Akademiska laboratoriet   ID: AL5478-2
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Utförandebeskrivning   Fastställd av: Olle Korsgren

Digital bildanalys av isolerat pankreasmaterial med QWin Digital image analysis of isolated pancreatic tissue with Qwin 710967

DIGITAL IMAGE ANALYSIS OF ISOLATED PANCREATIC TISSUE

Abbreviations, Codes, and Definitions

Routine	Computer macro, series of commands or processes
IEQ	Islet equivalents
DIA	Digital imaging analysis

Safety Routines

All work with human material always carries a risk for transferring disease, see Skyddsföreskrift laboratoriearbete KITM AL4731 and Hygienregler för Akademiska sjukhuset AL9112).

Procedure

1. Open the program Leica QWin V3. If the program alerts "Security device not found – continuing in Demo Mode" ensure that the purple QWin/Runner USB key is placed in a USB port, then exit and restart the program.

    Opening routine/macro

2. Click on the "QUIPS" menu and choose "Run Current Routine…" or simply click the "Run Current Routine…" button on the toolbar at the top of the window. This will prompt the last routine used to begin anew. The routine used to analyze pancreas tissue in the islet lab is "**Islet Analysis Macro**". If this does not work, click on the "QUIPS" menu and choose "Open and Run Routine…" and choose **Islet Analysis Macro**. This can be found at på **F:/cellulärimmunologi/Ö-lab/Bilder – humanisoleringar/Makro**. This option can be used to select a different routine.

3. Enter the initials of the person analyzing the photos. Click OK or hit enter.

4. A dialog asking for calibration information will pop up. Choose the appropriate option. For the islet lab, you should choose Leica. **If** Local Calibration is chosen make sure to have the calibration value (number of microns per pixel) available, enter it in the field and click Apply.

5. A window will open to select the picture to be analyzed. If analyzing pictures taken with the DFC490 camera in the islet lab the file path is "F:/cellulärimmunologi/Ö-lab/Bilder – human isoleringar/" followed by the H number and what day it was taken (e.g. "Iso" for the day of the isolation, "Medbyt" for Medium change and "Tx" for

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the day of transplantation). Choose the picture to be analyzed, copy the file name and click Open or hit Enter.

6. Paste the sample information into the Input SAMPLE IDs and click OK or hit Enter to begin the analysis.

Selecting Islets and All Tissue

NOTE: At any time to remove the last selection simply right click once. To remove all selected tissue and start over right click two times.

7. Adjust the picture to an appropriate size using the magnifying glass buttons and choose some DTZ stained islet tissue with the mouse. After the first selection, check the "**Accumulate**" box to continue to build upon what has already been selected. Select as much islet tissue as can be chosen without choosing too much non-islet tissue. If there are small lines of pixels around exocrine tissue it is ok. If over selection has occurred and restarting is needed, right click twice and *deselect* "Accumulate". In the case of restarting, begin with this step again until islets are selected to satisfaction.

8. Once all possible islet tissue has been selected click OK or hit enter. The green areas indicating the selected islets will change to blue and a dialog stating "**Detect ALL tissue**" comes up.

9. Select all remaining tissue. Again take special care not to over select tissue. Click OK or hit enter when the appropriate amount of tissue has been selected.

Editing Selection

10. The next option is to edit All Tissue. Remove bubbles, areas of non-tissue that were detected, etc. The **Reject** function is automatically chosen and to delete an object from analysis hold down the left button on the mouse and draw a ring around the object to be edited. If the **Delete** function is used simply click on the feature to be deleted. Click OK when ready to move on.

11. An erode function is performed to delete small groups of pixels or to remove thin strips of pixels. This results in the image rounding ill-defined/fuzzy borders and removing thin strips of pixels. The user does not need to do anything but may notice the changes.

12. After the erode there is an option to edit the islet-selected tissue. Choose from the list in the window for the desired operation. Each operation is described on page 113 of the Leica QWin User Guide – English PDF found on the Leica QWin Image Processing and Analysis Software CD.

Briefly,

Accept – Define an area to be accepted, as from a poorly chosen Reject area. All area outside will be rejected. Opposite of the Reject function. The use of this button can result in losing all selected islet tissue and its use is not recommended.

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Draw –Mark areas to be included in islet calculations. Use this function to connect islets that were poorly segmented.

Keep – Marks features for acceptance.

Reject – Define an area to be rejected. All marked areas inside this area will be marked for deletion. It is possible to go directly to the Accept feature to reclaim any area mistakenly marked for deletion. Once another editing option beside Accept is chosen the marked areas are deleted and can only be reclaimed by Draw or Undo.

Erase – Deletes tissue from islet calculations

Delete – Deletes tissue from islet calculations

Cut – Removes pixels from calculations, for example very useful to separate islet tissue that has not been segmented properly

Line – Creates a line counted as islet tissue

Vector – Does not function as intended in the manual. It creates a straight line counted as islet tissue

Undo – The Undo button removes all editing performed under the editing step, not just the last operation.

13. Once the editing is done click OK or hit enter. Edit all non-islet tissue in the same manner as the islets. Once the editing is done click OK or hit enter.

Interpreting the results

14. The program will perform a series of calculations and a Results window will pop up displaying important information.

15. There are a number of options to see how well tissue was selected. They are as follows:

Image Only – Displays only the original image

Overlay – Displays the analyzed image with pixels determined as islets in green and those of all other tissue in blue

Outlines – Displays the analyzed image with green outlines for islet tissue and blue outlines for non-islet tissue.

Classify – Opens another window to see the size of each islet in a certain range of equivalent diameters. It is possible to customize the size and number of "bins" or groups analyzed. All bins will have the same size of the range of pixels. As default there are 12 bins from 0-600 µm with the range of each bin being 50 µm.

All objects under 20 µm are deleted from calculations. When "Classify" is selected a color-coded histogram as well as a numerical histogram become visible. If Classify or Measure is pressed more than once the numbers may be incorrect.

Validate – Equivalent diameter sizes of each islet object are shown on the image under the lowest, right most pixel of each object.

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Merge and Copy – Merges graphics to picture to save image for archiving

Save As... – Saves current displayed image and requires a name to be given

Quit – Quits program analyzing allows exiting Qwin or makes software available to take more pictures.

Next image> – Opens window to select another image to be analyzed

16. Before closing or analyzing the next picture select first "Outlines" and then "Save As...". Give the picture the same name as the original but add "outlines" to indicate that it has been analyzed.

17. If there are more pictures to be analyzed select "Next image>". Begin analysis again with step 7.

18. When finished analyzing pictures make sure to copy the data into an excel file to be saved in the same folder as the pictures. The copy option can be found in the "Edit" menu option in the Results window. It is possible to copy the data after each analysis even if all pictures have not been analyzed. Paste the data into an Excel file and save the file with the H-number followed by "bildanalys" for the file name (eg H1234 bildanalys).

19. Enter the Purity V % values for each fraction into the database according to NICS användarhandledning AL5193 (NICS user tutorial).

Calculations and Protocol

The total islet equivalents (IEQ) calculated according to the following formula:

$$IEQ = \frac{\text{Percent purity x the volume of the pellet expressed in } \mu l \text{ x } 1000}{230}$$

The equation is built into the NICS database for calculating islet equivalents.

Archiving

Protocol information is archived at least 10 years.

Equipment

Computer with Leica Qwin software installed

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Leica Qwin/Runner USB software key (purple)

Introduction

Estimation of total islet equivalents (IEQ) from islet isolation comes from the purity and the volume of pancreas material. Endocrine and exocrine pancreas material can be photographed and characterized using digital imaging analysis (DIA). Purity estimation, size distribution, and total tissue volume are a couple of the many parameters that are made more objective using computer based image analysis. The purity concerns the portion of the isolated pancreas material that is made of islets of Langerhans.

References

Skyddsföreskrift laboratoriearbete KITM AL4731

Hygienregler för Akademiska sjukhuset AL9112.

Fotografering av öar med Leica DFC490 (Islet photography with Leica DFC490) AL5475

NICS användarhandledning AL5193