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Vesselucida 360 Protocol for Segmenting and Analyzing Human Islet Microvasculature

Forked from a private protocol

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Protocol status: Working
We use this protocol and it's working

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Set-up

1 Download and install Vesselucida 360 application (RRID:SCR_017320).

Software	
Vesselucida 360	NAME
MBF Bioscience	DEVELOPER

2 Launch the application and set-up SciCrunch connection.

2.1 Go to **https://scicrunch.org/** and click REGISTER in the top menu. Fill out the registration form to create an account. From "My account", click API KEYS. Click GENERATE AN API KEY. Copy the key and return to Vesselucida 360.

3 Select the "SPARC Vocabulary Services" icon from the *Trace* ribbon.

3.1 Right-click to paste the API key in the dialog, then click **SET API KEY**.

Get Started

4 Open microscopy image data (2D or 3D) in the application.

5 When the SPARC Vocabulary Services dialog appears, select the appropriate items from the drop-down lists and enter the appropriate information regarding the subject to be segmented. Then select **BEGIN**.

5.1 **Subject information.**
Indicate the species, subject ID, sex, and age of the subject. All entered metadata will be stored o the XML data file. Independent from the cirteria for anatmoic terms selected.

Note the "blinded to condition" options for sex and age.

5.2 **Criteria for anatomic terms.**
ORGAN: Select the organ term list you are interested in annotating with.
SPECIES: The species you are interested in annotating with.
PARCELLATION: The atlas parcellation scheme you are interested in annotating with. If no parcellation exists for the organ and species criteria, select *Species Independent* from the drop-down.

Vascular Reconstruction: 3D Semi-Automatic Tracing Methods

6 In the 3D Environment window, click the **VESSEL** icon to display the Trace Vessels panel.

7 Under TRACING MODE, select **USER-GUIDED**.

7.1 Select a TRACING METHOD: **Directional Kernels, Rayburst Crawl,** or **Voxel Scooping.**

Note: The three tracing methods are based on algorithms that work by tracing along the vessel one point at a time, and by producing three measurments at each point: (X,Y,Z) coordinate, thickness, estimated position of the next point.

8 Hover over the area of the vessel where you want to place the first point. Click to place the first point. A sphrere represents the first point. Continue to hover over the process. A series of circles is displayed, representing the path detected by the software. When you reach the end of the vessel, click to place the last point. Continue. Right-click once to end the branch.The traced branch is displayed in 3D.

9 To create a bifurcating vessel, hover over an existing traced branch (branch A) at the location where the process bifurcates in the image data. A partial sphere appears on branch A (indicating a potential connecting point). Click to place the bifurcation point. Hover over the area of the bifurcating vessel and trace as you did in step 8.

Note: all vessel branches can be traced in either direction, in any order.

Vascular Reconstruction: 3D Automatic Tracing Methods

10 In the 3D Environment window, click the **VESSEL** icon to display the Trace Vessels panel.

11 Under TRACING MODE, select **AUTOMATIC**.

12 Select a TRACING METHOD: **Directional Kernels, Rayburst Crawl,** or **Voxel Scooping.**

Note: The three tracing methods are based on algorithms that work by tracing along the vessel one point at a time, and by producing three measurments at each point: (X,Y,Z) coordinate, thickness, estimated position of the next point.

13 Click the **SHOW SETTINGS** button.

13.1 In the DISPLAY SEEDS tab, click the **DISPLAY SEEDS** button.

Optional: To change the seed color, use the color picker next to the HIDE SEEDS button. To adjust the number of seeds, use the slider and the density buttons.

13.2 In the REFINE SEEDS tab, click the **VALIDATE SEEDS** button.

Optional: to decrease the number of unwanted seeds, increase the REFINE FILTER value. Add or remove seeds manually.

13.3 In the TRACE tab, adjust the *SENSITIVITY, GAP TOLERANCE, REMOVE TRACES SHORTER THAN, AND CONNECT BRANCH SEGMENTS* settings. Then click the **TRACE** button to start the automatic trace.

Editing Vessels

14 **Selecting Vessels.**
To select a single vessel, click the vessel.
To select all the vessels, click the SELECT ALL button.
To select several adjacent vessels, press the CTRL key and draw a marquee around the vessels of interest.
To deselect, click away from the selected object.

15 **Selecting Points.**
To select a signle point on a vessel, click the POINTS button then click the point of interest.
To select multiple, adjacent points, draw a marquee around the points of interest.
To deselect, click away from the selected options.

16 **Connecting and Detaching Vessels.**

Connecting Vessels

1. Zoom in and rotate as needed.
2. Click the CONNECT button. Vessels are now represented by series of points along colored lines.

3. Click and drag an end point toward the area you want to connect to. The end point briefly turns green and a green line appears.
4. Release the mouse button to connect the segments.

Detaching Vessels

1. Click a vessel to select it.
2. Click the DETACH button.
3. Click on the segment to detach.

- If you click a single segment, the program splits the segment into two segments.
- If you click a bifurcation, the program splits the segment into three segments.

17 **Deleting Vessels**

1. Click a vessel to select it or click the SELECT ALL button.
2. Press the DELETE key or click the REMOVE button. You can't undo this action!

Contouring Islets

18 **Generating Serial Z Sections**

1. In the 2D window, Click TRACE > SECTIONS> SERIAL SECTION MANAGER (SSM).
2. Open the image organizer, IMAGE> ORGANIZER.
3. Right-click on the image and select CREATE SECTIONS FROM THIS STACK. This will generate a section in the SSM at each Z plane of the image.

Contouring Islets

Starting at the first Z section that the islet comes into view...

1. In the SSM, select SHOW CURRENT SECTION.
2. Select TRACE> CONTOUR> CONTOUR SELECTION.
3. From the CONTOUR SELECTION menu, choose the contour name "Set of pancreatic islets".
4. Select a tracing method by right-clicking in the tracing window. The method chosen is strictly a matter of preference. The tracing method can be changed at any time during the tracing by right-clicking in the tracing window.
5. Contour the islet region: Click to place points along the boundaries of a region of interest; when you're done, right-click and select CLOSE CONTOUR.

- To adjust the thickness of the contour, scroll the mouse wheel.
- If the contour is larger than the field of view, it is easiest to trace if MOVE> TOOLS> AUTOMOVE is turned on.
- If you make a mistake, press Ctrl-Z to erase the last drawn points one by one back to the beginning of the contour.

This was carried out for every 4th Z section that the islet(s) were present in.

Place contours into sets

1. Select TRACE> SELECT OBJECTS.
2. Hold control and click to select each contour making up one islet.
3. Right-click and select PLACE CONTOURS INTO SET.
4. Name the set with the islet ID and select OK

Save Data File

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1. Select FILE>SAVE AS> DATA FILE to save the file with a new name, the same name but with different options, or with a different file type.
2. Name the datafile with the same name as the associated image.
3. Choose XML from the Save as type dropdown.
4. Use FILE> SAVE> DATA FILE without asking for a filename, if the file already has a name.

Analysis of Reconstruction

20 Download and install Vesselucida Explorer application (RRID:SCR_017674).

Software	
Vesselucida Explorer	NAME
MBF Bioscience	DEVELOPER

Launch the application and open the XML reconstruction data file.

21 **Segment Details and Network Analyses**

1. In the TRACED STRUCTURES window, select all Directed Networks (vessels).
2. Select ANALYZE> NETWORKS.
3. From the Network Analysis window check ALL NETWORK SUMMARY from the Network tab and SEGMENT DETAILS from the Segment Details tab. Click OK to run the analysis.
4. Right-click and select SAVE TO EXEL for both result tables.

22 **Segment in Closed Surface**

1. In the TRACED STRUCTURES window, select one islet set. Hold control and select all Directed Networks (vessels).
2. Select ANALYZE> CLOSED SURFACE ANALYSIS
3. Right-click the result table and select SAVE TO EXEL

23 **3D Contour Summary and Contour Details**

1. In the TRACED STRUCTURES window, select one islet set.
2. Select ANALYZE> MARKERS AND REGION
3. From the Markers and Regions Analysis window check CONTOUR DETAILS and 3D CONTOUR SUMMARY from the Segment Details tab. Click OK to run the analysis.
4. Right-click the result table and select SAVE TO EXEL

Repeat this process for each islet set.