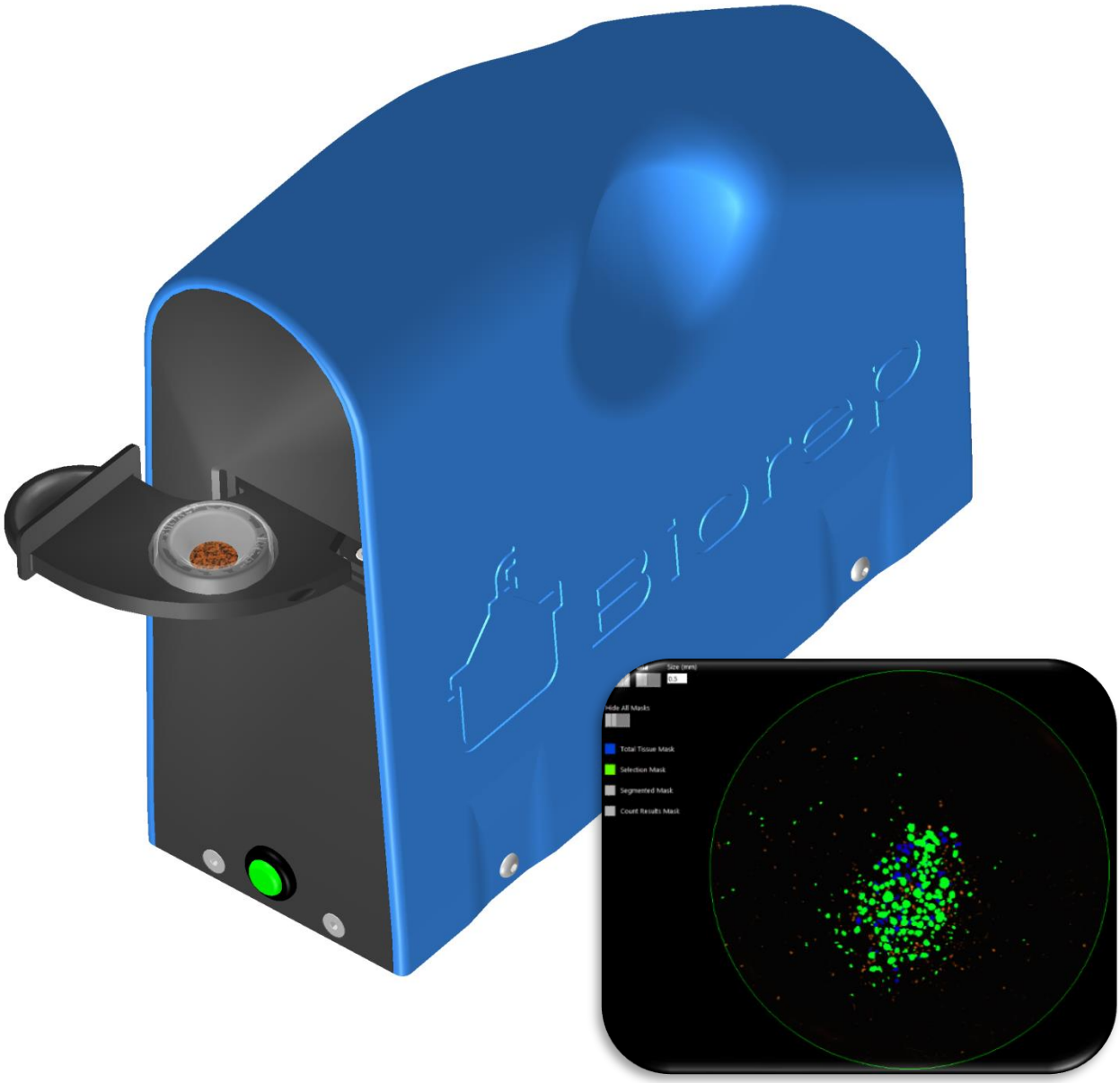
AUTOMATIC ISLET CELL COUNTER 4

BIOREP Ref. ICC-04

USER MANUAL



BIOREP®
TECHNOLOGIES

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1 Getting Started

WARNING:

Please, read this section before unpacking, installing or operating this machine.

1.1 Symbols Used in this Manual

The lightning flash with arrowhead symbol, within an equilateral triangle, is intended to alert the user to the presence of dangerous voltage within the machine's enclosure that may be of sufficient magnitude to constitute a risk of electric shock.

The exclamation point within an equilateral triangle is intended to alert the user to the presence of important information in the literature that accompanies the device.

1.2 Machine Information

In the spaces provided below, record the Model and Serial No. located on the bottom of your machine.

Model No. _____ Serial No._____

RETAIN THIS INFORMATION FOR FUTURE REFERENCE.

1.3 Contact Information

Biorep Technologies, Inc.
15804 NW 57th Ave
Miami Lakes, FL 33014
info@biorep.com
www.biorep.com
Tel: 305-330-4449
Fax: 305-330-4402

1.4 Safety Information

PLEASE READ AND OBSERVE ALL WARNINGS AND INSTRUCTIONS GIVEN IN THIS USER'S MANUAL AND THOSE MARKED ON THE UNIT.
RETAIN THIS BOOKLET FOR FUTURE REFERENCE.

DO NOT REMOVE THE MACHINE'S COVER OR YOU MAY BE EXPOSED TO DANGEROUS VOLTAGE. REFER SERVICING TO QUALIFIED PERSONNEL ONLY.

TO REDUCE THE RISK OF FIRE OR ELECTRIC SHOCK, DO NOT EXPOSE THIS DEVICE TO WATER OR MOISTURE.

READ AND FOLLOW THESE INSTRUCTIONS:

1. Keep these instructions for future reference and heed all warnings stated in this manual.
2. Protect the power cord, the power entry module, and the plug from being walked on or pinched to avoid damage.
3. Refer all servicing of the machine to qualified personnel. Servicing is required when the apparatus has been damaged in any way.

1.5 Packing List

The following items are included with the purchase of an Islet Counter:

Qty	Reference	Description
1	ICC4	Islet counter V3 vision system
1	ICC4-LT	Islet counter Laptop
1	TRD815WHT-3	Category 5E Patch Cable, RJ45 / RJ45
1	LFS123000D-A8B	Power Supply
1	ICC4-UM	User Manual
1	ICC-DISH	Islet Counter Dish pack (10)

Table 1: Packing List

2 Introduction

This User Manual is intended to be used by scientists, researchers, and technicians who have been trained in islet isolation procedures and technologies. This document contains the necessary information for installing and operating the islet counter vision system and its software.

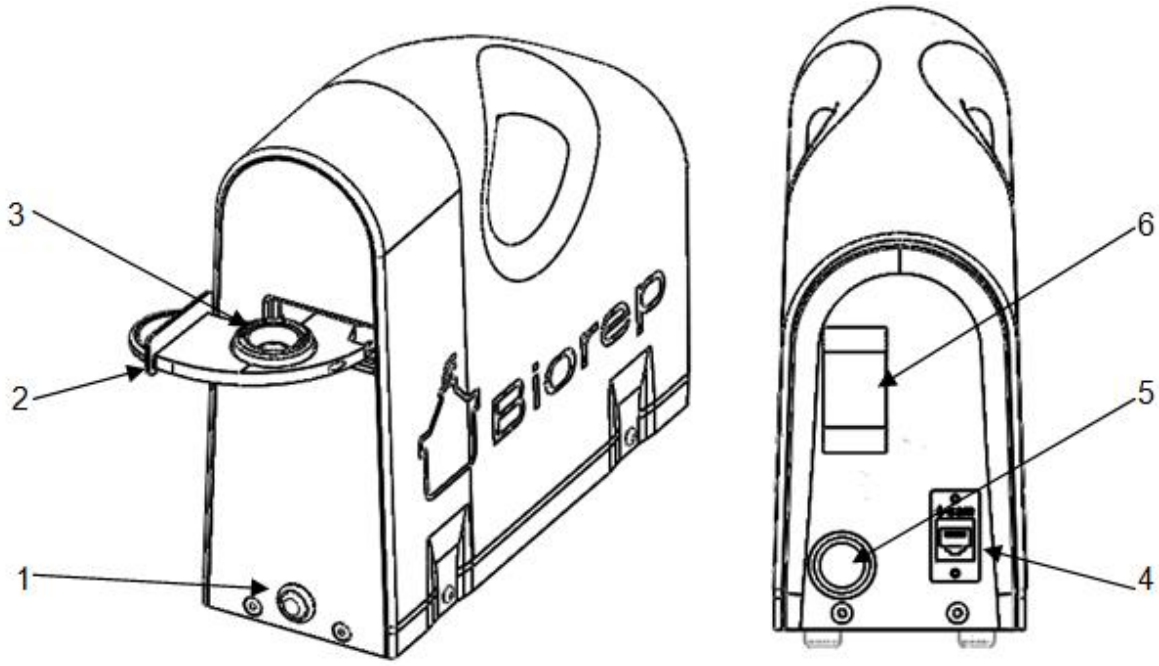
This Automatic Cell Counter has been developed to assist in the quantification of islet and other cell samples taken from isolated preparations; it is a useful tool to consistently determine yield of islet isolations or to quantify samples for Perifusion experiments.

The ICC was created to address the two main limitations of human counting: Speed and variability. Leveraging on machine vision technologies, the ICC can perform counts and generate a report in minutes including metrics like IEQ, area cover and purity. Trained human counters need several minutes to perform manual counts, and are usually required to fill the required documentation by hand to be later digitized. In addition, it is known that despite standardization efforts, islet counts can vary dramatically from one human counter to the other. The adoption of machine vision technologies reduces variability and ensures that the same counting method is used every time. An ICC in your lab will count with the same method to any other ICC out there, making results more comparable across research centers and hopefully more significant for peer reviewed publications.

Other software based cell counting technologies require training and rely on user experience and input to perform accurate counts; therefore, they are also subject to user judgment and variability. Furthermore, these software packages are independent of imaging hardware (such as microscopes, illumination and cameras), creating additional sources of variability.

The ICC is a self-contained machine vision system, that includes both hardware and software. This ensures that magnification, field of view, illumination, focus, and other optical variables are constant, making the measurements repeatable and reliable. The ICC software is powerful, yet its user interface is designed for intuitive workflow. Ease of use was a top priority when developing the ICC software; Now in its 4th generation, the software still provides the same easy wizard-driven interface to start counting on day one, plus super-user driven upgrades that allow advanced in-process manipulation of selection and segmentation results, powerful measuring tools, in-depth particle stats and reporting, and even customized selection settings for other cell types.
Islet counting has never been this easy.

3 Machine Features Identification



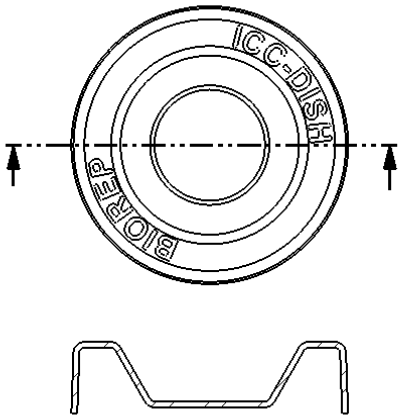
1. **Power switch / Power indicator LED**
2. **Sample tray**
3. **ICC Dish nest**
4. **RJ45/USB3 connector (to laptop)**
5. **Power supply connector**
6. **Illumination Switch (Disabled, intended for future expansion)**

Not much right?...As opposed to conventional microscopes, the ICC Vision system does not allow adjustments to be made by the user. This is actually a good thing. The vision system is carefully calibrated at the factory to be in focus, to have a consistent illumination, and to have a pre-set magnification factor. It is this careful calibration that allows automated counts to be repeatable and comparable across machines and across research centers.

4 Accessories

*(Some of these items must be ordered separately. For ordering information please consult our webpage **www.biorep.com** or contact one of our sales representatives at 305-330-4449.)*

4.1 Islet Cell Counter Dish (ICC-DISH)



The ICC-Dish was specifically designed for use with the ICC vision system. The central well is designed to provide enough area to allow the sample to be spread out to minimize errors due to aggregation, but compact enough to allow for high resolution imaging of the region of interest. The deep well is designed to minimize the risk of spillage during sampling and staining. The dish is accurately positioned with a matching nest geometry on the ICC tray.

5 Set-up of the ICC

5.1 ICC Connections

The ICC only requires 2 cables: Power and communications.

The external power supply (included) is connected to the standard 2mm round DC power connector. The power supply can be connected to a 110 or 220V outlet (outlet adapter not included)

Connection between the laptop and the vision system is done through a standard CAT5 network cable (included).

5.2 Islet Sample Preparation

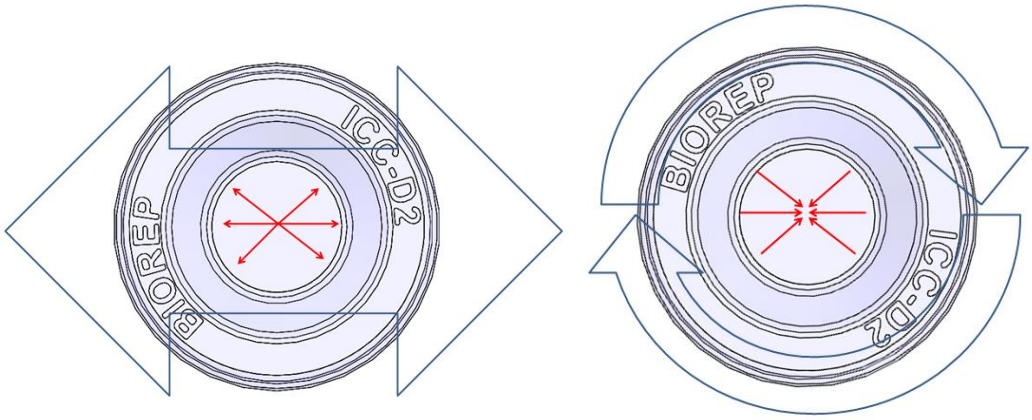
The recommended islet sample volume is 100 µL.
For best results follow these best practices:

If counting stained preparations, add 250 µL of DTZ solution (See recommended DTZ solution preparation in the next section). If counting an unstained preparation, add 250 µL of buffer solution.

The bottom of the dish must be completely covered in fluid. Partial fluid coverage creates shadows at droplet boundaries that cause artifacts that affect detection and count performance. The background of the image should be as a uniform as possible. A fill volume between 350 and 400 µL (Total) is recommended to ensure the dish surface is completely covered. Some slight leveling of the dish might be required to distribute the fluid uniformly.

Do not overfill the dish. Overfilling above 500 µL may have adverse effects on the illumination and therefore count accuracy. Avoid air bubbles in the dish. Large air bubbles cause similar problems to those of partial fluid coverage. Small bubbles might cause localized errors and may result in false positives.

The cell mass should be spread out as evenly as possible in the dish, but away from the edges. Agglomeration or "clustering" makes software segmentation harder and the results less reliable. Cells close to the edges risk being omitted. Gently shaking the dish, side to side in one direction, helps spread the cells on the dish. Swirling the dish in a small circle has the opposite effect and will tend to agglomerate the cells in the middle of the dish. Use these two techniques to achieve a good spread within the region of interest (ROI).



DO NOT LEAVE A FILLED ICC DISH STANDING FOR MORE THAN 20 MINUTES. Once a sample has been prepared it should be counted as soon as staining is stable. A standing dish may be subject to evaporation and other effects that mat have a detrimental effect on the count quality. LED Reflection artifacts may appear (as a dotted circular pattern on the ROI periphery) if the sample is left open to the environment for prolonged periods. If this happens, add 100uL of buffer solution and re-count.

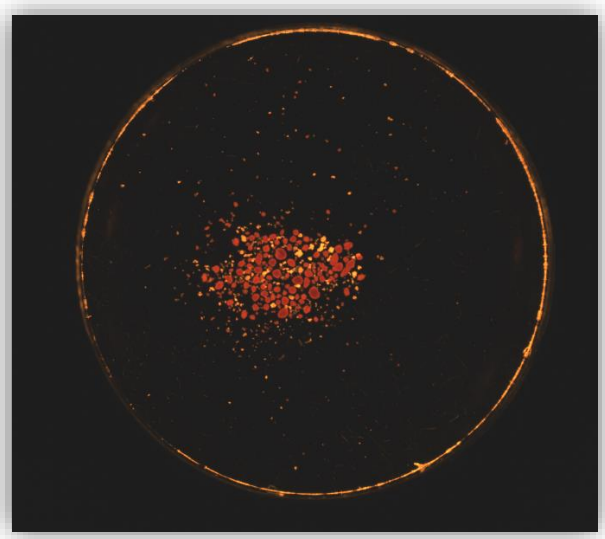
5.3 Sample Staining

Staining the sample with dithizone (DTZ) drastically improves the count accuracy by enhancing contrast between acinar tissue and islets. Whenever dealing with machine vision, repeatability is a key factor. Staining with DTZ has to be done in a repeatable and controlled fashion. For best results, the DTZ formulation/composition, the amount of DTZ added to the dish, and the uptake time allowed for the islets to pick up the stain need to be consistent. We recommend the following best practices:

Dithizone (DTZ) Solution Recipe

a. Dissolve 100 mg Dithizone in 20 ml Dimethyl sulfoxide (DMSO) and mix
b. Add 30 ml HBSS
c. Filter using a 0.2 or 0.4 μm bottle top filter
d. Add 50 ml HBSS
e. Label appropriately and use on the day of preparation
f. Store at room temperature (20-28°C).

DTZ uptake rates vary between samples. The value of the count may change as the cells get stained and it may take between 5 to10 minutes before staining stabilizes to perform an accurate count. The ICC automatically analyzes the sample in real time and will let you know when staining is stable and the sample is ready to count (see section 6.5). An average sample should look something like the image below.



6 Using the ICC

6.1 Power Up

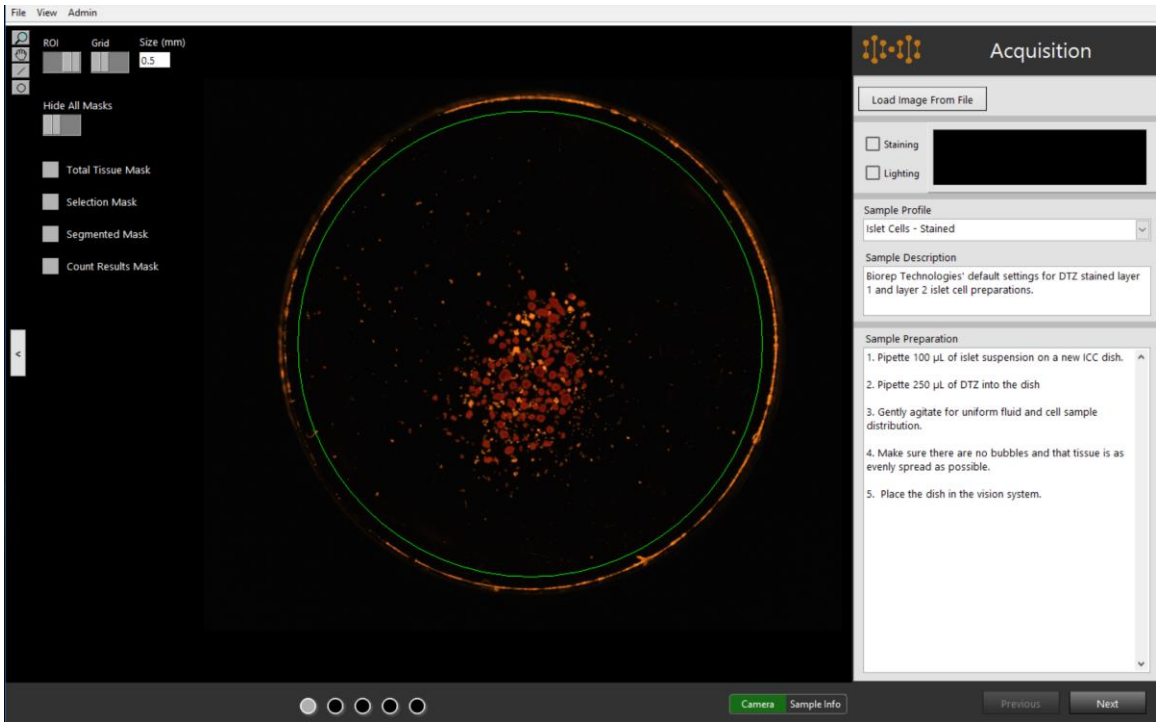
The main power is the green (and only) button on the front panel of the machine. Connect the power cord (provided in the accessory box) to the module, plug it into a 110 VAC (or 220 VAC) outlet and push the button. A green LED indicator should turn on.

Make sure the network/USB cable is connected to both the laptop and the vision system. Power up the ICC laptop. Boot into windows and double click on the ICC icon on the desktop.

6.2 Login

The ICC application starts with a splash screen. If everything is connected properly, the application will start acquiring a live image automatically. If the vision system is not detected, the splash screen will provide the option to EXIT or to SIMULATE an acquisition from file.

6.3 Acquisition



The GUI provides a live video feed of the sample so you can check the cell distribution within the Region of Interest (ROI), defined by a green circle within a safe margin from the dish edge. Remember that you want to spread the tissue as much as possible within the boundaries of the ROI; only the cells within the ROI will be counted.

LEFT- IMAGE CONTROLS: Selecting the **loupe** icon above the image will enable zooming. Zoom in by clicking on the region you want to explore. You can zoom out with SHIFT+click. If you wish to return to full screen view, right click and select “zoom to fit”. The **hand** tool allows you to pan a zoomed image. The line tool allows you to measure length between two points. The circle tool is inactive in this screen. Additional tools, like ROI toggling and a grid overlay are available by expanding the pane with the arrow button on the left margin.

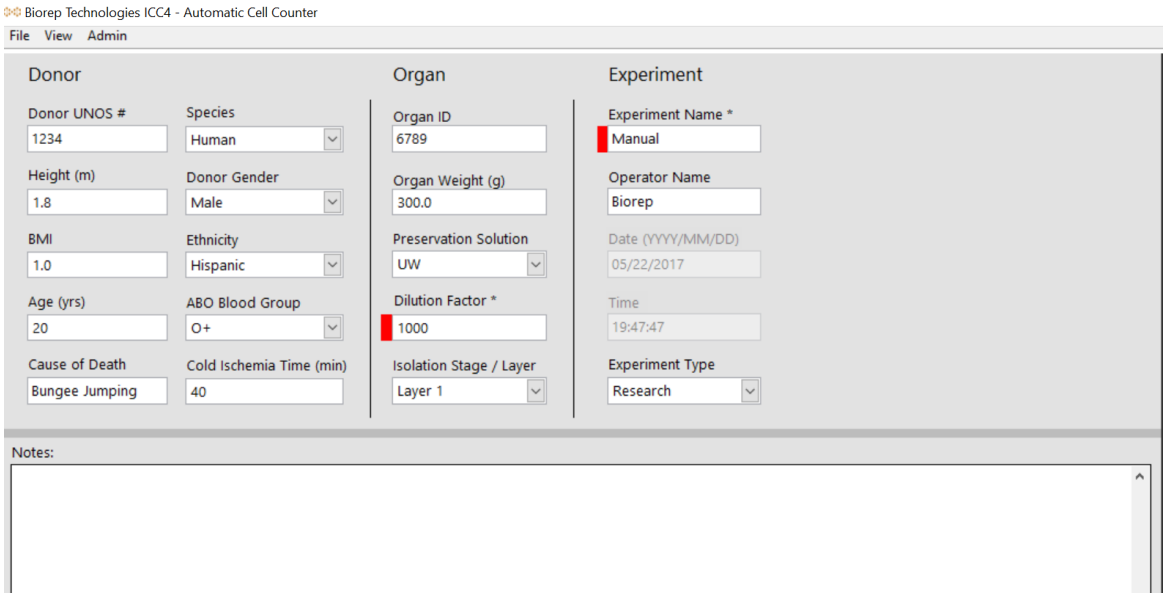
RIGHT- ACQUISITION PANELS: The top panel displays live sample analysis of lighting and staining parameters. These are monitored in real time and automatically display a check mark when both are stable and within the required range for accurate counting. Attempting a count when either of these fails may return inaccurate values, and requires confirmation of a pop-up warning.

Sample Profile can be selected by the user from a drop-down menu. The default mode is stained islets, but profile can be changed to unstained islets or to other user-defined profiles**. **Sample Description** displays the type of sample to be analyzed with active selection profile. The **Sample Preparation** panel describes all steps required to prepare the sample for counting with the active profile. The instructions change depending if your sample is stained or unstained, and the user can define their own preparation protocols when crating new profiles**.

***Please contact tech support if you would like to add a customized setting.*

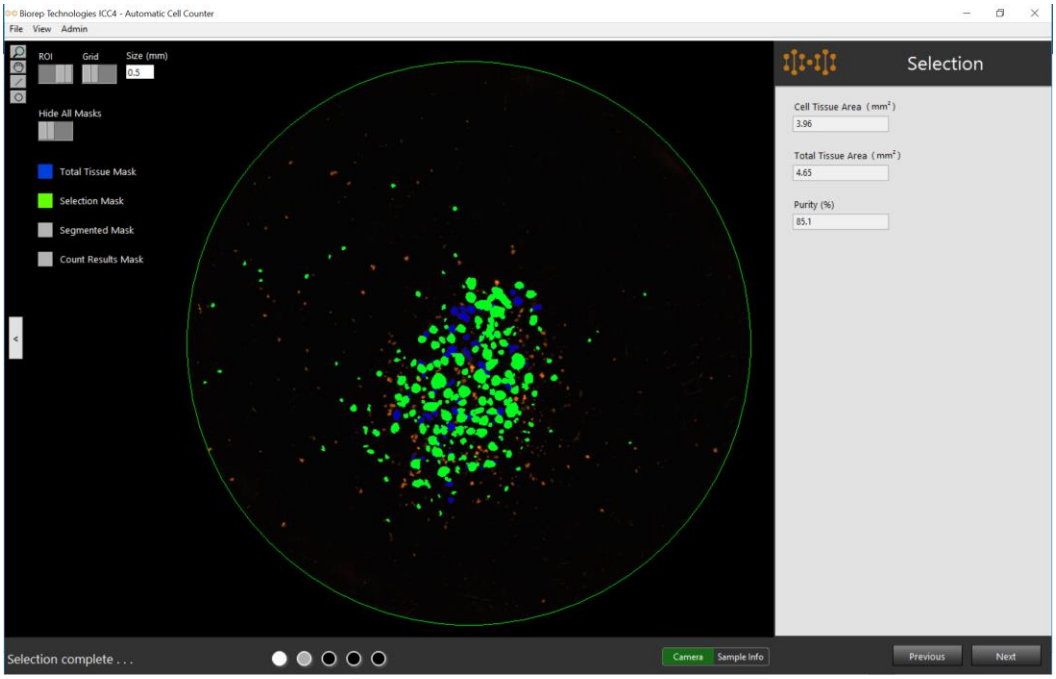
SAMPLE INFO: Click the button on the bottom bar to enter all sample/operator related info.

On the **Sample Info** page you can enter the information related to the sample being counted. Donor information, organ information, etc., can be filled with the keyboard or by selecting one of the choices in the drop-down menus. It is very important that you check the dilution factor is correct before proceeding. The machine has a default setting of 1000 (100uL out of 100ml).



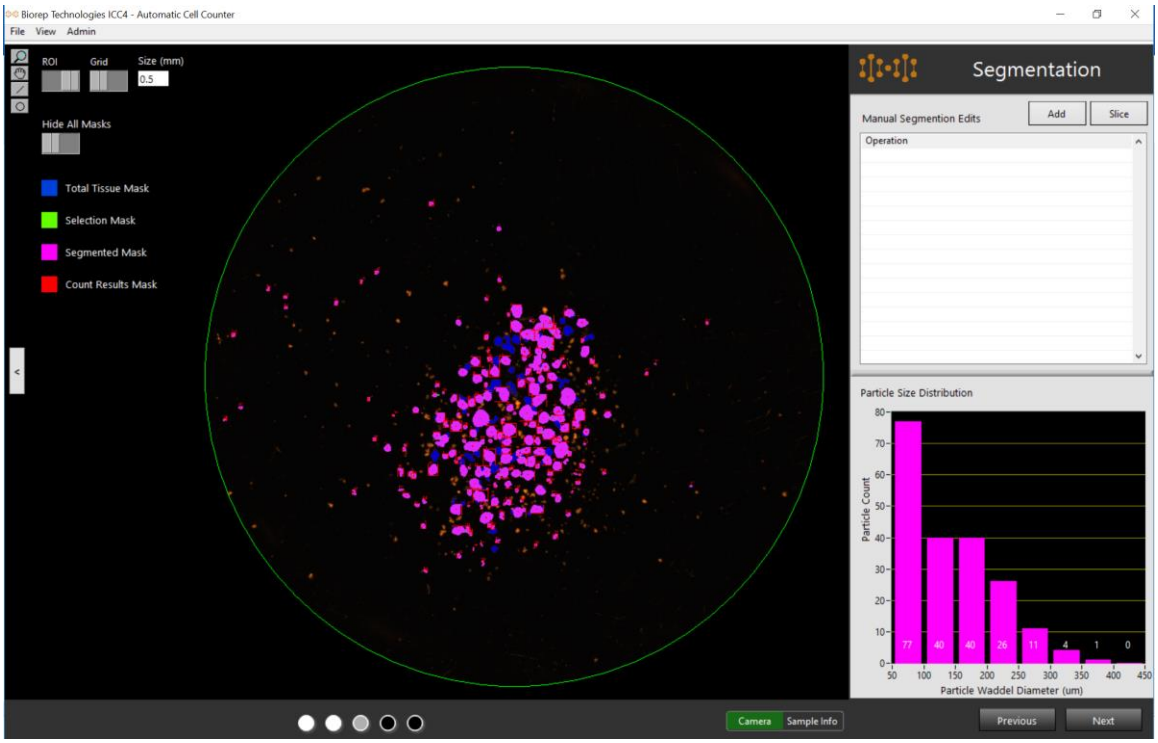
Press “Next” to continue to the Selection step.

6.4 Selection



The selection page displays color overlays of the detected tissue according with the filter parameters of the “Sample profile” selected. The **GREEN** overlay shows selected cell tissue, or tissue to be counted. The **BLUE** overlay displays all other tissue or particles present in the dish that fall within the inclusion criteria determined by filters in the profile. A quick calculation of cell tissue area and total tissue area are displayed, as well as their ratio as a measure of purity (%). Overlays can be turned on and off by toggling their icons in the dynamic pane on the left.

6.5 Segmentation



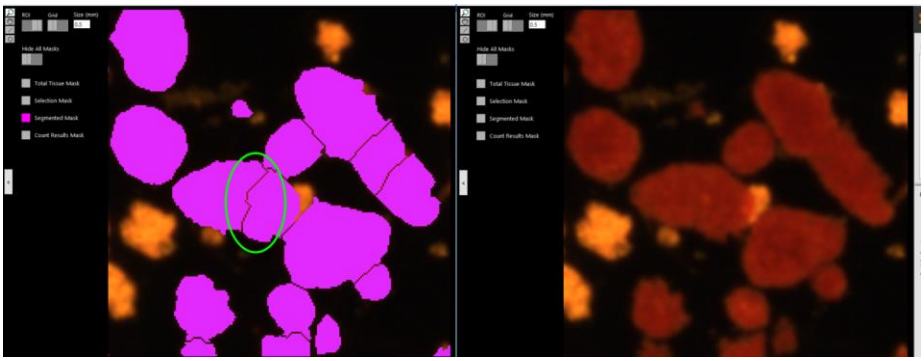
Segmentation is the step that attempts to determine particle boundaries from a digital selection map containing overlapping or touching particles. This is one of the most challenging aspects of automatic cell counting, especially with islets. Islets are not individual cells, but rather cell clusters that can vary significantly in size. The system needs to detect and account for islets ranging from 50um in diameter to 500um in diameter or more, within a random, overlapping and agglomerated sample. This means a difference of up to 100 times the area between the smallest and largest particles can occur. The ICC software uses proprietary algorithms to separate and sort particles in size groups, per the Edmonton protocol[1]. This distribution is used to determine IEQ results.

Segmentation results are presented as a **PINK** overlay on the selection map. This overlay will show the “cut-lines” where the software has determined there should be a boundary between touching particles. However, the algorithm accuracy depends greatly on sample quality. Samples with high degree of aggregation (overlapping or touching particles) and high degrees of impurity may be more challenging to segment and can lead to false positives (mistakenly splitting a single cell) or false negatives (failing to separate joined cells). In a sample with many particles (>200), segmentation errors will not contribute significantly to total error, and results should be within the acceptable error margin for most cases. However, for smaller or more critical samples where error needs to be minimized, the user can now edit the segmentation to split or join cells.

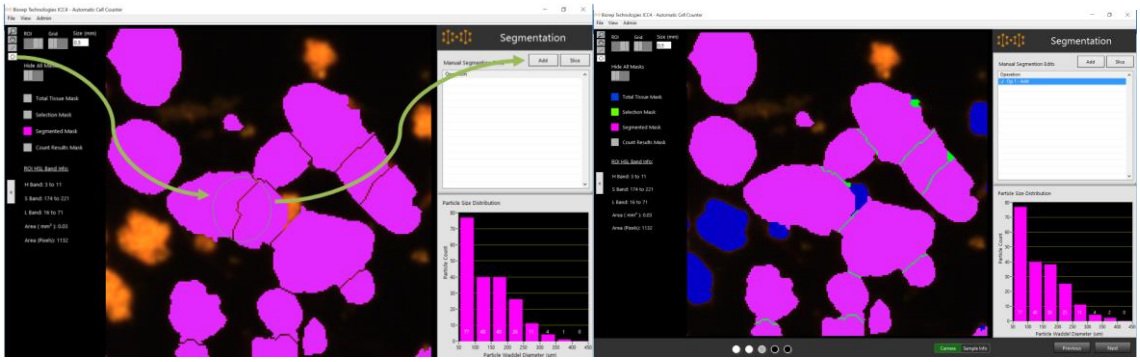
6.5.1 EDITING SEGMENTATION.

A new feature of the ICC4 is the ability for the user to edit the segmentation results. Edits may involve removing a cut-line, creating a new cut-line between cells and even selecting un-selected cells.

CORRECTING FALSE POSITIVES (removing a cut line): The user can correct a false positive by “adding” to the segmentation mask. Consider the following example, where a cut line created by the segmentation algorithm may be splitting a single large elongated cell.

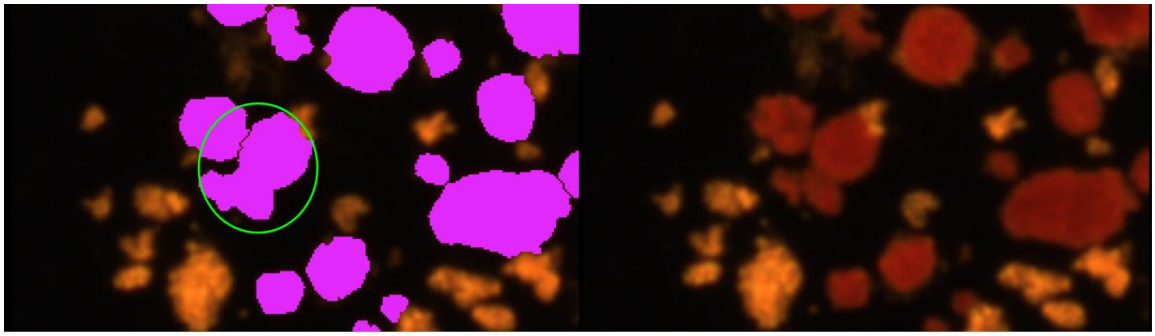


To remove the cut-line, the user can use the circle tool to create a region that bridges the two cell halves. The region is added to the segmentation mask by clicking “Add” in the Manual Segmentation Edits.

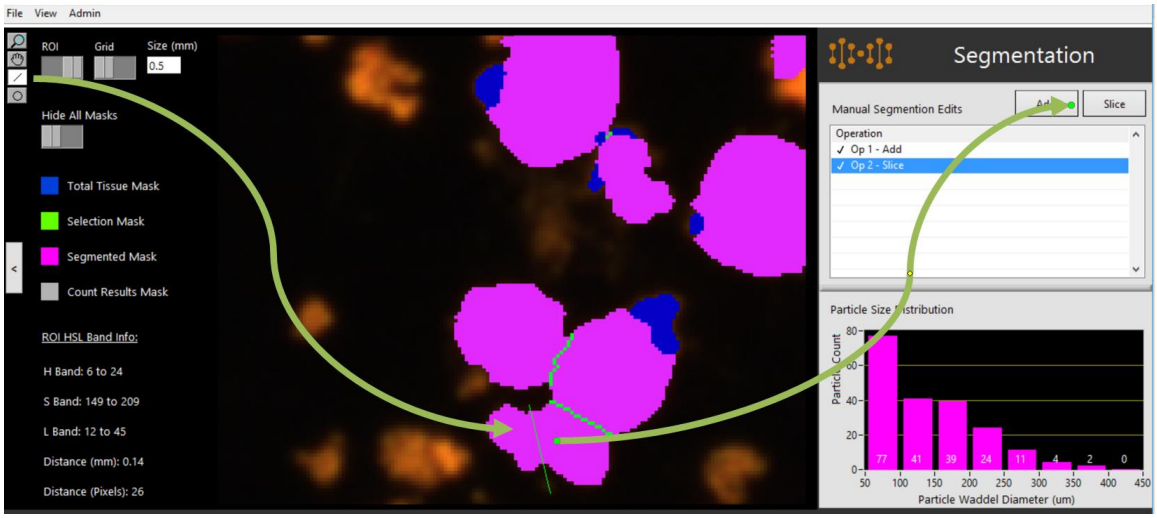


The operation will be listed in the right pane, and the particle size distribution will be updated accordingly. Use the same method to add cells that have been omitted by the software.

CORRECTING FALSE NEGATIVES (Adding a cut line): A cut line can be added to separate overlapping cells not detected by the software. Consider the following example where overlapping cells were not segmented and selected as a single cell:



The single cluster is made of three smaller islets, so we would like to add two cut lines to separate them. Use the line tool to draw the boundary line between cells and then select “Slice”.



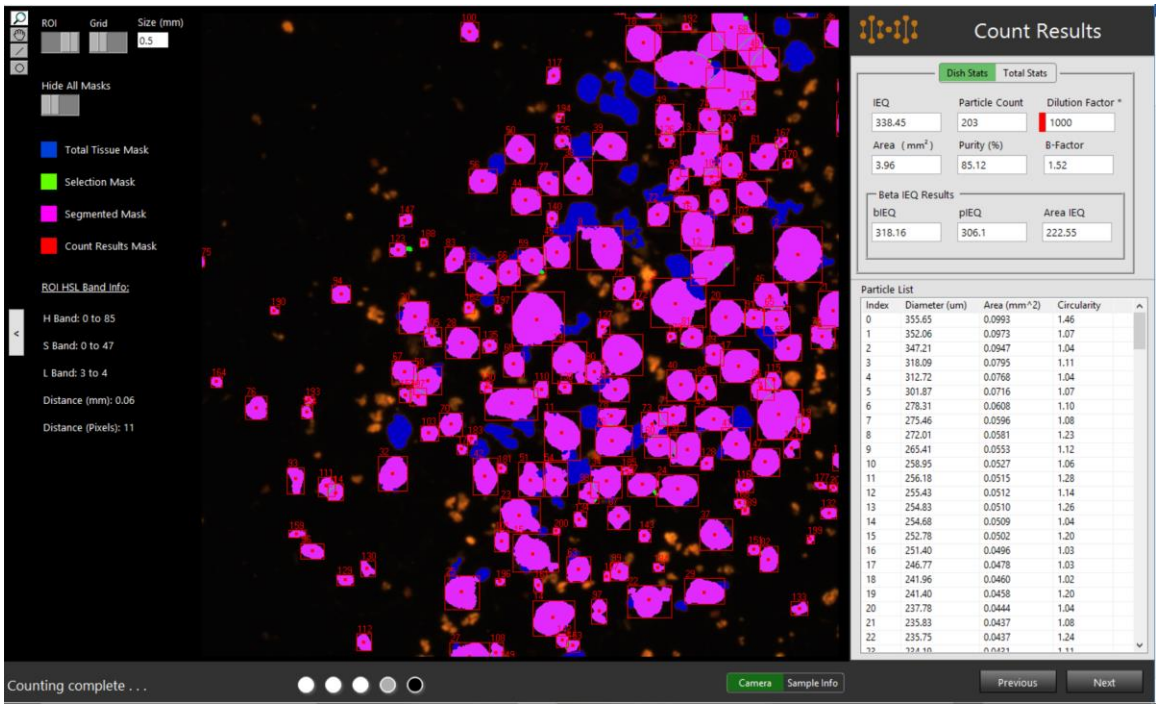
The operation will be listed in the right pane, and the particle size distribution will be updated accordingly.

***IMPORTANT**: Manual editing of automatic segmentation will make results user-dependent. It must be understood that automatic counting is not expected to be more accurate than any single expert human counter, but to count more repeatably and consistently than human counters as a group. Although automatic counting may not be 100% accurate, the methods used, and therefore the error margin, are the same across islet counters deployed world-wide. Samples that have manual segmentation edits will be noted as such in the report.*

After segmentation, the sample is ready to be counted. Check the “Next” button to generate a count.

6.6 Count Results

The count results displayed are the following:



IEQ displays the total islet equivalent in the preparation as calculated from the size groups per the Edmonton protocol.

PARTICLE COUNT displays the approximate number of individual islets present in the dish, without taking their size into consideration

DILUTION FACTOR is displayed as entered in the information fields on the Sample Info page. It can be edited at this point and it immediately affects the TOTAL STATS displayed.

AREA provides the islet area cover in millimeters squared. This can be a good alternate metric of islet quantity, which is not subject to size grouping like IEQ, and is less sensitive to cell aggregation.

PURITY provides a ratio between the area covered by islets and the total area covered by tissue in the sample

B-factor. The B-factor is an index of islet size distribution. A B-factor of 1 means that there is no significant impact of size distribution. As the B-factor increases above 1, it signals that large particles are predominant. If small islets are prevalent, the B-factor will be smaller than 1. It is up to the researcher to use and determine any acceptance criteria based on the B-factor.

A great new feature for the ICC4 is the ability to go through the list of detected particles. Clicking on the desired particle will automatically center the image on it. Zoom in and click on different particles on the list to see how it works. The list is arranged by size, starting with the larges, allowing the user to easily review the more critical contributors to the result.

6.6.1 Beta IEQ Results.

The main islet quantification index reported by the system is the IEQ, calculated by the machine following the standard method developed for human counting more than 20 years ago. The method for human counting was developed to achieve a compromise between accuracy and efficiency. Measuring and analyzing each individual islet would have been impractical in an isolation setting, and calculating exact areas of islets would prove practically impossible to do manually. Thus, the size-group binning method provided a expedient way for humans to quantify islet samples, and also established a standardized way to do this across the islet research community.
The ICC reproduces the human IEQ counting method so its results are immediately comparable and useful in current isolation and research SOPs using IEQ as the standard metric. However, the vision system is capable of quantifying islets in different, more precise ways, not available to the human counter before.

IEQ: The original, and generally accepted, metric for islet volume quantification described in Ricordi 1990 [1]. The following table is an excerpt from the paper showing the coefficients used in ICC4:

islet diameter range (μ)	mean volume (μ^3)	conversion into islets of 150 μ diameter
50-100	294,525	n/ 6.00
100-150	1,145,373	n/ 1.50
150-200	2,977,968	n× 1.7
200-250	6,185,010	n× 3.5
250-300	11,159,198	n× 6.3
300-350	18,293,231	n×10.4
350-400	27,979,808	n×15.8

Tab. 1 - Determination of islet volume for each 50 μ diameter range and relative conversion

bIEQ: This is a quantification very similar to IEQ, but with slightly corrected multiplication factors, based on better understanding of particle distribution as shown by Buchwald 2009 [2]

Table 2. Currently Used and Proposed IEQ Conversion Factors C_{IEQ} per Standard Islet Size Groups

Islet Size Group (d, µm)	C_{IEQ} Current SOP	C_{IEQ} Proposed
<50		(0.01)
50–100	0.167	0.13
101–150	0.667	0.58
151–200	1.685	1.55
201–250	3.500	3.27
251–300	6.315	5.96
301–350	10.352	9.83
351–400	15.833	17.60
>400	(22.750)	

pIEQ: This is a quantification of total IEQ based on the sum of the individual IEQ of each particle. In other words, particles are not grouped in size bins, but rather each individual particles' IEQ is calculated and added to get a total pIEQ. This method is very impractical for human counters, but available with vision systems.

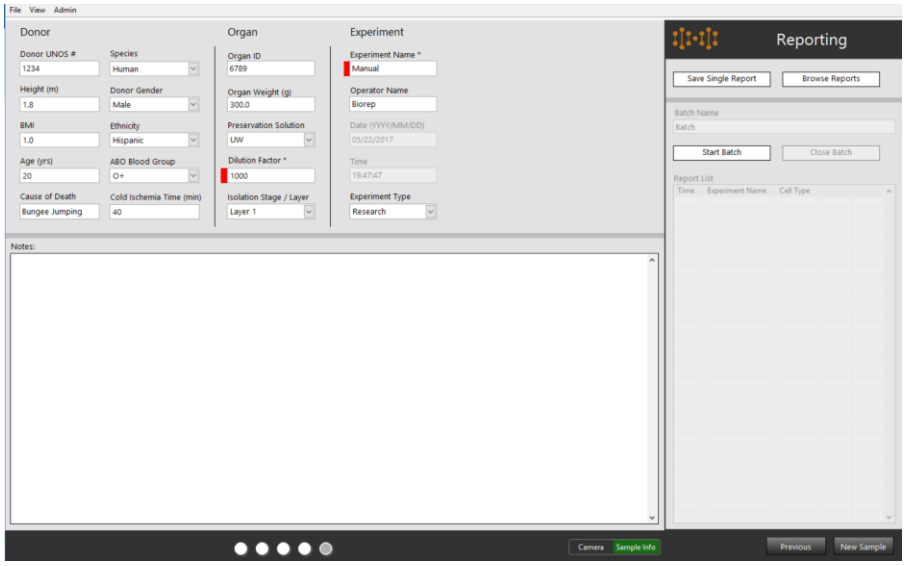
pIEQ = SUM for all particles[Volume of Particle/Volume of 150 micron Islet)

ArealEQ (aIEQ): This method reports the **islet area cover** in IEQ terms. The system computes the total area covered by islets, and then, to convey results in a familiar value, reports the number of unit IEQs (islets of 150µm) required to create the same area cover. Area IEQ is the most efficient and accurate metric to calculate for a vision system. It is also the least sensitive to segmentation and binning error. Although this method may be the least "standard" of the indexes, it leverages the ability of the vision system to precisely compute area cover; this is something previously impossible and unavailable to the human counter. The correlation of islet Area cover to volumetric content is yet to be determined, but it should provide an useful index [3] and we encourage the user to experiment with it and report their findings.

aIEQ = Islet Area Cover / Cross-sectional Area of 150 micron Iselt

6.7 Reporting

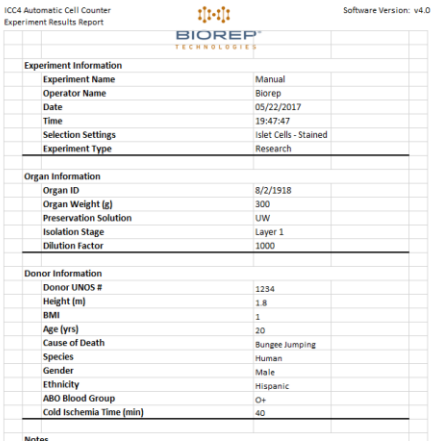
For every count, a full resolution image of the sample and a comprehensive report can be generated and saved in the ICC4 folder on C:\Users\Biorep\Documents\ICC4 Report Data. The report contains all the information, results and relevant count statistics of the count as well as some graphical presentations of the IEQ results.



The reporting page allows the user to review the donor, organ and experiment info, and add some notes relevant to the sample or count. The user can then generate a single report for that sample, or start a Batch of reports. A Batch of reports is useful when counting several samples of the same preparation/layer. The batch report will provide you with individual metrics of each sample as well as average metrics for the batch, allowing for a more detailed and significant analysis of the larger preparation, since sampling variability plays an effect on count results.

Click “Browse Reports” to open the folder containing all reports.

The 1st page of the report contains the general count, user and donor inforrmation, such as time and date, operator name and organ ID. It also contains the general notes filed where you can type in observations not captured in the default form. Note the note at the bottom: EXPERIMENT INCLUDES GMENTATION EDITS. This will be present whenever the user has atered the automatic segmentation results.



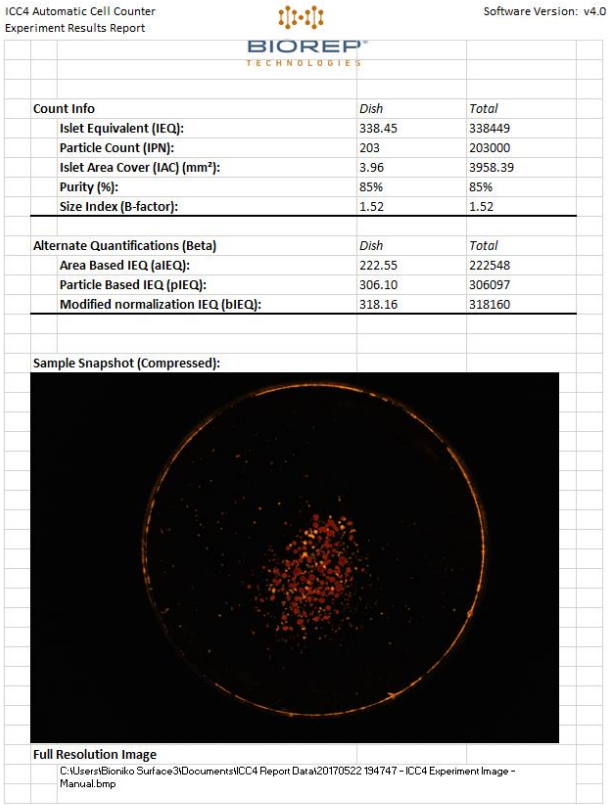
ICC4 Automatic Cell Counter
Experiment Results Report

Software Version: v4.0

BIOREP TECHNOLOGIES

Experiment Information	
Experiment Name	Manual
Operator Name	Biorep
Date	05/22/2017
Time	19:47:47
Selection Settings	Islet Cells - Stained
Experiment Type	Research
Organ Information	
Organ ID	8/2/1918
Organ Weight (g)	300
Preservation Solution	UW
Isolation Stage	Layer 1
Dilution Factor	1000
Donor Information	
Donor UNOS #	1234
Height (m)	1.8
BMI	1
Age (yrs)	20
Cause of Death	Bungee Jumping
Species	Human
Gender	Male
Ethnicity	Hispanic
ABO Blood Group	O+
Cold Ischemia Time (min)	40

Notes



ICC4 Automatic Cell Counter
Experiment Results Report

Software Version: v4.0

BIOREP TECHNOLOGIES

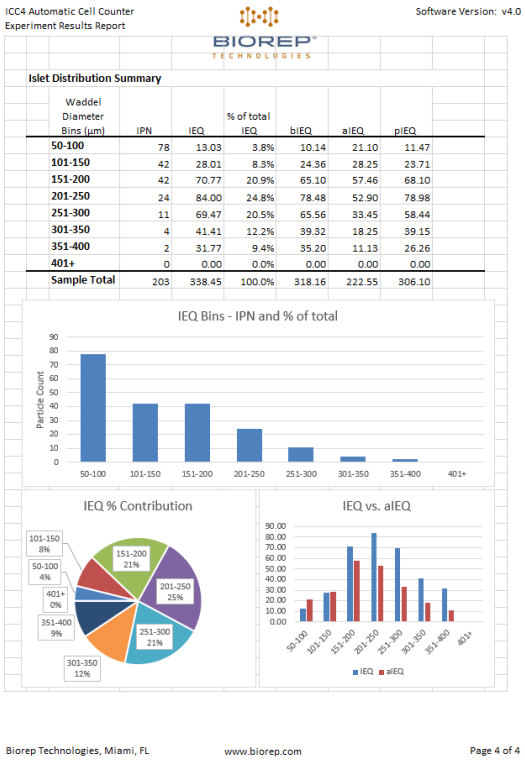
Count Info	*Dish*	*Total*
Islet Equivalent (IEQ):	338.45	338449
Particle Count (IPN):	203	203000
Islet Area Cover (IAC) (mm²):	3.96	3958.39
Purity (%):	85%	85%
Size Index (B-factor):	1.52	1.52
Alternate Quantifications (Beta)	*Dish*	*Total*
Area Based IEQ (aIEQ):	222.55	222548
Particle Based IEQ (pIEQ):	306.10	306097
Modified normalization IEQ (bIEQ):	318.16	318160

Sample Snapshot (Compressed):

Full Resolution Image

C:\Users\Bioniko Surface3\Documents\ICC4 Report Data\20170522 194747 - ICC4 Experiment Image - Manual.bmp

The 2nd page displays the count metric, including IEQ, IPN, Area, Purity and the alternate quantifications like aIEQ. It also displays a low-res image of the sample for reference. A Full-resolution image is saved alongside the report as a separate file with the same time-stamp/filename



ICC4 Automatic Cell Counter
Experiment Results Report

Software Version: v4.0

BIOREP TECHNOLOGIES

Islet Distribution Summary

Waddel Diameter Bins (µm)	IPN	IEQ	% of total IEQ	bIEQ	aIEQ	pIEQ
50-100	78	13.03	3.8%	10.14	21.10	11.47
101-150	42	28.01	8.3%	24.36	28.25	23.71
151-200	42	70.77	20.9%	65.10	57.46	68.10
201-250	24	84.00	24.8%	78.48	52.90	78.98
251-300	11	69.47	20.5%	65.56	33.45	58.44
301-350	4	41.41	12.2%	39.32	18.25	39.15
351-400	2	31.77	9.4%	35.20	11.13	26.26
401+	0	0.00	0.0%	0.00	0.00	0.00
Sample Total	203	338.45	100.0%	318.16	222.55	306.10

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The 3rd page contains the count statistics, including graphical visualizations of the results. The top table shows the numerical results per islet group.

IPN/BIN Bar graph shows the numerical quantity of particles in each IEQ size group. Generally speaking, the quantity is inversely proportional to the size group. The smaller the islets, the more there are.

% CONTRIBUTION Pie chart, shows how much of the total IEQ is contributed by each IEQ size group. This allows to users to see that even though the smaller cells are way more numerous, their contribution to the total is relatively small.

IEQ vs. aIEQ – It has been discussed in this manual how the aIEQ metric is more suited for automated vision systems, such as the ICC4. The comparative bar graph allows the user to compare both metrics in an effort to provide ways to incorporate the aIEQ metric in the workflow. The example shows how the aIEQ metric is more conservative, and may be more consistent with recent findings in volumetric estimations of islets [4].

6.8 Raw data file

The Islet counter also outputs a raw table of all particles and their parameters. The file is generated in the same excel report, under an additional worksheet/tab.

For reference, the image has the following dimensions in pixels (pixel size parameter for your system can be found in the “Pixel Size Calibration Page”).

o Width = 3,840 pixels = approx 20.43mm,

o Height = 2,748 pixels = approx 14.62 mm,

File does not display units. See list below for applicable units.

The CSV file has the following data:

 Particle Number - consecutive - Unique particle number I.D.

 Center of Mass X - mm - X coordinate of center of mass of particle

 Center of Mass Y - mm - Y coordinate of center of mass of particle

 Bounding Rect Left - mm - Left side of the bounding rectangle (smallest rectangle with sides parallel to the x-axis and y-axis that completely encloses the particle.)

 Bounding Rect Top - mm - Top side of the bounding rectangle

 Bounding Rect Right - mm - Right side of the bounding rectangle

 Bounding Rect Bottom - mm - Bottom side of the bounding rectangle

 Area - mm^2 - Measured area of particle

 Orientation - deg - The angle of the line (from horizontal) with the lowest moment of inertia.

 Bounding Rect Width - mm - Width of the bounding rectangle

 Bounding Rect Height - mm - Height of the bounding rectangle

 Number of Holes - integer - Number of holes in the particle

 Heywood Circularity Factor - index - Perimeter divided by the circumference of a circle with the same area. The closer the shape of a particle is to a disk, the closer the Heywood circularity factor is to 1. The larger the number, the more irregular the perimeter.

 Waddel Disk Diameter - mm - Diameter of a disk with the same area as the particle. This is the metric used to assign particles to different size groups per human counting protocol.

 Hydraulic Radius - mm - Particle area divided by the particle perimeter.

7 Customer Service

If you encounter any problems, please contact customer support at:

Biorep Technologies, Inc.
15804 NW 57th Ave
Miami Lakes, FL 33014
sales@biorep.com
www.biorep.com
Tel: 305-330-4449
Fax: 305-330-4402

8 References

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