Field Service Support

Genesis System Technical Overview
1. REVISION HISTORY

<table>
<thead>
<tr>
<th>REVISION</th>
<th>AUTHOR</th>
<th>DESCRIPTION OF CHANGE</th>
<th>DATE</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Cyrus Bilimoria/John Fitzpatrick</td>
<td>Initial Release</td>
<td>27Jun19</td>
</tr>
<tr>
<td>01</td>
<td>John Fitzpatrick</td>
<td>• Updated formatting to align with the support documentation</td>
<td>13Aug19</td>
</tr>
</tbody>
</table>

2. PURPOSE

2.1 The purpose of this document is to provide a technical overview of the Genesis System that includes a brief description of its role utility in a range of applications as well as a detailed look at the system’s components and architecture.

3. SCOPE

3.1 This procedure applies to any person involved with servicing the Genesis System.

4. RESPONSIBILITY

4.1 Field Service is responsible for the maintenance of this procedure.

5. TRAINING

5.1 Personnel performing servicing of the Genesis System should be have read and understood this document.

6. SAFETY

6.1 No special precautions are required.

7. ENVIRONMENTAL CONTROLS

7.1 No special environmental controls are required.

8. PERSONNEL PROTECTIVE EQUIPMENT (PPE)

8.1 No PPE required.

9. DEFINITIONS

N/A

10. RELATED DOCUMENTS

N/A

11. EQUIPMENT/ MATERIALS

12. OVERVIEW

12.1 This document provides the following:

12.1.1 Introduction to the Genesis System
12.1.2 Celsingle™ Technology
12.1.3 Celselect™ Technology
12.1.4 Genesis Architecture
13. TECHNICAL OVERVIEW

13.1 Introduction to the Genesis System

The Genesis System enables scientists to analyze molecular signatures from hundreds to millions of cells, uniquely allowing the processing of two Slide Technologies for improved results in a range of applications such as single-cell cytometry, single-cell proteogenomics, single-cell transcriptomics, rare-cell enumeration, and immune monitoring. The system provides a practical, scalable, and efficient approach to analyze rare cells. It is open and customizable to support method and application development as well as protocol optimization. The throughput is flexible across one or two slides per run, which includes a semi-automated workflow. The user just has to load consumables, reagents and run selected protocol on the Genesis. The Genesis System is customizable and supports methods development or protocol optimization for end-users specialized single cell applications.

The Genesis System supports a wide range of single cell applications with its Celsingle and Celselect technologies.

13.2 Celsingle Technology

Celsingle Slides capture and isolate single cells with a gentle, gravity-based approach. Discrete microwells allow a single cell to be paired with a unique cellular barcode and unique molecular indices for desired applications, such as gene expression or protein quantitation. The Celsingle process is open to support users that want to optimize, modify, or create new single-cell applications. The scalability of this approach is unmatched and has the potential to provide a 10x increase in cell throughput compared with other technologies. The Celsingle Slide design enables unique possibilities, including visualization and quality control of cells prior to lysis, as well as treatment or challenge of cells.

The key benefits of the Celsingle technology include:

- Solid or Liquid
- Dissociated Tissue
- Single Cell Suspension

Optional Microscopy for QC or Validation of Sample

Sequencing or Microscopy

- Cell Barcode Counting
- Read Alignment
- UMI Counting
- Filtering
- Cell/Gene Matrix File
- Run Report
• Unmatched sample and cell throughput flexibility — supports experiments from hundreds to millions of cells
• Semi-automated workflow on-instrument lysis and reverse transcription
• Deep and accurate view of cell populations with >70% capture efficiency of input cells
• Open system enables development and optimization of custom single-cell protocols
• Open format enables optional visual QC, confirmation of cell capture rates, and cell characterization by microscopy

13.2.1 Celsingle Applications

13.2.1.1 Single-Cell Cytometry
This is high-parameter protein analysis that scales from hundreds to millions of single cells with the Genesis system. This will mean limitless protein detection, with no overlap, no laser droplets or heavy metals and no gating calibration.

13.2.1.2 Single-Cell Proteogenomics
This enables characterization of both RNA and protein at the single cell resolution. This delivers best cell population resolution, limitless protein detection with combined RNA measurement with reduced risk of batch effects with higher cell throughput with no lasers or heavy metals.

13.2.1.3 Single-Cell Transcriptomics
This is deep cell population resolution using the Genesis system, which can scale millions of cells and this can interrogate large number of cells at once with a capture efficiency. This can achieve cost effective tissue atlases with high cell numbers.

13.2.2 Celsingle Design
The Celsingle slide is a macrowell vessel that utilizes gravitational forces to capture and isolate single cells. The slides are approximately the same length and width of a standard microscope slide. Each Celsingle Slide has an array of hexagonal wells etched on the polymer surface with manifold mounted around it. See Figure 1.

Figure 1: Celsingle Slide illustrating Hexagonal Wells
The number of wells per slide ranges from 100,000 through 250,000. The wells function to capture and isolate the single cells while the manifold contains the flow of reagents across the slide and supports the slide’s integration with the Genesis system. The dimensions of each well are designed to capture one cell and one bead. The possibility of more than one cell is possible but will be predicated on the loading density of the cells. There is also the likelihood of a well to remain empty, thus when beads are added wells will contain a bead or more than one bead, but no cell. This scenario does not have a biological affect since no cell is present with the bead. The end result with the Celsingle design is a one-cell, one-bead pairing. Celsingle Slides are optically transparent allowing for users to view the slide using a microscope at various steps allowing for optional QC measures to be implemented into protocols.

On the benchtop, after cells and beads have been loaded onto a Celsingle Slide, a lid is placed over the wells of the slide and the slide is mounted onto a slide station. See Figure 2. A funnel is positioned in the inlet of the slide’s manifold and the slide station is mounted in place on the Genesis System. See Figure 2. Waste jar tubing is connected to the manifold outlet and then the Genesis System is prepared for operation. With either 1 or 2 lanes of the Genesis System prepared for a run, using the Genesis System touchscreen interface a user can initiate a standard, Celsee certified protocol, or a custom-made protocol (if the end-user has purchased the Genesis System Protocol Builder).

13.3 Celselect Technology

Celselect Slides capture and isolate individual immune cells, rare cells or CTCs based on their size utilizing microfluidics and 56,400 individual microchambers. Isolated cells can be stained for immunofluorescent applications such as enumeration, FISH, or enriched for downstream methods such as cell-culture or single-cell genomics. The high capture efficiency and customizability paired with excellent sensitivity and specificity make the Celselect Slides the ideal approach to follow tumor
progression and response to therapy. The Celselect Technology is open to support users that want to optimize, modify, or create new protocols or custom reagent formulations.

Key benefits of the Celselect technology include:

- Compatible with samples like blood, pleural fluid, urine, and dissociated tissues
- Automated and highly-efficient isolation and immune-staining of circulating tumor cells (CTCs) from whole blood
- Increased sensitivity for the enumeration of CTCs, as low as 1 in 1,000,000 cells
- Remove >99% of RBCs and WBCs
- Rapid 6–minute capture of rare cells from any tumor type for downstream analysis
- On-slide immunostaining or FISH analysis

13.3.1 Celselect Applications

13.3.1.1 Rare-Cell Enumeration

This application captures rare cells which enables accurate enumeration and downstream analysis. This removes greater than 99% of RBCs and WBCs cells.

13.3.1.2 Rare-Cell Enrichment

This application captures and retrieves viable rare cells from blood, tissue and urine in less than six minutes. This is a highly efficient capture of rare cells and these viable cells can be used for imaging, cell culture, or downstream genomic analysis.

13.3.2 Celselect Design

The Celselect Slide is vessel that utilizes microfluidic forces to capture and isolate target cells. Like the Celsingle Slides, the Celselect Slides are approximately the same length and width of a standard microscope slide. Within each Celselect Slide exists a microfluidic pathway with a series of chambers that function to filter out unwanted cell types and
capture target cells based on cell size and deformation properties. See Figure 3. Celselect slides are also optically transparent, thereby allowing users to image, analyze, and characterize cells within the slides. Additionally, captured cells can be retrieved from the Celselect slide and then used for downstream applications such as cell culture.

On the benchtop, Celselect slides first must be mounted on to a slide station with a Celsect manifold and funnel. Next the slide is primed by pumping a buffer solution through the microfluidic chambers using the Celsee Priming Genie. The prepared slides for a Celsect protocol are mounted in slide stations which are secured in the Genesis System lanes. Each lane is connected to the respective waste jar via tubing connected to the slide manifold outlets. Using the Genesis System’s touchscreen interface, a user can initiate either a standard, Celsee certified protocol, or a custom-made protocol (if the end-user has purchased the Genesis System Protocol Builder).
13.4 **Genesis System Architecture**

The Genesis System is a benchtop system that hosts several different mechanicals, PCB’s, fluidic and pneumatic components that all integrate and function to support the Celsingle and Celselect Technologies. The Genesis System measures 16.5 inches tall, 17.5 inches in width, 23 inches in depth and weighs 44 lbs. It is powered by a 24V power supply and has a retractable lid that users can open to reveal 2 lanes, each lane can support one Celsingle or Celselect Slide at a time. See *Figure 5*.

The exterior shell of the Genesis System can be removed by unfastening 6 hex screws to provide access to the all components of the system. See *Figure 6 and 7*. 

*Figure 4: Celselect Slide Station on the Genesis*

*Figure 5: Genesis with Open Lid*
At the front of the Genesis System’s base plate exists the screen bezel mounting plate which supports the mother board, LCD touchscreen display, door lock, audio speaker and other ancillary components. Fixed on standoffs in middle of the base plate is the lane mounting plate which supports both lane assemblies. Each lane assembly includes a Peltier element with fan, piercer block, actuator, pinch valve, level sensor, motor cartridge, and lane plate. Beneath the lane...
mounting plate on the right side are manifold valves and peristaltic pump for each lane. Behind the lane mounting plate is a collection of printed circuit boards. Secured directly to the base plate on the bottom are two TEC boards, above them is the daughter board with direct header connected freedom board. Above the daughter board on standoffs is the AUX board. Located at the back of the base plate are two cooling fans, a power terminal, a USB port, an ethernet port, and a power switch.

The Genesis System uses the on-board computer integrating Raspberry Pi3 B+, touch display and Arch Linux operating system to orchestrate all components. Each lane can process one Celsingle or Celsect slide at a time. The on-board computer SD memory card (16GB) stores instrument software, test gui, calibration files, log files, and protocols. External interfaces include ethernet port for networking capabilities and remote VM support. Also, USB 2.0 to support external keyboard or USB drive for importing or exporting files. In conjunction with the Raspberry Pi3, daughter board, AUX board, and freedom board is to drive all automated mechanical components. The Genesis System hardware architecture is displayed in the flow chart below. Figure 8 displays the relevant components of the Genesis system and indicates how they interact with one another.

![Figure 8: Genesis System Hardware Architecture](image)

Each component of the Genesis System function to support Celsee applications using a defined protocol. Essentially the Genesis System performs three core functions that impact a given run: 1) dispenses reagents 2) generates a pressure differential 3) controls temperature of a slide (~2 – 100°C). Actuator and piercer assemblies dispense reagents, peristaltic pump generate pressure differentials, and Peltier elements drive temperature changes. Various other components support these functions and the Celsingle and Celsect applications. Below is a table that defines each relevant component of the Genesis System and describes its location and function.
## Genesis System Technical Overview

<table>
<thead>
<tr>
<th>Component</th>
<th>Type</th>
<th>Function</th>
<th>Location</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent Cartridges</td>
<td>Consumable</td>
<td>Holds up to 10 reagents for dispensing into funnel</td>
<td>On motor cartridge spindle</td>
<td>2</td>
</tr>
<tr>
<td>Reagent funnel</td>
<td>Consumable</td>
<td>Reagents go into funnel ready to be dispensed on slide</td>
<td>Attached to slide</td>
<td>2</td>
</tr>
<tr>
<td>Reagent Lid</td>
<td>Reusable</td>
<td>Lid to cover the Celsingle slides to support the Celsingle protocols</td>
<td>Positioned above the micro well of the Celsingle slide</td>
<td>2</td>
</tr>
<tr>
<td>Slide (Celsingle or Celselect)</td>
<td>Consumable</td>
<td>Main technology involved with the isolation of single cells</td>
<td>Attached to slide manifold and slide station</td>
<td>2</td>
</tr>
<tr>
<td>Reagent Tubing</td>
<td></td>
<td>Transfer of reagents from slide to reservoir</td>
<td>Attached to slide manifold</td>
<td>2</td>
</tr>
<tr>
<td>Peristaltic Pump</td>
<td>Electromechanical</td>
<td>Supply a pressure difference across a slide</td>
<td>Attached to base plate of Genesis</td>
<td>2</td>
</tr>
<tr>
<td>Level sensor</td>
<td>Electronic</td>
<td>Monitors level of reagents in inlet funnel</td>
<td>Attached just behind the spindle</td>
<td>2</td>
</tr>
<tr>
<td>Peltier</td>
<td>Electronic</td>
<td>Drives temperature change across slides</td>
<td>Located beneath the slide in each lane</td>
<td>2</td>
</tr>
<tr>
<td>Freedom Board</td>
<td>Electronic</td>
<td>Control</td>
<td>Attached on the Daughter board</td>
<td>1</td>
</tr>
<tr>
<td>Power Supply</td>
<td>Electronic</td>
<td>Supply’s power to all boards, electronics and Genesis instrument</td>
<td>Located in series with the Genesis power cable</td>
<td>1</td>
</tr>
<tr>
<td>Aux Board</td>
<td>Electronic</td>
<td>Controls components of Genesis</td>
<td>Mounted on the Daughter board</td>
<td>1</td>
</tr>
<tr>
<td>Motor</td>
<td>Electromechanical</td>
<td>Spins the reagent cartridges</td>
<td>Attached on the bottom of lane mounting plate</td>
<td>2</td>
</tr>
<tr>
<td>Home Sensor</td>
<td>Electronic</td>
<td>Detects the position of the cartridge</td>
<td>Mounted on the bottom of the lane mounting plate above the motor</td>
<td>2</td>
</tr>
<tr>
<td>Linear Actuator</td>
<td>Electromechanical</td>
<td>Extends and retracts the piercer plate</td>
<td>Attached on lane assembly</td>
<td>2</td>
</tr>
<tr>
<td>Daughter Board</td>
<td>Electronic</td>
<td>Controls components of Genesis such as UV LED, Audio</td>
<td>Mounted on standoffs on the base plate</td>
<td>1</td>
</tr>
<tr>
<td>LCD Touch Screen for Raspberry Pi</td>
<td>Electronic</td>
<td>Display information and user interface with Raspberry Pi</td>
<td>Attached to front of instrument</td>
<td>1</td>
</tr>
<tr>
<td>Computer 1.4GHz 1GB Raspberry Pi</td>
<td>Electronic</td>
<td>Functions as the Mother Board and the Genesis’s computer</td>
<td>Attached to front of instrument near screen</td>
<td>1</td>
</tr>
<tr>
<td>Manifold Valve</td>
<td>Electromechanical</td>
<td>Used to regulate pressure within the vacuum tubing, and pressure sensor lines</td>
<td>Attached on base plate</td>
<td>2</td>
</tr>
<tr>
<td>Pinch Valve</td>
<td>Electromechanical</td>
<td>Opens or closes pressure to the slide</td>
<td>Located in series between the waste jar and slide</td>
<td>2</td>
</tr>
<tr>
<td>Spindle Flag</td>
<td>Mechanical</td>
<td>Used to determine the relative position of the spindle</td>
<td>Located in spindle of motor</td>
<td>2</td>
</tr>
<tr>
<td>16GB SD Card MLC</td>
<td>Electronic</td>
<td>Attached to the Raspberry pi</td>
<td>Attached on Raspberry pi board</td>
<td>1</td>
</tr>
<tr>
<td>Internal Fan</td>
<td>Electromechanical</td>
<td>Reduces temperature within the Genesis</td>
<td>Attached to the rear of the Genesis base plate</td>
<td>4</td>
</tr>
<tr>
<td>Audio Speaker 4watt 2 Ohms</td>
<td>Electronic</td>
<td>Used for key sounds with Raspberry pi</td>
<td>Attached on front plate of Genesis</td>
<td>1</td>
</tr>
<tr>
<td>Battery Lithium Battery 3 Volt 3022</td>
<td>Consumable</td>
<td>Runs internal clock</td>
<td>Located on Aux board</td>
<td>1</td>
</tr>
<tr>
<td>LED 55lm 12mm bolt</td>
<td>Electronic</td>
<td>Displays that there is power to the instrument</td>
<td>Attached on front plate of Genesis</td>
<td>1</td>
</tr>
<tr>
<td>Assembly sensor door Genesis</td>
<td>Electromechanical</td>
<td>Locks the front door</td>
<td>Attached Genesis Screen bezel mounting plate</td>
<td>1</td>
</tr>
</tbody>
</table>