell number, and the specific increase of cell area (Caldwell and Lawrence, 1986; Lawrence *et al.*,

1989). These parameters were measured using image analysis techniques. It was shown that using the specific increase in cell area or size may give misleading results due to cells becoming smaller with each generation due to environmental conditions.

Direct observation of sessile cell growth has been reported (Lawrence *et al.*, 1987b). In these studies, a characteristic growth pattern of *Pseudomonas fluorescens* was determined. Planktonic cells approach the surface and attach followed by rotational motion for up to half an hour with rotation gradually ceasing, resulting in a longitudinal irreversible attachment. Rotational behavior was also seen with detaching or emigrating cells which move to a nearby location and repeat the behavior of rotation and attachment. Subsequent to longitudinal attachment, cell division occurred resulting in two daughter cells 0.2 to 0.3 μm apart. After 3 to 4 generations, other movement was seen such as vibration, directional movement and eventually emigration. The standard colonization pattern reported was that *P. fluorescens* first colonizes a surface in single cells, 8 to 16 cell microcolonies arise from cell division, followed by emigration of the daughter cells to form 8 to 16 cell microcolonies elsewhere.

Further research into the development of *P. fluorescens* biofilms focused on the "recolonization" behavior of both motile and non-motile emigrating cells. It was reported that the average recolonization distance for motile cells was 47 μm which was 3 times greater than for non-motile cells. This characteristic behavior allows for the redistribution of cells across a substratum to virtually all vacant

spaces and is a key phase in the development of a confluent biofilm (Korber *et al.*, 1989)

Another report of direct observation of growth and behavior of biofilm cells described 4 types of cell movement: packing, spreading, shedding and rolling (Lawrence and Caldwell, 1987a). This work involved the use of natural stream populations cultivated in flow cell reactors. The packing behavior arose from progeny cells remaining very close to the parent cell location which resulted in the formation of discrete, tightly packed microcolonies. Spreading of daughter cells by distances of 5 –20 μm created diffuse microcolonies. Shedding behavior involved a parent cell being attached to the substratum while each daughter cell oriented away from the substratum and detached upon the completion of division. Rolling cells maintained contact with the surface but were not stationary nor did they form microcolonies. Exponential growth was observed in the microcolonies of the packing and spreading cells, but not for rolling or shedding cells. The authors state that these colonization maneuvers may be species characteristics and can only be discerned by continuous observation.

A related report addressed growth of marine bacteria on a surface bound substrate (Power and Marshall, 1988). The pseudomonad studied underwent growth and division while attached to the surface. Attached cells were observed to increase in size and then divide, with the two daughter cells moving away from each other. The cells tended to move great distances, up to 50 times their own length before completing another division cycle. The migration rate was relatively

slow with a mean of 0.15 $\mu\text{m}/\text{min}$. Detachment of cells from the substratum was also observed and suspected to be related to the unavailability of sorbed nutrients.

Modeling

Modeling of biofilm growth is a fundamental area of research. Cellular automata (CA) is a well-defined method (Wuensche, 1999) and has been described in the context of biofilms by (Picioreanu *et al.*, 1998a; Picioreanu *et al.*, 1998b; Wimpenny and Colasanti, 1997b; Wimpenny and Colasanti, 1997a). CA is a discrete dynamic array composed of "cells". Each cell or compartment in the model is capable of being updated simultaneously according to rules set for the particular model. In the case of modeling biofilms, these compartments may contain bacterial cells, bulk fluid, EPS or another material. The rules governing a biofilm CA are designed to simulate cell growth, detachment, and other biofilm processes based on varying conditions within the biofilm as dictated by user specified rules.

One aspect unique aspect of biofilm CA models is that the rules governing substrate concentrations are based on differential equations where the predicted concentration gradient drives the instantaneous changes. However, cell growth and movement and detachment is still modeled in discrete steps (Picioreanu *et al.*, 1998a; Picioreanu *et al.*, 1998b). An interesting application of this type of modeling has been presented (Wimpenny and Colasanti, 1997b; Wimpenny and Colasanti, 1997a). In these models, the relative substrate concentration has

significant effects on the morphology of the predicted biofilm. When very high substrate concentrations are used, a dense, uniform biofilm is predicted. On the contrary, when a low substrate concentration in the bulk is modeled, a sparse dendritic structure results.

CA models are important in biofilm research because, when sufficiently developed, they can be used to complement laboratory findings. In some cases, the model can be used to formulate research hypotheses (Wimpenny and Colasanti, 1997b). The findings described herein can be used to further define cell behavior rules within a biofilm and contribute to the refinement of modeling input parameters.

Purpose

This research examined the following behaviors exhibited by individual cells growing in a biofilm environment on a glass substratum: growth, movement and emigration. Others have conducted similar research, however their work involved sessile cells on a sparsely colonized surface (Arturo, 1998; Power and Marshall, 1988; Lawrence *et al.*, 1987b; Lawrence and Caldwell, 1987a). This research examines cells within a 3-10 μm thick biofilm composed of *Pseudomonas aeruginosa* PA01. The planktonic inocula used for the experiments were cultivated in a chemostat reactor and then transferred to a parallel plate flow cell reactor for observation with confocal scanning laser microscopy.

Materials and Methods

Overview

The early stages of biofilm development were studied by observing individual cells growing within a monospecies biofilm matrix inside a flow cell reactor. Flow cell inocula were grown in chemostats with glucose as the carbon source. The same nutrient media was utilized in both chemostat and flow cell reactors.

The purpose of this research was to quantify rates of replication, motion, and detachment of individual cells. This was accomplished by tracking the position of specific cells and their progeny over the course of each of three experiments and determining the time to replication, or detachment, and the distance moved for each cell. Images of bacterial cells within a biofilm were acquired using a confocal scanning microscope. Data from the images was extracted using image analysis software.

Organism

A parent strain and a construct of *Pseudomonas aeruginosa* PA01 were used in this study. *Pseudomonas aeruginosa* is a motile, aerobic, Gram negative rod. The parent strain contained a plasmid conveying a resistance to 150 mg/l of carbenicillin. The construct strain contained the same plasmid, but production of a green fluorescent protein (GFP) was also encoded. The GFP was constitutively

expressed, thus enabling each parent and daughter cell to be "self labeling". The excitation wavelength of the GFP was 488 nm and the emission maxima was at 512 nm. These organisms were obtained from Dr. Mike Franklin, at Montana State University.

Cultures of the parent strain of the organism (PA01-GFP-) were kept separate from the GFP construct strain (PA01-GFP+) until the inoculation of the parallel plate flow cell reactor. A much greater ratio of parent cells to GFP construct cells was used in the flow cell reactor. The resulting biofilm was dilute mix of labeled, or visible, cells which made it possible to identify and track individual cells and their progeny within a biofilm matrix. The ratio was approximately 100:1 GFP- to GFP+.

Stock Culture

Stock cultures of both the parent strain and construct (PA01-GFP- and PA01-GFP+) were prepared by streaking for isolated colonies on R2A agar (Difco) which contained 150 mg/l of carbenicillin (Sigma). Isolated colonies were then transferred to fresh R2A/carbenicillin agar and incubated until a confluent lawn was present. The culture was harvested, placed in a 2% peptone- 20% glycerol solution and stored in 2.0-ml vials at -70 C. For each experiment, approximately 1.0 ml of frozen stock culture was used as an initial inoculum for each organism.

Equipment

The equipment used in this study included media and waste containers, assorted tubing, two chemostat reactors, a parallel plate flow cell reactor, an inoculation vessel, and a bubble trap. These components are illustrated in Figures 1-4 and described in following paragraphs.

The nutrient media for each experiment was prepared and sterilized separately from the remainder of the items as explained in the following section. The various vessels and tubing were cleaned by washing with soap and water and sterilized by autoclaving dry for 20 minutes at 121 C and 20 psi. The tubing upstream from each of the reactors was replaced for each experiment.

Media

Table 3.1 - Media Composition

Constituent	Amount (grams per liter)
K_2HPO_4	0.70
KH_2HPO_4	0.30
$(NH_4)_2SO_4$	0.10
$MgSO_4 \cdot 7H_2O$	0.01
Glucose	0.40

The same nutrient media was used in the chemostat, flow cell, and batch reactor. The constituents are listed above in Table 3.1. The potassium salts and

ammonium sulfate (Fisher) were added to glass carboys, an appropriate amount of reverse osmosis water was added and the containers were sealed and autoclaved for 15 minutes per liter. The solution was cooled to room temperature before the remaining constituents were added. Proper amounts of glucose (Fisher) and magnesium sulfate (Aldrich) were dissolved in approximately 25 ml of reverse osmosis water and added to solution via syringe and a 0.2-micron syringe filter.

Chemostat Operation

Two chemostat reactors were used to grow inocula for the experiments. The chemostats were each operated at room temperature with a volume of 500 ml and a 5 hour residence time. The cultures in the reactors were well mixed by a magnetic stir bar and plate and aerated by the laboratory pressurized air system and bacterial air vents (Gelman Sciences). A schematic diagram of the system is shown in Figure 3.1. After sterilizing all reactor components the system was assembled. Each reactor was filled with sterile nutrient media, inoculated with 1.0 ml of the appropriate frozen stock culture (PA01-GFP- or PA01-GFP+) and operated in batch mode for 24 hours. The pumps were then turned on to supply sterile media to the reactors and the effluent was pumped to a waste container. The bacteria were grown in a chemostat environment for 48 hours, or about 10 residence times.

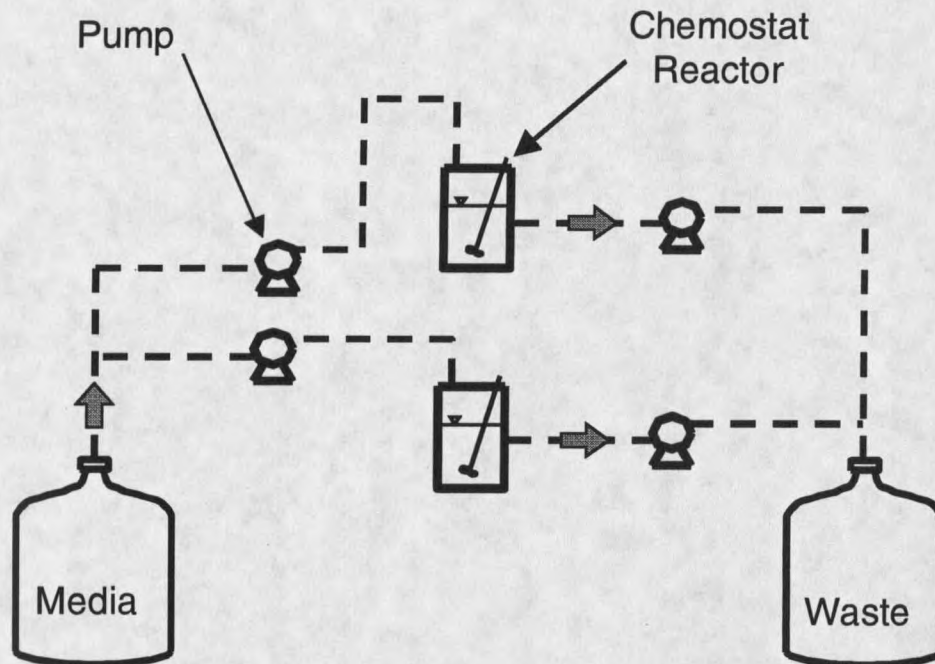


Figure 3.1. Schematic of two chemostat reactor system.

Flow Cell Cleaning

Cells growing in an attached or sessile state were observed on the "wet side" of the coverglass in a parallel plate flow cell reactor (Figure 3.2). Cleaning of the flow cell prior to each experiment was carried out as follows. The polycarbonate base was submersed in 95% ethanol for 30 minutes and then subjected to UV light for 30 minutes. Each experiment utilized a new glass coverslip that was washed twice with antibacterial soap and rinsed twice with 95% ethanol. The coverslip was then autoclaved for 20 minutes and dried at 115 C for 24 hours. Other assorted materials (tubing, coverplate, gasket, screw and nuts) were autoclaved for 20

minutes. After all parts of flow cell were sterilized or cleaned, the flow cell was assembled inside a biological hood.

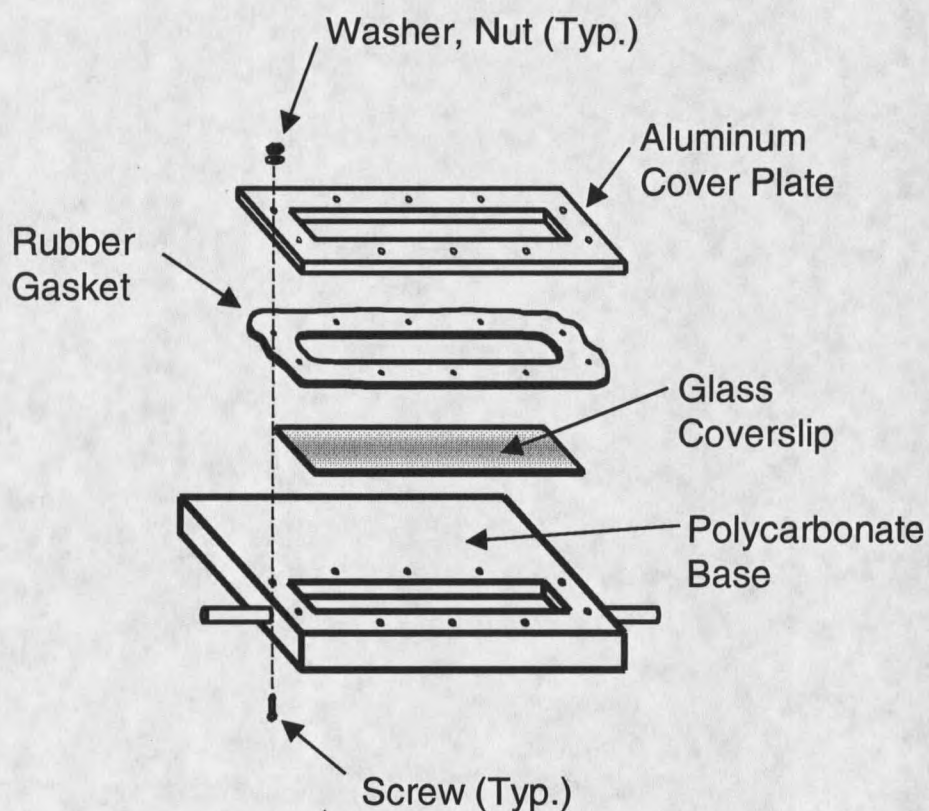


Figure 3.2. Parallel plate flow cell reactor.

Flow Cell Operation

The entire flow cell reactor system (see Figure 3.3) was set up on a laboratory cart to facilitate transportation to and from the microscope lab where the experiments were completed.

Prior to inoculation, the flow cell and all upstream tubing were filled with sterile media to purge air from the system. Using a sterile syringe, 20 ml of parent strain inoculum (PA01-GFP-) was withdrawn from a 48-hour chemostat culture and placed into the inoculation vessel along with 0.5 ml of PA01-GFP+ inoculum. The inoculum was rapidly mixed using a magnetic stir bar and plate to break up cell clumps.

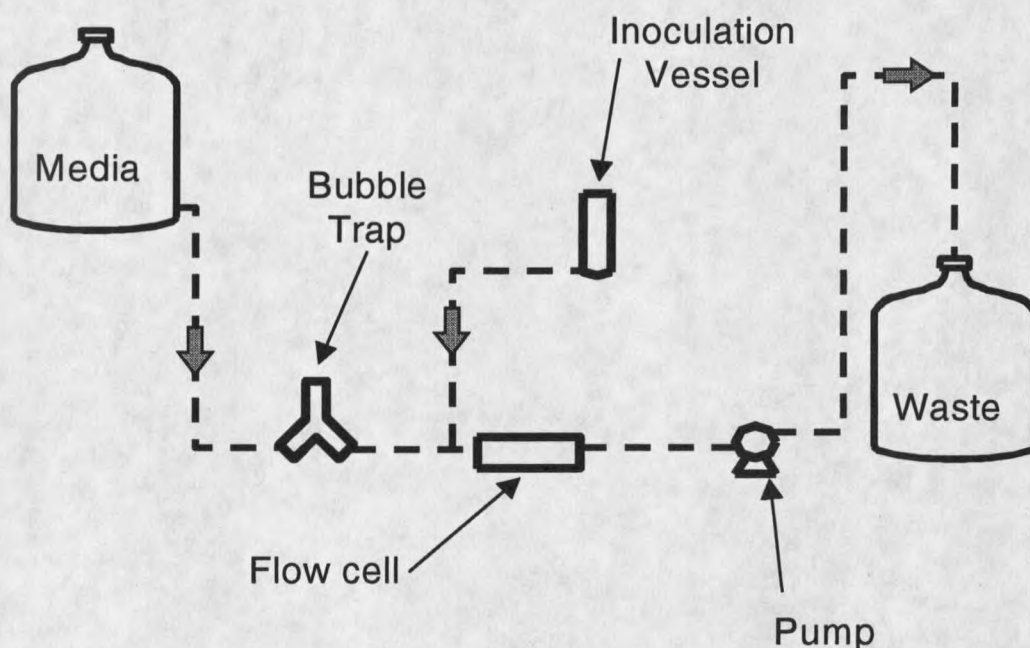


Figure 3.3. Schematic of flow cell reactor system.

The entire volume of the stirred inoculum was pumped through the flow cell in a series of three pulses. Approximately one third of the inoculum (7.0 ml) was drawn through the flow cell and flow was stopped for 5 minutes. The second and final 7.0 ml were pumped in a similar manner, with 5-minute stagnation periods before

resuming flow. This method of introducing cells into the reactor was used to ensure that high initial cell densities on the coverglass were reached.

After the entire volume of inoculum had passed through the reactor and the final 5 minute no-flow period expired, flow of sterile, cell free media commenced. The flow rate was set at 2.0 ml/minute which is laminar flow ($Re \sim 5.5$) with a flow channel of 12 mm x 1.5 mm in cross section and 37 mm in length.

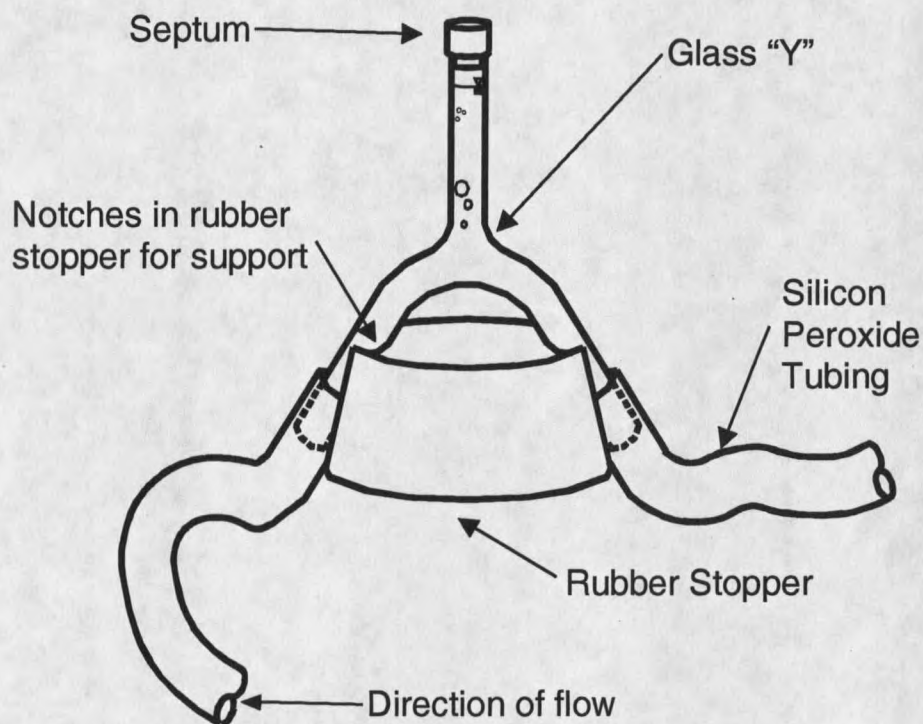


Figure 3.4. Bubble trap.

In order to prevent bubbles from entering the flow cell, the media container was elevated so that the upper surface of the media was approximately 0.5 meter higher than the flow cell with the media effluent port at the bottom of the container.

The effluent line from the flow cell was at an elevation approximately level with the reactor. By aligning the system in this manner, the elevation of the media reservoir served to slightly pressurize the reactor, preventing the formation of bubbles in the flow cell. A bubble trap (Figure 3.4) was also used downstream of the media reservoir to remove any large bubbles that may have entered the line during the initial start up or media container exchange. The pump is located downstream from the flow cell to minimize the amount of upstream tubing subject to initial cell inoculation. In this arrangement, the pump serves to hold flow back at the proper rate; without the pump in place, the media container would drain completely by gravity in a matter of minutes.

Data Acquisition

After inoculation of the flow cell reactor, the adhered cells were subjected to uninterrupted, cell free nutrient flow for a period of 24 hours. This amount of time was sufficient to allow a biofilm of 3 to 10 microns in depth to form. At the end of this incubation period, a particular field of view was chosen for observation. The flow cell reactor was left in place on the microscope stage and not moved for the duration of the experiment. The cells in this field of view were then observed every 15 minutes for a period.

At each interval, the desired field of view was first checked for focus by eye with transmitted white light. Subsequently, the image was captured using the laser

light source. This method greatly reduced the amount of time the sample was subjected to bleaching and heating from exposure to the laser beam. Nutrient flow was halted temporarily for each image to prevent pulsing of the coverglass.

Each of the cells in the initial field of view, or image, was identified and assigned an object number using image analysis software, as is described in the following section. The original cells and their progeny were tracked over the course of the experiment using the sets of images and the object numbers. The time to division or detachment, the distance moved for each cell, and the growth of the population was determined from the sequence of images.

Image Capture

TIF format images of PA01-GFP+ cells growing in a biofilm were produced using a Leica TCS-NT Confocal Microscope System. The fluorescence emitted from the GFP identified the location individual cells. Excitation of the protein was achieved with the 488-nm laser light source. The fluorescent emission, maxima at 512 nm, was registered after passing through a BF filter block which only allowed the passage of light between 500 and 550 nm. PA01-GFP- organisms were also present in the reactor, but were not visible due to the absence of GFP.

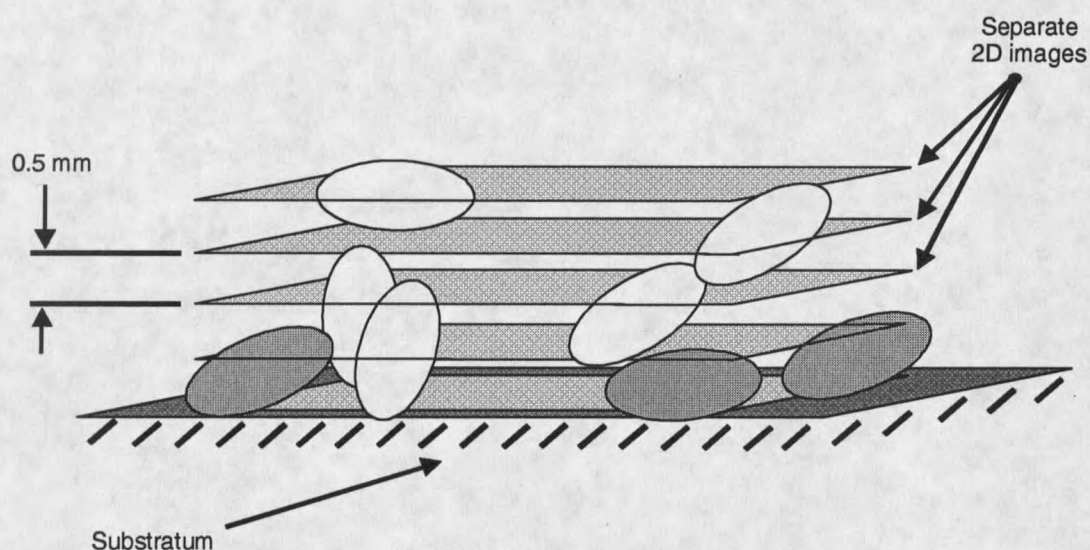


Figure 3.5. Schematic of composition of three-dimensional image, darker shaded cells represent PA01-GFP-.

Images were acquired at ~ 0.5 micron elevation intervals starting at, and proceeding away from, the “wet” surface of the coverglass (Figure 3.5). These “optical slices” were compiled to produce a three dimensional picture of cells within the biofilm matrix using software provided by Leica. A composite image, or “top down view”, of images from each elevation at one time point was also stored in TIF format (Figure 3.6.)

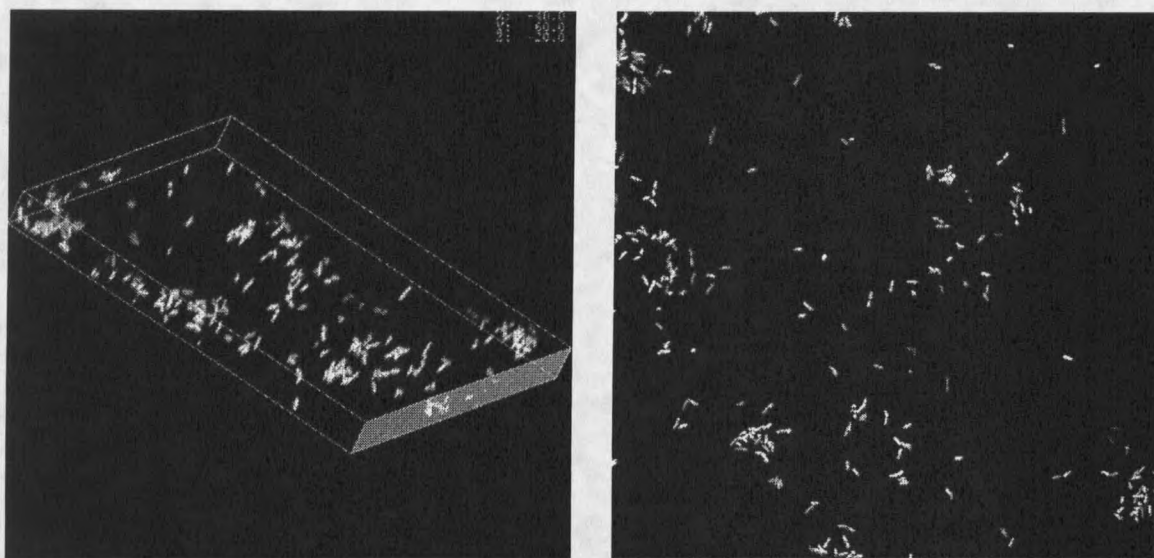


Figure 3.6. Example 2D and 3D images. Left image is a rotated, three dimensional compilation and right image is a composite, top down, view of the same three dimensional image composed of 18 separate 2D optical slices. Dimensions of box in left image: $100 \times 100 \times 18.5 \mu\text{m}$. Dimensions of right image: $100 \times 100 \mu\text{m}$.

Image Analysis

Extracting the data, or the x,y,z coordinates of each cell in the images was undertaken in two steps. The first step required that individual cells be uniquely identified and the two dimensional x,y coordinates of each cell be determined. The second step produced the third, or z coordinate of each cell.

To determine x,y coordinates of each cell, the composite image files (shown at right in Figure 6) were imported into an image analysis software package, Image-Pro Plus, Version 3.00.00 (Media Cybernetics). The software was calibrated based on the actual dimensions ($100 \mu\text{m} \times 100 \mu\text{m}$ for images captured

using 100X, 1.4 N.A., oil immersion objective) of each image. The auto-count feature was set to identify all objects (cells) within a specified area range, usually 0.5 to 2.5 μm^2 . The software automatically assigned each cell an object number, and x and y coordinates, in μm , with the origin being the upper left corner of the image. Occasionally, several cells were overlapping and had to be manually tagged. In this event, the software still took record of the x,y coordinates of the object, however it was the researcher's task to identify and tag the locations of each of the overlapping cells.

To determine the z coordinate of each cell, all the images in a particular stack were examined. An elevation of zero was assigned to the image nearest the location of the substratum. Each cell was in focus in a minimum of 2 adjacent images and it was therefore necessary to determine which image best reflected the location of the centroid of each cell. The absolute elevation of each cell was based on the image number in the stack and the distance between images.

Data Analysis and Results

Cell Movement

The final result of the image analysis process described previously was a list of cells for each particular image containing their assigned object number and x,y and z coordinates. An example of this is shown in Table 3.2; the microscope stage vertical step size and coordinate dimensions are in microns. The next step was to assign each cell a unique identification (i.e. A, B, C...) and then determine which cell/object number (i.e. 1, 2, 3...) from each image corresponded to that ID. The result was a chart similar to that of Table 3.3.

Table 3.2. Object Numbers and Coordinates

Image	1			
Filename	98112109.tif			
7 of 8	objects in range			
6	added by manually splitting objects			
step size	0.486			
Obj.#		Center-X	Center-Y	Center-Z
1		51.95	46.46	0.486
2		51.78	48.37	0.6075
3		53.16	48.67	0.486
4		54.46	49.38	0.486
6		52.57	52.61	0.6075
7		56.79	53.66	0.8505
8		53.57	53.95	0.6075
T1		50.24	50.83	0.972
T2		51.61	50.34	0.486
T3		53.76	50.24	1.458
T4		53.67	50.93	0.6075

Table 3.3. Object Tracking

Image Time (min)	0	1	2	3	4	5	6	7	8	9	10	11
Cell ID	Object Numbers (T1 = manual assignment; -1 = detachment; blank = not appeared yet)											
A	1	1	1	1	1	1	1	1	1	1	1	1
B												
C	2	2	2	2	3	3	3	3	4	4	4	4
D												
E	3	3	3	3	2	2	2	2	2	3	3	3
F												
G		4	4	T7	4	5	4	4	3	2	2	2
H												
I	5	6	6	5	6	9	10	8	9	T7	10	10
J												
K			7	6	7	10	11	9	10	9	11	11
L												
M	6	7	8	8	9	-1	-1	-1	-1	-1	-1	-1
N	7	8	9	7	8	11	12	10	11	10	12	12
O	T1	T1	T1	T1	T1	7	8	6	7	8	9	8
P						8	9	7	8	7	8	9
Q	T2	T2	T2	T2	T2	T1	T1	T1	T1	T1	6	6
R											T1	T1
S	T3	T6	T5	T3	T6	T5	T5	T5	T5	T6	T5	T6
T												
U							T6	T6	T6	T4	T6	T4
V												
W	T4	T3	T3	T6	T3	T2	T2	T2	5	5	5	5
X												
Y									T2	T2	T2	T2
Z	T5	T4	T4	T5	T4	T3	T3	T3	T3	T3	T3	T3
AA												
AB	T6	T5	T6	T4	T5	T4	T4	T4	T4	T5	T4	T5
AC												

Once the data were arranged in such fashion, each table was imported from Microsoft Excel 97 into Microsoft Access 97. Due to the microscope stage moving laterally by small amounts during the course of each experiment, mathematical corrections had to be made based upon the magnitude and direction of the image shifts. These correction distances were determined visually and a list, one correction for each image, was created and applied to all cell coordinates. The data was then sorted first by cell ID and second by time to produce a format such

as Table 3.4 which included the corresponding image number and time of observation.

Table 3.4. Movement Data

Cell ID	Image	Time (min)	Object Number	X-coord	Y-coord	Z-coord
C	0	0	2	51.67	48.11	0.61
C	1	13	2	51.78	48.174	0.61
C	2	26	2	51.808	48.041	0.61
C	3	43	2	51.701	48.133	0.49
C	4	58	3	51.63	48.151	0.49
C	5	72	3	51.626	48.321	0.49
C	6	88	3	51.569	48.28	0.73
C	7	103	3	51.439	48.15	0.85
C	8	118	4	51.49	48.212	0.73
C	9	133	4	51.401	48.129	0.24
C	10	148	4	50.991	48.085	0.73
C	11	163	4	50.992	48.136	0.97
C	12	179	4	50.982	48.142	0.73
C	13	193	4	51.114	47.658	0.73
C	14	209	4	51.014	47.698	0.73
C	15	223	4	51.183	47.702	0.73
C	16	237	4	51.329	47.797	0.73
C	17	253	4	51.309	47.752	0.73
C	18	269	4	51.254	47.677	0.73
C	19	283	4	51.044	47.657	0.73
C	20	306	4	51.032	47.535	0.61
C	21	318	4	50.82	47.431	0.73
C	22	334	4	50.853	47.397	0.97
C	23	349	5	50.846	47.408	1.22
C	24	363	4	50.82	47.692	0.97
C	25	381	4	50.285	48.089	1.09
C	26	397	4	50.351	47.964	1.215
C	27	413	4	50.254	48.05	0.97
C	28	424	5	50.178	48.104	1.22
C	29	438	5	50.548	47.576	2.67
C	30	453	4	50.204	46.945	2.67
C	31	468	3	49.957	46.717	2.67
C	32	483	4	50.106	46.79	2.92
C	33	498	T10	50.437	46.825	2.19
C	34	513	T11	50.54	47.017	2.67
C	35	528	T10	50.438	47.023	2.92
C	36	543	T9	50.147	47.017	2.43
C	37	558	T9	49.953	46.923	2.19
C	38	573	T1	49.953	46.626	2.43
C	39	588	T1	49.856	46.825	2.43
C	40	605	2	49.658	46.825	1.94

From the arrangement of data as presented above, the Euclidean distance each cell moved between each observation periods was determined. Cells which emigrated during the experiment were grouped separately from those that did not. The magnitude of all the distances moved were then sorted from least to greatest, and a cumulative distribution was plotted for emigrating and remaining cells based on the log 10 of the distance. The statistical analyses were completed by Dr. Martin Hamilton and the distributions are shown in Figures 3.7 and 3.8. The remaining cell movement distribution was fit by a student's t distribution while the distribution of emigrating cell movements was approximated best with a normal distribution. A list of descriptive statistics for each is included in Table 3.5 and a plot of both is presented in Figure 3.9 for comparison.

Table 3.5. Descriptive Statistics

Variable	N	Mean	Median	St. Dev
Remain log 10 (Dist/time)	1659	-1.7607	-1.7514	.4075
Detach log 10 (Dist/time)	287	-1.5992	-1.6091	.4595

Cumulative Distribution of "Remain" Cell Movements

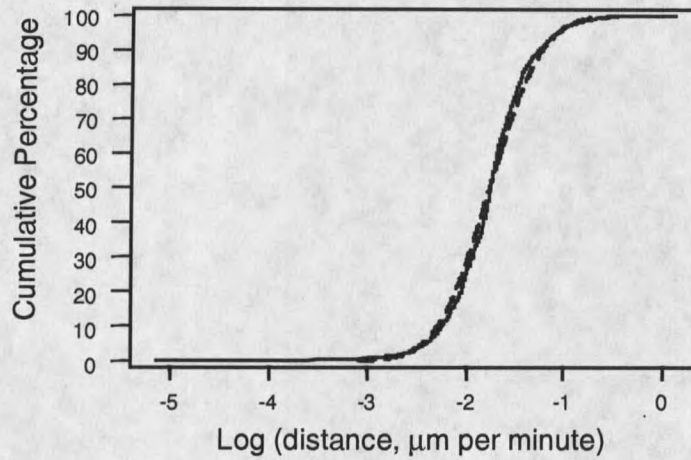


Figure 3.7. Distribution of movements of remaining cells (dashed line represents t(5) distribution; solid line represents actual data)

Cumulative Distribution of "Emigrate" Cell Movements

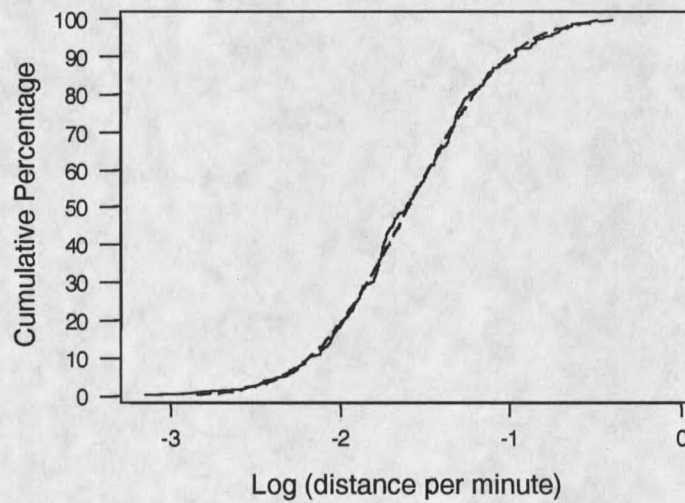


Figure 3.8. Distribution of movements of emigrating cells (dashed line represents normal distribution; solid line represents actual data)

It is instructive to present these results in an additional format. When examining the differences between the two distributions, it is informative to consider the following descriptors of each distribution. These are based on movement distances for 15-minute intervals:

1. The median of each distribution:
0.26 microns for remaining cells
0.38 microns for emigrating cells
2. 0.51 microns is the 80th percentile of the remaining distribution.
0.51 microns is the 61st percentile of the emigrating distribution.
0.92 microns is the 80th percentile of the emigrating distribution.
3. 0.76 microns is the 90th percentile of the remaining distribution.
0.76 microns is the 75th percentile of the emigrating distribution.
1.46 microns is the 90th percentile of the emigrating distribution.

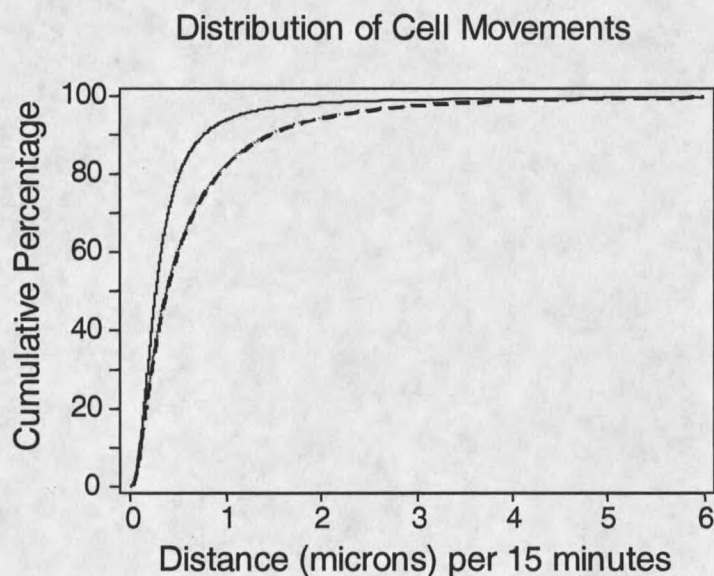


Figure 3.9. Fitted Cumulative Distributions of Cell Movements – Solid line for remaining cells (based on $t(5)$ distribution for $\log_{10}$ (distance)); Dashed line for emigrating cells (based on a normal distribution for $\log_{10}$ (distance)).

Growth and Emigration

A similar method as described above was used to determine growth and emigration results. The analysis was somewhat simpler in that only the times to either division or detachment were tracked. Again, the times were sorted according to magnitude and arranged in a cumulative distribution. However, a large portion of the data is considered censored (either beginning before the period of observation or continuing past). Therefore, a non-parametric distribution analysis using the Kaplan-Meier method was performed with Minitab 12.1 statistical software. This type of data analysis is common in the pharmaceutical industry where the survival of patients is the variable. In these analyses, the window of observation can not encompass all participants so a non-parametric, right-censored distribution (Kaplan-Meier) analysis is used. In these results, the median of the distribution is the most informative parameter. The median time for cells to divide was 6.6 hours and the median time for cells to emigrate was 2.0 hours. Graphs of each distribution are shown below in Figures 3.10 and 3.11.

Distribution of Emigration Times

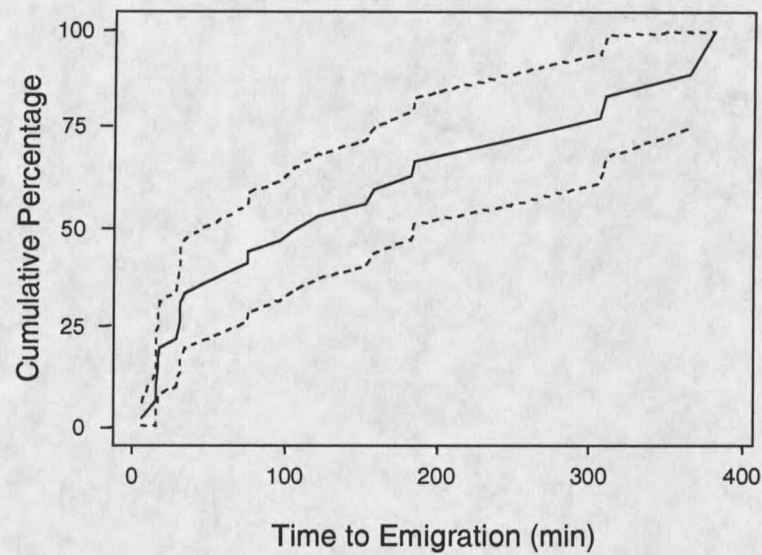


Figure 3.10. Cumulative distribution of emigration times. Dashed line represents 95% confidence interval.

Distribution of Division Times

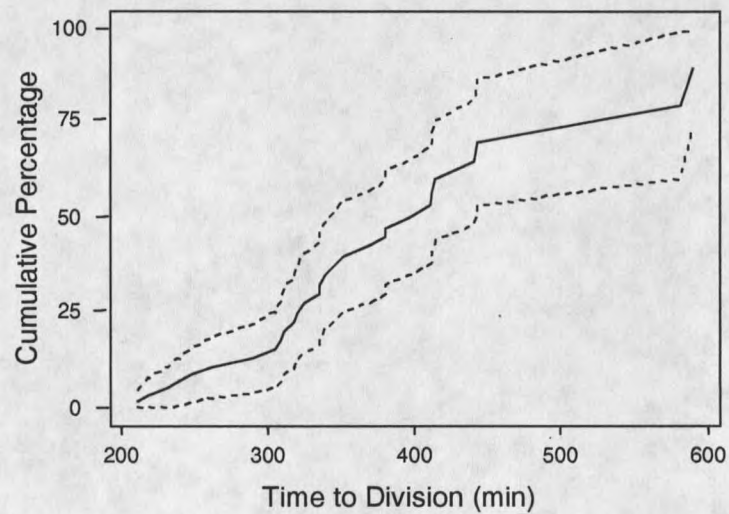


Figure 3.11. Cumulative distribution of division times. Dashed line represents 95% confidence interval.

A third parameter measured in these experiments is the probability of a cell to emigrate. A tree diagram depicting lineage and fate of all observed cells is included as Appendix A. There were two ways to analyze this data. The first is to examine only division events and obtain the percentage of divisions that resulted in at least one cell emigrating. This method produced a 26% rate of emigration. However, this is in all likelihood a low estimate because all cells that were observed during the last time period were considered to have no chance of emigrating. Based on the behavior exhibited by others, many of these cells surely emigrated at some point after the experiment ended.

The other way of obtaining rate of emigration is to compare the number of cells emigrating with the total number of cells observed. This takes into account all the initially observed cells that detached before division occurred. This method yielded a 44% chance of emigration.

Discussion

The purpose of this research was to describe the behavior of individual cells within a biofilm matrix. The parameters chosen to accomplish this were: time to division, time to emigrate, movement distributions of emigrating and dividing cells, and probability of emigration following a division event. The quantification of these activities can be used in determining input parameters for modeling of biofilm behavior.

The median observed time to division for secondary biofilm cells was 396.5 minutes or 6.6 hours. This corresponds to a specific growth rate of 0.105 hr^{-1} (Madigan *et al.*, 1997). In a similar, unpublished work, Arturo determined the median time to division of the same organism in a biofilm state to be 260 min or 4.33 hours, corresponding to a specific growth rate of 0.16 hr^{-1} (Arturo, 1998). This seems contradictory since the very same experimental set up was used for each experiment; however, the previous research examined the growth of primary cells as they emerged from their lag stage. Therefore, these growth rates were not measured from the same stage of biofilm development, and this might explain the difference. Also, Arturo sought out more active cells to observe and this might lead to a higher growth rate being measured. The median time to detachment measured by the current research was 121 minutes or 2.0 hours. The previous work measured this to be 90 min or 1.5 hours (Arturo, 1998). Again, the same possible explanations for this difference can be offered.

The cumulative distributions compiled for both the emigrating and remaining cells show that these cells, although enmeshed in a 3-8 micron thick biofilm matrix are still quite active in terms of movement. The distributions show that the cells which emigrate are significantly more dynamic than the cells which do not. It is suspected that the difference is even more striking than what these results indicate. As explained earlier, all cells which remained in view through the end of the observation period were analyzed as "remain" and not "emigrant" cells. Undoubtedly, some of these cells emigrated at some point after the experiment

ended based on the behavior evidenced by their predecessors. However, their movement was grouped with the remain cells for lack of an alternative.

A normal distribution fit the emigration data quite well. A $t(5)$ distribution fit the "remain" data better than a normal distribution. This is a very interesting result. When two different normally distributed sets of data are compiled into one, the resulting distribution is likely to be a t distribution (Hamilton, 1999). Therefore, it is supposed that the "remain" distribution is actually a mix of two normally distributed data sets. This theory fits well with the idea that some of the cells are indeed emigrant cells, but sure evidence of this was unattainable due to the period of observation coming to an end. From these results, it is concluded that cells destined to emigrate display significantly more motion than those destined to remain in place.

Arturo also examined the movement of biofilm cells. His findings indicate a median distance moved in 15 minutes is 1.15 microns and the 90th percentile is 3.82 microns (Arturo, 1998). For "remain" cells observed in the current research, the median movement for 15 minutes is 0.26 microns and the 90th percentile is 0.76 microns. For cells which will emigrate, the median is 0.38 microns and the 90th percentile is 1.46 microns. These results perfectly illustrate what has been observed during the course of performing the experiments: Once primary cells begin to replicate, they spread out upon the surface. Since there are not many other cells (i.e. biofilm is less than a monolayer at this point) to hinder their movement, the primary cells exhibit a significant amount of locomotion. As the

biofilm surpasses the monolayer stage, the cells are still quite active, but are not as free to move around. These distances moved over 15 minute intervals, show that cells in a several micron thick biofilm do not move as much as those on a sparsely covered substratum.

Similar movement of cells has been observed and quantified by others (Power and Marshall, 1988). *Pseudomonas* JD8 was studied in biofilms that were less than a monolayer in thickness. Cells moved distances of up to 150 microns before completion of a division cycle. The average movement distance for a 15-minute period measured was 2.25 microns. The nutrient and substrata conditions were different than in the work described in this manuscript, however, it is evident that the movement being is described and quantified is very similar (Power and Marshall, 1988).

In a study examining the replication patterns of various biofilm organisms from a natural stream population, the same behavior is seen (Lawrence and Caldwell, 1987a). The term given for this type of movement is "spreading". This spreading behavior is displayed by *Pseudomonas aeruginosa* PA01 both when enmeshed in a biofilm matrix and on a sparsely colonized surface. During the time frame of the experiments described herein, there was no significant microcolony development with respect to daughter cells being located in close proximity to parent cell location. The spreading behavior persists into a maturing biofilm, although hindered by the biofilm matrix. This behavior also contributes to a rather flat, uniform biofilm structure during the early stages of accumulation.

Two methods for quantifying the tendency of a cell to emigrate have been described in this report and lead to estimates of 26% and 44%; neither method is satisfactory. The higher rate is more representative of the activity witnessed in these experiments. A more detailed analysis and a larger data set is needed to further refine this parameter. However, these results do suggest that emigration with *P. aeruginosa* PA01 is a significant event in biofilm development: Clearly 1/3 of new cells do not stay located near the parent cell. This result lends support to the finding by O'Toole and Kolter (1998a) that microcolonies develop as result of "recruitment" and not lineage of cellular division.

This study made an effort to quantify the rates of movement of bacterial cells, the time to division or emigration of a cell and the probability of a cell to emigrate. These results can be useful inputs for rules governing cell behavior in biofilm models.

CHAPTER 4

CONCLUSION

This research project was an effort to evaluate the importance of the initial events in biofilm formation. This purpose was accomplished by monitoring the behavior of both the cells which transition from a planktonic state to a biofilm state and the progeny which arise from this original population. The original population of biofilm cells, primary cells, actually undergo a change in phenotype as they become sessile organisms. An apparent lag time in replication is associated with the attachment event.

Once primary cells emerge from this lag stage, they proceed to populate their chosen substratum and form a biofilm. During this period of biofilm growth, it is evident that *Pseudomonas aeruginosa* PA01 cells retain their ability to move around within the biofilm. This constant movement of cells away from their "birth" location serves to disperse the cells to colonize all reaches of a substratum.

This study showed that biofilm development is a very dynamic process with cells varying in growth rate depending upon their point of origin. Cells also vary in the amount of locomotion they exhibit depending upon whether the cell is destined to emigrate or remain in the general area. The most surprising result from this

project was that there were no forming microcolonies observed. All cells as they replicated, moved away from their original location. Therefore, the cells which appear to be closely associated with one another in these biofilms were not necessarily of the same lineage. GFP+ cells which were clumped together were more likely a result of either colonization or recolonization events, rather than subsequent generations.

Another useful result of this project was the quantification of cell behaviors such as time to division and emigration, movement rates, and probability of detachment. This information can be directly used to refine modeling parameters or to compare with modeling results.

Finally, the methods used in this research will be invaluable when used to investigate other biofilm questions. For instance, the same techniques can be used to assess movement rates of non-motile bacteria in biofilms. This will elucidate how much movement is a result of a "popcorn" effect and not from motility. Also, reattachment of GFP cells can be visualized on a non-GFP biofilm. This type of work will allow researchers to investigate how organisms become entrained into an established biofilm. Another very interesting question to investigate is whether or not detached biofilm phenotype cells endure a similar lag phase upon reattachment. This type of research could possibly vary the length of times that "biofilm" cells were in a planktonic environment before a surface associated lag time occurs.

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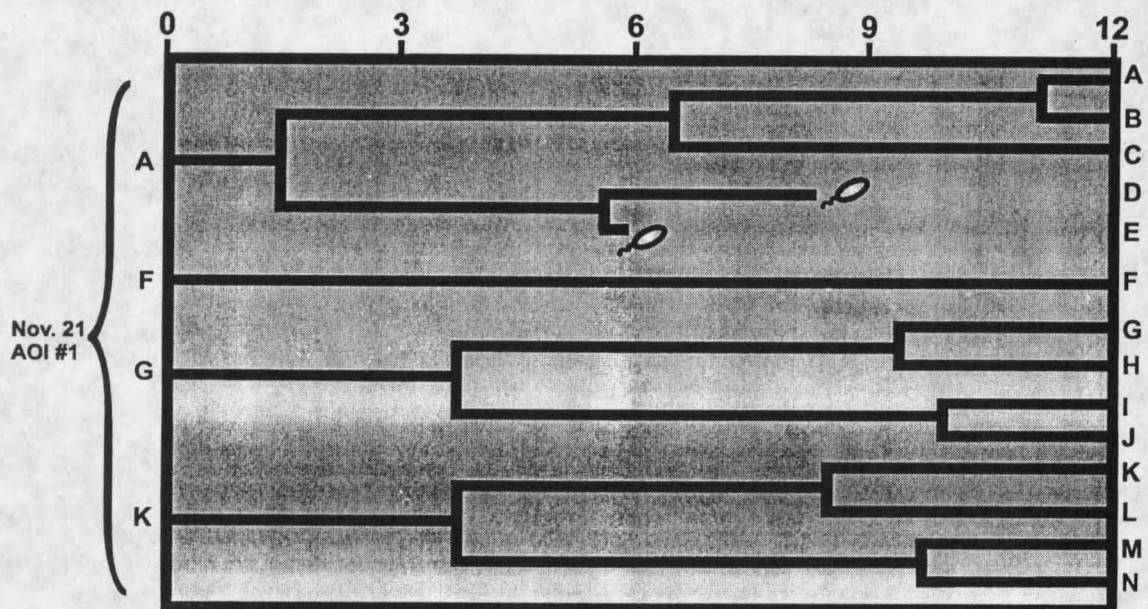
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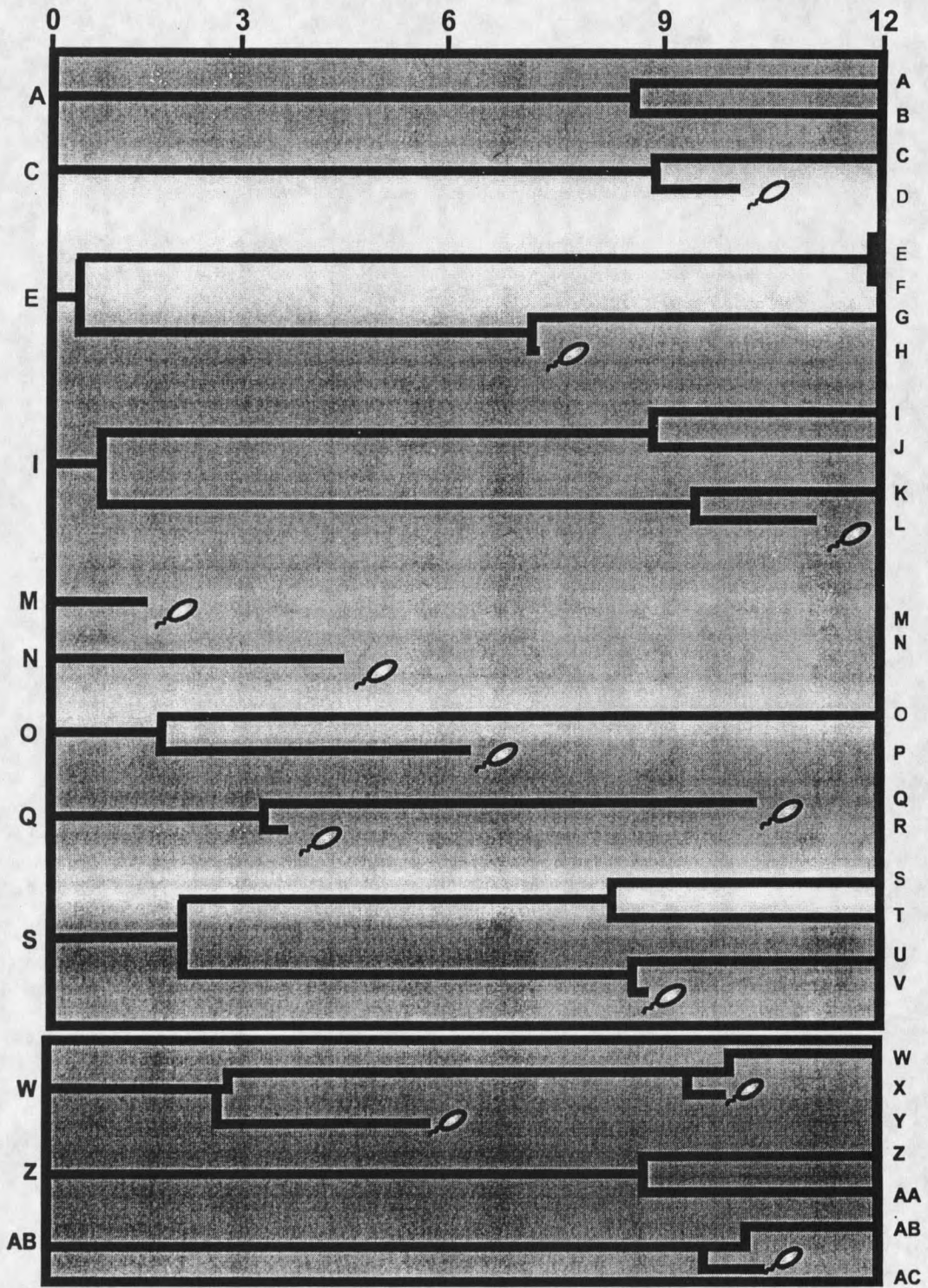
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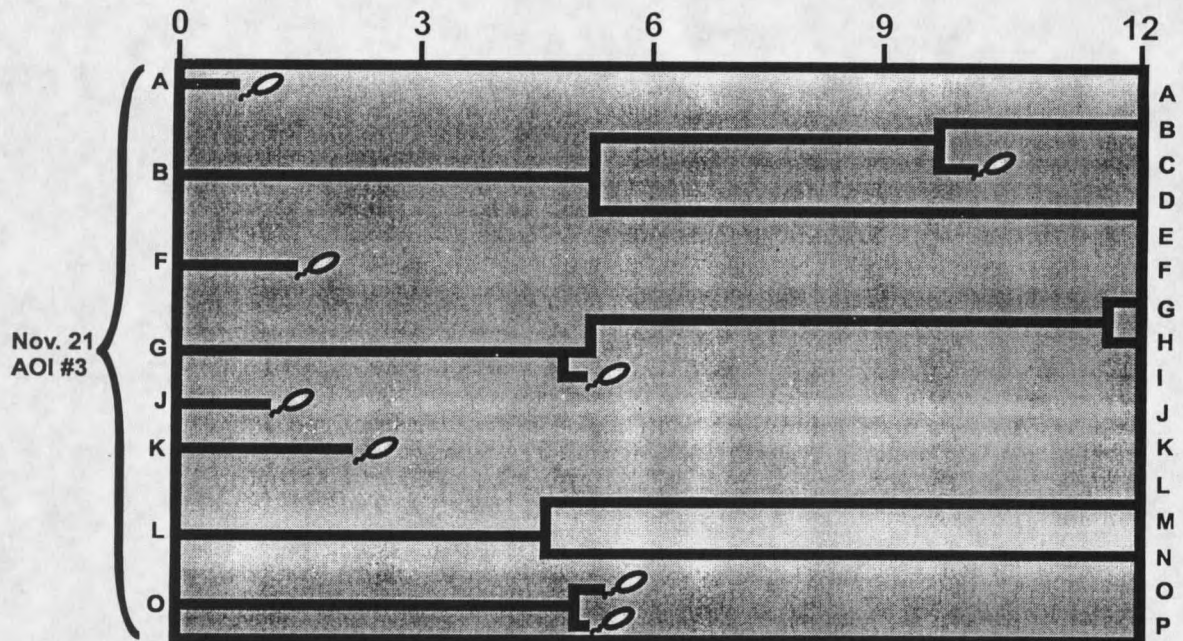
APPENDICES

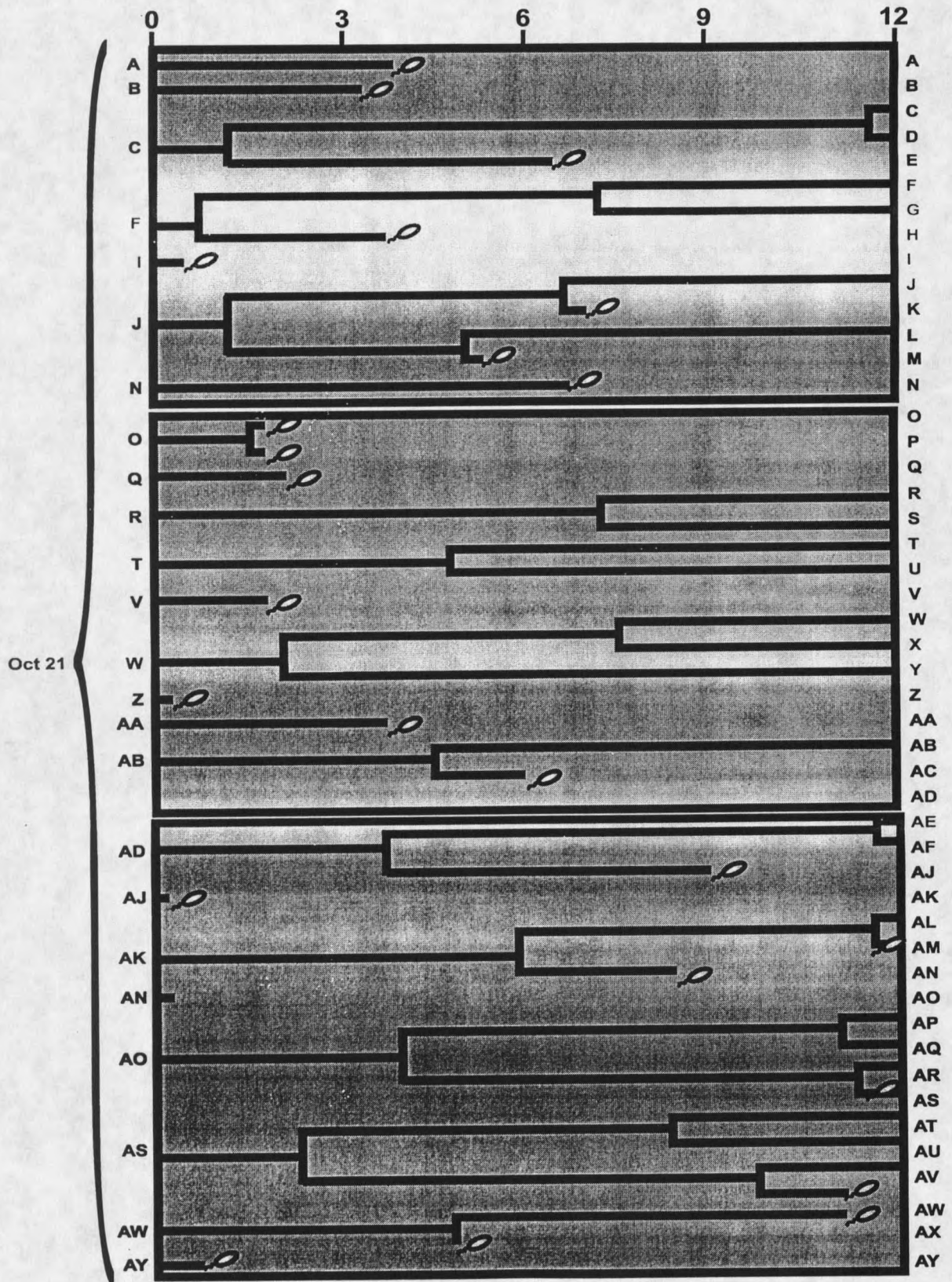
APPENDIX A – TREE DIAGRAMS



Nov. 21
AOI #2







APPENDIX B – OBJECT NUMBERS AND COORDINATES

0						1					
Initial Cells from Image t98112108						98112109					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
4 of 4 objects in range						4 of 4 objects in range					
0 added by manually splitting objects						added by manually splitting objects					
surface 4.5						surface 2.5					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.57	11.38	8.62	0.1215	4.75	1	1.69	11.42	8.84	0.243	3
2	0.91	13.46	11.70	0.1215	4.75	2	0.91	13.45	11.85	0.243	3
3	0.74	27.44	12.74	0.3645	5.25	3	0.78	27.39	13.46	0.486	3.5
4	0.80	18.44	15.17	0	4.5	4	0.99	18.36	15.10	0	2.5

2						3					
98112110						98112111					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
4 of 4 objects in range						4 of 5 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1.5						surface 2.5					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.72	11.27	9.25	0.1215	1.75	1	1.75	11.01	9.29	0.3645	3.25
2	0.94	13.40	12.21	0.1215	1.75	2	0.95	13.10	12.21	0.243	3
3	0.90	27.07	13.67	0.1215	1.75	3	0.85	26.81	13.69	0.243	3
4	0.88	17.92	15.42	0	1.5	5	0.93	17.48	15.13	0	2.5

4							5							
98112112							98112113							
Intensity	80...255						Intensity	80...255						
Area:	.01 - 7.5						Area:	.01 - 7.5						
5 of 5	objects in range						5 of 6	objects in range						
	added by manually splitting objects							added by manually splitting objects						
surface	3.5						surface	4						
step	0.486						step	0.486						
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		
1	0.92	10.47	9.50	0.1215	3.75		1	0.92	10.08	9.37	0.1215	4.25		
2	0.93	11.96	11.19	0.1215	3.75		2	0.92	11.53	10.39	0.1215	4.25		
3	0.92	13.42	12.96	0.243	4		4	0.94	13.21	12.86	0.1215	4.25		
4	0.93	27.13	14.16	0	3.5		5	1.02	26.91	14.15	0	4		
5	0.96	18.05	15.62	0	3.5		6	0.94	17.26	15.43	0	4		

6							7						
98112114							98112115						
Intensity 80...255							Intensity 80...255						
Area: .01 - 7.5							Area: .01 - 7.5						
5 of 5 objects in range							5 of 5 objects in range						
added by manually splitting objects							added by manually splitting objects						
surface 2							surface 1						
step 0.486							step 0.486						
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.93	9.78	9.74	0.243	2.5		1	0.99	9.64	9.73	0.1215	1.25	
2	0.91	12.08	11.39	0.486	3		2	0.89	11.62	11.32	0.1215	1.25	
3	0.97	12.99	13.34	0.243	2.5		3	0.88	12.45	13.29	0	1	
4	1.12	26.63	14.34	0	2		4	0.98	26.63	14.72	0.243	1.5	
5	0.79	18.89	18.31	0.972	4		5	1.13	17.62	17.46	0	1	

8						9					
98112116						98112117					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
5 of 5 objects in range						5 of 6 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 2.5						surface 3					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.96	9.94	8.94	0.3645	3.25	1	0.96	9.34	9.01	0.6075	4.25
2	1.02	12.07	10.57	0	2.5	2	1.00	12.15	10.54	0.6075	4.25
3	1.01	12.88	12.57	0	2.5	3	0.91	12.33	12.85	0	3
4	1.12	27.25	14.26	0	2.5	4	1.11	26.61	14.44	0	3
5	1.28	19.91	16.96	0.3645	3.25	6	1.01	17.21	15.87	0.1215	3.25

10						11					
98112118						98112119					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
5 of 5 objects in range						5 of 5 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.99	9.33	8.58	0.6075	2.25	1	1.11	9.71	8.38	0.486	3
2	1.14	11.87	10.12	0.243	1.5	2	1.14	12.26	9.68	0	2
3	1.01	12.57	12.65	0.243	1.5	3	1.06	12.94	12.30	0	2
4	1.30	16.11	13.78	0.243	1.5	4	1.41	26.97	13.72	0	2
5	1.26	26.56	14.12	0.243	1.5	5	1.31	18.34	15.02	0	2

12						13					
98112120						98112121					
Intensity	80...255					Intensity	80...255				
Area:	.01 - 7.5					Area:	.01 - 7.5				
7 of 9	objects in range					7 of 8	objects in range				
added by manually splitting objects						added by manually splitting objects					
surface	3					surface	1.5				
step	0.486					step	0.486				
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.19	9.67	8.68	0.6075	4.25	1	1.25	9.92	8.90	0.486	2.5
2	1.19	12.36	10.10	0.6075	4.25	2	0.64	16.03	10.45	0.1215	1.75
4	0.98	12.93	12.62	0.243	3.5	3	1.21	11.89	11.81	0.1215	1.75
5	0.64	16.42	12.18	0.243	3.5	4	1.00	13.18	13.01	0.1215	1.75
6	0.62	17.41	13.32	0.243	3.5	5	0.73	26.40	14.14	0.1215	1.75
7	0.69	26.49	13.49	0.243	3.5	6	0.72	27.94	14.81	0.1215	1.75
8	0.70	27.66	14.30	0.243	3.5	7	0.58	17.85	16.30	0.486	2.5

14						15					
98112122						98112123					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
7 of 7 objects in range						7 of 7 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 2.5						surface 0.5					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.29	9.86	9.00	0.3645	3.25	1	1.36	10.11	8.27	0.729	2
2	0.63	15.15	9.61	0	2.5	2	0.80	15.98	8.55	0.243	1
3	1.18	11.81	12.16	0.3645	3.25	3	1.20	12.26	11.36	0.729	2
4	0.99	13.13	13.19	0	2.5	4	0.94	13.74	12.53	0.243	1
5	0.67	26.39	14.42	0	2.5	5	0.79	27.25	13.98	0.486	1.5
6	0.74	27.85	14.91	0	2.5	6	0.79	28.34	14.11	0.243	1
7	0.62	18.02	18.14	0.3645	3.25	7	0.74	19.18	18.01	0.486	1.5

16						17					
98112124						98112125					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
7 of 7 objects in range						7 of 7 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 6						surface 2.5					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.36	9.55	8.34	0.486	7	1	1.47	9.35	8.17	0.3645	3.25
2	0.79	15.73	8.89	0	6	2	0.82	15.36	9.03	0	2.5
3	1.18	10.54	9.60	0	6	3	1.32	11.14	10.54	0.729	4
4	0.93	13.62	12.84	0	6	4	0.98	13.56	12.57	0.1215	2.75
5	0.80	27.01	14.53	0.486	7	5	0.75	27.35	13.26	0.486	3.5
6	0.77	28.17	14.42	0	6	6	0.79	28.09	14.18	0	2.5
7	0.79	19.94	18.50	0	6	7	0.87	19.71	18.27	0	2.5

18						19					
98112126						98112127					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
8 of 9 objects in range						7 of 7 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 3.5						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.55	8.77	8.11	0.3645	4.25	1	1.65	8.92	8.16	0.243	2.5
2	0.86	14.45	10.23	0.243	4	2	0.83	13.35	10.29	0.1215	2.25
3	0.63	10.09	10.44	1.0935	5.75	3	0.50	9.72	11.15	0.486	3
4	0.73	11.51	11.20	-0.122	3.25	4	0.93	13.14	12.67	0	2
5	1.01	13.19	12.61	0.243	4	5	0.80	26.95	13.46	0.729	3.5
6	0.81	26.64	13.59	0.972	5.5	6	0.86	27.78	14.31	0	2
7	0.79	27.66	14.23	0	3.5	7	0.89	19.15	18.10	0	2
8	0.81	18.90	17.92	0.486	4.5						

20						21					
98112128						98112129					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
7 of 7 objects in range						8 of 8 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1.5						surface 1					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.96	11.61	7.85	0.486	2.5	1	0.92	10.57	7.83	0.1215	1.25
2	0.72	12.59	10.45	0.3645	2.25	2	0.71	12.35	8.78	0.486	2
3	1.18	15.29	11.25	0	1.5	3	0.64	12.52	10.40	0.243	1.5
4	1.12	16.06	12.54	0	1.5	4	1.11	15.05	11.36	0	1
5	0.97	30.56	13.24	0.6075	2.75	5	0.94	15.74	12.85	0	1
6	0.97	30.64	14.21	0	1.5	6	0.95	30.33	13.48	0.729	2.5
7	1.09	22.14	18.14	0	1.5	7	0.84	30.35	14.47	0	1
						8	1.01	21.83	18.48	0.1215	1.25

22						23					
98112130						98112131					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
8 of 8 objects in range						8 of 8 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 3.5					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.88	9.34	6.59	0.1215	1.25	1	0.89	9.07	7.28	0.243	4
2	0.96	11.53	7.65	0.3645	1.75	2	0.97	11.10	8.64	0.729	5
3	0.71	11.43	9.05	0.243	1.5	3	1.14	13.71	10.40	0	3.5
4	1.06	13.93	10.08	0	1	4	0.56	11.00	10.05	0.6075	4.75
5	0.87	14.61	11.63	0	1	5	0.86	14.37	12.24	0	3.5
6	1.02	29.34	12.37	0.729	2.5	6	0.99	29.10	13.00	1.215	6
7	0.86	29.12	13.29	0	1	7	0.97	28.87	13.91	0	3.5
8	1.04	20.27	16.38	0	1	8	1.08	20.21	15.76	0	3.5

24							25						
98112132							98112133						
Intensity	80...255						Intensity	80...255					
Area:	.01 - 7.5						Area:	.01 - 7.5					
8 of 8	objects in range						8 of 9	objects in range					
	added by manually splitting objects							added by manually splitting objects					
surface	0						surface	1.5					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	1.43	9.38	7.69	0	0		1	1.11	8.50	7.27	0.243	2	
2	0.81	12.43	9.06	0.243	0.5		2	1.25	12.04	8.80	0.243	2	
3	1.39	13.78	10.07	0.243	0.5		3	1.40	13.15	10.03	0.243	2	
4	0.60	11.13	11.10	0.486	1		4	0.64	10.18	10.61	0.972	3.5	
5	1.20	14.43	12.71	0	0		5	0.94	13.82	12.20	0.486	2.5	
6	1.07	29.33	13.33	0.729	1.5		6	1.11	28.49	12.83	1.701	5	
7	1.08	28.97	14.34	0	0		7	1.18	28.33	13.84	0.243	2	
8	1.16	20.09	16.82	0	0		8	1.18	19.60	17.23	1.215	4	

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30						31					
98112138						98112139					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
8 of 8 objects in range						9 of 9 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 1					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.40	8.52	9.57	0	1	1	1.49	8.31	9.70	0	1
2	0.86	13.62	10.71	0.486	2	2	0.88	13.19	10.72	0	1
3	1.60	11.70	11.16	0.486	2	3	1.59	11.38	11.18	0.243	1.5
4	0.78	12.87	13.20	0	1	4	0.77	12.68	13.60	0.243	1.5
5	0.81	13.86	14.40	0	1	5	0.95	13.56	14.50	0	1
6	1.49	28.94	14.89	1.458	4	6	0.83	29.49	14.84	0.972	3
7	1.35	28.21	16.17	0.243	1.5	7	0.80	27.91	14.88	0.972	3
8	1.71	20.57	18.17	0.1215	1.25	8	1.42	27.89	16.22	0	1
						9	1.73	20.10	17.25	0.243	1.5

32						33					
98112140						98112141					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
10 of 10 objects in range						11 of 12 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 1					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.57	7.66	9.83	0.1215	1.25	1	1.65	8.17	9.55	0.243	1.5
2	0.58	11.58	10.20	0.729	2.5	2	0.65	12.00	9.86	1.215	3.5
3	1.70	10.64	11.15	0.486	2	3	1.74	11.15	11.05	0.486	2
4	0.78	12.09	13.84	0.486	2	4	0.81	12.38	13.46	0.486	2
5	0.96	12.99	14.58	0.243	1.5	5	0.94	13.31	14.32	0.243	1.5
6	0.86	29.13	14.81	0.972	3	6	0.87	29.72	14.46	1.0935	3.25
7	0.80	27.47	14.99	1.215	3.5	8	0.80	27.81	14.73	1.3365	3.75
8	1.47	27.25	16.26	0.1215	1.25	9	0.75	26.78	16.03	0.243	1.5
9	0.88	19.26	17.82	0.243	1.5	10	0.85	17.79	16.36	0.729	2.5
10	0.94	21.13	18.11	0	1	11	0.79	28.35	16.06	0.243	1.5
						12	0.91	22.28	17.48	0.486	2

34						35					
98112142						98112143					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
11 of 11 objects in range						11 of 11 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.76	8.66	9.18	0	1	1	1.83	8.56	8.91	0.243	2.5
2	0.65	12.57	9.28	0.729	2.5	2	0.65	12.34	8.94	0.972	4
3	1.90	12.06	10.78	0.243	1.5	3	2.03	12.19	10.53	0.243	2.5
4	0.95	12.87	13.00	0.486	2	4	1.05	12.68	12.50	0.243	2.5
5	0.90	13.84	13.82	0	1	5	0.91	13.70	13.66	0.243	2.5
6	0.90	30.64	13.97	0.729	2.5	6	0.91	30.42	13.73	0.729	3.5
7	0.83	28.21	14.17	1.458	4	7	0.61	28.15	14.04	1.944	6
8	1.64	20.26	16.27	0.486	2	8	0.81	20.13	14.67	1.3365	4.75
9	0.79	27.33	15.62	0	1	9	0.86	27.18	15.41	0.243	2.5
10	0.80	28.92	15.61	0	1	10	0.87	28.77	15.42	0.243	2.5
11	0.85	23.18	17.11	0.972	3	11	0.80	23.98	17.10	1.215	4.5

36						37					
98112144						98112145					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
14 of 15 objects in range						12 of 12 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 2.5						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.89	8.90	9.27	0.243	3	1	0.88	7.98	8.42	0.243	2.5
2	0.62	12.66	9.19	1.0935	4.75	2	0.75	12.52	9.09	0.243	2.5
3	2.17	12.55	10.75	0.243	3	3	1.03	9.51	9.45	0.972	4
4	0.98	12.91	12.95	0.486	3.5	4	2.09	12.31	10.64	0.243	2.5
5	0.97	13.84	13.89	0	2.5	5	1.03	12.77	12.88	0.486	3
6	0.81	28.04	13.87	2.187	7	6	0.86	13.64	13.67	0.243	2.5
7	1.06	30.96	13.94	0.486	3.5	7	1.10	30.85	13.67	0.729	3.5
8	0.34	20.04	14.53			8	0.78	26.83	14.31	1.458	5
9	0.38	20.30	15.15			9	1.10	20.53	15.40	1.215	4.5
10	0.53	20.95	15.42	0.486	3.5	10	0.97	27.26	15.42	0.243	2.5
11	0.93	27.52	15.63	0	2.5	11	0.97	28.84	15.40	0.243	2.5
12	0.91	29.11	15.62	0	2.5	12	0.98	24.74	16.62	0.729	3.5
13	0.38	20.58	16.19								
15	0.97	24.75	16.86	1.215	5						

38						39					
98112146						98112147					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
14 of 15 objects in range						12 of 13 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						substatum 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.90	7.82	8.81	0.243	1.5	1	0.92	7.41	8.93	0.243	2.5
2	1.04	9.37	9.52	0.243	1.5	2	1.10	8.99	9.97	0.243	2.5
3	0.82	12.50	9.65	0.972	3	3	0.87	11.99	9.87	0.972	4
4	2.29	12.38	11.16	0.243	1.5	4	2.23	11.99	11.38	0.243	2.5
5	1.05	12.49	13.29	0.486	2	5	1.18	12.00	13.57	0.486	3
6	0.83	13.56	14.09	0.243	1.5	7	0.78	13.25	14.49	0	2
7	0.23	20.01	13.52			8	1.18	30.23	14.33	0.729	3.5
8	1.19	30.69	14.03	1.215	3.5	9	0.81	28.05	14.86	1.944	6
9	0.82	28.20	14.73	1.944	5	10	1.01	26.84	16.26	0.243	2.5
10	0.15	19.72	14.31			11	1.17	19.10	16.46	1.458	5
11	1.31	20.37	15.71	0.729	2.5	12	0.97	28.37	16.15	0.243	2.5
12	1.03	27.15	15.86	0	1	13	1.14	24.59	17.56	0.486	3
13	0.93	28.75	15.80	0	1						
15	1.07	24.84	17.06	0.729	2.5						

40					
98112148					
Intensity	80...255				
Area:	.01 - 7.5				
11 of 12	objects in range				
added by manually splitting objects					
ubstatum	1				
step	0.486				
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.89	7.39	8.77	0.729	2.5
2	1.11	9.31	9.81	0.729	2.5
3	1.06	12.05	9.91	0.972	3
4	2.57	11.99	11.57	0.243	1.5
5	1.06	12.18	13.74	0.729	2.5
6	1.26	31.39	13.64	0.486	2
8	0.75	13.41	14.66	0.243	1.5
9	0.63	27.53	14.91	2.673	6.5
10	0.99	26.93	16.36	0.243	1.5
11	1.04	28.48	16.25	0.243	1.5
12	1.20	24.62	17.68	0.486	2

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6							7						
98112114							98112115						
Intensity 80...255							Intensity 80...255						
Area: .01 - 2.5							Area: .01 - 2.5						
9 of 12 objects in range							9 of 10 objects in range						
6 added by manually splitting objects							6 added by manually splitting objects						
surface 1.5							surface 0						
step 0.486							step 0.486						
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.73	51.54	47.89	0.486	2.5		1	0.73	51.55	47.71	0.486	1	
2	0.65	52.33	49.25	0.729	3		2	0.69	52.14	49.23	0.8505	1.75	
3	0.83	51.08	50.04	0.729	3		3	0.86	50.95	49.91	0.8505	1.75	
4	0.68	53.92	50.66	1.215	4		4	0.70	55.06	49.87	0.729	1.5	
8	0.59	50.52	52.29	0.729	3		6	0.75	50.40	52.35	0.8505	1.75	
9	0.67	49.08	52.45	0.729	3		7	0.72	49.08	52.39	0.8505	1.75	
10	0.80	52.63	53.70	0.6075	2.75		8	0.85	52.45	53.61	0.729	1.5	
11	0.71	51.50	54.41	0.486	2.5		9	0.75	51.39	54.33	0.486	1	
12	0.84	53.01	55.27	0.486	2.5		10	0.85	52.88	55.17	0.6075	1.25	
T1		51.12	51.71	0.486	2.5		T1		51.12	51.91	0.729	1.5	
T2		52.79	51.51	1.701	5		T2		52.98	51.42	1.458	3	
T3		53.27	52.30	0.486	2.5		T3		53.27	52.00	0.8505	1.75	
T4		52.20	52.79	0.486	2.5		T4		52.00	52.69	0.729	1.5	
T5		51.12	52.98	2.187	6		T5		51.03	53.18	2.3085	4.75	
T6		52.59	52.79	1.701	5		T6		52.30	52.59	1.944	4	

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10							11						
98112118							98112119						
Intensity	80...255						Intensity	80...255					
Area:	.01 - 2.5						Area:	.01 - 2.5					
11 of 12	objects in range						11 of 12	objects in range					
6	added by manually splitting objects						6	added by manually splitting objects					
surface	1						surface	2					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.75	51.57	46.85	0.243	1.5		1	0.84	52.30	46.49	0.243	2.5	
2	0.76	55.64	48.06	0.243	1.5		2	0.83	56.81	47.10	0.729	3.5	
3	0.73	52.16	48.35	0.486	2		3	0.75	52.61	48.10	0.729	3.5	
4	0.88	50.60	49.16	0.729	2.5		4	0.86	51.09	48.82	0.972	4	
5	0.73	53.18	49.91	0.486	2		5	0.75	53.45	49.58	0.486	3	
6	0.62	50.81	50.61	0.243	1.5		6	0.68	51.21	50.34	0.243	2.5	
8	0.64	48.90	51.47	0.486	2		8	0.76	50.93	51.26	0.729	3.5	
9	0.75	50.65	51.75	0.729	2.5		9	0.62	48.90	51.27	0.972	4	
10	0.87	52.68	52.94	0.486	2		10	0.89	53.07	52.63	0.486	3	
11	0.77	51.49	53.69	0.243	1.5		11	0.86	51.92	53.39	0.243	2.5	
12	0.95	53.12	54.43	0.243	1.5		12	1.01	53.55	54.14	0.243	2.5	
T1		51.51	51.91	0.972	3		T1		52.10	51.12	0.486	3	
T2		53.18	51.12	2.187	5.5		T2		53.67	50.83	1.944	6	
T3		53.37	51.32	0.243	1.5		T3		53.76	51.03	0.243	2.5	
T4		52.20	52.20	0.243	1.5		T4		52.98	51.42	1.701	5.5	
T5		51.32	52.88	1.701	4.5		T5		52.69	51.71	0.243	2.5	
T6		52.49	51.91	1.701	4.5		T6		51.81	52.30	1.458	5	

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28							29						
98112136							98112137						
Intensity	80...255						Intensity	80...255					
Area:	.5 - 2.5						Area:	.01 - 7.5					
8 of 12	objects in range						13 of 17	objects in range					
8	added by manually splitting objects						6	added by manually splitting objects					
surface	2						surface	0					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.69	53.47	47.60	0.486	3		1	0.80	53.73	47.95	0.486	1	
2	0.70	52.82	48.97	0.486	3		2	0.74	52.78	49.06	0.486	1	
3	0.85	57.38	48.87	0.486	3		3	0.89	57.32	49.11	0.486	1	
4	1.59	53.69	49.98	0.729	3.5		4	1.61	53.58	50.28	0.972	2	
5	1.37	51.84	50.45	1.215	4.5		5	0.79	52.21	50.02	2.673	5.5	
6	1.80	54.73	51.11	0.486	3		7	1.59	54.96	51.11	0.486	1	
10	1.38	54.18	54.61	2.187	6.5		8	0.83	51.50	51.22	0.972	2	
12	1.44	47.80	56.49	0.972	4		9	0.64	55.15	52.30	0.486	1	
T1		54.64	52.59	0.972	4		11	0.78	54.35	54.60	1.215	2.5	
T2		53.57	53.37	0.486	3		12	0.59	52.50	54.82	0.972	2	
T3		52.88	53.18	0.486	3		14	0.76	53.69	55.87	2.916	6	
T4		52.49	54.74	0.729	3.5		15	1.55	47.61	56.73	0.972	2	
T5		53.37	54.25	2.187	6.5		17	1.09	58.90	59.54	0.729	1.5	
T6		53.57	52.39	1.944	6		T1		52.98	53.18	0.729	1.5	
T7		52.69	53.76	1.944	6		T2		53.67	53.47	0.729	1.5	
T8		52.88	52.39	6.561	15.5		T3		54.15	52.69	1.944	4	
							T4		53.57	54.45	2.187	4.5	
							T5		52.98	53.57	2.187	4.5	
							T6		53.47	51.42	6.075	12.5	

30							31						
98112138							98112139						
Intensity	80...255						Intensity	80...255					
Area:	.5 - 2.5						Area:	.5 - 2.5					
12 of 16	objects in range						7 of 10	objects in range					
6	added by manually splitting objects							added by manually splitting objects					
surface	1						surface	0.5					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.84	53.23	47.99	0.486	2		1	0.83	52.82	47.97	0.486	1.5	
2	0.78	52.40	49.35	0.486	2		2	0.90	52.04	49.45	0.486	1.5	
3	0.98	57.00	49.29	0.486	2		3	0.81	51.13	49.65	2.673	6	
4	0.80	51.67	49.78	2.673	6.5		4	0.97	56.70	49.38	0.486	1.5	
5	1.66	53.15	50.52	0.729	2.5		5	1.51	52.84	50.41	0.729	2	
6	0.83	51.25	51.26	1.458	4		6	0.93	51.15	50.95	1.215	3	
7	1.66	54.69	51.32	0.729	2.5		7	1.48	54.43	51.38	0.729	2	
10	0.65	54.88	52.61	0.486	2		9	0.68	54.58	52.69	0.486	1.5	
13	0.77	55.52	54.65	1.215	3.5		10	0.70	54.95	54.77	1.701	4	
14	0.64	52.20	55.09	0.729	2.5		11	0.64	51.85	55.12	0.729	2	
15	1.58	47.51	56.77	1.215	3.5		12	0.81	54.95	55.77	0.972	2.5	
16	0.79	54.74	56.20	0.972	3		13	0.81	47.60	56.44	1.458	3.5	
T1		53.37	53.67	0.486	2		14	0.77	46.31	57.50	0.729	2	
T2		52.69	53.27	0.486	2		T1		53.18	53.76	0.729	2	
T3		53.76	52.88	1.458	4		T2		52.39	52.88	0.729	2	
T4		53.18	54.64	2.187	5.5		T3		53.57	52.79	1.458	3.5	
T5		52.69	54.06	2.187	5.5		T4		53.08	54.64	2.187	5	
T6		52.88	52.10	6.075	13.5		T5		52.69	53.76	2.43	5.5	
							T6		52.79	51.51	6.318	13.5	

32						33					
98112140						98112141					
Intensity 80...255						Intensity 80...255					
Area: .5 - 2.5						Area: .01 - 7.5					
12 of 15 objects in range						10 of 12 objects in range					
7 added by manually splitting objects						10 added by manually splitting objects					
surface 1						surface 1					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.93	52.30	47.99	0.486	2	1	0.92	52.70	47.65	0.486	2
2	0.95	51.56	49.54	0.486	2	3	1.11	56.34	49.10	0.486	2
3	1.03	56.13	49.47	0.486	2	4	0.94	55.12	50.82	0.486	2
4	0.64	50.79	49.82	2.916	7	5	1.14	50.68	51.42	0.6075	2.25
5	1.48	52.32	50.56	1.215	3.5	6	0.79	54.29	52.53	0.486	2
7	0.96	50.76	51.00	1.458	4	7	0.87	54.64	54.99	1.458	4
9	0.75	54.01	52.76	0.486	2	8	0.75	51.61	55.02	0.729	2.5
10	0.87	54.36	55.04	1.215	3.5	9	0.97	54.68	56.03	0.972	3
11	0.67	51.26	55.22	0.729	2.5	11	1.32	47.77	56.82	2.187	5.5
12	0.91	54.66	56.25	0.972	3	12	0.86	45.73	57.46	0.486	2
13	1.26	47.28	57.59	2.187	5.5	T1		52.98	53.47	0.486	2
15	0.90	45.48	57.66	0.486	2	T2		52.20	52.49	0.486	2
T1		52.49	53.76	0.486	2	T3		53.57	52.20	1.458	4
T2		51.81	52.79	0.729	2.5	T4		52.69	54.06	1.944	5
T3		53.18	52.79	1.215	3.5	T5		52.69	52.98	2.43	6
T4		52.49	54.55	1.944	5	T6		53.37	50.93	4.617	10.5
T5		52.10	53.57	2.43	6	T7		53.47	51.32	0.486	2
T6		52.59	51.22	6.075	13.5	T8		52.59	50.64	0.729	2.5
T7		53.76	51.32	0.729	2.5	T9		51.91	49.17	0.486	2
						T10		51.61	49.66	2.187	5.5

34							35						
98112142							98112143						
Intensity	80...255						Intensity	80...255					
Area:	.01 - 7.5						Area:	.01 - 7.5					
9 of 10	objects in range						9 of 10	objects in range					
11	added by manually splitting objects						10	added by manually splitting objects					
surface	1						surface	2					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	0.91	53.13	47.13	0.486	2		1	1.00	52.96	46.82	0.486	3	
3	1.12	56.86	48.58	0.486	2		3	1.18	56.83	48.30	0.486	3	
4	0.90	55.46	50.31	0.486	2		4	1.10	55.24	50.12	0.729	3.5	
5	0.76	54.81	52.04	0.486	2		5	0.94	53.81	50.60	0.486	3	
6	1.00	55.35	53.46	0.6075	2.25		6	0.92	54.67	51.87	0.486	3	
7	0.78	52.09	54.64	0.729	2.5		7	1.02	55.12	53.94	1.701	5.5	
8	1.00	55.21	55.51	0.8505	2.75		8	0.80	52.03	54.46	0.729	3.5	
9	0.93	48.12	56.36	2.5515	6.25		9	0.95	55.43	55.13	1.215	4.5	
10	0.86	46.25	57.02	0.486	2		10	0.93	46.13	56.80	0.486	3	
T1		53.67	52.20	0.486	2		T1		53.76	52.10	0.486	3	
T2		53.37	53.96	0.486	2		T2		53.18	53.76	0.486	3	
T3		54.06	51.71	1.215	3.5		T3		54.06	51.61	1.458	5	
T4		53.18	53.67	2.187	5.5		T4		53.18	53.67	2.187	6.5	
T5		53.18	52.49	2.43	6		T5		53.18	52.59	2.673	7.5	
T6		52.59	52.10	0.486	2		T6		51.81	53.67	2.43	7	
T7		53.86	50.83	0.486	2		T7		52.30	52.20	1.215	4.5	
T8		53.08	49.95	0.729	2.5		T8		52.98	49.85	0.729	3.5	
T9		53.67	50.44	4.9815	11.25		T9		52.20	48.58	0.486	3	
T10		52.39	48.97	0.486	2		T10		52.10	48.88	2.916	8	
T11		52.30	49.07	2.673	6.5								

36							37						
98112144							98112145						
Intensity	80...255						Intensity	80...255					
Area:	.01 - 7.5						Area:	.01 - 7.5					
10 of 12	objects in range						5 of 6	objects in range					
9	added by manually splitting objects							added by manually splitting objects					
surface	2.5						surface	2					
step	0.486						step	0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	
1	1.01	53.49	46.93	0.486	3.5		1	1.05	53.17	46.69	0.486	3	
3	1.17	56.92	48.48	0.486	3.5		3	1.21	56.60	48.35	0.486	3	
4	0.98	54.09	50.76	0.486	3.5		4	0.92	50.92	49.76	2.916	8	
5	1.16	51.48	51.97	4.617	12		5	0.87	54.75	51.86	0.486	3	
6	0.89	55.00	52.12	0.486	3.5		6	1.08	54.11	54.18	1.701	5.5	
7	0.94	55.71	53.69	1.458	5.5		7	0.84	52.12	54.58	0.729	3.5	
8	0.67	55.11	53.82	1.458	5.5		8	1.09	55.53	54.91	0.972	4	
9	0.79	52.30	54.74	0.729	4		9	0.98	46.11	56.81	0.486	3	
11	1.05	55.65	55.19	1.215	5		T1		53.86	52.20	0.486	3	
12	0.95	46.39	57.06	0.486	3.5		T2		53.27	53.76	0.486	3	
T1		54.06	52.39	0.729	4		T3		54.06	51.71	0.972	4	
T2		53.47	54.06	0.729	4		T4		53.18	53.47	2.187	6.5	
T3		54.35	51.81	1.215	5		T5		52.98	52.59	2.43	7	
T4		53.47	53.86	2.187	7		T6		52.59	52.00	0.972	4	
T5		53.47	52.79	2.673	8		T7		52.98	49.66	0.8505	3.75	
T6		52.69	52.39	1.458	5.5		T8		53.57	50.73	0.486	3	
T7		53.27	49.95	0.729	4		T9		51.81	48.78	2.187	6.5	
T8		52.59	48.88	0.486	3.5		T10		52.30	48.78	0.486	3	
T9		52.20	49.07	2.43	7.5								

[illegible]

40					
98112148					
Intensity	80...255				
Area:	.01 - 7.5				
11 of 12	objects in range				
added by manually splitting objects					
ubstatum	1				
step	0.486				
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.04	52.92	47.55	0.729	2.5
2	1.18	51.32	49.66	1.944	5
3	1.22	51.99	49.67	0.729	2.5
4	1.18	56.28	49.17	0.729	2.5
5	0.99	52.77	49.79	0.729	2.5
7	0.93	54.33	52.74	0.729	2.5
8	1.11	54.30	54.96	1.458	4
9	1.26	55.61	55.70	0.729	2.5
10	0.82	51.72	55.36	1.215	3.5
11	0.92	45.81	57.73	0.729	2.5
T1		52.49	52.59	0.972	3
T2		52.69	51.61	1.701	4.5
T3		53.37	51.61	0.729	2.5
T4		53.47	52.59	1.215	3.5
T5		53.18	54.55	0.486	2
T6		53.18	53.96	2.43	6
T7		52.10	53.76	1.701	4.5

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[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

18						19					
98112126						98112127					
Intensity:	80...255					Intensity:	80...255				
Area:	.01 - 2.5					Area:	.01 - 2.5				
12 of 13	objects in range					7 of 10	objects in range				
added by manually splitting objects						added by manually splitting objects					
surface	3.5					surface	2				
step	0.486					step	0.486				
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.74	94.72	80.12	0.972	5.5	1	0.76	94.74	79.96	0.972	4
2	0.71	97.03	81.16	1.215	6	2	0.69	97.04	81.15	1.3365	4.75
3	0.53	96.19	81.62	0.972	5.5	3	0.53	96.13	81.57	0.972	4
4	0.76	94.10	82.04	1.701	7	4	0.75	94.31	82.19	1.944	6
5	0.66	95.61	84.55	1.215	6	6	0.70	95.44	84.54	1.3365	4.75
6	0.32	94.63	86.52	-1.704		7	0.60	91.90	85.97	1.701	5.5
7	0.33	92.69	86.53	-1.704		8	0.54	92.74	88.04	-0.972	
8	0.08	89.17	86.41	-1.704							
9	0.37	91.08	87.12	2.43	8.5						
10	0.38	91.85	87.23	-1.704							
11	0.40	93.89	87.18	-1.704							
12	0.45	90.19	87.38	2.187	8						

[illegible]

[illegible]

[illegible]

26							27							
98112134							98112135							
Intensity	80...255						Intensity	80...255						
Area:	.01 - 7.5						Area:	.01 - 7.5						
5 of 6	objects in range						5 of 6	objects in range						
	added by manually splitting objects							added by manually splitting objects						
surface	2						surface	3						
step	0.486						step	0.486						
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		
1	1.04	95.43	80.01	0.972	4		1	1.18	95.35	80.55	0.972	5		
2	0.85	98.02	81.44	1.3365	4.75		2	0.83	98.02	81.80	1.458	6		
3	0.76	97.06	81.99	0.972	4		3	0.84	97.04	82.44	0.972	5		
4	1.20	96.57	83.34	0.972	4		4	1.28	96.46	83.87	0.972	5		
5	0.82	97.86	85.26	0.972	4		5	0.81	97.64	85.96	0.972	5		

28						29					
98112136						98112137					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
5 of 6 objects in range						5 of 6 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 2						surface 0.75					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.27	95.33	81.06	0.972	4	1	1.27	95.41	81.09	0.972	2.75
2	0.97	97.94	82.52	1.458	5	2	1.00	98.00	82.57	1.458	3.75
3	0.75	96.84	83.14	0.972	4	3	1.40	95.77	83.47	1.458	3.75
4	1.31	96.30	84.51	0.972	4	4	0.84	96.99	83.21	0.972	2.75
5	0.64	97.14	86.52	1.701	5.5	5	0.61	97.14	86.65	1.3365	3.5

Nov21-Cell Tracking(AOI#3)
Object #'s

30							31							
98112138							98112139							
Intensity 80...255							Intensity 80...255							
Area: .01 - 7.5							Area: .01 - 7.5							
8 of 8 objects in range							7 of 10 objects in range							
added by manually splitting objects							added by manually splitting objects							
surface 0.5							surface 1							
step 0.486							step 0.486							
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		Obj.#	Area	Center-X	Center-Y	Center-Z	Frame		
1	1.36	95.09	81.43	0.972	2.5		4	0.93	93.10	81.26	-0.486			
2	1.07	97.70	82.88	1.458	3.5		2	1.42	94.89	81.30	0.729	2.5		
3	1.01	95.08	83.00	2.916	6.5		3	0.59	91.61	81.69	3.402	8		
4	0.83	96.62	83.47	0.972	2.5		6	0.97	97.39	82.78	1.215	3.5		
5	0.78	96.83	86.92	1.458	3.5		7	1.34	92.50	82.76	-0.486			
							8	1.00	96.36	83.51	0.729	2.5		
							9	0.75	96.65	86.94	1.215	3.5		

Nov21-Cell Tracking(AOI#3)
Object #'s

32						33					
98112140						98112141					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
6 of 7 objects in range						6 of 7 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 1					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.09	95.11	79.46	2.43	6	1	0.75	95.28	80.55	0.972	3
2	0.65	94.88	80.94	0.972	3	2	0.64	94.02	81.14	1.458	4
3	0.82	93.70	81.78	0.972	3	3	0.98	96.30	81.86	2.916	7
4	1.04	96.74	82.93	1.458	4	4	1.18	97.00	82.66	1.458	4
5	0.88	95.68	83.61	0.972	3	5	0.97	96.00	83.36	0.972	3
6	0.80	96.22	87.05	1.458	4	6	0.78	96.43	86.88	1.458	4

Nov21-Cell Tracking(AOI#3)
Object #'s

34						35					
98112142						98112143					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
5 of 6 objects in range						5 of 6 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 1						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.75	95.77	80.06	0.972	3	1	0.77	95.65	79.80	0.972	4
2	1.59	97.56	80.70	2.187	5.5	2	1.33	97.06	81.18	2.187	6.5
3	1.07	97.50	82.22	1.701	4.5	3	1.27	97.39	82.01	1.701	5.5
4	1.06	96.52	82.95	0.972	3	4	1.12	96.39	82.80	0.972	4
5	0.83	96.95	86.51	1.5795	4.25	5	0.82	96.82	86.32	1.701	5.5

Nov21-Cell Tracking(AOI#3)
Object #'s

36						37					
98112144						98112145					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
5 of 6 objects in range						5 of 6 objects in range					
added by manually splitting objects						added by manually splitting objects					
surface 2.5						surface 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	1.66	97.71	80.64	2.3085	7.25	1	0.84	95.68	79.80	0.972	4
2	0.86	95.92	80.05	1.0935	4.75	2	1.62	97.52	80.47	2.43	7
3	1.11	97.72	82.19	1.701	6	3	1.18	97.44	82.01	1.701	5.5
4	1.21	96.76	83.04	1.0935	4.75	4	1.20	96.42	82.80	0.972	4
5	0.89	97.22	86.46	1.5795	5.75	5	0.97	96.96	86.24	1.458	5

Nov21-Cell Tracking(AOI#3)
Object #'s

38						39					
98112146						98112147					
Intensity 80...255						Intensity 80...255					
Area: .01 - 7.5						Area: .01 - 7.5					
2 of 4 objects in range						5 of 7 objects in range					
3 added by manually splitting objects						added by manually splitting objects					
surface 1						substatum 2					
step 0.486						step 0.486					
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame	Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.86	95.60	80.27	0.972	3	1	0.91	95.21	80.66	0.972	4
3	0.94	96.95	86.70	1.458	4	3	1.46	96.73	81.59	2.43	7
T1		97.26	81.82	3.402	8	4	0.86	97.11	82.47	0.972	4
T2		97.16	82.70	1.701	4.5	5	1.85	96.10	83.68	0.972	4
T3		96.38	83.28	0.972	3	6	1.00	96.61	87.09	1.458	5
						T1		96.48	83.87	2.43	7

Nov21-Cell Tracking(AOI#3)
Object #'s

40					
98112148					
Intensity	80...255				
Area:	.01 - 7.5				
11 of 12	objects in range				
	added by manually splitting objects				
ubstatum	1				
step	0.486				
Obj.#	Area	Center-X	Center-Y	Center-Z	Frame
1	0.88	95.35	80.70	1.0935	3.25
2	0.99	97.50	81.80	3.402	8
3	0.73	97.04	82.58	1.215	3.5
4	0.59	99.05	82.87	2.3085	5.75
5	0.64	95.34	83.37	1.0935	3.25
6	1.05	96.83	83.89	1.0935	3.25
7	0.89	96.67	87.25	1.701	4.5
T1		96.87	83.87	2.43	6

98102204					98102205				
Intensity: 90...255					Range: 90...255				
Area: .01 - 7.5					Area: 0.01 - 7.5				
25 of 28 objects in range					21 of 22 objects in range				
5,12,20,26 added by manually splitting objects					2,5,7,11,13 added by manually splitting objects				
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.45	43.37	0.35	0.5	1	0.75	43.70	2.71	0.5
2	0.71	43.74	1.55	1	2	0.78	43.94	4.11	1
3	1.24	66.10	45.22	0	3	1.50	66.33	46.80	0
4	1.33	66.40	48.30	0	4	1.70	66.77	50.14	0
5	0.23	66.81	48.59	1.25	5	0.62	67.49	50.10	1.25
6	1.17	70.53	49.70	0	6	1.45	70.59	51.40	0
7	0.68	71.30	49.13	0	7	0.81	71.18	51.09	0
8	1.36	72.04	50.13	0	8	1.48	71.76	52.28	0.5
9	0.67	71.36	50.73	0.25	9	0.82	68.14	52.29	0.25
10	0.68	67.84	50.84	0	10	0.75	66.72	52.75	1
11	0.65	66.48	50.98	0.75	11	0.92	12.46	53.25	0
12	0.88	12.24	51.64	0	12	1.48	73.19	53.75	0
13	1.31	72.96	52.08	0	13	0.81	71.16	53.56	0.5
14	0.75	67.30	52.34	0	14	0.86	13.40	54.35	0.75
15	0.75	13.25	52.70	0.5	15	0.81	65.69	54.84	0.5
16	0.66	65.89	52.93	0.25	16	0.94	62.74	56.28	0.5
17	0.82	61.14	53.95	0	17	0.84	60.76	56.89	0.25
18	0.73	68.63	53.91	0	18	0.97	63.02	57.45	1.5
19	0.63	60.64	55.37	0	19	1.52	74.65	59.00	0.25
20	0.88	63.17	55.92	1	20	0.50	83.92	93.00	1.5
21	0.58	61.70	55.96	1	21	0.65	85.86	95.01	0.5
22	1.21	74.54	57.59	0.5					
23	0.22	84.07	91.95	0.5					
24	0.35	84.19	92.72	1					

Oct21-Cell Tracking
Object #s

98102206					98102207				
Range:		90...255			Range:		90...255		
Area:		0.01 - 7.5			Area:		0.01 - 7.5		
21 of 22		objects in range			21 of 23		objects in range		
2,5,7,11,13,18		added by manually splitting objects			1,2,5,7,8,10,14		added by manually splitting objects		
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.75	44.27	2.58	0	1	0.73	44.91	3.35	0
2	0.76	44.44	3.97	0.5	2	0.76	45.09	4.68	0.5
3	1.56	66.88	46.67	0	3	1.56	67.42	47.40	0
4	0.85	67.78	49.30	0	4	0.90	68.38	50.00	0
5	1.60	71.14	51.28	0	5	1.50	71.72	51.97	0
6	0.87	66.95	50.65	0	6	0.84	67.55	51.36	0
7	0.72	71.77	50.89	0	7	0.87	72.44	51.44	0
8	1.29	72.15	51.48	1	8	1.30	72.87	51.95	0.75
9	0.75	68.74	52.41	0	9	0.80	69.36	53.13	0
10	0.82	67.37	52.70	1	10	0.84	13.71	53.62	0
11	0.87	13.05	53.02	0	11	1.61	74.30	54.30	0
12	1.53	73.70	53.62	0	12	0.68	67.92	53.68	1.25
13	0.91	71.72	53.51	0.25	13	0.90	72.03	54.59	0.5
14	0.87	14.01	54.16	0.5	14	0.92	14.74	54.70	0.5
15	0.80	66.15	54.78	0.5	15	0.46	67.54	54.50	0.5
16	0.94	63.72	56.37	0.5	16	0.76	66.36	55.33	0.5
17	0.86	61.33	56.70	0	17	0.83	64.24	56.76	0.75
18	0.98	64.88	57.63	1.25	18	0.89	61.95	57.46	0
19	1.54	75.14	58.98	0	19	1.03	64.86	58.55	1.25
20	0.54	84.23	93.38	1	21	1.57	75.82	59.73	0.5
21	0.47	85.96	95.19	1	22	0.50	84.96	94.13	1.5

98102208					98102209				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
22 of 23	objects in range				23 of 24	objects in range			
6,8,9,10,15, 16	added by manually splitting objects				1...11, 14,15,17	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.71	45.19	3.50	0.25	1	0.77	45.33	3.79	0
2	0.67	45.47	4.85	0.75	2	0.76	45.64	5.07	1
3	0.64	67.05	46.92	0.25	3	0.81	67.27	47.19	0.5
4	0.83	68.00	48.12	0.25	4	0.99	68.23	48.32	0.25
5	0.80	68.59	50.22	0	5	0.99	68.77	50.50	0
6	0.75	71.95	51.31	0	6	0.98	72.20	51.50	0
7	0.87	67.69	51.54	0	7	0.85	73.62	51.78	0.75
8	1.47	73.19	52.42	0.5	8	1.02	67.80	51.75	0
9	0.95	72.50	51.82	0	9	1.13	72.80	52.03	0
10	0.86	71.77	53.08	0	10	0.98	72.02	53.35	0
11	0.75	69.54	53.32	0.25	11	0.83	73.69	53.53	1.5
12	1.64	74.41	54.43	0.25	12	1.00	69.76	53.62	0.25
13	0.78	13.96	53.87	0	13	1.08	67.66	53.66	1.75
14	0.88	67.95	54.15	1	14	1.87	74.58	54.75	0.25
15	1.18	73.07	54.85	2	15	0.89	14.17	54.14	0
16	0.83	14.91	54.98	0.5	16	1.10	67.29	54.94	0.25
17	0.79	66.79	55.18	0.5	17	0.90	15.14	55.26	0.5
18	1.24	66.06	57.28	0	18	1.18	72.71	55.58	2
19	1.00	64.36	57.39	0.5	19	1.25	64.62	57.68	0.5
20	0.81	62.12	57.53	0	20	1.50	66.17	57.52	0
21	1.57	75.95	59.94	0.5	21	1.16	62.53	57.77	0
22	0.51	85.09	94.53	1.5	22	1.83	76.15	60.08	0.5
					23	0.67	85.55	94.38	1.5

98102210					98102211				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				21 of 22	objects in range			
1... 10,12,13,15,16	added by manually splitting objects				3,4,5,7,6,8,9,11,14,10,12,15	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.75	45.82	4.19	0.25	1	0.65	45.94	4.47	0.5
2	0.77	46.01	5.50	0.75	2	0.62	45.88	5.84	1
3	0.74	67.53	47.52	0.5	3	0.75	67.75	47.75	0.5
4	1.00	68.53	48.64	0	4	0.94	68.78	48.79	0
5	0.99	69.02	50.84	0	5	0.91	69.25	51.07	0
6	1.06	72.33	51.86	0	6	0.90	72.58	52.11	0
7	1.03	68.02	52.08	0	7	0.96	68.35	52.33	0
8	1.16	73.10	52.31	0.25	8	1.08	73.36	52.61	0.25
9	1.04	72.36	53.53	0	9	0.91	72.61	53.71	0
10	1.03	69.68	53.93	0	10	0.94	69.76	54.13	0
11	1.98	74.90	55.08	0.25	11	0.92	75.05	54.44	0.5
12	0.90	14.68	54.48	0	12	0.64	68.94	55.17	1.5
13	1.07	68.28	54.98	1.5	13	0.75	15.79	55.67	1.25
14	1.18	72.31	55.67	1.5	14	0.91	75.39	56.12	0
15	0.82	15.41	55.64	0.75	15	0.88	67.96	55.74	0.25
16	0.85	67.26	55.77	0.5	16	1.56	73.05	56.49	0
17	1.56	66.43	57.79	0	17	1.34	66.76	58.11	0
18	1.26	64.85	58.00	0.5	18	1.22	65.16	58.28	0.5
19	1.21	62.85	58.05	0	19	1.03	63.12	58.31	0
20	1.88	76.41	60.30	0.5	20	1.80	76.71	60.57	0.75
21	0.52	86.23	94.59	1.5	21	0.68	86.57	94.33	1.75

Oct21-Cell Tracking
Object #s

98102212					98102213				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				21 of 22	objects in range			
1...10,15	added by manually splitting objects				3...9,11,15,16,17	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.62	46.12	5.09	0.5	1	0.61	46.58	5.89	0.5
2	0.61	46.07	6.39	1	2	0.60	46.36	7.17	1
3	0.75	67.92	48.28	0.5	3	0.80	68.37	49.16	0.5
4	0.97	68.98	49.39	0	4	1.00	69.40	50.23	0
5	1.00	69.41	51.66	0	5	1.01	69.80	52.55	0
6	0.98	72.76	52.71	0	6	1.03	73.15	53.60	0
7	0.98	68.55	52.92	0	7	1.06	68.95	53.75	0
8	1.11	73.58	53.15	0.25	8	1.18	73.98	53.94	0.25
9	0.98	72.76	54.37	0	9	1.01	73.12	55.30	0
10	1.12	68.52	55.02	1	10	1.05	70.39	55.64	0
11	0.97	69.96	54.75	0	11	1.11	68.93	56.11	1.25
12	0.91	75.21	55.06	0.5	12	0.91	75.84	56.06	0.5
13	0.76	16.28	56.10	0.75	13	0.66	16.71	56.66	1
14	0.97	75.55	56.69	0	14	1.00	75.98	57.59	0
15	0.90	68.09	56.32	0.25	15	0.98	68.32	57.36	0.5
16	1.51	66.97	58.71	0	16	1.52	67.35	59.60	0
17	1.23	65.38	58.86	0.75	17	1.23	65.79	59.69	0.75
18	1.12	63.33	58.84	0	18	1.18	63.73	59.79	0
19	0.90	76.68	60.68	0.5	19	0.98	77.79	61.36	0.5
20	0.95	77.13	61.82	0.25	20	0.96	77.56	62.68	0.25
21	0.65	86.92	94.81	1.5	21	0.65	87.28	96.31	1.5

98102214					98102215				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
20 of 21	objects in range				21 of 22	objects in range			
3...9,16,17	added by manually splitting objects				1...11,16,17,19,20	added by manually splitting objects			
1	added by manual tag								
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.57	46.95	6.45	0.5	1	0.61	47.44	6.92	0.5
2	0.59	46.72	7.70	1	2	0.58	47.11	8.18	1
3	0.75	69.07	49.67	0	3	0.80	69.59	50.03	0
4	0.80	69.63	50.66	0.75	4	0.78	70.11	51.03	0.75
5	1.14	70.31	52.90	0	5	1.03	70.73	53.39	0
6	1.06	69.28	54.22	0	6	1.08	73.87	54.52	0
7	1.09	73.41	54.04	0	7	1.13	69.85	54.76	0
8	1.19	74.33	54.39	0.25	8	1.26	74.79	54.92	0.25
9	1.10	73.50	55.67	0	9	1.12	73.95	56.09	0
10	1.04	69.44	56.33	0.75	10	1.15	71.24	56.56	0
11	1.10	70.87	56.16	0	11	1.04	69.79	56.93	0.75
12	0.98	76.17	56.52	0.5	12	0.96	76.81	56.99	0.5
13	0.69	16.96	57.17	1.25	13	0.60	17.35	57.76	1
14	0.97	76.27	57.97	0	14	1.04	76.84	58.39	0
15	1.12	68.83	57.96	0.25	15	1.17	69.31	58.38	0.25
16	1.51	67.74	60.01	0	16	1.59	68.17	60.36	0
17	1.20	66.11	60.06	1	17	1.30	66.51	60.54	1
18	1.18	64.02	60.18	0	18	1.24	64.45	60.54	0
19	1.50	77.82	62.86	0	19	0.89	78.08	63.38	0.25
20	0.72	87.65	96.61	1.5	20	0.78	77.33	64.04	0.75
T1		77.71	62.85	1	21	0.59	87.79	97.20	1.5

98102216					98102217				
Range:	90...256				Range:	90...255			
Area:	0.01 - 7.6				Area:	0.01 - 7.5			
21 of 23	objects in range				22 of 23	objects in range			
2...8,15,16, 18,19,20	added by manually splitting objects				2,3,6,7,8,9, 14	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.54	47.27	6.53	0.25	1	0.50	47.23	6.21	0.25
2	0.98	69.59	49.91	0	2	1.01	69.59	49.62	0
3	0.91	69.65	50.94	0.75	3	0.97	69.59	50.62	0.75
4	1.10	70.57	53.25	0	4	1.12	70.60	52.91	0
5	1.18	69.57	54.50	0	5	1.20	69.39	54.03	0.25
6	1.08	73.68	54.29	0	6	1.09	73.70	53.99	0
7	1.27	74.77	54.82	0.25	7	1.30	74.80	54.55	0.25
8	1.13	73.75	55.92	0	8	1.31	73.80	55.61	0.25
9	1.20	69.61	56.77	0.75	9	1.15	69.70	56.31	1
10	1.15	71.22	56.43	0	10	1.09	71.46	56.20	0
11	1.06	76.55	56.84	0.5	11	0.98	77.22	56.66	0.75
12	0.63	17.17	57.47	1.25	12	0.59	17.04	57.25	1.5
13	0.98	76.61	58.26	0	13	1.00	76.63	57.97	0.25
14	1.08	68.83	58.15	0.25	14	1.15	68.89	57.65	0.5
15	1.60	68.05	60.22	0	15	1.62	68.15	59.94	0
16	1.34	66.25	60.32	1	16	0.75	66.23	59.67	0.25
17	1.26	64.38	60.41	0	17	1.35	64.49	60.08	0
18	0.56	76.63	62.73		18	0.58	66.93	60.47	1.5
19	1.02	77.89	63.25	0.25	19	0.94	77.97	63.19	0.5
20	1.09	76.82	63.65	1	20	0.89	76.14	64.13	1.25
22	0.84	87.69	97.71	1.25	21	0.69	87.29	98.29	1.75
					22	0.16	88.25	98.64	

98102218					98102219				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
19 of 20	objects in range				19 of 20	objects in range			
1,2,4,5,6,7, 11,13,15	added by manually splitting objects				1,2,7,11,13, 15,16	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.02	69.46	50.93	0	1	0.90	70.03	50.31	0
2	0.96	69.48	51.86	0.75	2	0.83	70.25	51.21	0.75
3	1.13	70.66	54.24	0	3	1.12	71.16	53.70	0
4	1.15	73.58	55.28	0	4	1.14	74.14	54.70	0
5	1.35	74.59	55.91	0.25	5	1.37	75.15	55.38	0.25
6	1.26	73.63	56.96	0.25	6	1.38	74.17	56.35	0
7	1.28	69.37	57.64	1	7	1.24	69.80	57.04	1
8	1.18	71.33	57.57	0	8	1.18	71.95	57.02	0
9	1.05	77.08	57.99	0.75	9	1.11	77.56	57.41	0.5
10	1.05	76.48	59.24	0.25	10	1.04	77.12	58.68	0.25
11	1.22	68.83	59.07	0.5	11	1.03	69.34	58.55	0.5
12	0.86	68.45	60.58	0	12	0.81	68.97	59.94	0
13	0.73	66.29	60.97	0.5	13	0.67	66.67	60.65	1
14	1.38	64.37	61.38	0	14	1.38	64.93	60.93	0
15	0.52	66.89	61.68	2	15	0.55	67.42	61.19	2
16	0.86	67.65	61.96	0	16	0.83	68.28	61.29	0
17	0.92	78.02	64.59	0.75	17	1.18	75.96	63.81	0.75
18	0.88	75.26	64.62	1	18	0.97	78.27	64.08	0.75
19	0.83	87.82	98.46	2.25	19	0.70	88.17	97.85	2.25

98102220					98102221				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
19 of 20	objects in range				22 of 23	objects in range			
3,5,6,10,13,15	added by manually splitting objects				1,2,10,13,17,18,21,22	added by manually splitting objects			
1	added by manually tagging object								
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.86	70.23	50.11	0	1	1.03	69.87	50.01	0
2	1.25	70.90	53.31	0	2	0.75	69.88	50.79	1
3	1.28	73.93	54.20	0	3	1.15	70.77	53.45	0
4	1.37	75.14	54.85	0.25	4	1.12	75.07	54.44	0.5
5	1.50	74.01	55.89	0	5	1.15	73.86	54.35	0
6	1.30	69.75	56.33	0.75	6	1.40	73.91	56.07	0
7	1.29	71.77	56.50	0	7	0.58	69.89	56.20	0.75
8	1.20	77.38	56.96	0.5	8	1.21	71.75	56.51	0
9	1.14	76.80	58.18	0	9	1.14	77.25	57.10	0.5
10	0.64	69.72	57.84	0.5	10	0.54	69.70	57.39	2
11	0.54	68.19	58.06	0	11	1.08	76.68	58.27	0.25
12	0.87	68.70	59.47	0	12	0.44	67.88	58.24	0.5
13	0.70	66.52	60.16	0.5	13	0.39	69.54	58.21	0.75
14	1.52	64.79	60.48	0	14	0.76	68.60	59.63	0
15	0.51	67.46	60.28	1.75	15	0.71	66.40	60.42	0.75
16	0.95	67.89	60.88	0	16	1.35	64.73	60.60	0
17	1.09	78.19	63.41	0.5	17	0.40	67.31	60.80	2.25
18	1.17	75.77	63.47	0.75	18	0.73	67.92	61.02	0
19	1.05	88.69	98.01	1.5	19	0.99	78.24	63.62	0.5
T1		70.58	50.24	0.75	20	1.00	75.56	63.70	1
					21	0.51	87.93	97.75	1.5
					22	0.50	88.94	98.24	3

98102222					98102223				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 24	objects in range				22 of 23	objects in range			
2,11...14,17 ,19,20	added by manually splitting objects				1,2,10,12,1 4,15,17,18	added by manually splitting objects			
1,2	added by manual tag								
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.72	70.02	51.59	0	1	0.97	70.34	46.82	0
2	0.61	70.24	52.47	1.75	2	1.31	71.12	47.52	1.5
3	1.04	70.96	55.17	0.25	3	1.25	71.49	50.50	0.25
4	0.61	75.78	55.15	0.25	4	0.75	76.24	50.41	0.5
5	0.90	73.97	56.14	0.5	5	1.20	74.37	51.42	0
6	0.47	75.04	56.85	0.75	6	0.44	76.31	51.37	1.5
7	1.17	74.06	57.93	0.5	7	1.48	74.46	53.21	0.25
8	0.64	70.31	57.91	1	8	0.67	70.56	53.28	1
9	1.10	71.80	58.34	0.25	9	1.25	72.23	53.71	0.25
10	1.12	77.40	58.95	0.75	10	1.22	77.77	54.15	0.75
11	0.50	69.91	59.42	2.5	11	0.51	70.37	54.70	2
12	0.99	76.78	60.01	0.5	12	1.20	77.23	55.27	0.5
13	0.36	67.54	59.62	1	13	0.38	67.97	54.70	0.75
14	0.49	69.38	60.13	0.75	14	0.57	69.84	55.52	1
15	0.76	68.73	61.35	0	15	0.80	69.14	56.61	0
16	1.12	64.81	62.21	0	16	1.50	65.23	57.49	0
17	0.62	66.41	62.33	1	17	0.53	67.54	57.09	2
18	0.07	66.92	62.17		18	0.66	66.89	57.61	1.25
19	0.87	67.84	62.84	0	19	0.97	68.39	58.08	0
20	0.47	66.52	63.23	2.75	20	1.12	78.68	60.65	0.75
23	0.94	78.34	65.35	0.75	21	1.14	75.64	61.44	1
24	0.99	75.48	65.81	1.25	22	0.39	88.24	94.88	2
T1	X	87.49	99.71	2					

98102224					98102225				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				20 of 21	objects in range			
1,2,10,12,14,15,17,18	added by manually splitting objects				4,5,9,12,17,18	added by manually splitting objects			
					1	added by manual tag			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.03	53.82	41.14	0	1	1.67	53.83	42.56	0
2	1.07	53.95	42.08	1.25	2	1.06	54.75	45.78	0.25
3	1.18	55.19	44.59	0.25	3	0.66	59.60	45.78	0.25
4	0.70	59.93	44.87	0.25	4	1.00	57.75	46.92	0.25
5	1.10	58.05	45.81	0.25	5	1.20	58.13	47.86	0.5
6	1.46	57.93	47.61	0	6	0.51	53.39	48.04	1
7	0.62	54.03	46.92	0.75	7	1.27	55.44	49.08	0.25
8	1.28	55.70	48.05	0.25	8	1.24	61.15	49.37	0.75
9	1.27	61.20	48.46	0.75	9	0.64	54.04	49.78	1.5
10	0.66	54.02	48.98	2	10	0.36	51.21	50.04	1
11	0.32	51.48	48.94	0.75	11	1.19	60.57	50.70	0.25
12	1.21	60.79	49.62	0.25	12	0.60	53.42	50.55	0.5
13	0.62	53.17	49.90	0.75	13	0.92	52.48	52.00	0
14	0.83	52.63	51.03	0	14	0.75	48.92	52.06	0
15	1.61	48.64	51.71	0	15	0.71	50.92	52.27	0.75
16	0.72	51.08	51.10	0.75	16	0.72	47.77	53.40	0
17	1.03	51.94	52.48	0	17	0.81	51.77	53.49	0
18	0.46	50.80	52.57	3	18	0.49	50.63	53.62	2.5
19	1.00	62.31	55.11	1	19	1.05	61.98	55.98	0.5
20	1.26	59.41	55.17	1	20	1.25	59.41	55.94	1
21	0.49	71.43	88.42	1.5	21	0.49	71.43	89.44	1.75
					T1		54.06	42.72	1.5

98102226					98102227				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
20 of 21	objects in range					objects in range			
9,11,16,17	added by manually splitting objects				1,2,4,5,9,11 ,15..18	added by manually splitting objects			
1	added by manual tag								
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.76	53.47	42.36	0	1	0.88	53.21	41.65	0
2	1.28	54.29	45.50	0.25	2	0.90	54.07	42.14	1.75
3	0.68	59.35	45.67	0.25	3	1.48	54.15	45.21	0
4	1.20	57.36	46.82	0	4	0.81	59.45	45.11	0.25
5	1.49	58.59	47.83	0.5	5	1.40	57.59	46.62	0
6	0.63	53.04	47.80	1	6	0.81	53.02	47.50	1.5
7	1.44	54.89	48.84	0	7	1.55	55.02	48.22	0
8	0.72	53.55	49.50	1.25	8	0.77	53.51	48.82	1.5
9	1.26	60.63	49.37	0.75	9	1.25	61.14	49.16	1
10	0.39	50.93	49.90	0.75	10	0.54	50.88	49.49	0.5
11	1.18	60.24	50.57	0.25	11	1.27	60.30	50.19	0.25
12	0.98	52.09	51.90	0	12	1.55	55.30	50.16	0.25
13	0.82	48.47	51.99	0	13	1.05	52.21	51.50	0
14	0.80	50.60	52.22	1	14	0.82	50.17	51.54	0.5
15	0.76	47.50	53.33	0	15	0.93	48.61	51.76	0.25
16	0.96	51.37	53.35	0	16	0.86	47.48	52.97	0
17	0.38	50.27	54.20	3.25	17	1.12	51.31	52.98	0
18	1.31	58.82	55.87	1	18	0.81	50.07	53.89	3.5
19	1.06	61.69	56.06	0.75	19	1.17	61.82	55.66	0.75
20	0.53	71.15	89.46	1.75	20	1.40	59.09	55.59	1.25
T1		53.76	42.62	1.75	21	0.87	71.26	88.92	1.5

98102228					98102229				
Range:	90...255				Range:	90...256			
Area:	0.01 - 7.5				Area:	0.01 - 7.6			
20 of 21	objects in range				20 of 21	objects in range			
11,13-17	added by manually splitting objects				14...17	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.31	53.65	41.95	0	1	1.38	53.33	43.28	0
2	0.82	59.80	45.17	0.25	2	0.86	59.29	46.45	0.25
3	1.42	54.53	45.65	0	3	1.50	54.21	47.02	0.25
4	0.64	58.56	46.26	0.25	4	0.65	58.29	47.57	0.25
5	0.68	57.41	47.49	0.25	5	0.70	57.09	48.63	0.25
6	0.71	53.44	47.76	1.5	6	0.64	53.13	49.02	1.5
7	1.55	55.26	48.52	0	7	1.58	54.86	49.69	0.25
8	0.81	53.76	49.28	1.5	8	0.96	49.33	50.43	0
9	1.30	60.76	49.16	1	9	1.51	59.68	50.20	1.5
10	0.51	51.23	49.67	0.75	10	0.64	53.50	50.77	1.5
11	1.25	60.59	50.37	0.25	11	0.59	50.96	51.08	0.75
12	1.15	52.51	51.70	0	12	1.33	60.14	51.72	0.25
13	0.93	50.35	52.36	0.5	13	1.13	52.13	53.09	0
14	0.92	48.87	51.99	0.5	14	0.99	48.41	53.33	0.5
15	0.83	47.95	53.11	0	15	0.94	47.62	54.51	0
16	0.97	51.71	53.13	0.25	16	0.94	51.29	54.41	0.25
17	0.53	50.78	53.88	3.75	17	0.57	50.49	55.19	3.5
18	1.22	62.32	55.80	1	18	1.19	61.79	57.06	0.75
19	1.36	59.23	55.79	1.25	19	1.34	58.86	57.17	1.25
20	0.49	71.28	89.67	2.25	20	0.54	71.39	91.23	2.25

98102230					98102231				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				21 of 22	objects in range			
16,18,15,17	added by manually splitting objects				16,18,15,17	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.39	53.53	42.24	0	1	1.36	53.90	42.23	0
2	0.89	59.78	45.33	0.25	2	0.85	60.21	45.31	0.25
3	0.75	55.23	45.82	0	3	0.78	55.62	45.80	0
4	0.78	53.70	46.24	0.25	4	0.71	58.84	46.65	0.25
5	0.68	58.56	46.71	0	5	0.81	54.01	46.28	0.25
6	0.74	53.33	47.93	1.25	6	0.75	53.62	48.01	1.5
7	0.70	55.78	48.49	0	7	0.66	56.19	48.38	0.25
8	0.77	54.22	48.69	0	8	0.76	54.57	48.63	0.25
9	1.50	60.46	49.31	1	9	0.70	49.07	49.25	2.75
10	0.36	49.03	49.58	2.25	10	0.81	61.60	49.30	0.75
11	0.67	53.63	49.69	2	11	0.75	60.07	49.32	1.25
12	1.30	60.29	50.68	0	12	0.72	54.13	49.64	2.25
13	0.61	51.02	50.17	0.5	13	0.68	51.36	50.12	0.5
14	1.09	52.31	52.04	0	14	1.37	60.60	50.75	0
15	1.00	48.61	52.31	0.5	15	1.09	52.73	51.95	0
16	1.13	51.48	53.40	0	16	0.95	48.95	52.40	0.5
17	0.95	47.72	53.55	0	17	0.96	51.88	53.37	0
18	0.60	51.09	54.25	3.5	18	0.92	48.18	53.53	0
19	1.16	61.58	56.28	1	19	0.60	51.72	54.10	3.75
20	1.48	59.07	56.22	1	20	1.18	62.15	56.41	1.25
21	0.60	71.45	90.13	2.25	21	1.31	59.18	56.67	1.5
					22	0.63	72.33	90.41	2.25

98102232					98102233				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				21 of 22	objects in range			
3,5,16..19	added by manually splitting objects				8,13,6,715..18	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.44	53.66	41.71	0.25	1	1.47	53.69	41.59	0
2	0.87	60.02	44.78	0.5	2	0.89	60.06	44.68	0.25
3	0.87	55.38	45.34	0.25	3	0.79	55.33	45.15	0
4	0.72	58.87	46.15	0.25	4	0.89	53.67	45.62	0
5	0.87	53.76	45.78	0.25	5	0.66	58.84	46.03	0
6	0.71	53.38	47.44	1.25	6	0.71	53.39	47.24	1
7	0.74	55.66	47.45	0.25	7	0.85	54.31	47.93	0.5
8	0.85	54.33	48.11	0.5	8	0.91	59.74	48.69	1.5
9	0.73	59.66	48.82	1.5	9	0.79	53.91	49.05	2
10	0.73	53.84	49.30	2.25	10	0.79	61.94	48.99	0.25
11	0.76	61.86	49.12	0.5	11	0.61	48.70	49.02	3.5
12	0.56	48.77	49.36	3.25	12	0.67	51.15	49.49	0.5
13	1.38	60.43	50.20	0.5	13	1.33	60.44	49.94	0.25
14	0.70	51.12	49.69	0.5	14	1.26	52.34	51.25	0
15	1.24	52.45	51.51	0	15	1.01	48.69	51.70	0.5
16	1.01	48.68	51.98	0.75	16	0.97	47.92	52.84	0
17	1.12	51.61	52.86	0	17	1.18	51.49	52.78	0.25
18	1.06	47.92	53.08	0	18	0.79	52.16	53.77	3.5
19	0.67	51.76	53.60	3.75	19	1.21	61.97	55.80	1
20	1.21	62.02	55.84	1	20	1.73	58.43	56.63	0.5
21	1.55	58.64	56.78	1.25	21	0.52	72.19	56.13	3.75
22	0.92	72.38	90.27	2.25	22	0.66	70.72	88.75	-
					23	0.31	71.51	89.54	1.5
					24	0.38	70.74	89.82	-

Oct21-Cell Tracking
Object #'s

98102234					98102235				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
24 of 25	objects in range				22 of 23	objects in range			
6,7,8,13,15,17,18	added by manually splitting objects					added by manually splitting objects			
1	added by manual tag								
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.47	53.66	41.60	0	1	1.49	53.63	41.65	0
2	0.92	60.18	44.68	0.5	2	0.92	60.27	44.68	0.5
3	0.83	55.36	45.16	0	3	0.94	55.31	45.17	0
4	0.63	58.85	45.94	0.25	4	0.94	53.64	45.82	0.25
5	0.92	53.69	45.77	0.25	5	0.71	58.81	46.04	0
6	0.65	53.29	47.30	1.25	6	0.57	53.25	47.17	1.25
7	0.82	54.26	47.90	0.25	7	0.84	54.16	47.85	0.25
8	0.81	59.72	48.99	1.5	8	0.54	48.78	48.71	3.5
9	0.60	48.66	48.88	3.5	9	0.90	62.27	48.99	0.25
10	0.85	62.10	48.97	0.5	10	0.72	59.64	48.92	1.25
11	0.71	53.70	49.19	1.25	11	0.80	53.65	49.13	0.75
12	0.74	51.09	49.44	0.5	12	0.77	50.70	49.59	0.5
13	1.25	60.32	50.10	0	13	1.51	60.28	50.10	0.25
14	1.29	52.39	51.36	0	14	1.18	52.47	51.38	0
15	1.16	48.56	51.85	0.5	15	1.17	48.57	52.19	0.5
16	0.20	51.42	52.35		16	1.03	51.04	52.64	0.75
17	1.00	47.81	52.97	0	17	0.65	51.57	53.28	3.5
18	1.10	51.33	52.94	0.5	18	1.18	62.03	55.91	1
19	0.33	51.60	53.58		19	0.87	58.60	56.22	1
20	1.11	62.13	55.92	1	20	0.97	57.91	57.73	0
21	0.80	59.02	55.79	1	21	0.48	72.53	57.88	3
22	0.52	71.47	56.98	2.75	22	0.85	72.74	89.85	2
23	0.94	58.14	57.16	0.25					
24	0.64	71.74	89.91	2.5					
T1		51.51	52.98	3.5					

Oct21-Cell Tracking
Object #'s

98102236					98102237				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
21 of 22	objects in range				23 of 24	objects in range			
16,18,15,17	added by manually splitting objects				6,8,13,15,16	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.56	53.56	41.70	0	1	1.55	53.58	41.71	0
2	0.99	59.81	44.62	0	2	1.01	59.92	44.57	0
3	0.94	55.26	45.14	0	3	0.95	55.28	45.20	0
4	1.07	53.59	45.85	0.25	4	1.04	53.60	45.94	0.25
5	0.79	58.67	46.08	0	5	0.72	58.72	46.07	0
6	0.49	53.33	47.33	1.25	6	0.62	53.45	47.32	1.5
7	0.77	54.10	47.86	0.25	7	0.82	59.62	47.94	2
8	0.95	62.28	48.94	0.25	8	0.76	54.21	47.82	0
9	0.58	48.87	49.05	4	9	0.88	62.47	49.05	0.5
10	0.94	60.14	49.26	1.5	10	0.87	53.80	49.21	0.75
11	0.75	53.66	49.27	0.75	11	1.47	60.24	50.18	0
12	1.46	60.16	50.14	0	12	0.78	50.72	49.66	0.5
13	0.70	50.57	49.73	0.5	13	1.25	52.34	51.42	0
14	1.16	52.48	51.33	0	14	1.22	48.38	52.54	0.25
15	1.19	48.39	52.36	0.25	15	1.04	50.91	52.74	0.5
16	1.13	51.05	52.58	0.5	16	0.97	51.44	53.69	3
17	0.89	51.86	53.52	3.25	17	1.31	61.92	55.76	1
18	1.32	61.59	55.74	1	18	0.84	59.04	55.73	1
19	0.82	58.98	55.95	1	19	0.49	73.28	57.55	1.25
20	0.61	73.53	56.28	0.75	20	1.00	58.21	58.80	0
21	1.02	57.98	58.44	0	21	0.66	70.60	89.58	-
22	0.81	72.52	90.03	1.5	22	0.28	71.62	90.06	3
					23	0.53	72.71	90.41	-

98102238					98102239				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
22 of 23	objects in range				22 of 23	objects in range			
6,7,13,16,17	added by manually splitting objects				3,5,6,7,13,15,16	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.56	53.55	41.68	0	1	1.60	53.56	41.74	0
2	1.05	59.85	44.52	0.25	2	1.05	59.89	44.49	0.25
3	1.04	55.16	45.24	0	3	1.09	55.20	45.23	0
4	0.75	58.67	46.08	0	4	0.79	58.68	46.04	0
5	1.07	53.52	46.05	0.25	5	1.17	53.49	46.14	0.25
6	0.67	53.44	47.32	1.5	6	0.65	53.49	47.39	1.5
7	0.74	54.21	47.86	0	7	0.67	54.27	47.83	0.25
8	0.83	58.41	48.44	2	8	0.93	62.42	48.98	0.75
9	0.97	62.52	49.07	0.5	9	0.85	53.71	49.17	1
10	0.78	53.74	49.22	1	10	0.76	60.26	49.16	2.25
11	0.83	50.92	49.60	0.5	11	1.60	60.12	50.16	0.5
12	1.51	60.16	50.17	0	12	0.90	50.89	49.77	0.5
13	1.19	52.25	51.41	0	13	1.39	52.29	51.44	0
14	1.11	49.18	51.61	0.75	14	1.25	48.96	52.54	1
15	0.02	52.25	51.84		15	1.33	51.36	52.68	0.25
16	1.24	51.11	52.70	0.5	16	0.86	51.41	53.85	3.25
17	0.70	51.21	53.67	3	17	0.51	61.68	55.03	1.25
18	1.27	61.80	55.82	1	18	0.95	59.08	55.66	1.25
19	0.89	58.94	55.79	1	19	0.71	62.07	56.30	0.25
20	0.55	73.09	57.30	0.75	20	0.63	73.36	57.46	1.5
21	0.97	58.35	58.65	0	21	1.03	58.49	58.73	0.25
22	0.96	70.82	89.54	1.25	22	0.78	70.99	89.26	2.5

98102240					98102241				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
23 of 23	objects in range				23 of 23	objects in range			
6,7,13,15,16	added by manually splitting objects				3,4,6,7,10,11,13,15,16	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.60	53.61	41.69	0	1	1.63	53.61	41.65	0
2	1.12	59.87	44.43	0.25	2	1.06	59.80	44.39	0.25
3	1.13	55.22	45.22	0	3	1.18	55.18	45.24	0
4	0.83	58.70	46.05	0.25	4	1.20	53.60	46.03	0.25
5	1.22	53.55	46.11	0.5	5	0.77	58.68	46.07	0
6	0.63	53.56	47.33	1.5	6	0.68	53.65	47.34	1.5
7	0.80	54.36	47.84	0.25	7	0.73	54.36	47.86	0
8	0.99	53.65	49.19	1	8	0.98	62.50	49.10	0.75
9	0.94	62.36	49.03	0.75	9	0.98	53.61	49.17	0.75
10	0.80	60.38	49.24	1.5	10	0.89	60.26	49.11	1.75
11	1.60	60.13	50.13	0.25	11	1.61	60.14	50.11	0.25
12	0.84	51.14	49.74	0.5	12	0.91	51.29	49.73	0.25
13	1.50	52.34	51.50	0	13	1.49	52.38	51.45	0
14	1.20	49.00	52.44	1	14	1.29	48.86	52.33	1
15	1.27	51.37	52.74	0.5	15	1.31	51.47	52.61	0.25
16	0.86	51.28	53.85	3.5	16	1.08	51.16	53.82	3.75
17	0.50	61.67	55.32	1	17	0.52	61.71	55.37	1
18	0.94	59.04	55.77	1.25	18	0.97	59.02	55.84	1
19	0.63	62.11	56.51	0.5	19	0.72	62.29	56.55	0
20	0.87	74.07	58.24	0.25	20	0.78	73.87	58.23	0.5
21	1.06	58.53	58.79	0.25	21	1.19	58.25	58.97	0
22	0.99	70.54	91.61	1.5	22	1.68	72.60	90.21	1

98102242					98102243				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
23 of 23	objects in range				22 of 24	objects in range			
3,5,7,8,13,15,16	added by manually splitting objects				6,7,13,15,17	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.62	54.02	42.62	0	1	1.67	54.23	43.12	0
2	1.08	60.18	45.35	0.25	2	1.18	60.36	45.89	1.25
3	1.14	55.59	46.24	0	3	1.24	55.85	46.82	0
4	0.85	59.04	47.06	0.25	4	1.16	54.33	47.18	0.75
5	1.23	53.99	47.10	0.5	5	0.81	59.23	47.58	0.25
6	1.14	57.65	48.74	1.75	6	0.73	54.07	48.77	1.75
7	0.60	53.45	48.55	1.75	7	0.74	55.11	49.05	0.5
8	0.85	54.69	48.74	0.25	8	0.90	58.20	50.06	2.5
9	0.98	54.06	50.02	1	9	0.98	54.18	50.43	1.25
10	0.97	62.90	50.10	1	10	1.04	63.05	50.69	1
11	1.62	60.54	51.02	0.5	11	1.67	60.67	51.59	0.5
12	0.90	51.88	50.79	0.5	12	0.92	52.23	51.29	0.5
13	1.40	52.96	52.34	0	13	1.66	53.08	53.08	0
14	1.38	49.44	53.37	1.25	14	1.30	49.84	54.15	2.25
15	1.40	51.88	53.58	0.5	15	1.38	52.11	54.22	0.5
16	1.01	51.68	54.76	4	17	1.40	51.89	55.53	5
17	1.06	59.41	56.72	1.25	18	1.06	59.59	57.09	1.25
18	0.70	62.82	57.59	0.5	19	0.74	63.11	58.13	0.5
19	0.70	61.58	57.60	2.5	20	0.67	61.76	58.16	1
20	0.94	75.26	59.73	0	21	1.23	58.93	60.57	0
21	1.18	58.60	60.15	0	22	0.92	75.64	60.49	0.5
22	1.07	73.57	91.77	1.5	23	1.12	72.28	93.40	1

Oct21-Cell Tracking
Object #'s

98102244					98102245				
Range:	90...255				Range:	90...255			
Area:	0.01 - 7.5				Area:	0.01 - 7.5			
22 of 23	objects in range				22 of 23	objects in range			
13,14,15,16	added by manually splitting objects				14,16,17,18	added by manually splitting objects			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	1.68	54.40	43.69	0	1	1.69	54.63	44.11	0
2	1.15	60.73	46.47	0.5	2	1.13	60.85	47.00	0.5
3	1.30	56.07	47.37	0	3	1.24	56.35	47.78	0
4	1.09	54.53	47.64	1.25	4	1.14	54.70	48.15	1.25
5	0.77	59.43	48.12	0.5	5	0.77	59.64	48.62	0
6	0.70	54.37	49.15	2.25	6	0.64	54.47	49.69	1.5
7	0.70	55.62	50.12	1.25	7	0.88	55.81	50.68	1.25
8	0.72	58.48	50.70	2	8	0.73	59.11	50.93	3.5
9	0.92	54.35	51.03	1.25	9	1.11	63.37	51.49	1
10	0.97	63.25	51.20	1.5	10	1.03	54.49	51.48	1.25
11	1.67	60.87	52.10	0.5	11	1.69	61.08	52.53	0.5
12	0.93	52.41	51.81	0.75	12	0.94	52.70	52.34	0.5
13	0.74	54.14	53.16	0.5	13	0.80	54.37	53.81	0.5
14	1.25	51.59	53.86	1.5	14	0.70	52.66	54.24	0
15	0.62	52.59	53.86	0	15	1.42	50.09	54.93	1.5
16	1.59	52.31	54.67	0.75	16	0.64	52.77	54.79	1.25
17	0.40	51.97	55.96	4.25	17	0.70	51.88	55.55	0.5
18	1.08	59.62	57.83	1.5	18	1.17	52.01	56.74	4.5
19	0.70	63.27	58.73	0.75	19	1.08	59.84	58.34	1.5
20	1.28	59.21	61.20	0	20	0.74	63.35	59.34	0.5
21	0.96	76.12	61.41	0.5	21	1.30	59.43	61.67	0
22	0.89	71.99	91.82	3.75	22	0.94	76.66	62.17	0.5

98102246					98102247				
Range:	90...255				Range:	90...255			
Area:	0.01 - 3.0				Area:	0.01 - 2.5			
20 of 23	objects in range				19 of 21	objects in range			
1,5	added by manually splitting objects				9,12	added by manually splitting objects			
1 to 5	added by manual tag				1 to 4	added by manual tag			
Obj.#	Area	Center-X	Center-Y	Center-Z	Obj.#	Area	Center-X	Center-Y	Center-Z
1	0.79	54.49	43.68	0	1	0.83131	54.8003	44.0342	0
2	0.83	55.11	45.20	0	2	0.83	55.50	45.68	0
4	1.17	60.98	47.28	0.5	3	1.21	61.16	47.74	0.5
5	1.31	56.48	48.19	0.5	4	1.34	56.68	48.62	0.5
6	1.11	54.91	48.58	1.5	5	1.20	55.19	48.94	1.5
7	0.74	59.82	48.94	0	6	0.82	60.09	49.43	0
8	0.72	54.99	50.10	2	7	0.78	55.29	50.47	2
9	0.90	56.32	51.13	1.5	8	0.93	56.51	51.27	1.75
10	0.86	59.35	51.01	3.5	9	0.75	59.77	51.77	4
11	1.23	63.42	51.99	1.25	10	1.22	63.87	52.56	1.5
12	1.06	54.71	51.66	1.5	11	1.01	55.07	51.97	1.5
13	1.72	61.26	52.82	0.5	12	1.83	61.47	53.19	0.5
14	1.01	52.85	52.58	0.75	13	1.02	53.18	53.00	0.5
15	0.75	54.39	54.27	0.75	14	0.81	54.73	54.56	0.5
17	0.70	51.12	54.96	1.5	16	0.64	50.38	55.91	1
18	0.67	49.64	55.59	1.5	17	1.11	61.37	58.90	1
19	1.08	60.17	58.74	2	18	0.76	64.07	60.34	0.5
20	0.75	63.83	59.77	1	19	1.34	59.81	62.48	0
21	1.30	59.57	62.05	0	20	1.07	81.99	66.17	0.5
22	1.34	78.61	64.34	0	T1		52.98	55.03	0
T1		52.88	54.64	0	T2		53.18	55.42	1.5
T2		52.88	54.94	1.25	T3		53.08	55.82	4.5
T3		52.49	55.42	4	T4		52.49	57.09	5
T4		52.20	55.91	1					
T5		52.20	56.60	5					

APPENDIX C – OBJECT TRACKING INFORMATION

Image	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>
Time	0	13	26	43	58	72	88	103	118	133	148	163	179	193	209	223
Cell ID	98112108	98112109	98112110	98112111	98112112	98112113	98112114	98112115	98112116	98112117	98112118	98112119	98112120	98112121	98112122	98112123
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B																
C																
D					2	2	2	2	2	2	2	2	2	3	3	3
E																
F	2	2	2	2	3	4	3	3	3	3	3	3	4	4	4	4
G	3	3	3	3	4	5	4	4	4	4	5	4	7	5	5	5
H																
I																
J													8	6	6	6
K	4	4	4	5	5	6	5	5	5	6	4	5	5	2	2	2
L																
M													6	7	7	7
N																

Image	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Time	237	253	269	283	306	318	334	349	363	381.001	397.001	413.001	424	438	453	468
Cell ID	98112124	98112125	98112126	98112127	98112128	98112129	98112130	98112131	98112132	98112133	98112134	98112135	98112136	98112137	98112138	98112139
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B																
C						2	2	2	2	2	2	2	2	2	3	3
D	3	3	3	3	2	3	3	4	4	4	4	4	-1	-1	-1	-1
E			4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
F	4	4	5	4	4	5	5	5	5	5	5	5	5	5	5	5
G	5	5	6	5	5	6	6	6	6	6	6	6	6	6	6	6
H																7
I	6	6	7	6	6	7	7	7	7	7	7	7	8	7	7	8
J																
K	2	2	2	2	3	4	4	3	3	3	3	3	3	3	2	2
L													4	4	4	4
M	7	7	8	7	7	8	8	8	8	8	8	9	9	8	8	9
N																

Image	32	33	34	35	36	37	38	39	40
Time	483	498	513	527.999	542.999	557.999	572.998	587.998	605
Cell ID	98112140	98112141	98112142	98112143	98112144	98112145	98112146	98112147	98112148
A	1	1	1	1	1	1	1	1	1
B						3	2	2	2
C	3	3	3	3	3	4	4	4	4
D	-1	-1	-1	-1	-1	-1	-1	-1	-1
E	-1	-1	-1	-1	-1	-1	-1	-1	-1
F	5	5	5	5	5	6	6	7	8
G	6	6	6	6	6	8	9	9	9
H	7	8	7	7	7	7	8	8	6
I	8	9	9	9	11	10	12	10	10
J		11	10	10	12	11	13	12	11
K	2	2	2	2	2	2	3	3	3
L	4	4	4	4	4	5	5	5	5
M	9	10	8	8	8,9,10,13	9	7,10,11	11	-1
N	10	12	11	11	15	12	15	13	12

Nov21-Cell Tracking(AOI#1)
Object Tracking

Image Time Cell ID	<u>0</u> 0 98112108	<u>1</u> 13 98112109	<u>2</u> 26 98112110	<u>3</u> 43 98112111	<u>4</u> 58 98112112	<u>5</u> 72 98112113	<u>6</u> 88 98112114	<u>7</u> 103 98112115	<u>8</u> 118 98112116	<u>9</u> 133 98112117	<u>10</u> 148 98112118	<u>11</u> 163 98112119	<u>12</u> 179 98112120	<u>13</u> 193 98112121	<u>14</u> 209 98112122
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B															
C	2	2	2	2	3	3	3	3	4	4	4	4	4	4	4
D															
E	3	3	3	3	2	2	2	2	2	3	3	3	3	3	3
F															
G		4	4	T7	4	5	4	4	3	2	2	2	2	2	2
H															
I	5	6	6	5	6	9	10	8	9	T7	10	10	10	11	11
J															
K			7	6	7	10	11	9	10	9	11	11	11	12	13
L															
M	6	7	8	8	9	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
N	7	8	9	7	8	11	12	10	11	10	12	12	12	13	12
O	T1	T1	T1	T1	T1	7	8	6	7	8	9	8	8	9	8
P						8	9	7	8	7	8	9	9	10	10
Q	T2	T2	T2	T2	T2	T1	T1	T1	T1	T1	6	6	6	6	6
R											T1	T1	-1	-1	-1
S	T3	T6	T5	T3	T6	T5	T5	T5	T5	T6	T5	T6	T5	T4	T1
T															
U							T6	T6	T6	T4	T6	T4	T3	T2	T3
V															
W	T4	T3	T3	T6	T3	T2	T2	T2	5	5	5	5	5	5	5
X															
Y									T2	T2	T2	T2	T1	7	9
Z	T5	T4	T4	T5	T4	T3	T3	T3	T3	T3	T3	T3	T2	T1	T4
AA															
AB	T6	T5	T6	T4	T5	T4	T4	T4	T4	T5	T4	T5	T4	T3	T2
AC															

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Nov21-Cell Tracking(AOI#2)
Object Tracking

Image	<u>15</u>	<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>
Time	223	237	253	269	283	306	318	334	349	363	381.001	397.001	413.001	424	438
Cell ID	98112123	98112124	98112125	98112126	98112127	98112128	98112129	98112130	98112131	98112132	98112133	98112134	98112135	98112136	98112137
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
B															
C	4	4	4	4	4	4	4	4	5	4	4	4	4	2	2
D														5	5
E	3	3	3	3	2	3	3	3	4	3	3	3	3	4	8
F															4
G	2	2	2	2	3	2	2	2	2	2	2	2	2	3	3
H									3	-1	-1	-1	-1	-1	-1
I	11	8	10	12	10	11	9	7	9	9	7	T1	7	10	11
J															14
K	12	9	11	14	11	14	10	8	10	10	8	9	9	12	15
L															
M	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
N	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
O	T1	T7	T4	T4	T4	T4	T4	T4	T4	T5	T4	T5	T5	T3	T1
P	10	7	9	10	9	10	-1	-1	-1	-1	-1	-1	-1	-1	-1
Q	6	6	7	8	7	T6	T6	T6	T6	T6	T6	T7	T6	T8	T6
R	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
S	T2	T6	T5	T5	T5	T5	T5	T5	T5	T4	T5	T3	8	T4	12
T													T1	T5	T4
U	T4	T4	T2	T2	T2	T2	T2	T2	T2	T2	T2	T6	T4	T7	T5
V														T6	-1
W	5	T1	5	7	6	5	5	5	7	5	5	5	5	6	7
X															
Y	7	T2	6	5	5	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Z	T5	T3	T1	T1	T1	T1	T1	T1	T1	T1	T1	T2	T2	T1	9
AA															T3
AB	T3	T5	T3	T3	T3	T3	T3	T3	T3	T3	T3	T4	T3	T2	T2
AC															

Image	30	31	32	33	34	35	36	37	38	39	40
Time	453	468	483	498	513	527.999	542.999	557.999	572.998	587.998	605
Cell ID	98112138	98112139	98112140	98112141	98112142	98112143	98112144	98112145	98112146	98112147	98112148
A	1	1	1	1	1	1	1	1	1	1	1
B	2	2	2	T9	T10	T9	T8	T10	T2	T2	3
C	4	3	4	T10	T11	T10	T9	T9	T1	T1	2
D	6	6	7	5	-1	-1	-1	-1	-1	-1	-1
E	5	5	5	T8	T8	T8	T7	T7	T3	T3	5
F											T2
G	3	4	3	3	3	3	3	3	3	3	4
H	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
I	13	10	10	7	6	7	7,8	6	6	5	8
J	16	12	12	9	8	9	11	8	8	6	9
K	15	14	15	12	10	10	12	9	9	8	11
L		13	13	11	9	T6	5	4	-1	-1	-1
M	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
N	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
O	T2	T2	T2	T2	T6	T7	T6	T6	T5	T4	T1
P	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Q	T6	T6	T6	T6	T9	-1	-1	-1	-1	-1	-1
R	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
S	14	11	11	8	7	8	9	7	7	7	10
T	T4	T4	T4	T4	T4	T4	T4	T4	T7	T8	T6
U	T5	T5	T5	T5	T5	T5	T5	T5	T6	T7	T7
V	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
W	7	7	T7	T7	T7	5	4	T8	T4	T5	T3
X				4	4	4	-1	-1	-1	-1	-1
Y	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Z	10	9	9	6	5	6	6	5	5	4	7
AA	T3	T3	T3	T3	T3	T3	T3	T3	4	T6	T4
AB	T1	T1	T1	T1	T2	T2	T2	T2	T8	T9	T5
AC					T1	T1	T1	T1	-1	-1	-1

Nov21-Cell Tracking(AOI#2)
Object Tracking

Image	<u>0</u>	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>
Time	0	13	26	43	58	72	88	103	118	133	148	163	179	193	209	223
Cell ID	98112108	98112109	98112110	98112111	98112112	98112113	98112114	98112115	98112116	98112117	98112118	98112119	98112120	98112121	98112122	98112123
A	1	1	1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1	1
C																
D																
E																
F	3	3	3	2	3	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
G	4	4	4	3	2	2	2	2	2	2	2	2	2	2	2	2
H																
I																
J	5	8	5	7	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
K	6	5	6	4	4	3	4	3	-1	-1	-1	-1	-1	-1	-1	-1
L	7	6	7	5	5	4	3	4	5	3	3	3	3	3	3	3
M																
N																
O	8	7	8	6	6	5	5	6	3,4,6	4	4	4	4	4	4	4
P																5

Image	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Time	237	253	269	283	306	318	334	349	363	381.001	397.001	413.001	424	438	453	468
Cell ID	98112124	98112125	98112126	98112127	98112128	98112129	98112130	98112131	98112132	98112133	98112134	98112135	98112136	98112137	98112138	98112139
A	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2
C		3	3	3	3	3	3	3	3	4	3	3	3	4	4	8
D																
E																
F	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
G	2	2	2	2	2	2	2	2	2	3	2	2	2	2	2	6
H																
I		4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
J	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
K	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
L	3	5	4	4	4	4	5	4	4	5	4	4	4	3	3	3
M																
N	4	6	5	6	5	5	6	5	5	6	5	5	5	5	5	9
O	5	7	9	7	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
P			12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Image	<u>32</u>	<u>33</u>	<u>34</u>	<u>35</u>	<u>36</u>	<u>37</u>	<u>38</u>	<u>39</u>	<u>40</u>
Time	483	498	513	527.999	542.999	557.999	572.998	587.998	605
Cell ID	98112140	98112141	98112142	98112143	98112144	98112145	98112146	98112147	98112148
A	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	2	1	1	1	2	1	1	1	1
C	3	2	-1	-1	-1	-1	-1	-1	-1
D	5	5	4	4	4	4	T3	5	5
E									6
F	-1	-1	-1	-1	-1	-1	-1	-1	-1
G	4	4	3	3	3	3	T2	4	3
H								T1	T1
I	-1	-1	-1	-1	-1	-1	-1	-1	-1
J	-1	-1	-1	-1	-1	-1	-1	-1	-1
K	-1	-1	-1	-1	-1	-1	-1	-1	-1
L	1	3	2	2	1	2	T1	3	2
M									4
N	6	6	5	5	5	5	3	6	7
O	-1	-1	-1	-1	-1	-1	-1	-1	-1
P	-1	-1	-1	-1	-1	-1	-1	-1	-1

Image	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Time	0	22	37	50	66	81	97	112	126	142	157	172	189	204	220
Cell ID	98102204	98102205	98102206	98102207	98102208	98102209	98102210	98102211	98102212	98102213	98102214	98102215	98102216	98102217	98102218
A	1	1	1	1	1	1	1	1	1	1	1	1	1	1	-1
B	2	2	2	2	2	2	2	2	2	2	2	2	-1	-1	-1
C	3	3	3	3	3	3	3	3	3	3	3	3	2	2	1
D															
E					4	4	4	4	4	4	4	4	3	3	2
F	4	4	4	4	5	5	5	5	5	5	5	5	4	4	3
G															
H			5	6	7	8	7	7	7	7	6	7	5	5	-1
I	5	5	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
J	6	6	6	5	6	6	6	6	6	6	7	6	6	6	4
K															
L					10	10	9	9	9	9	8	8	7	7	5
M															
N	7	7	7	7	9	9	8	8	8	8	9	9	8	8	6
O	8	8	8	8	8	7	-1	-1	-1	-1	-1	-1	-1	-1	-1
P						11	-1	-1	-1	-1	-1	-1	-1	-1	-1
Q	9	13	13	13	15	18	14	16	-1	-1	-1	-1	-1	-1	-1
R	10	9	9	9	11	12	10	10	11	10	11	10	10	10	8
S															
T	11	10	10	12	14	13	13	12	10	11	10	11	9	9	7
U															
V	12	11	11	10	13	15	12	-1	-1	-1	-1	-1	-1	-1	-1
W	13	12	12	11	12	14	11	11	12	12	12	12	11	11	9
X															
Y								14	14	14	14	14	13	13	10
Z	14	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AA	15	14	14	14	16	17	15	13	13	13	13	13	12	12	-1
AB	16	15	15	16	17	16	16	15	15	15	15	15	14	14	11
AC															
AD	17	16	16	17	19	19	18	18	17	17	17	17	16	18	15
AE															
AF														16	13
AJ	18	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AK	19	17	17	18	20	21	19	19	18	18	18	18	17	17	14
AL															

Image	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Time	0	22	37	50	66	81	97	112	126	142	157	172	189	204	220
Cell ID	98102204	98102205	98102206	98102207	98102208	98102209	98102210	98102211	98102212	98102213	98102214	98102215	98102216	98102217	98102218
AM															
AN	20	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AO	21	18	18	19	18	20	17	17	16	16	16	16	15	15	12
AP															
AQ															
AR															16
AS	22	19	19	21	21	22	20	20	19	19	T1	20	20	20	18
AT															
AU									20	20	19	19	19	19	17
AV															
AW	23	20	20	22	22	23	21	21	21	21	20	21	22	21	19
AX															
AY	24	21	21	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Image	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Time	235	251	267	285	295	313	330	346	361	376	391	407	420	436	451
Cell ID	98102219	98102220	98102221	98102222	98102223	98102224	98102225	98102226	98102227	98102228	98102229	98102230	98102231	98102232	98102233
A	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D															
E	2	T1	2	2	2	2	T1	T1	2	-1	-1	-1	-1	-1	-1
F	3	2	3	3	3	3	2	2	3	3	3	3	3	3	3
G															
H	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
I	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
J	4	3	5	5	5	5	4	4	5	4	4	5	4	4	5
K															
L	5	4	4	4	4	4	3	3	4	2	2	2	2	2	2
M				6	6	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
N	6	5	6	7	7	6	5	5	12	-1	-1	-1	-1	-1	-1
O	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
P	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Q	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
R	8	7	8	9	9	8	7	7	7	7	7	7	7	7	21
S												8	8	8	7
T	7	6	7	8	8	7	6	6	6	6	6	6	6	6	6
U			10	11	11	10	9	8	8	8	10	11	12	10	9
V	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
W	9	8	9	10	10	9	8	9	9	9	9	9	10	11	10
X													11	9	8
Y	10	9	11	12	12	12	11	11	11	11	12	12	14	13	13
Z	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AA	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AB	11	11	12	13	13	11	10	10	10	10	11	13	13	14	12
AC		10	13	14	14	13	12	-1	-1	-1	-1	-1	-1	-1	-1
AD	15	15	17	20	17	18	18	17	18	17	17	18	19	19	18
AE															
AF	13	13	15	17	18	16	15	14	14	13	8	10	9	12	11
AJ	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AK	14	14	16	16	16	15	14	13	15	14	14	15	16	16	15
AL															

Image	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
Time	235	251	267	285	295	313	330	346	361	376	391	407	420	436	451
Cell ID	98102219	98102220	98102221	98102222	98102223	98102224	98102225	98102226	98102227	98102228	98102229	98102230	98102231	98102232	98102233
AM							16	15	16	15	15	17	18	18	16
AN	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AO	12	12	14	15	15	14	13	12	13	12	13	14	15	15	14
AP															
AQ	16	16	18	19	19	17	17	16	17	16	16	16	17	17	17
AR															
AS	17	18	20	24	21	20	20	18	20	19	19	20	21	21	20
AT															
AU	18	17	19	23	20	19	19	19	19	18	18	19	20	20	19
AV															
AW	19	19	21	T1	22	21	21	20	21	20	20	21	22	22	22,23,24
AX			22	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AY	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

Image	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Time	466	482	495	511	525	542	555	571	587	601	616	632	647	661
Cell ID	98102234	98102235	98102236	98102237	98102238	98102239	98102240	98102241	98102242	98102243	98102244	98102245	98102246	98102247
A	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
B	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
C	1	1	1	1	1	1	1	1	1	1	1	1	1	1
D													2	2
E	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
F	3	3	3	3	3	3	3	3	3	3	3	3	5	4
G	5	4	4	4	5	5	5	4	5	4	4	4	6	5
H	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
I	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
J	4	5	5	5	4	4	4	5	4	5	5	5	7	6
K	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
L	2	2	2	2	2	2	2	2	2	2	2	2	4	3
M	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
N	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
O	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
P	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
Q	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
R	22	21	20	19	20	20	20	20	20	22	21	22	22	20
S	7	7	7	8	7	7	7	7	8	7	7	7	9	8
T	6	6	6	6	6	6	6	6	7	6	6	6	8	7
U	11	11	11	10	10	9	8	9	9	9	9	10	12	11
V	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
W	10	9	8	9	9	8	9	8	10	10	10	9	11	10
X	8	10	10	7	8	10	10	10	6	8	8	8	10	9
Y	13	13	12	11	12	11	11	11	11	11	11	11	13	12
Z	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AA	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AB	12	12	13	12	11	12	12	12	12	12	12	12	14	13
AC	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AD	T1	17	17	16	17	16	16	16	16	17	17	18	T3	T3
AE													T5	T4
AF	9	8	9	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AJ	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AK	15	15	15	14	14	14	14	14	14	14	14	15	18	16
AL													17	-1

Image	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Time	466	482	495	511	525	542	555	571	587	601	616	632	647	661
Cell ID	98102234	98102235	98102236	98102237	98102238	98102239	98102240	98102241	98102242	98102243	98102244	98102245	98102246	98102247
AM	17	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AN	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AO	14	14	14	13	13	13	13	13	13	13	13	13	15	14
AP											15	14	T1	T1
AQ	18	16	16	15	16	15	15	15	15	15	16	16	T2	T2
AR											17	T4	-1	-1
AS	21	19	19	18	19	18	18	18	17	18	18	19	19	17
AT	23	20	21	20	21	21	21	21	21	21	20	21	21	19
AU	20	18	18	17	18	19	19	19	18	19	19	20	20	18
AV						17	17	17	19	20	-1	-1	-1	-1
AW	24	22	22	21,22,23	22	22	22	22	22	23	22	-1	-1	-1
AX	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
AY	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1

APPENDIX D – MOVEMENT DATA

MOVEMENT DATA FOR NOV21-aoi#1 DATA SET

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
A	0	0	1	11.38	8.62	0.12
A	1	13	1	11.42	8.644	0.24
A	2	26	1	11.368	8.761	0.12
A	3	43	1	11.401	8.703	0.36
A	4	58	1	10.47	8.131	0.12
A	5	72	1	10.276	8.001	0.12
A	6	88	1	10.269	7.98	0.24
A	7	103	1	10.129	7.97	0.12
A	8	118	1	9.94	7.962	0.36
A	9	133	1	9.731	7.739	0.61
A	10	148	1	9.721	7.505	0.61
A	11	163	1	9.612	7.696	0.49
A	12	179	1	9.572	7.702	0.61
A	13	193	1	9.724	7.238	0.49
A	14	209	1	9.664	7.338	0.36
A	15	223	1	9.523	7.292	0.73
A	16	237	1	9.159	7.167	0.49
A	17	253	1	8.959	7.192	0.36
A	18	269	1	8.574	6.937	0.36
A	19	283	1	8.724	6.987	0.24
A	20	306	1	8.482	6.775	0.49
A	21	318	1	7.54	6.461	0.12
A	22	334	1	7.483	6.297	0.12
A	23	349	1	7.506	6.498	0.24
A	24	363	1	7.62	6.712	0
A	25	381	1	7.425	6.879	0.24
A	26	397	1	7.291	6.744	0.24
A	27	413	1	7.214	6.82	0.24
A	28	424	1	7.208	6.864	0.24
A	29	438	1	7.238	6.886	0
A	30	453	1	7.054	6.735	0
A	31	468	1	7.137	6.767	0
A	32	483	1	6.976	6.8	0.12
A	33	498	1	6.997	6.715	0.24
A	34	513	1	6.9	7.127	0
A	35	528	1	6.898	7.053	0.24
A	36	543	1	6.847	7.217	0.24
A	37	558	1	6.123	6.563	0.24
A	38	573	1	5.963	6.366	0.24
A	39	588	1	5.846	6.095	0.24
A	40	605	1	5.728	5.935	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
B	37	558	3	7.653	7.593	0.97
B	38	573	2	7.513	7.076	0.24
B	39	588	2	7.426	7.135	0.24
B	40	605	2	7.648	6.975	0.73
C	21	318	2	9.32	7.411	0.49
C	22	334	2	9.673	7.357	0.36
C	23	349	2	9.536	7.858	0.73
C	24	363	2	10.67	8.082	0.24
C	25	381	2	10.965	8.409	0.24
C	26	397	2	10.991	8.124	0.49
C	27	413	2	10.404	8.28	0.24
C	28	424	2	10.288	8.214	0.49
C	29	438	2	10.488	8.296	0.49
C	30	453	3	10.234	8.325	0.49
C	31	468	3	10.207	8.247	0.24
C	32	483	3	9.956	8.12	0.49
C	33	498	3	9.977	8.215	0.49
C	34	513	3	10.3	8.727	0.24
C	35	528	3	10.528	8.673	0.24
C	36	543	3	10.497	8.697	0.24
C	37	558	4	10.453	8.783	0.24
C	38	573	4	10.523	8.716	0.24
C	39	588	4	10.426	8.545	0.24
C	40	605	4	10.328	8.735	0.24
D	4	58	2	11.96	9.821	0.12
D	5	72	2	11.726	9.021	0.12
D	6	88	2	12.569	9.63	0.49
D	7	103	2	12.109	9.56	0.12
D	8	118	2	12.07	9.592	0
D	9	133	2	12.541	9.269	0.61
D	10	148	2	12.261	9.045	0.24
D	11	163	2	12.162	8.996	0
D	12	179	2	12.262	9.122	0.61
D	13	193	3	11.694	10.148	0.12
D	14	209	3	11.614	10.498	0.36
D	15	223	3	11.673	10.382	0.73
D	16	237	3	10.149	8.427	0
D	17	253	3	10.749	9.562	0.73
D	18	269	3	9.894	9.267	1.09
D	19	283	3	9.524	9.977	0.49
D	20	306	2	9.462	9.375	0.36
D	21	318	3	9.49	9.031	0.24
D	22	334	3	9.573	8.757	0.24
D	23	349	4	9.436	9.268	0.61

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
D	24	363	4	9.37	10.122	0.49
D	25	381	4	9.105	10.219	0.97
D	26	397	4	9.371	10.074	0.49
D	27	413	4	9.344	10.05	0.73
E	18	269	4	11.314	10.027	-0.12
F	0	0	2	13.46	11.7	0.12
F	1	13	2	13.45	11.654	0.24
F	2	26	2	13.498	11.721	0.12
F	3	43	2	13.491	11.623	0.24
F	4	58	3	13.42	11.591	0.24
F	5	72	4	13.406	11.491	0.12
F	6	88	3	13.479	11.58	0.24
F	7	103	3	12.939	11.53	0
F	8	118	3	12.88	11.592	0
F	9	133	3	12.721	11.579	0
F	10	148	3	12.961	11.575	0.24
F	11	163	3	12.842	11.616	0
F	12	179	4	12.832	11.642	0.24
F	13	193	4	12.984	11.348	0.12
F	14	209	4	12.934	11.528	0
F	15	223	4	13.153	11.552	0.24
F	16	237	4	13.229	11.667	0
F	17	253	4	13.169	11.592	0.12
F	18	269	5	12.994	11.437	0.24
F	19	283	4	12.944	11.497	0
F	20	306	4	12.932	11.465	0
F	21	318	5	12.71	11.481	0
F	22	334	5	12.753	11.337	0
F	23	349	5	12.806	11.458	0
F	24	363	5	12.67	11.732	0
F	25	381	5	12.745	11.809	0.49
F	26	397	5	12.751	11.624	0.24
F	27	413	5	12.664	11.73	0.24
F	28	424	5	12.478	11.654	0.12
F	29	438	5	12.538	11.736	0.24
F	30	453	5	12.394	11.565	0
F	31	468	5	12.387	11.567	0
F	32	483	5	12.306	11.55	0.24
F	33	498	5	12.137	11.485	0.24
F	34	513	5	12.08	11.767	0
F	35	528	5	12.038	11.803	0.24
F	36	543	5	11.787	11.837	0
F	37	558	6	11.783	11.813	0.24
F	38	573	6	11.703	11.646	0.24

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
F	39	588	7	11.686	11.655	0
F	40	605	8	11.748	11.825	0.24
G	0	0	3	27.44	12.74	0.36
G	1	13	3	27.39	13.264	0.49
G	2	26	3	27.168	13.181	0.12
G	3	43	3	27.201	13.103	0.24
G	4	58	4	27.13	12.791	0
G	5	72	5	27.106	12.781	0
G	6	88	4	27.119	12.58	0
G	7	103	4	27.119	12.96	0.24
G	8	118	4	27.25	13.282	0
G	9	133	4	27.001	13.169	0
G	10	148	5	26.951	13.045	0.24
G	11	163	4	26.872	13.036	0
G	12	179	7	26.392	12.512	0.24
G	13	193	5	26.204	12.478	0.12
G	14	209	5	26.194	12.758	0
G	15	223	5	26.663	13.002	0.49
G	16	237	5	26.619	13.357	0.49
G	17	253	5	26.959	12.282	0.49
G	18	269	6	26.444	12.417	0.97
G	19	283	5	26.754	12.287	0.73
G	20	306	5	27.432	12.165	0.61
G	21	318	6	27.3	12.111	0.73
G	22	334	6	27.483	12.077	0.73
G	23	349	6	27.536	12.218	1.22
G	24	363	6	27.57	12.352	0.73
G	25	381	6	27.415	12.439	1.7
G	26	397	6	27.571	12.274	1.46
G	27	413	6	27.454	12.46	1.7
G	28	424	6	27.458	12.354	1.7
G	29	438	6	27.508	12.396	1.7
G	30	453	6	27.474	12.055	1.46
G	31	468	6	28.317	11.907	0.97
G	32	483	6	28.446	11.78	0.97
G	33	498	6	28.547	11.625	1.09
G	34	513	6	28.88	11.917	0.73
G	35	528	6	28.758	11.873	0.73
G	36	543	6	25.987	11.817	2.19
G	37	558	8	24.973	12.453	1.46
G	38	573	9	26.343	12.286	1.94
G	39	588	9	26.486	12.025	1.94
G	40	605	9	25.868	12.075	2.67
H	31	468	7	26.737	11.947	0.97

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
H	32	483	7	26.786	11.96	1.22
H	33	498	8	26.637	11.895	1.34
H	34	513	7	26.45	12.117	1.46
H	35	528	7	26.488	12.183	1.94
H	36	543	7	28.907	11.887	0.49
H	37	558	7	28.993	11.813	0.73
H	38	573	8	28.833	11.586	1.22
H	39	588	8	28.666	11.495	0.73
H	40	605	6	29.728	10.805	0.49
I	12	179	8	27.562	13.322	0.24
I	13	193	6	27.744	13.148	0.12
I	14	209	6	27.654	13.248	0
I	15	223	6	27.753	13.132	0.24
I	16	237	6	27.779	13.247	0
I	17	253	6	27.699	13.202	0
I	18	269	7	27.464	13.057	0
I	19	283	6	27.584	13.137	0
I	20	306	6	27.512	13.135	0
I	21	318	7	27.32	13.101	0
I	22	334	7	27.263	12.997	0
I	23	349	7	27.306	13.128	0
I	24	363	7	27.21	13.362	0
I	25	381	7	27.255	13.449	0.24
I	26	397	7	27.221	13.254	0.24
I	27	413	7	27.034	13.45	0.24
I	28	424	8	26.938	13.374	0.24
I	29	438	7	26.938	13.446	0.24
I	30	453	7	26.744	13.335	0.24
I	31	468	8	26.717	13.287	0
I	32	483	8	26.566	13.23	0.12
I	33	498	9	25.607	13.195	0.24
I	34	513	9	25.57	13.567	0
I	35	528	9	25.518	13.553	0.24
I	36	543	11	25.467	13.577	0
I	37	558	10	25.403	13.563	0.24
I	38	573	12	25.293	13.416	0
I	39	588	10	25.276	13.425	0.24
I	40	605	10	25.268	13.525	0.24
J	33	498	11	27.177	13.225	0.24
J	34	513	10	27.16	13.557	0
J	35	528	10	27.108	13.563	0.24
J	36	543	12	27.057	13.567	0
J	37	558	11	26.983	13.543	0.24
J	38	573	13	26.893	13.356	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
J	39	588	12	26.806	13.315	0.24
J	40	605	11	26.818	13.415	0.24
K	0	0	4	18.44	15.17	0
K	1	13	4	18.36	14.904	0
K	2	26	4	18.018	14.931	0
K	3	43	5	17.871	14.543	0
K	4	58	5	18.05	14.251	0
K	5	72	6	17.456	14.061	0
K	6	88	5	19.379	16.55	0.97
K	7	103	5	18.109	15.7	0
K	8	118	5	19.91	15.982	0.36
K	9	133	6	17.601	14.599	0.12
K	10	148	4	16.501	12.705	0.24
K	11	163	5	18.242	14.336	0
K	12	179	5	16.322	11.202	0.24
K	13	193	2	15.834	8.788	0.12
K	14	209	2	14.954	7.948	0
K	15	223	2	15.393	7.572	0.24
K	16	237	2	15.339	7.717	0
K	17	253	2	14.969	8.052	0
K	18	269	2	14.254	9.057	0.24
K	19	283	2	13.154	9.117	0.12
K	20	306	3	12.162	10.175	0
K	21	318	4	12.02	9.991	0
K	22	334	4	12.073	9.787	0
K	23	349	3	12.146	9.618	0
K	24	363	3	12.02	9.092	0.24
K	25	381	3	12.075	9.639	0.24
K	26	397	3	12.011	9.564	0.24
K	27	413	3	11.974	9.42	0.24
K	28	424	3	11.638	9.034	0.12
K	29	438	3	12.078	8.396	0.49
K	30	453	2	12.154	7.875	0.49
K	31	468	2	12.017	7.787	0
K	32	483	2	10.896	7.17	0.73
K	33	498	2	10.827	7.025	1.22
K	34	513	2	10.81	7.227	0.73
K	35	528	2	10.678	7.083	0.97
K	36	543	2	10.607	7.137	1.09
K	37	558	2	10.663	7.233	0.24
K	38	573	3	10.643	7.206	0.97
K	39	588	3	10.426	7.035	0.97
K	40	605	3	10.388	7.075	0.97
L	28	424	4	11.368	10.514	0.12

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
L	29	438	4	11.638	10.586	0.36
L	30	453	4	11.404	10.365	0
L	31	468	4	11.507	10.667	0.24
L	32	483	4	11.406	10.81	0.49
L	33	498	4	11.207	10.625	0.49
L	34	513	4	11.11	10.947	0.49
L	35	528	4	11.018	10.643	0.24
L	36	543	4	10.857	10.897	0.49
L	37	558	5	10.913	11.023	0.49
L	38	573	5	10.633	10.846	0.49
L	39	588	5	10.436	10.735	0.49
L	40	605	5	10.518	10.905	0.73
M	12	179	6	17.312	12.342	0.24
M	13	193	7	17.654	14.638	0.49
M	14	209	7	17.824	16.478	0.36
M	15	223	7	18.593	17.032	0.49
M	16	237	7	19.549	17.327	0
M	17	253	7	19.319	17.292	0
M	18	269	8	18.704	16.747	0.49
M	19	283	7	18.954	16.927	0
M	20	306	7	19.012	17.065	0
M	21	318	8	18.8	17.111	0.12
M	22	334	8	18.413	16.087	0
M	23	349	8	18.646	14.978	0
M	24	363	8	18.33	15.842	0
M	25	381	8	18.525	16.839	1.22
M	26	397	8	19.271	18.044	0.97
M	27	413	9	17.984	15.91	0.24
M	28	424	9	17.468	15.914	1.22
M	29	438	8	18.388	15.846	0.97
M	30	453	8	19.104	15.335	0.12
M	31	468	9	18.927	14.317	0.24
M	32	483	9	18.576	14.79	0.24
M	33	498	10	16.617	13.525	0.73
M	34	513	8	18.5	14.217	0.49
M	35	528	8	18.468	12.813	1.34
M	36	543	10	18.897	13.367	0.49
M	37	558	9	18.673	13.543	1.22
M	38	573	11	18.513	13.266	0.73
M	39	588	11	17.536	13.625	1.46
N	32	483	10	20.446	15.08	0
N	33	498	12	21.107	14.645	0.49
N	34	513	11	21.42	15.057	0.97
N	35	528	11	22.318	15.243	1.22

[illegible]

MOVEMENT DATA FOR NOV21-aoi#2 DATA SET

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
A	0	0	1	51.94	46.31	0.49
A	1	13	1	51.95	46.264	0.49
A	2	26	1	51.978	46.251	0.49
A	3	43	1	52.001	46.083	0.49
A	4	58	1	51.93	46.161	0.49
A	5	72	1	51.876	45.981	0.36
A	6	88	1	52.029	46.13	0.49
A	7	103	1	52.039	45.95	0.49
A	8	118	1	52.03	45.972	0.49
A	9	133	1	51.961	46.069	0.12
A	10	148	1	51.961	45.775	0.24
A	11	163	1	52.202	45.806	0.24
A	12	179	1	52.212	45.852	0.24
A	13	193	1	52.124	45.588	0.24
A	14	209	1	52.034	45.868	0.24
A	15	223	1	52.243	45.712	0.24
A	16	237	1	52.209	45.737	0.24
A	17	253	1	52.319	45.702	0.24
A	18	269	1	52.054	45.627	0.24
A	19	283	1	51.934	45.597	0.24
A	20	306	1	51.762	45.545	0.24
A	21	318	1	51.35	45.761	0.24
A	22	334	1	51.603	45.647	0.24
A	23	349	1	51.676	45.698	0.24
A	24	363	1	51.43	46.032	0.24
A	25	381	1	51.515	46.119	0.24
A	26	397	1	51.561	46.014	0.243
A	27	413	1	51.524	46.08	0.24
A	28	424	1	51.808	45.254	0.49
A	29	438	1	52.068	45.506	0.49
A	30	453	1	51.764	45.155	0.49
A	31	468	1	51.647	45.037	0.49
A	32	483	1	51.616	44.96	0.49
A	33	498	1	51.527	44.815	0.49
A	34	513	1	51.37	45.077	0.49
A	35	528	1	51.298	44.963	0.49
A	36	543	1	51.437	44.877	0.49
A	37	558	1	51.313	44.833	0.49
A	38	573	1	51.263	44.666	0.49
A	39	588	1	51.206	44.705	0.49
A	40	605	1	51.258	44.715	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
AA	29	438	T3	52.488	50.246	1.94
AA	30	453	T3	52.294	50.045	1.46
AA	31	468	T3	52.397	49.857	1.46
AA	32	483	T3	52.496	49.76	1.22
AA	33	498	T3	52.397	49.365	1.46
AA	34	513	T3	52.3	49.657	1.22
AA	35	528	T3	52.398	49.753	1.46
AA	36	543	T3	52.297	49.757	1.22
AA	37	558	T3	52.203	49.853	0.97
AA	38	573	4	52.003	49.606	0.73
AA	39	588	T6	51.616	49.855	0.97
AA	40	605	T4	51.808	49.755	1.22
AB	0	0	T6	52.59	51.51	0.49
AB	1	13	T5	52.59	51.314	0.61
AB	2	26	T6	52.588	51.511	0.49
AB	3	43	T4	52.591	51.323	0.36
AB	4	58	T5	52.59	51.121	0.24
AB	5	72	T4	52.496	51.121	0.24
AB	6	88	T4	52.689	51.03	0.49
AB	7	103	T4	52.489	50.93	0.73
AB	8	118	T4	52.59	51.122	0.61
AB	9	133	T5	52.591	51.029	0.24
AB	10	148	T4	52.591	51.125	0.24
AB	11	163	T5	52.592	51.026	0.24
AB	12	179	T4	52.492	51.412	0.24
AB	13	193	T3	52.684	50.928	1.7
AB	14	209	T2	52.684	51.028	0.24
AB	15	223	T3	52.783	51.022	0.24
AB	16	237	T5	52.689	51.127	0.24
AB	17	253	T3	52.689	51.022	0.24
AB	18	269	T3	52.494	51.127	0.24
AB	19	283	T3	52.594	51.127	0.36
AB	20	306	T3	52.392	51.125	0.36
AB	21	318	T3	52.39	50.931	0.24
AB	22	334	T3	52.493	50.927	0.24
AB	23	349	T3	52.296	51.128	0.24
AB	24	363	T3	52.39	51.222	0.24
AB	25	381	T3	52.295	51.119	0.36
AB	26	397	T4	52.301	51.024	0.486
AB	27	413	T3	52.104	51.12	0.24
AB	28	424	T2	51.908	51.024	0.49
AB	29	438	T2	52.008	51.026	0.73
AB	30	453	T1	51.904	50.835	0.49
AB	31	468	T1	52.007	50.827	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
AB	32	483	T1	51.806	50.73	0.49
AB	33	498	T1	51.807	50.635	0.49
AB	34	513	T2	51.61	51.907	0.49
AB	35	528	T2	51.518	51.903	0.49
AB	36	543	T2	51.417	52.007	0.73
AB	37	558	T2	51.413	51.903	0.49
AB	38	573	T8	51.323	51.806	0.49
AB	39	588	T9	51.316	51.715	0.49
AB	40	605	T5	51.518	51.715	0.49
AC	34	513	T1	51.91	50.147	0.49
AC	35	528	T1	52.098	50.243	0.49
AC	36	543	T1	52.007	50.337	0.73
AC	37	558	T1	52.003	50.343	0.49
B	28	424	2	51.158	46.624	0.49
B	29	438	2	51.118	46.616	0.49
B	30	453	2	50.934	46.515	0.49
B	31	468	2	50.867	46.517	0.49
B	32	483	2	50.876	46.51	0.49
B	33	498	T9	50.737	46.335	0.49
B	34	513	T10	50.63	46.917	0.49
B	35	528	T9	50.538	46.723	0.49
B	36	543	T8	50.537	46.827	0.49
B	37	558	T10	50.443	46.923	0.49
B	38	573	T2	50.343	46.826	0.49
B	39	588	T2	50.246	46.925	0.61
B	40	605	3	50.328	46.835	0.73
C	0	0	2	51.67	48.11	0.61
C	1	13	2	51.78	48.174	0.61
C	2	26	2	51.808	48.041	0.61
C	3	43	2	51.701	48.133	0.49
C	4	58	3	51.63	48.151	0.49
C	5	72	3	51.626	48.321	0.49
C	6	88	3	51.569	48.28	0.73
C	7	103	3	51.439	48.15	0.85
C	8	118	4	51.49	48.212	0.73
C	9	133	4	51.401	48.129	0.24
C	10	148	4	50.991	48.085	0.73
C	11	163	4	50.992	48.136	0.97
C	12	179	4	50.982	48.142	0.73
C	13	193	4	51.114	47.658	0.73
C	14	209	4	51.014	47.698	0.73
C	15	223	4	51.183	47.702	0.73
C	16	237	4	51.329	47.797	0.73
C	17	253	4	51.309	47.752	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
C	18	269	4	51.254	47.677	0.73
C	19	283	4	51.044	47.657	0.73
C	20	306	4	51.032	47.535	0.61
C	21	318	4	50.82	47.431	0.73
C	22	334	4	50.853	47.397	0.97
C	23	349	5	50.846	47.408	1.22
C	24	363	4	50.82	47.692	0.97
C	25	381	4	50.285	48.089	1.09
C	26	397	4	50.351	47.964	1.215
C	27	413	4	50.254	48.05	0.97
C	28	424	5	50.178	48.104	1.22
C	29	438	5	50.548	47.576	2.67
C	30	453	4	50.204	46.945	2.67
C	31	468	3	49.957	46.717	2.67
C	32	483	4	50.106	46.79	2.92
C	33	498	T10	50.437	46.825	2.19
C	34	513	T11	50.54	47.017	2.67
C	35	528	T10	50.438	47.023	2.92
C	36	543	T9	50.147	47.017	2.43
C	37	558	T9	49.953	46.923	2.19
C	38	573	T1	49.953	46.626	2.43
C	39	588	T1	49.856	46.825	2.43
C	40	605	2	49.658	46.825	1.94
D	29	438	8	49.838	48.776	0.97
D	30	453	6	49.784	48.425	1.46
D	31	468	6	49.977	48.017	1.22
D	32	483	7	50.076	47.97	1.46
D	33	498	5	49.507	48.585	0.61
E	0	0	3	53.9	48.77	0.49
E	1	13	3	53.16	48.474	0.49
E	2	26	3	53.008	48.561	0.49
E	3	43	3	53.241	47.993	0.49
E	4	58	2	52.94	47.861	0.49
E	5	72	2	53.116	47.761	0.49
E	6	88	2	52.819	47.49	0.73
E	7	103	2	52.629	47.47	0.85
E	8	118	2	52.67	47.492	0.73
E	9	133	3	52.521	47.649	0.12
E	10	148	3	52.551	47.275	0.49
E	11	163	3	52.512	47.416	0.73
E	12	179	3	52.492	47.302	0.73
E	13	193	3	52.644	47.028	0.49
E	14	209	3	52.664	47.258	0.49
E	15	223	3	52.853	47.322	0.49

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
E	16	237	3	52.829	47.427	0.49
E	17	253	3	52.749	47.352	0.49
E	18	269	3	52.474	47.197	0.73
E	19	283	2	52.484	47.237	0.24
E	20	306	3	52.392	47.285	0.36
E	21	318	3	52.18	47.141	0.49
E	22	334	3	52.193	47.077	0.49
E	23	349	4	52.156	47.338	0.24
E	24	363	3	52.26	47.362	0.61
E	25	381	3	52.265	47.199	0.61
E	26	397	3	52.341	47.104	0.729
E	27	413	3	52.294	47.26	0.73
E	28	424	4	52.028	47.634	0.73
E	29	438	4	51.918	47.836	0.97
E	30	453	5	51.684	47.685	0.73
E	31	468	5	51.667	47.477	0.73
E	32	483	5	51.636	47.53	1.22
E	33	498	T8	51.417	47.805	0.73
E	34	513	T8	51.32	47.897	0.73
E	35	528	T8	51.318	47.993	0.73
E	36	543	T7	51.217	47.897	0.73
E	37	558	T7	51.123	47.803	0.85
E	38	573	T3	51.123	47.796	0.97
E	39	588	T3	51.026	47.895	0.97
E	40	605	5	51.108	46.955	0.73
F	40	605	T2	51.028	48.775	1.7
G	1	13	4	54.46	49.184	0.49
G	2	26	4	54.438	49.101	0.49
G	3	43	T7	54.451	49.463	0.73
G	4	58	4	54.48	49.451	0.73
G	5	72	5	54.376	49.081	0.97
G	6	88	4	54.409	48.9	1.22
G	7	103	4	55.549	48.11	0.73
G	8	118	3	56.31	47.632	0.49
G	9	133	2	56.171	47.399	0.12
G	10	148	2	56.031	46.985	0.24
G	11	163	2	56.712	46.416	0.73
G	12	179	2	56.532	46.682	0.49
G	13	193	2	55.154	46.318	0.24
G	14	209	2	55.444	46.638	0.24
G	15	223	2	55.553	46.212	0.24
G	16	237	2	56.409	46.347	0.73
G	17	253	2	56.649	46.392	0.36
G	18	269	2	56.594	46.477	0.24

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
G	19	283	3	56.694	47.027	0.73
G	20	306	2	56.482	46.475	0.24
G	21	318	2	56.27	46.411	0.24
G	22	334	2	56.273	46.297	0.36
G	23	349	2	55.606	45.748	0.24
G	24	363	2	55.86	46.092	0.24
G	25	381	2	55.825	46.219	0.24
G	26	397	2	55.981	46.124	0.243
G	27	413	2	55.874	46.29	0.24
G	28	424	3	55.718	46.524	0.49
G	29	438	3	55.658	46.666	0.49
G	30	453	3	55.534	46.455	0.49
G	31	468	4	55.527	46.447	0.49
G	32	483	3	55.446	46.44	0.49
G	33	498	3	55.167	46.265	0.49
G	34	513	3	55.1	46.527	0.49
G	35	528	3	55.168	46.443	0.49
G	36	543	3	54.867	46.427	0.49
G	37	558	3	54.743	46.493	0.49
G	38	573	3	54.643	46.346	0.49
G	39	588	3	54.596	46.285	0.49
G	40	605	4	54.618	46.335	0.73
H	23	349	3	56.896	46.508	0.24
I	0	0	5	52.56	52.43	0.49
I	1	13	6	52.57	52.414	0.61
I	2	26	6	53.168	52.111	0.49
I	3	43	5	53.131	52.053	0.49
I	4	58	6	52.99	52.021	0.49
I	5	72	9	53.036	51.941	0.49
I	6	88	10	53.119	51.94	0.61
I	7	103	8	52.939	51.85	0.73
I	8	118	9	52.93	51.922	0.49
I	9	133	T7	52.981	51.909	0.36
I	10	148	10	53.071	51.865	0.49
I	11	163	10	52.972	51.946	0.49
I	12	179	10	53.002	51.942	0.73
I	13	193	11	53.084	51.698	0.36
I	14	209	11	52.964	51.938	0.49
I	15	223	11	53.163	51.912	0.49
I	16	237	8	53.059	52.197	0.36
I	17	253	10	53.069	52.082	0.36
I	18	269	12	52.854	52.207	0.49
I	19	283	10	52.874	52.137	0.49
I	20	306	11	52.762	52.195	0.36

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
I	21	318	9	52.61	52.261	0.24
I	22	334	7	53.113	52.417	0.73
I	23	349	9	53.066	52.328	0.97
I	24	363	9	53.72	52.122	0.73
I	25	381	7	53.295	52.089	0.61
I	26	397	T1	53.081	51.714	0.972
I	27	413	7	53.294	51.6	0.97
I	28	424	10	52.518	52.264	2.19
I	29	438	11	52.688	52.156	1.22
I	30	453	13	54.054	51.815	1.22
I	31	468	10	53.777	51.837	1.7
I	32	483	10	53.676	52.01	1.22
I	33	498	7	53.467	52.155	1.46
I	34	513	6	53.59	51.407	0.61
I	35	528	7	53.458	52.083	1.7
I	36	543	7	53.657	51.637	1.46
I	37	558	6	52.253	52.323	1.7
I	38	573	6	52.703	52.046	1.22
I	39	588	5	52.476	51.895	1.22
I	40	605	8	52.638	52.125	1.46
J	29	438	14	52.028	53.426	2.92
J	30	453	16	53.274	53.365	0.97
J	31	468	12	53.777	52.837	0.97
J	32	483	12	53.976	53.22	0.97
J	33	498	9	53.507	53.195	0.97
J	34	513	8	53.45	53.457	0.85
J	35	528	9	53.768	53.273	1.22
J	36	543	11	53.597	53.137	1.22
J	37	558	8	53.673	53.053	0.97
J	38	573	8	53.853	52.796	1.46
J	39	588	6	53.706	52.345	0.97
J	40	605	9	53.948	52.865	0.73
K	2	26	7	51.828	52.731	0.49
K	3	43	6	51.981	52.743	0.24
K	4	58	7	51.93	52.671	0.36
K	5	72	10	51.906	52.561	0.36
K	6	88	11	51.989	52.65	0.49
K	7	103	9	51.879	52.57	0.49
K	8	118	10	51.82	52.682	0.49
K	9	133	9	51.901	52.599	0.12
K	10	148	11	51.881	52.615	0.24
K	11	163	11	51.822	52.706	0.24
K	12	179	11	51.722	52.702	0.24
K	13	193	12	51.884	52.628	0.24

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
K	14	209	13	49.994	53.728	0.36
K	15	223	12	48.403	56.212	0.49
K	16	237	9	46.949	55.647	0.73
K	17	253	11	46.709	55.572	0.73
K	18	269	14	46.044	55.367	0.49
K	19	283	11	45.954	55.227	0.49
K	20	306	14	45.772	55.045	0.36
K	21	318	10	45.44	55.001	0.49
K	22	334	8	45.423	54.727	0.49
K	23	349	10	45.406	54.828	0.73
K	24	363	10	45.62	55.092	0.61
K	25	381	8	45.315	55.149	0.61
K	26	397	9	45.371	55.044	0.729
K	27	413	9	46.564	54.46	1.22
K	28	424	12	46.138	54.144	0.97
K	29	438	15	45.948	54.286	0.97
K	30	453	15	46.044	53.935	1.22
K	31	468	14	45.137	54.567	0.73
K	32	483	15	44.796	54.63	0.49
K	33	498	12	44.557	54.625	0.49
K	34	513	10	44.49	54.967	0.49
K	35	528	10	44.468	54.943	0.49
K	36	543	12	44.337	55.007	0.49
K	37	558	9	44.253	54.953	0.49
K	38	573	9	44.163	54.866	0.49
K	39	588	8	44.116	54.805	0.49
K	40	605	11	44.148	54.895	0.73
L	31	468	13	46.427	53.507	1.46
L	32	483	13	46.596	54.56	2.19
L	33	498	11	46.597	53.985	2.19
L	34	513	9	46.36	54.307	2.55
L	35	528	T6	50.148	51.813	2.43
L	36	543	5	49.427	49.917	4.62
L	37	558	4	49.063	47.903	2.92
M	0	0	6	56.37	53.74	1.46
M	1	13	7	56.79	53.464	0.85
M	2	26	8	56.658	53.471	1.46
M	3	43	8	55.901	53.563	1.7
M	4	58	9	56.99	53.571	1.46
N	0	0	7	53.5	53.76	0.49
N	1	13	8	53.57	53.754	0.61
N	2	26	9	53.608	53.711	0.49
N	3	43	7	53.571	53.673	0.49
N	4	58	8	53.46	53.581	0.36

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
N	5	72	11	53.446	53.491	0.36
N	6	88	12	53.499	53.51	0.49
N	7	103	10	53.369	53.41	0.61
N	8	118	11	53.42	53.492	0.49
N	9	133	10	53.431	53.419	0.12
N	10	148	12	53.511	53.355	0.24
N	11	163	12	53.452	53.456	0.24
N	12	179	12	53.602	53.512	0.24
N	13	193	13	53.714	52.968	0.36
N	14	209	12	51.344	53.208	2.92
O	0	0	T1	50.34	50.64	0.73
O	1	13	T1	50.24	50.634	0.97
O	2	26	T1	50.338	50.631	0.97
O	3	43	T1	50.341	50.533	0.73
O	4	58	T1	50.44	50.441	0.97
O	5	72	7	50.756	50.331	0.73
O	6	88	8	51.009	50.53	0.73
O	7	103	6	50.889	50.59	0.85
O	8	118	7	50.95	50.682	0.73
O	9	133	8	50.931	50.669	0.49
O	10	148	9	51.041	50.675	0.73
O	11	163	8	50.832	50.576	0.73
O	12	179	8	51.012	50.602	0.73
O	13	193	9	51.004	50.098	0.49
O	14	209	8	51.004	50.318	0.49
O	15	223	T1	51.413	50.532	0.73
O	16	237	T7	51.319	50.637	0.49
O	17	253	T4	51.319	50.632	0.73
O	18	269	T4	51.024	50.637	0.73
O	19	283	T4	51.024	50.637	0.49
O	20	306	T4	51.122	50.535	0.49
O	21	318	T4	51.22	50.831	0.49
O	22	334	T4	51.123	50.637	0.49
O	23	349	T4	51.126	50.728	0.49
O	24	363	T5	51.42	50.442	0.24
O	25	381	T4	51.315	50.829	0.36
O	26	397	T5	51.421	50.634	0.486
O	27	413	T5	51.224	50.73	0.24
O	28	424	T3	51.218	50.834	0.49
O	29	438	T1	51.318	50.736	0.73
O	30	453	T2	51.224	50.435	0.49
O	31	468	T2	51.217	49.947	0.73
O	32	483	T2	51.126	49.76	0.73
O	33	498	T2	51.027	49.655	0.49

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
O	34	513	T6	50.83	50.047	0.49
O	35	528	T7	50.638	50.343	1.22
O	36	543	T6	50.637	50.337	1.46
O	37	558	T6	50.733	50.143	0.97
O	38	573	T5	50.833	49.656	0.49
O	39	588	T4	50.736	49.755	0.73
O	40	605	T1	50.828	49.755	0.97
P	5	72	8	49.566	50.571	0.49
P	6	88	9	49.569	50.69	0.73
P	7	103	7	49.569	50.63	0.85
P	8	118	8	49.39	50.572	0.73
P	9	133	7	49.461	50.409	0.12
P	10	148	8	49.291	50.395	0.49
P	11	163	9	48.802	50.586	0.97
P	12	179	9	48.852	50.662	0.97
P	13	193	10	48.924	50.328	0.97
P	14	209	10	48.804	50.418	0.97
P	15	223	10	49.073	50.342	0.85
P	16	237	7	49.039	50.477	0.97
P	17	253	9	48.999	50.442	0.85
P	18	269	10	48.534	50.597	1.09
P	19	283	9	48.414	50.527	1.22
P	20	306	10	48.042	50.555	1.09
Q	0	0	T2	51.61	50.15	0.49
Q	1	13	T2	51.61	50.144	0.49
Q	2	26	T2	51.608	50.151	0.49
Q	3	43	T2	51.611	50.143	0.49
Q	4	58	T2	51.61	50.051	0.49
Q	5	72	T1	51.516	49.951	0.49
Q	6	88	T1	51.609	49.95	0.49
Q	7	103	T1	51.609	50.15	0.73
Q	8	118	T1	51.51	50.142	0.73
Q	9	133	T1	51.511	50.149	0.24
Q	10	148	6	51.201	49.535	0.24
Q	11	163	6	51.112	49.656	0.24
Q	12	179	6	51.152	49.692	0.24
Q	13	193	6	51.184	49.258	0.24
Q	14	209	6	51.124	49.368	0.24
Q	15	223	6	51.303	49.352	0.24
Q	16	237	6	51.289	49.467	0.24
Q	17	253	7	51.209	49.422	0.24
Q	18	269	8	51.014	49.417	0.24
Q	19	283	7	51.014	49.427	0.24
Q	20	306	T6	50.342	48.875	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
Q	21	318	T6	50.64	50.051	1.22
Q	22	334	T6	51.123	50.827	4.86
Q	23	349	T6	51.416	50.828	4.86
Q	24	363	T6	51.22	51.022	5.1
Q	25	381	T6	50.925	51.419	4.98
Q	26	397	T7	50.051	50.044	4.86
Q	27	413	T6	51.224	49.66	4.62
Q	28	424	T8	51.218	50.044	6.56
Q	29	438	T6	51.808	48.976	6.08
Q	30	453	T6	51.414	49.265	6.08
Q	31	468	T6	51.617	48.577	6.32
Q	32	483	T6	51.906	48.19	6.08
Q	33	498	T6	52.197	48.095	4.62
Q	34	513	T9	51.91	48.387	4.98
R	10	148	T1	51.901	50.835	0.97
R	11	163	T1	52.002	50.436	0.49
S	0	0	T3	52.49	50.93	2.07
S	1	13	T6	52.59	51.024	2.43
S	2	26	T5	52.398	50.931	2.19
S	3	43	T3	52.301	50.923	2.19
S	4	58	T6	52.1	51.021	2.19
S	5	72	T5	52.106	50.931	1.94
S	6	88	T5	51.609	51.22	2.19
S	7	103	T5	51.519	51.42	2.31
S	8	118	T5	51.61	51.612	2.19
S	9	133	T6	51.811	51.809	1.46
S	10	148	T5	51.711	51.805	1.7
S	11	163	T6	51.712	51.616	1.46
S	12	179	T5	51.902	51.512	1.94
S	13	193	T4	51.804	51.218	1.22
S	14	209	T1	51.614	51.418	1.22
S	15	223	T2	51.803	51.512	0.97
S	16	237	T6	51.709	51.707	1.09
S	17	253	T5	51.709	51.712	0.97
S	18	269	T5	51.614	51.707	1.22
S	19	283	T5	51.614	51.707	1.22
S	20	306	T5	51.902	51.615	1.09
S	21	318	T5	51.71	51.811	0.97
S	22	334	T5	51.813	51.707	0.97
S	23	349	T5	51.706	51.608	1.22
S	24	363	T4	51.32	52.102	1.22
S	25	381	T5	51.125	52.299	1.09
S	26	397	T3	51.611	51.804	1.215
S	27	413	8	51.024	52.36	0.49

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
S	28	424	T4	50.828	52.394	0.73
S	29	438	12	50.838	52.376	0.97
S	30	453	14	50.734	52.255	0.73
S	31	468	11	50.677	52.187	0.73
S	32	483	11	50.576	52.19	0.73
S	33	498	8	50.437	52.185	0.73
S	34	513	7	50.33	52.587	0.73
S	35	528	8	50.368	52.603	0.73
S	36	543	9	50.247	52.687	0.73
S	37	558	7	50.263	52.723	0.73
S	38	573	7	50.113	52.596	0.97
S	39	588	7	50.046	52.575	0.97
S	40	605	10	50.058	52.525	1.22
T	27	413	T1	51.714	51.61	1.94
T	28	424	T5	51.708	51.904	2.19
T	29	438	T4	51.908	52.006	2.19
T	30	453	T4	51.714	51.805	2.19
T	31	468	T4	51.907	51.707	2.19
T	32	483	T4	51.806	51.52	1.94
T	33	498	T4	51.517	51.225	1.94
T	34	513	T4	51.42	51.617	2.19
T	35	528	T4	51.518	51.813	2.19
T	36	543	T4	51.417	51.807	2.19
T	37	558	T4	51.323	51.613	2.19
T	38	573	T7	50.933	51.516	2.43
T	39	588	T8	51.126	51.125	2.19
T	40	605	T6	51.518	51.125	2.43
U	6	88	T6	53.079	51.03	1.7
U	7	103	T6	52.789	50.83	1.94
U	8	118	T6	52.98	50.832	1.94
U	9	133	T4	52.881	50.539	1.7
U	10	148	T6	52.881	50.835	1.7
U	11	163	T4	52.882	50.736	1.7
U	12	179	T3	52.882	50.732	1.7
U	13	193	T2	52.784	50.338	0.24
U	14	209	T3	52.884	50.538	1.7
U	15	223	T4	52.883	50.242	1.46
U	16	237	T4	52.399	50.247	1.22
U	17	253	T2	52.589	50.242	1.22
U	18	269	T2	52.594	50.147	1.46
U	19	283	T2	52.594	50.247	1.22
U	20	306	T2	52.392	50.245	1.34
U	21	318	T2	52.3	50.341	4.37
U	22	334	T2	52.293	50.147	1.22

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
U	23	349	T2	52.396	50.248	1.46
U	24	363	T2	52.49	50.342	0.97
U	25	381	T2	52.005	50.339	1.82
U	26	397	T6	52.001	50.244	1.701
U	27	413	T4	51.714	50.34	1.7
U	28	424	T7	51.028	51.414	1.94
U	29	438	T5	51.318	51.126	2.19
U	30	453	T5	51.224	51.225	2.19
U	31	468	T5	51.517	50.827	2.43
U	32	483	T5	51.416	50.54	2.43
U	33	498	T5	51.517	50.145	2.43
U	34	513	T5	51.42	50.437	2.43
U	35	528	T5	51.518	50.733	2.67
U	36	543	T5	51.417	50.737	2.67
U	37	558	T5	51.123	50.733	2.43
U	38	573	T6	50.443	50.736	1.94
U	39	588	T7	50.146	50.835	1.22
U	40	605	T7	50.438	50.925	1.7
V	28	424	T6	51.908	50.044	1.94
W	0	0	T4	53.57	49.95	1.46
W	1	13	T3	53.76	50.044	1.46
W	2	26	T3	53.668	50.051	1.46
W	3	43	T6	53.571	49.853	1.46
W	4	58	T3	53.18	50.051	1.46
W	5	72	T2	53.276	49.951	1.46
W	6	88	T2	53.279	49.75	1.7
W	7	103	T2	53.469	49.66	1.46
W	8	118	5	53.79	49.112	0.49
W	9	133	5	53.801	48.969	0.12
W	10	148	5	53.571	48.835	0.49
W	11	163	5	53.352	48.896	0.49
W	12	179	5	53.592	48.852	0.49
W	13	193	5	53.664	48.348	0.24
W	14	209	5	53.794	48.608	0.24
W	15	223	5	54.033	48.622	0.36
W	16	237	T1	53.959	48.777	0.24
W	17	253	5	53.719	48.582	0.36
W	18	269	7	53.474	48.697	0.49
W	19	283	6	53.494	48.647	0.49
W	20	306	5	53.512	48.475	0.24
W	21	318	5	53.39	48.441	0.24
W	22	334	5	53.413	48.227	0.24
W	23	349	7	53.426	48.298	0.24
W	24	363	5	53.62	48.482	0.24

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
W	25	381	5	53.475	48.639	0.24
W	26	397	5	53.491	48.494	0.3645
W	27	413	5	53.374	48.58	0.24
W	28	424	6	53.068	48.764	0.49
W	29	438	7	53.298	48.666	0.49
W	30	453	7	53.224	48.485	0.73
W	31	468	7	53.257	48.447	0.73
W	32	483	T7	53.076	48.29	0.73
W	33	498	T7	52.297	48.485	0.49
W	34	513	T7	52.1	48.777	0.49
W	35	528	5	52.148	48.743	0.49
W	36	543	4	52.037	48.707	0.49
W	37	558	T8	51.713	48.873	0.49
W	38	573	T4	51.713	48.776	0.49
W	39	588	T5	51.616	48.775	0.49
W	40	605	T3	51.708	48.775	0.73
X	33	498	4	53.947	47.985	0.49
X	34	513	4	53.7	48.257	0.49
X	35	528	4	53.578	48.263	0.73
Y	8	118	T2	53.57	50.342	2.43
Y	9	133	T2	53.571	49.849	1.94
Y	10	148	T2	53.571	50.045	2.19
Y	11	163	T2	53.572	50.146	1.94
Y	12	179	T1	53.472	50.242	2.67
Y	13	193	7	55.064	49.758	3.65
Y	14	209	9	54.844	50.438	3.4
Y	15	223	7	55.253	49.862	3.16
Y	16	237	T2	54.839	49.367	3.65
Y	17	253	6	54.649	48.962	3.89
Y	18	269	5	55.054	48.427	3.4
Y	19	283	5	54.954	48.467	3.4
Z	0	0	T5	53.67	50.73	0.61
Z	1	13	T4	53.67	50.734	0.61
Z	2	26	T4	53.768	50.541	0.61
Z	3	43	T5	53.861	50.533	0.24
Z	4	58	T4	53.76	50.441	0.24
Z	5	72	T3	53.666	50.541	0.24
Z	6	88	T3	53.759	50.54	0.49
Z	7	103	T3	53.759	50.24	0.85
Z	8	118	T3	53.67	50.442	0.61
Z	9	133	T3	53.661	50.339	0.24
Z	10	148	T3	53.761	50.245	0.24
Z	11	163	T3	53.662	50.346	0.24
Z	12	179	T2	53.762	50.442	0.49

[illegible]

MOVEMENT DATA FOR NOV21-aoi#3 DATA SET

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
A	0	0	1	93.35	78.88	0.49
A	1	13	1	93.39	78.874	0.73
A	2	26	1	93.418	78.911	0.73
B	0	0	2	95.03	79.47	0.49
B	1	13	2	95.09	79.464	0.73
B	2	26	2	95.138	79.481	0.73
B	3	43	1	95.141	79.513	0.73
B	4	58	1	95.02	79.591	0.73
B	5	72	1	95.006	79.901	0.49
B	6	88	1	95.109	79.72	0.73
B	7	103	1	95.239	79.55	0.73
B	8	118	1	95.26	79.602	0.73
B	9	133	1	95.241	79.579	0.97
B	10	148	1	95.331	79.365	0.73
B	11	163	1	95.202	79.486	0.97
B	12	179	1	95.312	79.612	0.97
B	13	193	1	95.274	79.058	0.73
B	14	209	1	95.264	79.498	0.73
B	15	223	1	95.333	79.502	0.85
B	16	237	1	95.409	79.647	0.73
B	17	253	1	95.069	78.952	0.73
B	18	269	1	94.524	78.947	0.97
B	19	283	1	94.544	78.787	0.97
B	20	306	1	94.222	78.715	0.49
B	21	318	1	94.12	78.731	0.49
B	22	334	1	94.043	78.467	0.49
B	23	349	1	94.076	78.478	0.97
B	24	363	1	94.18	78.752	0.97
B	25	381	1	94.045	78.699	0.97
B	26	397	1	94.061	78.544	0.97
B	27	413	1	93.884	78.79	0.97
B	28	424	1	93.668	78.714	0.97
B	29	438	1	93.748	78.646	0.97
B	30	453	1	93.624	78.595	0.97
B	31	468	2	93.717	78.367	0.73
B	32	483	2	94.196	77.91	0.97
B	33	498	1	94.107	77.715	0.97
B	34	513	1	94.01	78.007	0.97
B	35	528	1	93.988	77.943	0.97
B	36	543	2	93.867	77.997	1.09
B	37	558	1	93.823	77.943	0.97

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
B	38	573	1	93.743	77.826	0.97
B	39	588	1	93.646	77.825	0.97
B	40	605	1	93.688	77.865	1.09
C	32	483	3	93.016	78.75	0.97
C	33	498	2	92.847	78.305	1.46
D	17	253	3	96.149	80.102	0.73
D	18	269	3	95.994	80.447	0.97
D	19	283	3	95.934	80.397	0.97
D	20	306	3	96.012	80.475	0.49
D	21	318	3	95.85	80.551	0.49
D	22	334	3	95.913	80.337	0.49
D	23	349	3	95.896	80.418	0.97
D	24	363	3	95.98	80.592	0.97
D	25	381	4	95.715	80.719	0.49
D	26	397	3	95.691	80.524	0.97
D	27	413	3	95.574	80.68	0.97
D	28	424	3	95.178	80.794	0.97
D	29	438	4	95.328	80.766	0.97
D	30	453	4	95.154	80.635	0.97
D	31	468	8	95.187	80.577	0.73
D	32	483	5	94.996	80.58	0.97
D	33	498	5	94.827	80.525	0.97
D	34	513	4	94.76	80.897	0.97
D	35	528	4	94.728	80.943	0.97
D	36	543	4	94.707	80.987	1.09
D	37	558	4	94.563	80.943	0.97
D	38	573	T3	94.523	80.836	0.97
D	39	588	5	94.536	80.845	0.97
D	40	605	5	93.678	80.535	1.09
E	40	605	6	95.168	81.055	1.09
F	0	0	3	94.52	80.19	0.49
F	1	13	3	94.48	80.224	0.85
F	2	26	3	94.218	79.931	1.94
F	3	43	2	94.251	80.063	2.07
F	4	58	3	94.81	80.551	1.34
G	0	0	4	96.32	80.21	0.49
G	1	13	4	96.39	80.204	0.73
G	2	26	4	96.348	80.201	0.73
G	3	43	3	96.311	80.163	1.22
G	4	58	2	96.24	80.011	0.97
G	5	72	2	96.226	80.051	0.73
G	6	88	2	96.379	79.94	1.22
G	7	103	2	96.419	80.15	0.73
G	8	118	2	96.41	80.202	0.97

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
G	9	133	2	96.421	80.149	1.22
G	10	148	2	96.441	80.115	0.97
G	11	163	2	96.342	80.116	1.46
G	12	179	2	96.342	80.372	1.46
G	13	193	2	96.344	79.758	1.58
G	14	209	2	96.364	80.138	1.46
G	15	223	2	96.573	80.122	1.46
G	16	237	2	96.499	80.377	1.22
G	17	253	2	96.989	79.812	0.97
G	18	269	2	96.834	79.987	1.22
G	19	283	2	96.844	79.977	1.34
G	20	306	2	96.662	79.965	0.85
G	21	318	2	96.61	79.931	0.73
G	22	334	2	96.683	79.697	0.73
G	23	349	2	96.716	79.808	1.22
G	24	363	2	96.85	79.922	1.34
G	25	381	3	96.655	80.129	1.46
G	26	397	2	96.651	79.974	1.34
G	27	413	2	96.554	80.04	1.46
G	28	424	2	96.278	80.174	1.46
G	29	438	2	96.338	80.126	1.46
G	30	453	2	96.234	80.045	1.46
G	31	468	6	96.217	79.847	1.22
G	32	483	4	96.056	79.9	1.46
G	33	498	4	95.827	79.825	1.46
G	34	513	3	95.74	80.167	1.7
G	35	528	3	95.728	80.153	1.7
G	36	543	3	95.667	80.137	1.7
G	37	558	3	95.583	80.153	1.7
G	38	573	T2	95.303	80.256	1.7
G	39	588	4	95.546	79.635	0.97
G	40	605	3	95.378	79.745	1.22
H	39	588	T1	94.916	81.035	2.43
H	40	605	T1	95.208	81.035	2.43
I	17	253	4	95.579	80.382	1.94
J	0	0	5	93.64	80.8	1.22
J	1	13	8	93.97	83.224	1.22
J	2	26	5	92.798	81.171	2.19
J	3	43	7	92.651	82.383	1.94
K	0	0	6	96	81.23	0.49
K	1	13	5	96.02	81.184	0.73
K	2	26	6	96.058	81.271	0.73
K	3	43	4	96.021	81.233	0.73
K	4	58	4	95.98	81.231	0.73

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
K	5	72	3	95.896	80.931	0.49
K	6	88	4	96.099	80.79	0.73
K	7	103	3	96.989	81.11	0.97
L	0	0	7	94.02	81.75	1.22
L	1	13	6	93.83	81.634	1.22
L	2	26	7	94.448	81.281	1.34
L	3	43	5	94.421	81.323	1.82
L	4	58	5	94.47	81.751	1.34
L	5	72	4	93.916	81.051	1.22
L	6	88	3	94.179	80.95	1.22
L	7	103	4	94.189	81.77	0.97
L	8	118	5	94.36	81.402	-1.22
L	9	133	3	94.371	81.699	1.46
L	10	148	3	94.301	81.865	1.46
L	11	163	3	94.242	82.126	1.7
L	12	179	3	94.662	82.032	1.46
L	13	193	3	94.964	82.038	1.46
L	14	209	3	95.014	82.248	1.46
L	15	223	3	94.753	81.562	1.7
L	16	237	3	94.309	81.257	1.46
L	17	253	5	94.449	81.102	1.7
L	18	269	4	93.904	80.867	1.7
L	19	283	4	94.114	81.017	1.94
L	20	306	4	94.692	82.275	0.73
L	21	318	4	94.63	82.341	0.49
L	22	334	5	95.083	81.927	0.49
L	23	349	4	95.326	81.988	0.97
L	24	363	4	95.25	82.092	0.97
L	25	381	5	95.245	82.129	0.49
L	26	397	4	95.201	81.874	0.97
L	27	413	4	94.994	82.11	0.97
L	28	424	4	94.638	82.164	0.97
L	29	438	3	94.108	81.026	1.46
L	30	453	3	93.614	80.165	2.92
L	31	468	3	90.437	78.757	3.4
L	32	483	1	94.426	76.43	2.43
L	33	498	3	95.127	79.025	2.92
L	34	513	2	95.8	78.647	2.19
L	35	528	2	95.398	79.323	2.19
L	36	543	1	95.657	78.587	2.31
L	37	558	2	95.663	78.613	2.43
L	38	573	T1	95.403	79.376	3.4
L	39	588	3	95.166	78.755	2.43
L	40	605	2	95.838	78.965	3.4

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z
M	40	605	4	97.388	80.035	2.31
N	15	223	4	95.493	82.942	0.85
N	16	237	4	95.249	82.977	0.97
N	17	253	6	95.719	83.162	0.97
N	18	269	5	95.414	83.377	1.22
N	19	283	6	95.244	83.367	1.34
N	20	306	5	95.582	83.225	0.73
N	21	318	5	95.37	83.501	0.73
N	22	334	6	95.563	83.377	0.85
N	23	349	5	96.146	83.368	0.97
N	24	363	5	96.16	83.562	0.97
N	25	381	6	96.245	83.849	0.49
N	26	397	5	96.491	83.794	0.97
N	27	413	5	96.174	84.2	0.97
N	28	424	5	95.478	84.174	1.7
N	29	438	5	95.478	84.206	1.34
N	30	453	5	95.364	84.085	1.46
N	31	468	9	95.477	84.007	1.22
N	32	483	6	95.536	84.02	1.46
N	33	498	6	95.257	84.045	1.46
N	34	513	5	95.19	84.457	1.58
N	35	528	5	95.158	84.463	1.7
N	36	543	5	95.167	84.407	1.58
N	37	558	5	95.103	84.383	1.46
N	38	573	3	95.093	84.256	1.46
N	39	588	6	95.046	84.255	1.46
N	40	605	7	95.008	84.415	1.7
O	0	0	8	91.69	81.98	0.97
O	1	13	7	92.05	82.324	0.97
O	2	26	8	91.868	81.921	1.22
O	3	43	6	91.891	81.883	1.7
O	4	58	6	91.66	82.381	1.22
O	5	72	5	91.656	82.441	0.97
O	6	88	5	91.679	82.11	1.58
O	7	103	6	93.239	82.76	1.7
O	8	118	4	91.42	80.782	0.97
O	9	133	4	91.501	84.989	1.46
O	10	148	4	92.091	85.195	2.19
O	11	163	4	92.472	85.216	2.43
O	12	179	4	92.322	86.912	2.19
O	13	193	4	93.354	84.658	1.94
O	14	209	4	92.594	85.848	2.19
O	15	223	5	93.423	86.222	2.43
O	16	237	5	91.779	85.677	2.19

[illegible]

MOVEMENT DATA FOR OCT21 DATA SET

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
A	98102204	0	1	43.37	0.35	0.5
A	98102205	22	1	43.407	1.341	0.5
A	98102206	37	1	43.488	0.918	0
A	98102207	50	1	43.444	1.297	0
A	98102208	66	1	43.626	1.349	0.25
A	98102209	81	1	43.473	1.248	0
A	98102210	97	1	43.767	1.355	0.25
A	98102211	112	1	43.496	1.342	0.5
A	98102212	126	1	43.578	1.571	0.5
A	98102213	142	1	43.55	1.589	0.5
A	98102214	157	1	43.529	1.66	0.5
A	98102215	172	1	43.53	1.641	0.5
A	98102216	189	1	43.555	1.447	0.25
A	98102217	204	1	43.711	1.42	0.25
AA	98102204	0	15	13.25	52.7	0.5
AA	98102205	22	14	13.107	52.981	0.75
AA	98102206	37	14	13.228	52.498	0.5
AA	98102207	50	14	13.274	52.647	0.5
AA	98102208	66	16	13.346	52.829	0.5
AA	98102209	81	17	13.283	52.718	0.5
AA	98102210	97	15	13.357	52.805	0.75
AA	98102211	112	13	13.346	52.542	1.25
AA	98102212	126	13	13.738	52.581	0.75
AA	98102213	142	13	13.68	52.359	1
AA	98102214	157	13	13.539	52.38	1.25
AA	98102215	172	13	13.44	52.481	1
AA	98102216	189	12	13.455	52.387	1.25
AA	98102217	204	12	13.521	52.46	1.5
AB	98102204	0	16	65.89	52.93	0.25
AB	98102205	22	15	65.397	53.471	0.5
AB	98102206	37	15	65.368	53.118	0.5
AB	98102207	50	16	64.894	53.277	0.5
AB	98102208	66	17	65.226	53.029	0.5
AB	98102209	81	16	65.433	52.398	0.25
AB	98102210	97	16	65.207	52.935	0.5
AB	98102211	112	15	65.516	52.612	0.25
AB	98102212	126	15	65.548	52.801	0.25
AB	98102213	142	15	65.29	53.059	0.5
AB	98102214	157	15	65.409	53.17	0.25
AB	98102215	172	15	65.4	53.101	0.25
AB	98102216	189	14	65.115	53.067	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AB	98102217	204	14	65.371	52.86	0.5
AB	98102218	220	11	65.604	53.009	0.5
AB	98102219	235	11	65.528	53.174	0.5
AB	98102220	251	11	64.573	53.075	0
AB	98102221	267	12	64.263	53.059	0.5
AB	98102222	285	13	63.923	52.582	1
AB	98102223	295	13	63.962	52.452	0.75
AB	98102224	313	11	64.09	52.459	0.75
AB	98102225	330	10	63.918	52.582	1
AB	98102226	346	10	64.029	52.539	0.75
AB	98102227	361	10	63.979	52.618	0.5
AB	98102228	376	10	63.938	52.7	0.75
AB	98102229	391	11	63.961	52.84	0.75
AB	98102230	407	13	63.923	53.298	0.5
AB	98102231	420	13	64.068	53.248	0.5
AB	98102232	436	14	63.925	53.209	0.5
AB	98102233	451	12	63.955	53.302	0.5
AB	98102234	466	12	63.895	53.252	0.5
AB	98102235	482	12	63.505	53.402	0.5
AB	98102236	495	13	63.375	53.542	0.5
AB	98102237	511	12	63.525	53.472	0.5
AB	98102238	525	11	63.725	53.412	0.5
AB	98102239	542	12	63.695	53.582	0.5
AB	98102240	555	12	63.945	53.552	0.5
AB	98102241	571	12	64.095	53.542	0.25
AB	98102242	587	12	64.294	53.723	0.5
AB	98102243	601	12	64.449	53.832	0.5
AB	98102244	616	12	64.531	53.863	0.75
AB	98102245	632	12	64.626	54.002	0.5
AB	98102246	647	14	64.482	53.851	0.75
AB	98102247	661	13	64.519	54.662	0.5
AC	98102220	251	10	66.103	52.855	0.5
AC	98102221	267	13	65.923	53.029	0.75
AC	98102222	285	14	65.763	53.092	0.75
AC	98102223	295	14	65.832	53.272	1
AC	98102224	313	13	65.78	53.419	0.75
AC	98102225	330	12	66.128	53.092	0.5
AD	98102204	0	17	61.14	53.95	0
AD	98102205	22	16	62.447	54.911	0.5
AD	98102206	37	16	62.938	54.708	0.5
AD	98102207	50	17	62.774	54.707	0.75
AD	98102208	66	19	62.796	55.239	0.5
AD	98102209	81	19	62.763	55.138	0.5
AD	98102210	97	18	62.797	55.165	0.5

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AD	98102211	112	18	62.716	55.152	0.5
AD	98102212	126	17	62.838	55.341	0.75
AD	98102213	142	17	62.76	55.389	0.75
AD	98102214	157	17	62.689	55.27	1
AD	98102215	172	17	62.6	55.261	1
AD	98102216	189	16	62.535	55.237	1
AD	98102217	204	18	63.411	55.68	1.5
AD	98102218	220	15	63.664	55.619	2
AD	98102219	235	15	63.608	55.814	2
AD	98102220	251	15	63.843	55.295	1.75
AD	98102221	267	17	63.693	55.619	2.25
AD	98102222	285	20	62.903	56.192	2.75
AD	98102223	295	17	63.532	54.842	2
AD	98102224	313	18	63.41	56.089	3
AD	98102225	330	18	63.338	56.162	2.5
AD	98102226	346	17	63.369	56.839	3.25
AD	98102227	361	18	63.169	57.018	3.5
AD	98102228	376	17	63.488	56.91	3.75
AD	98102229	391	17	63.491	56.95	3.5
AD	98102230	407	18	63.993	57.378	3.5
AD	98102231	420	19	64.428	57.228	3.75
AD	98102232	436	19	64.565	57.119	3.75
AD	98102233	451	18	64.965	57.582	3.5
AD	98102234	466	T1	64.315	56.792	3.5
AD	98102235	482	17	64.375	57.092	3.5
AD	98102236	495	17	64.665	57.332	3.25
AD	98102237	511	16	64.245	57.502	3
AD	98102238	525	17	64.015	57.482	3
AD	98102239	542	16	64.215	57.662	3.25
AD	98102240	555	16	64.085	57.662	3.5
AD	98102241	571	16	63.965	57.632	3.75
AD	98102242	587	16	64.094	57.693	4
AD	98102243	601	17	64.109	58.072	5
AD	98102244	616	17	64.091	58.013	4.25
AD	98102245	632	18	63.936	58.402	4.5
AD	98102246	647	T3	64.122	56.691	4
AD	98102247	661	T3	64.419	57.482	4.5
AE	98102246	647	T5	63.832	57.871	5
AE	98102247	661	T4	63.829	58.752	5
AF	98102217	204	16	62.711	54.88	0.25
AF	98102218	220	13	63.064	54.909	0.5
AF	98102219	235	13	62.858	55.274	1
AF	98102220	251	13	62.903	55.175	0.5
AF	98102221	267	15	62.783	55.239	0.75

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AF	98102222	285	17	62.793	55.292	1
AF	98102223	295	18	62.882	55.362	1.25
AF	98102224	313	16	63.69	54.619	0.75
AF	98102225	330	15	63.628	54.812	0.75
AF	98102226	346	14	63.699	54.859	1
AF	98102227	361	14	63.269	54.668	0.5
AF	98102228	376	13	63.058	55.39	0.5
AF	98102229	391	8	62.331	52.19	0
AF	98102230	407	10	61.933	52.708	2.25
AF	98102231	420	9	61.778	52.378	2.75
AF	98102232	436	12	61.575	52.879	3.25
AF	98102233	451	11	61.505	52.832	3.5
AF	98102234	466	9	61.465	52.692	3.5
AF	98102235	482	8	61.585	52.522	3.5
AF	98102236	495	9	61.675	52.862	4
AJ	98102204	0	18	68.63	53.91	0
AK	98102204	0	19	60.64	55.37	0
AK	98102205	22	17	60.467	55.521	0.25
AK	98102206	37	17	60.548	55.038	0
AK	98102207	50	18	60.484	55.407	0
AK	98102208	66	20	60.556	55.379	0
AK	98102209	81	21	60.673	55.228	0
AK	98102210	97	19	60.797	55.215	0
AK	98102211	112	19	60.676	55.182	0
AK	98102212	126	18	60.788	55.321	0
AK	98102213	142	18	60.7	55.489	0
AK	98102214	157	18	60.599	55.39	0
AK	98102215	172	18	60.54	55.261	0
AK	98102216	189	17	60.665	55.327	0
AK	98102217	204	17	60.971	55.29	0
AK	98102218	220	14	61.144	55.319	0
AK	98102219	235	14	61.118	55.554	0
AK	98102220	251	14	61.173	55.495	0
AK	98102221	267	16	61.113	55.419	0
AK	98102222	285	16	61.193	55.172	0
AK	98102223	295	16	61.222	55.242	0
AK	98102224	313	15	61.25	55.229	0
AK	98102225	330	14	61.628	54.602	0
AK	98102226	346	13	61.569	54.629	0
AK	98102227	361	15	61.709	54.888	0.25
AK	98102228	376	14	61.578	55.02	0.5
AK	98102229	391	14	61.411	55.09	0.5
AK	98102230	407	15	61.513	55.438	0.5
AK	98102231	420	16	61.658	55.528	0.5

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AK	98102232	436	16	61.485	55.499	0.75
AK	98102233	451	15	61.495	55.512	0.5
AK	98102234	466	15	61.365	55.662	0.5
AK	98102235	482	15	61.375	56.002	0.5
AK	98102236	495	15	61.195	56.172	0.25
AK	98102237	511	14	61.185	56.352	0.25
AK	98102238	525	14	61.985	55.422	0.75
AK	98102239	542	14	61.765	56.352	1
AK	98102240	555	14	61.805	56.252	1
AK	98102241	571	14	61.665	56.142	1
AK	98102242	587	14	61.854	56.303	1.25
AK	98102243	601	14	62.059	56.692	2.25
AK	98102244	616	14	63.711	55.913	1.5
AK	98102245	632	15	62.016	56.592	1.5
AK	98102246	647	18	61.272	56.861	1.5
AK	98102247	661	16	61.719	57.572	1
AL	98102246	647	17	62.752	56.231	1.5
AM	98102225	330	16	60.478	55.942	0
AM	98102226	346	15	60.599	55.969	0
AM	98102227	361	16	60.579	56.098	0
AM	98102228	376	15	60.658	56.14	0
AM	98102229	391	15	60.621	56.27	0
AM	98102230	407	17	60.623	56.678	0
AM	98102231	420	18	60.888	56.658	0
AM	98102232	436	18	60.725	56.599	0
AM	98102233	451	16	60.725	56.652	0
AM	98102234	466	17	60.615	56.782	0
AN	98102204	0	20	63.17	55.92	1
AO	98102204	0	21	61.7	55.96	1
AO	98102205	22	18	62.727	56.081	1.5
AO	98102206	37	18	64.098	55.968	1.25
AO	98102207	50	19	63.394	56.497	1.25
AO	98102208	66	18	64.496	55.129	0
AO	98102209	81	20	64.313	54.978	0
AO	98102210	97	17	64.377	54.955	0
AO	98102211	112	17	64.316	54.982	0
AO	98102212	126	16	64.428	55.191	0
AO	98102213	142	16	64.32	55.299	0
AO	98102214	157	16	64.319	55.22	0
AO	98102215	172	16	64.26	55.081	0
AO	98102216	189	15	64.335	55.137	0
AO	98102217	204	15	64.631	55.15	0
AO	98102218	220	12	65.224	54.519	0
AO	98102219	235	12	65.158	54.564	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AO	98102220	251	12	65.083	54.485	0
AO	98102221	267	14	64.983	54.449	0
AO	98102222	285	15	65.113	54.312	0
AO	98102223	295	15	65.132	54.362	0
AO	98102224	313	14	65.24	54.549	0
AO	98102225	330	13	65.188	54.542	0
AO	98102226	346	12	65.189	54.539	0
AO	98102227	361	13	65.309	54.628	0
AO	98102228	376	12	65.218	54.73	0
AO	98102229	391	13	65.131	54.85	0
AO	98102230	407	14	65.213	55.168	0
AO	98102231	420	15	65.438	55.078	0
AO	98102232	436	15	65.255	55.029	0
AO	98102233	451	14	65.145	55.062	0
AO	98102234	466	14	65.195	55.172	0
AO	98102235	482	14	65.275	55.192	0
AO	98102236	495	14	65.285	55.142	0
AO	98102237	511	13	65.145	55.232	0
AO	98102238	525	13	65.055	55.222	0
AO	98102239	542	13	65.095	55.252	0
AO	98102240	555	13	65.145	55.312	0
AO	98102241	571	13	65.185	55.262	0
AO	98102242	587	13	65.374	55.273	0
AO	98102243	601	13	65.299	55.622	0
AO	98102244	616	13	66.261	55.213	0.5
AO	98102245	632	13	66.296	55.472	0.5
AO	98102246	647	15	66.022	55.541	0.75
AO	98102247	661	14	66.069	56.222	0.5
AP	98102244	616	15	64.711	55.913	0
AP	98102245	632	14	64.586	55.902	0
AP	98102246	647	T1	64.512	55.911	0
AP	98102247	661	T1	64.319	56.692	0
AQ	98102218	220	16	64.424	55.899	0
AQ	98102219	235	16	64.468	55.914	0
AQ	98102220	251	16	64.273	55.895	0
AQ	98102221	267	18	64.303	55.839	0
AQ	98102222	285	19	64.223	55.802	0
AQ	98102223	295	19	64.382	55.832	0
AQ	98102224	313	17	64.55	55.999	0
AQ	98102225	330	17	64.478	56.032	0
AQ	98102226	346	16	64.469	55.989	0
AQ	98102227	361	17	64.409	56.108	0
AQ	98102228	376	16	64.418	56.16	0.25
AQ	98102229	391	16	64.291	56.17	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AQ	98102230	407	16	64.383	56.528	0
AQ	98102231	420	17	64.588	56.498	0
AQ	98102232	436	17	64.415	56.379	0
AQ	98102233	451	17	64.295	56.592	0.25
AQ	98102234	466	18	64.135	56.752	0.5
AQ	98102235	482	16	63.845	56.452	0.75
AQ	98102236	495	16	63.855	56.392	0.5
AQ	98102237	511	15	63.715	56.552	0.5
AQ	98102238	525	16	63.915	56.512	0.5
AQ	98102239	542	15	64.165	56.492	0.25
AQ	98102240	555	15	64.175	56.552	0.5
AQ	98102241	571	15	64.275	56.422	0.25
AQ	98102242	587	15	64.294	56.513	0.5
AQ	98102243	601	15	64.329	56.762	0.5
AQ	98102244	616	16	64.431	56.723	0.75
AQ	98102245	632	16	64.696	56.452	1.25
AQ	98102246	647	T2	64.512	56.211	1.25
AQ	98102247	661	T2	64.519	57.082	1.5
AR	98102245	632	17	63.806	57.212	0.5
AR	98102246	647	T4	63.832	57.181	1
AS	98102204	0	22	74.54	57.59	0.5
AS	98102205	22	19	74.357	57.631	0.25
AS	98102206	37	19	74.358	57.318	0
AS	98102207	50	21	74.354	57.677	0.5
AS	98102208	66	21	74.386	57.789	0.5
AS	98102209	81	22	74.293	57.538	0.5
AS	98102210	97	20	74.357	57.465	0.5
AS	98102211	112	20	74.266	57.442	0.75
AS	98102212	126	19	74.138	57.161	0.5
AS	98102213	142	19	74.76	57.059	0.5
AS	98102214	157	T1	74.289	58.06	1
AS	98102215	172	20	73.42	58.761	0.75
AS	98102216	189	20	73.105	58.567	1
AS	98102217	204	20	72.621	59.34	1.25
AS	98102218	220	18	72.034	58.559	1
AS	98102219	235	17	72.148	58.434	0.75
AS	98102220	251	18	72.153	58.485	0.75
AS	98102221	267	20	71.943	58.519	1
AS	98102222	285	24	71.863	58.772	1.25
AS	98102223	295	21	71.632	59.192	1
AS	98102224	313	20	72.02	58.689	1
AS	98102225	330	20	72.118	58.482	1
AS	98102226	346	18	71.919	58.509	1
AS	98102227	361	20	72.189	58.718	1.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AS	98102228	376	19	71.938	58.82	1.25
AS	98102229	391	19	71.861	58.93	1.25
AS	98102230	407	20	71.973	59.348	1
AS	98102231	420	21	71.888	59.798	1.5
AS	98102232	436	21	71.445	60.299	1.25
AS	98102233	451	20	71.235	60.442	0.5
AS	98102234	466	21	71.825	59.602	1
AS	98102235	482	19	71.405	60.032	1
AS	98102236	495	19	71.785	59.762	1
AS	98102237	511	18	71.845	59.542	1
AS	98102238	525	19	71.745	59.602	1
AS	98102239	542	18	71.885	59.472	1.25
AS	98102240	555	18	71.845	59.582	1.25
AS	98102241	571	18	71.825	59.652	1
AS	98102242	587	17	71.824	59.653	1.25
AS	98102243	601	18	71.809	59.632	1.25
AS	98102244	616	18	71.741	59.883	1.5
AS	98102245	632	19	71.766	60.002	1.5
AS	98102246	647	19	71.802	60.011	2
AS	98102247	661	17	72.709	60.562	1
AT	98102234	466	23	70.945	60.972	0.25
AT	98102235	482	20	70.715	61.542	0
AT	98102236	495	21	70.785	62.252	0
AT	98102237	511	20	71.015	62.612	0
AT	98102238	525	21	71.155	62.462	0
AT	98102239	542	21	71.295	62.542	0.25
AT	98102240	555	21	71.335	62.602	0.25
AT	98102241	571	21	71.055	62.782	0
AT	98102242	587	21	71.014	63.083	0
AT	98102243	601	21	71.149	63.112	0
AT	98102244	616	20	71.331	63.253	0
AT	98102245	632	21	71.356	63.332	0
AT	98102246	647	21	71.202	63.321	0
AT	98102247	661	19	71.149	64.142	0
AU	98102212	126	20	74.588	58.301	0.25
AU	98102213	142	20	74.53	58.379	0.25
AU	98102214	157	19	74.399	58.07	0
AU	98102215	172	19	74.17	58.101	0.25
AU	98102216	189	19	74.175	58.167	0.25
AU	98102217	204	19	74.451	58.4	0.5
AU	98102218	220	17	74.794	58.529	0.75
AU	98102219	235	18	74.458	58.704	0.75
AU	98102220	251	17	74.573	58.425	0.5
AU	98102221	267	19	74.623	58.439	0.5

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AU	98102222	285	23	74.723	58.312	0.75
AU	98102223	295	20	74.672	58.402	0.75
AU	98102224	313	19	74.92	58.629	1
AU	98102225	330	19	74.688	58.522	0.5
AU	98102226	346	19	74.789	58.699	0.75
AU	98102227	361	19	74.919	58.788	0.75
AU	98102228	376	18	75.028	58.83	1
AU	98102229	391	18	74.791	58.82	0.75
AU	98102230	407	19	74.483	59.408	1
AU	98102231	420	20	74.858	59.538	1.25
AU	98102232	436	20	74.825	59.359	1
AU	98102233	451	19	74.775	59.612	1
AU	98102234	466	20	74.935	59.732	1
AU	98102235	482	18	74.835	59.722	1
AU	98102236	495	18	74.395	59.552	1
AU	98102237	511	17	74.725	59.572	1
AU	98102238	525	18	74.605	59.632	1
AU	98102239	542	19	74.875	60.112	0.25
AU	98102240	555	19	74.915	60.322	0.5
AU	98102241	571	19	75.095	60.362	0
AU	98102242	587	18	75.234	60.523	0.5
AU	98102243	601	19	75.329	60.672	0.5
AU	98102244	616	19	75.391	60.783	0.75
AU	98102245	632	20	75.276	61.002	0.5
AU	98102246	647	20	75.462	61.041	1
AU	98102247	661	18	75.409	62.002	0.5
AV	98102239	542	17	74.485	58.842	1.25
AV	98102240	555	17	74.475	59.132	1
AV	98102241	571	17	74.515	59.182	1
AV	98102242	587	19	73.994	60.533	2.5
AV	98102243	601	20	73.979	60.702	1
AW	98102204	0	23	84.07	91.95	0.5
AW	98102205	22	20	83.627	91.631	1.5
AW	98102206	37	20	83.448	91.718	1
AW	98102207	50	22	83.494	92.077	1.5
AW	98102208	66	22	83.526	92.379	1.5
AW	98102209	81	23	83.693	91.838	1.5
AW	98102210	97	21	84.177	91.755	1.5
AW	98102211	112	21	84.126	91.202	1.75
AW	98102212	126	21	84.378	91.291	1.5
AW	98102213	142	21	84.25	92.009	1.5
AW	98102214	157	20	84.229	91.82	1.5
AW	98102215	172	21	83.88	91.921	1.5
AW	98102216	189	22	83.975	92.627	1.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
AW	98102217	204	21	83.771	93.5	1.75
AW	98102218	220	19	84.594	92.399	2.25
AW	98102219	235	19	84.358	92.474	2.25
AW	98102220	251	19	85.073	93.025	1.5
AW	98102221	267	21	84.313	92.569	1.5
AW	98102222	285	T1	83.873	92.672	2
AW	98102223	295	22	84.232	92.632	2
AW	98102224	313	21	84.04	91.939	1.5
AW	98102225	330	21	84.138	91.982	1.75
AW	98102226	346	20	84.249	92.099	1.75
AW	98102227	361	21	84.359	92.048	1.5
AW	98102228	376	20	83.988	92.7	2.25
AW	98102229	391	20	84.391	92.99	2.25
AW	98102230	407	21	84.353	93.258	2.25
AW	98102231	420	22	85.038	93.538	2.25
AW	98102232	436	22	85.185	93.789	2.25
AW	98102233	451	23	84.315	93.352	1.5
AW	98102234	466	24	84.545	93.722	2.5
AW	98102235	482	22	85.545	93.662	2
AW	98102236	495	22	85.325	93.842	1.5
AW	98102237	511	22	84.425	93.872	3
AW	98102238	525	22	83.625	93.352	1.25
AW	98102239	542	22	83.795	93.072	2.5
AW	98102240	555	22	83.345	95.422	1.5
AW	98102241	571	22	85.405	94.022	1
AW	98102242	587	22	85.984	94.703	1.5
AW	98102243	601	23	84.499	95.942	1
AW	98102244	616	22	84.111	93.873	3.75
AX	98102221	267	22	85.323	93.059	3
AY	98102204	0	24	84.19	92.72	1
AY	98102205	22	21	85.567	93.641	0.5
AY	98102206	37	21	85.178	93.528	1
B	98102204	0	2	43.74	1.55	1
B	98102205	22	2	43.647	2.741	1
B	98102206	37	2	43.658	2.308	0.5
B	98102207	50	2	43.624	2.627	0.5
B	98102208	66	2	43.906	2.699	0.75
B	98102209	81	2	43.783	2.528	1
B	98102210	97	2	43.957	2.665	0.75
B	98102211	112	2	43.436	2.712	1
B	98102212	126	2	43.528	2.871	1
B	98102213	142	2	43.33	2.869	1
B	98102214	157	2	43.299	2.91	1
B	98102215	172	2	43.2	2.901	1

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
C	98102204	0	3	66.1	45.22	0
C	98102205	22	3	66.037	45.431	0
C	98102206	37	3	66.098	45.008	0
C	98102207	50	3	65.954	45.347	0
C	98102208	66	3	65.486	44.769	0.25
C	98102209	81	3	65.413	44.648	0.5
C	98102210	97	3	65.477	44.685	0.5
C	98102211	112	3	65.306	44.622	0.5
C	98102212	126	3	65.378	44.761	0.5
C	98102213	142	3	65.34	44.859	0.5
C	98102214	157	3	65.649	44.88	0
C	98102215	172	3	65.68	44.751	0
C	98102216	189	2	65.875	44.827	0
C	98102217	204	2	66.071	44.83	0
C	98102218	220	1	66.234	44.869	0
C	98102219	235	1	66.218	44.934	0
C	98102220	251	1	66.613	45.125	0
C	98102221	267	1	66.253	44.829	0
C	98102222	285	1	66.403	44.552	0
C	98102223	295	1	66.332	44.572	0
C	98102224	313	1	66.43	44.659	0
C	98102225	330	1	66.538	45.102	0
C	98102226	346	1	66.569	44.999	0
C	98102227	361	1	66.309	44.778	0
C	98102228	376	1	66.358	44.98	0
C	98102229	391	1	66.331	45.04	0
C	98102230	407	1	66.433	45.368	0
C	98102231	420	1	66.608	45.358	0
C	98102232	436	1	66.465	45.229	0.25
C	98102233	451	1	66.495	45.402	0
C	98102234	466	1	66.465	45.412	0
C	98102235	482	1	66.435	45.462	0
C	98102236	495	1	66.365	45.512	0
C	98102237	511	1	66.385	45.522	0
C	98102238	525	1	66.355	45.492	0
C	98102239	542	1	66.365	45.552	0
C	98102240	555	1	66.415	45.502	0
C	98102241	571	1	66.415	45.462	0
C	98102242	587	1	66.434	45.553	0
C	98102243	601	1	66.449	45.662	0
C	98102244	616	1	66.521	45.743	0
C	98102245	632	1	66.556	45.772	0
C	98102246	647	1	66.122	44.951	0
C	98102247	661	1	66.139	45.692	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
D	98102246	647	2	66.742	46.471	0
D	98102247	661	2	66.839	47.342	0
E	98102208	66	4	66.436	45.969	0.25
E	98102209	81	4	66.373	45.778	0.25
E	98102210	97	4	66.477	45.805	0
E	98102211	112	4	66.336	45.662	0
E	98102212	126	4	66.438	45.871	0
E	98102213	142	4	66.37	45.929	0
E	98102214	157	4	66.209	45.87	0.75
E	98102215	172	4	66.2	45.751	0.75
E	98102216	189	3	65.935	45.857	0.75
E	98102217	204	3	66.071	45.83	0.75
E	98102218	220	2	66.254	45.799	0.75
E	98102219	235	2	66.438	45.834	0.75
E	98102220	251	T1	66.963	45.255	0.75
E	98102221	267	2	66.263	45.609	1
E	98102222	285	2	66.623	45.432	1.75
E	98102223	295	2	67.112	45.272	1.5
E	98102224	313	2	66.56	45.599	1.25
E	98102225	330	T1	66.768	45.262	1.5
E	98102226	346	T1	66.859	45.259	1.75
E	98102227	361	2	67.169	45.268	1.75
F	98102204	0	4	66.4	48.3	0
F	98102205	22	4	66.477	48.771	0
F	98102206	37	4	66.998	47.638	0
F	98102207	50	4	66.914	47.947	0
F	98102208	66	5	67.026	48.069	0
F	98102209	81	5	66.913	47.958	0
F	98102210	97	5	66.967	48.005	0
F	98102211	112	5	66.806	47.942	0
F	98102212	126	5	66.868	48.141	0
F	98102213	142	5	66.77	48.249	0
F	98102214	157	5	66.889	48.11	0
F	98102215	172	5	66.82	48.111	0
F	98102216	189	4	66.855	48.167	0
F	98102217	204	4	67.081	48.12	0
F	98102218	220	3	67.434	48.179	0
F	98102219	235	3	67.348	48.324	0
F	98102220	251	2	67.283	48.325	0
F	98102221	267	3	67.153	48.269	0
F	98102222	285	3	67.343	48.132	0.25
F	98102223	295	3	67.482	48.252	0.25
F	98102224	313	3	67.8	48.109	0.25
F	98102225	330	2	67.458	48.322	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
F	98102226	346	2	67.389	48.139	0.25
F	98102227	361	3	67.249	48.338	0
F	98102228	376	3	67.238	48.68	0
F	98102229	391	3	67.211	48.78	0.25
F	98102230	407	3	68.133	48.948	0
F	98102231	420	3	68.328	48.928	0
F	98102232	436	3	68.185	48.859	0.25
F	98102233	451	3	68.135	48.962	0
F	98102234	466	3	68.165	48.972	0
F	98102235	482	3	68.115	48.982	0
F	98102236	495	3	68.065	48.952	0
F	98102237	511	3	68.085	49.012	0
F	98102238	525	3	67.965	49.052	0
F	98102239	542	3	68.005	49.042	0
F	98102240	555	3	68.025	49.032	0
F	98102241	571	3	67.985	49.052	0
F	98102242	587	3	68.004	49.173	0
F	98102243	601	3	68.069	49.362	0
F	98102244	616	3	68.191	49.423	0
F	98102245	632	3	68.276	49.442	0
F	98102246	647	5	68.112	49.461	0.5
F	98102247	661	4	68.019	50.282	0.5
G	98102230	407	4	66.603	49.368	0.25
G	98102231	420	5	66.718	49.408	0.25
G	98102232	436	5	66.565	49.299	0.25
G	98102233	451	4	66.475	49.432	0
G	98102234	466	5	66.495	49.582	0.25
G	98102235	482	4	66.445	49.632	0.25
G	98102236	495	4	66.395	49.662	0.25
G	98102237	511	4	66.405	49.752	0.25
G	98102238	525	5	66.325	49.862	0.25
G	98102239	542	5	66.295	49.952	0.25
G	98102240	555	5	66.355	49.922	0.5
G	98102241	571	4	66.405	49.842	0.25
G	98102242	587	5	66.404	50.033	0.5
G	98102243	601	4	66.549	49.722	0.75
G	98102244	616	4	66.651	49.693	1.25
G	98102245	632	4	66.626	49.812	1.25
G	98102246	647	6	66.542	49.851	1.5
G	98102247	661	5	66.529	50.602	1.5
H	98102206	37	5	70.358	49.618	0
H	98102207	50	6	66.084	49.307	0
H	98102208	66	7	66.126	49.389	0
H	98102209	81	8	65.943	49.208	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
H	98102210	97	7	65.967	49.245	0
H	98102211	112	7	65.906	49.202	0
H	98102212	126	7	66.008	49.401	0
H	98102213	142	7	65.92	49.449	0
H	98102214	157	6	65.859	49.43	0
H	98102215	172	7	65.94	49.481	0
H	98102216	189	5	65.855	49.417	0
H	98102217	204	5	65.871	49.24	0.25
I	98102204	0	5	66.81	48.59	1.25
I	98102205	22	5	67.197	48.731	1.25
J	98102204	0	6	70.53	49.7	0
J	98102205	22	6	70.297	50.031	0
J	98102206	37	6	66.168	48.988	0
J	98102207	50	5	70.254	49.917	0
J	98102208	66	6	70.386	49.159	0
J	98102209	81	6	70.343	48.958	0
J	98102210	97	6	70.277	49.025	0
J	98102211	112	6	70.136	48.982	0
J	98102212	126	6	70.218	49.191	0
J	98102213	142	6	70.12	49.299	0
J	98102214	157	7	69.989	49.25	0
J	98102215	172	6	69.96	49.241	0
J	98102216	189	6	69.965	49.207	0
J	98102217	204	6	70.181	49.2	0
J	98102218	220	4	70.354	49.219	0
J	98102219	235	4	70.328	49.324	0
J	98102220	251	3	70.313	49.215	0
J	98102221	267	5	70.243	49.169	0
J	98102222	285	5	70.353	49.102	0.5
J	98102223	295	5	70.362	49.172	0
J	98102224	313	5	70.66	49.329	0.25
J	98102225	330	4	70.458	49.462	0.25
J	98102226	346	4	70.459	49.459	0
J	98102227	361	5	70.689	49.748	0
J	98102228	376	4	71.268	49.29	0.25
J	98102229	391	4	71.291	49.33	0.25
J	98102230	407	5	71.463	49.838	0
J	98102231	420	4	71.548	49.778	0.25
J	98102232	436	4	71.675	49.669	0.25
J	98102233	451	5	71.645	49.842	0
J	98102234	466	4	71.655	49.752	0.25
J	98102235	482	5	71.615	49.852	0
J	98102236	495	5	71.475	49.892	0
J	98102237	511	5	71.525	49.882	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
J	98102238	525	4	71.475	49.892	0
J	98102239	542	4	71.485	49.852	0
J	98102240	555	4	71.505	49.862	0.25
J	98102241	571	5	71.485	49.882	0
J	98102242	587	4	71.454	49.993	0.25
J	98102243	601	5	71.449	50.122	0.25
J	98102244	616	5	71.551	50.173	0.5
J	98102245	632	5	71.566	50.282	0
J	98102246	647	7	71.452	50.211	0
J	98102247	661	6	71.429	51.092	0
K	98102228	376	5	70.118	50.52	0.25
K	98102229	391	5	70.091	50.39	0.25
L	98102208	66	10	70.206	50.929	0
L	98102209	81	10	70.163	50.808	0
L	98102210	97	9	70.307	50.695	0
L	98102211	112	9	70.166	50.582	0
L	98102212	126	9	70.218	50.851	0
L	98102213	142	9	70.09	50.999	0
L	98102214	157	8	70.909	49.6	0.25
L	98102215	172	8	70.88	49.641	0.25
L	98102216	189	7	71.055	49.737	0.25
L	98102217	204	7	71.281	49.76	0.25
L	98102218	220	5	71.364	49.849	0.25
L	98102219	235	5	71.338	50.004	0.25
L	98102220	251	4	71.523	49.865	0.25
L	98102221	267	4	71.453	49.259	0.5
L	98102222	285	4	72.163	48.112	0.25
L	98102223	295	4	72.232	48.162	0.5
L	98102224	313	4	72.54	48.389	0.25
L	98102225	330	3	72.308	48.322	0.25
L	98102226	346	3	72.449	48.309	0.25
L	98102227	361	4	72.549	48.238	0.25
L	98102228	376	2	72.508	48.2	0.25
L	98102229	391	2	72.291	48.21	0.25
L	98102230	407	2	72.683	48.458	0.25
L	98102231	420	2	72.918	48.438	0.25
L	98102232	436	2	72.825	48.299	0.5
L	98102233	451	2	72.865	48.492	0.25
L	98102234	466	2	72.985	48.492	0.5
L	98102235	482	2	73.075	48.492	0.5
L	98102236	495	2	72.615	48.432	0
L	98102237	511	2	72.725	48.382	0
L	98102238	525	2	72.655	48.332	0.25
L	98102239	542	2	72.695	48.302	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
L	98102240	555	2	72.675	48.242	0.25
L	98102241	571	2	72.605	48.202	0.25
L	98102242	587	2	72.594	48.283	0.25
L	98102243	601	2	72.579	48.432	1.25
L	98102244	616	2	72.851	48.523	0.5
L	98102245	632	2	72.776	48.662	0.5
L	98102246	647	4	72.612	48.551	0.5
L	98102247	661	3	72.499	49.402	0.5
M	98102222	285	6	71.423	49.812	0.75
M	98102223	295	6	72.302	49.122	1.5
N	98102204	0	7	71.3	49.13	0
N	98102205	22	7	70.887	49.721	0
N	98102206	37	7	70.988	49.228	0
N	98102207	50	7	70.974	49.387	0
N	98102208	66	9	70.936	49.669	0
N	98102209	81	9	70.943	49.488	0
N	98102210	97	8	71.047	49.475	0.25
N	98102211	112	8	70.916	49.482	0.25
N	98102212	126	8	71.038	49.631	0.25
N	98102213	142	8	70.95	49.639	0.25
N	98102214	157	9	70.079	50.88	0
N	98102215	172	9	70.04	50.811	0
N	98102216	189	8	70.035	50.837	0
N	98102217	204	8	70.281	50.82	0.25
N	98102218	220	6	70.404	50.899	0.25
N	98102219	235	6	70.358	50.974	0
N	98102220	251	5	70.393	50.905	0
N	98102221	267	6	70.293	50.889	0
N	98102222	285	7	70.443	50.892	0.5
N	98102223	295	7	70.452	50.962	0.25
N	98102224	313	6	70.54	51.129	0
N	98102225	330	5	70.838	50.402	0.5
N	98102226	346	5	71.689	50.469	0.5
N	98102227	361	12	68.399	53.288	0.25
O	98102204	0	8	72.04	50.13	0
O	98102205	22	8	71.467	50.911	0.5
O	98102206	37	8	71.368	49.818	1
O	98102207	50	8	71.404	49.897	0.75
O	98102208	66	8	71.626	50.269	0.5
O	98102209	81	7	71.763	49.238	0.75
P	98102209	81	11	71.833	50.988	1.5
Q	98102204	0	9	71.36	50.73	0.25
Q	98102205	22	13	70.867	52.191	0.5
Q	98102206	37	13	70.938	51.848	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
Q	98102207	50	13	70.564	52.537	0.5
Q	98102208	66	15	71.506	52.699	2
Q	98102209	81	18	70.853	53.038	2
Q	98102210	97	14	70.257	52.835	1.5
Q	98102211	112	16	70.606	53.362	0
R	98102204	0	10	67.84	50.84	0
R	98102205	22	9	67.847	50.921	0.25
R	98102206	37	9	67.958	50.748	0
R	98102207	50	9	67.894	51.077	0
R	98102208	66	11	67.976	51.169	0.25
R	98102209	81	12	67.903	51.078	0.25
R	98102210	97	10	67.627	51.095	0
R	98102211	112	10	67.316	51.002	0
R	98102212	126	11	67.418	51.231	0
R	98102213	142	10	67.36	51.339	0
R	98102214	157	11	67.449	51.37	0
R	98102215	172	10	67.33	51.281	0
R	98102216	189	10	67.505	51.347	0
R	98102217	204	10	67.941	51.41	0
R	98102218	220	8	68.104	51.509	0
R	98102219	235	8	68.138	51.644	0
R	98102220	251	7	68.153	51.515	0
R	98102221	267	8	68.133	51.329	0
R	98102222	285	9	68.183	51.302	0.25
R	98102223	295	9	68.222	51.462	0.25
R	98102224	313	8	68.31	51.569	0.25
R	98102225	330	7	68.148	51.622	0.25
R	98102226	346	7	67.989	51.479	0
R	98102227	361	7	68.119	51.348	0
R	98102228	376	7	67.968	51.55	0
R	98102229	391	7	67.861	51.45	0.25
R	98102230	407	7	68.683	51.618	0
R	98102231	420	7	68.898	51.508	0.25
R	98102232	436	7	68.465	50.969	0.25
R	98102233	451	21	84.995	59.942	3.75
R	98102234	466	22	84.275	60.792	2.75
R	98102235	482	21	85.335	61.692	3
R	98102236	495	20	86.335	60.092	0.75
R	98102237	511	19	86.085	61.362	1.25
R	98102238	525	20	85.895	61.112	0.75
R	98102239	542	20	86.165	61.272	1.5
R	98102240	555	20	86.875	62.052	0.25
R	98102241	571	20	86.675	62.042	0.5
R	98102242	587	20	87.674	62.663	0

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
R	98102243	601	22	87.859	63.032	0.5
R	98102244	616	21	88.241	63.463	0.5
R	98102245	632	22	88.586	63.832	0.5
R	98102246	647	22	90.242	65.611	0
R	98102247	661	20	93.329	67.832	0.5
S	98102230	407	8	67.123	51.818	0
S	98102231	420	8	67.278	51.758	0.25
S	98102232	436	8	67.135	51.629	0.5
S	98102233	451	7	67.115	51.742	0.5
S	98102234	466	7	67.065	51.712	0.25
S	98102235	482	7	66.965	51.662	0.25
S	98102236	495	7	66.905	51.672	0.25
S	98102237	511	8	67.015	51.632	0
S	98102238	525	7	67.015	51.672	0
S	98102239	542	7	67.075	51.642	0.25
S	98102240	555	7	67.165	51.652	0.25
S	98102241	571	7	67.165	51.672	0
S	98102242	587	8	67.104	51.673	0.25
S	98102243	601	7	67.329	51.592	0.5
S	98102244	616	7	67.741	52.173	1.25
S	98102245	632	7	67.736	52.342	1.25
S	98102246	647	9	67.952	52.401	1.5
S	98102247	661	8	67.849	52.932	1.75
T	98102204	0	11	66.48	50.98	0.75
T	98102205	22	10	66.427	51.381	1
T	98102206	37	10	66.588	51.038	1
T	98102207	50	12	66.454	51.627	1.25
T	98102208	66	14	66.386	51.999	1
T	98102209	81	13	65.803	51.118	1.75
T	98102210	97	13	66.227	52.145	1.5
T	98102211	112	12	66.496	52.042	1.5
T	98102212	126	10	65.978	51.501	1
T	98102213	142	11	65.9	51.809	1.25
T	98102214	157	10	66.019	51.54	0.75
T	98102215	172	11	65.88	51.651	0.75
T	98102216	189	9	65.895	51.687	0.75
T	98102217	204	9	66.181	51.52	1
T	98102218	220	7	66.144	51.579	1
T	98102219	235	7	65.988	51.664	1
T	98102220	251	6	66.133	51.345	0.75
T	98102221	267	7	66.273	51.019	0.75
T	98102222	285	8	66.693	50.872	1
T	98102223	295	8	66.552	51.032	1
T	98102224	313	7	66.64	50.439	0.75

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
T	98102225	330	6	66.098	50.582	1
T	98102226	346	6	66.139	50.439	1
T	98102227	361	6	66.119	50.628	1.5
T	98102228	376	6	66.148	50.79	1.5
T	98102229	391	6	66.131	50.78	1.5
T	98102230	407	6	66.233	51.058	1.25
T	98102231	420	6	66.328	51.138	1.5
T	98102232	436	6	66.185	50.959	1.25
T	98102233	451	6	66.195	51.052	1
T	98102234	466	6	66.095	51.112	1.25
T	98102235	482	6	66.055	50.982	1.25
T	98102236	495	6	66.135	51.142	1.25
T	98102237	511	6	66.255	51.132	1.5
T	98102238	525	6	66.245	51.132	1.5
T	98102239	542	6	66.295	51.202	1.5
T	98102240	555	6	66.365	51.142	1.5
T	98102241	571	6	66.455	51.152	1.5
T	98102242	587	7	65.864	51.483	1.75
T	98102243	601	6	66.289	51.312	1.75
T	98102244	616	6	66.491	51.203	2.25
T	98102245	632	6	66.396	51.352	1.5
T	98102246	647	8	66.622	51.371	2
T	98102247	661	7	66.629	52.132	2
U	98102221	267	10	66.083	52.209	2
U	98102222	285	11	66.293	52.382	2.5
U	98102223	295	11	66.362	52.452	2
U	98102224	313	10	66.63	52.499	2
U	98102225	330	9	66.748	52.322	1.5
U	98102226	346	8	66.649	52.139	1.25
U	98102227	361	8	66.609	51.948	1.5
U	98102228	376	8	66.468	52.31	1.5
U	98102229	391	10	66.501	52.53	1.5
U	98102230	407	11	66.533	52.818	2
U	98102231	420	12	66.838	52.768	2.25
U	98102232	436	10	66.645	52.819	2.25
U	98102233	451	9	66.715	52.862	2
U	98102234	466	11	66.505	53.002	1.25
U	98102235	482	11	66.455	52.942	0.75
U	98102236	495	11	66.465	53.082	0.75
U	98102237	511	10	66.605	53.022	0.75
U	98102238	525	10	66.545	53.032	1
U	98102239	542	9	66.515	52.982	1
U	98102240	555	8	66.455	53.002	1
U	98102241	571	9	66.415	52.982	0.75

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
U	98102242	587	9	66.474	52.953	1
U	98102243	601	9	66.399	52.972	1.25
U	98102244	616	9	66.471	53.083	1.25
U	98102245	632	10	66.416	53.142	1.25
U	98102246	647	12	66.342	52.931	1.5
U	98102247	661	11	66.409	53.632	1.5
V	98102204	0	12	12.24	51.64	0
V	98102205	22	11	12.167	51.881	0
V	98102206	37	11	12.268	51.358	0
V	98102207	50	10	12.244	51.567	0
V	98102208	66	13	12.396	51.719	0
V	98102209	81	15	12.313	51.598	0
V	98102210	97	12	12.627	51.645	0
W	98102204	0	13	72.96	52.08	0
W	98102205	22	12	72.897	52.381	0
W	98102206	37	12	72.918	51.958	0
W	98102207	50	11	72.834	52.247	0
W	98102208	66	12	72.846	52.279	0.25
W	98102209	81	14	72.723	52.208	0.25
W	98102210	97	11	72.847	52.245	0.25
W	98102211	112	11	72.606	51.312	0.5
W	98102212	126	12	72.668	51.541	0.5
W	98102213	142	12	72.81	51.759	0.5
W	98102214	157	12	72.749	51.73	0.5
W	98102215	172	12	72.9	51.711	0.5
W	98102216	189	11	72.835	51.757	0.5
W	98102217	204	11	73.701	51.87	0.75
W	98102218	220	9	73.854	51.929	0.75
W	98102219	235	9	73.748	52.034	0.5
W	98102220	251	8	73.763	51.975	0.5
W	98102221	267	9	73.633	51.919	0.5
W	98102222	285	10	73.783	51.912	0.75
W	98102223	295	10	73.762	51.902	0.75
W	98102224	313	9	73.81	51.979	0.75
W	98102225	330	8	73.858	51.912	0.75
W	98102226	346	9	73.729	52.009	0.75
W	98102227	361	9	74.239	52.288	1
W	98102228	376	9	73.468	52.19	1
W	98102229	391	9	72.681	51.96	1.5
W	98102230	407	9	73.363	52.438	1
W	98102231	420	10	74.308	52.428	0.75
W	98102232	436	11	74.665	52.639	0.5
W	98102233	451	10	74.745	52.802	0.25
W	98102234	466	10	74.905	52.782	0.5

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
W	98102235	482	9	75.075	52.802	0.25
W	98102236	495	8	75.085	52.752	0.25
W	98102237	511	9	75.275	52.862	0.5
W	98102238	525	9	75.325	52.882	0.5
W	98102239	542	8	75.225	52.792	0.75
W	98102240	555	9	75.165	52.842	0.75
W	98102241	571	8	75.305	52.912	0.75
W	98102242	587	10	75.314	53.033	1
W	98102243	601	10	75.269	53.232	1
W	98102244	616	10	75.371	53.253	1.5
W	98102245	632	9	75.296	53.152	1
W	98102246	647	11	75.052	53.261	1.25
W	98102247	661	10	75.209	54.222	1.5
X	98102231	420	11	72.778	52.448	1.25
X	98102232	436	9	72.465	52.339	1.5
X	98102233	451	8	72.545	52.502	1.5
X	98102234	466	8	72.525	52.802	1.5
X	98102235	482	10	72.445	52.732	1.25
X	98102236	495	10	72.945	53.072	1.5
X	98102237	511	7	72.425	51.752	2
X	98102238	525	8	71.215	52.252	2
X	98102239	542	10	73.065	52.972	2.25
X	98102240	555	10	73.185	53.052	1.5
X	98102241	571	10	73.065	52.922	1.75
X	98102242	587	6	70.064	51.673	1.75
X	98102243	601	8	70.419	52.602	2.5
X	98102244	616	8	70.601	52.753	2
X	98102245	632	8	71.036	52.592	3.5
X	98102246	647	10	70.982	52.281	3.5
X	98102247	661	9	71.109	53.432	4
Y	98102211	112	14	72.946	52.992	0
Y	98102212	126	14	73.008	53.171	0
Y	98102213	142	14	72.95	53.289	0
Y	98102214	157	14	72.849	53.18	0
Y	98102215	172	14	72.93	53.111	0
Y	98102216	189	13	72.895	53.177	0
Y	98102217	204	13	73.111	53.18	0.25
Y	98102218	220	10	73.254	53.179	0.25
Y	98102219	235	10	73.308	53.304	0.25
Y	98102220	251	9	73.183	53.195	0
Y	98102221	267	11	73.063	53.089	0.25
Y	98102222	285	12	73.163	52.972	0.5
Y	98102223	295	12	73.222	53.022	0.5
Y	98102224	313	12	73.4	53.139	0.25

CellID	Image	Time(min)	Object#	X-coord	Y-coord	Z-coord
Y	98102225	330	11	73.278	53.242	0.25
Y	98102226	346	11	73.339	53.209	0.25
Y	98102227	361	11	73.399	53.318	0.25
Y	98102228	376	11	73.298	53.4	0.25
Y	98102229	391	12	73.141	53.48	0.25
Y	98102230	407	12	73.193	53.808	0
Y	98102231	420	14	73.308	53.878	0
Y	98102232	436	13	73.235	53.719	0.5
Y	98102233	451	13	73.245	53.752	0.25
Y	98102234	466	13	73.125	53.912	0
Y	98102235	482	13	73.085	53.912	0.25
Y	98102236	495	12	72.965	53.952	0
Y	98102237	511	11	73.045	53.992	0
Y	98102238	525	12	72.965	53.982	0
Y	98102239	542	11	72.925	53.972	0.5
Y	98102240	555	11	72.935	53.942	0.25
Y	98102241	571	11	72.945	53.922	0.25
Y	98102242	587	11	72.954	53.953	0.5
Y	98102243	601	11	72.889	54.132	0.5
Y	98102244	616	11	72.991	54.153	0.5
Y	98102245	632	11	73.006	54.192	0.5
Y	98102246	647	13	72.892	54.091	0.5
Y	98102247	661	12	72.809	54.852	0.5
Z	98102204	0	14	67.3	52.34	0

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