function output = vesselrender

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% vesselrender.m
% Copyright 2010-2012,
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% Last modified 7/8/2012
% Renders a vessel network in MATLAB R2008a (MathWorks) using exported
% filament coordinates from Imaris 7.4.2 (Bitplane) and the
% tracks calculated by "calctracks.m". Filament coordinates can be
% exported using ImarisXT.
% Program can run in three modes (selected using dialog box):
% A) Plot Vessels - Plots a 3D vessel network in MATLAB
                   Also gives the option to plot a reference plane
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    Inputs:
응
    -Directory containing vessel coordinates
    -Directory containing filament coordinates that define reference plane
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    or alternatively the equation of a plane. Used for rotation of data
     and plotting of the plane. Only needed if plotting plane.
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    Outputs:
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    -3D plot of vessels and plane
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% B) Analyze Density - Calculates vessel density as a function of
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                      perpendicular distance from a reference plane
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    Inputs:
응
    -Directory containing vessel coordinates
    -Directory containing filament coordinates that define reference plane
응
응
     or alternatively the equation of a plane
    -Directory for saving output files
응
응
     -xmin/xmax/ymin/ymax/zmin/zmax for the volume to be analyzed
응
    -Sampling frequency (axial distance between adjacent cross-sections)
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응
    Outputs:
     "FigXXX.fig" - MATLAB figures displaying cross-sectional planes
응
       through the plotted 3D volume
응
응
     "VesselArea.txt" - csv formatted file containing vessel cross-
응
       sectional areas as a function of distance from reference plane
     "TotalArea.txt" - csv formatted file containing cross-sectional areas
응
응
       of total volume as a function of distance from reference plane
     "AllAreaData.txt" - csv formatted file containing cross-sectional
응
응
      areas of the vessels and total volume, as well as their ratio,
9
       as a function of distance from reference plane
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      "DensityProfile.fig" - MATLAB figure plotting the vessel volume
        density as a function of distance from reference plane
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% C) Analyze Angle - Calculates angle between vessel tracks and a reference
                     plane
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    Inputs:
응
    -Directory containing vessel coordinates
응
    -Directory containing filament coordinates that define reference plane
     or alternatively the equation of a plane
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    -Directory for saving output files
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용
    Outputs:
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     "AngleProfile.fig" - MATLAB figure displaying a scatterplot of
응
       angles from reference plane as a function of distance from plane
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     "AngleData.txt" - csv formatted file containing angles from reference
       plane as a function of distance from plane
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     "CumAngle.fig" - MATLAB figure displaying the cumulative probability
응
       distribution of measured angles
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     "CumAngle.txt" - csv formatted file containing the cumulative
       probability distribution of measured angles
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% Part 1 - Select run-mode and define reference plane
% Start by setting the defaults
modetest=0;
analysismode=0;
planetest=1;
fitplanetest=0;
% Determine whether to plot vessels, analyze density, or analyze planarity.
% Default is to plot vessels.
button = questdlg('What do you want to do with vessel coordinates?','Select Mode','Plot ✓
Vessels', 'Analyze Density', 'Analyze Planarity', 'Plot Vessels');
if button
   modetest=button(1) == 'A';
end
if modetest
   analysismode=button(9) == 'P';
else
% If plotting vessels, decide whether to plot reference plane. Default is yes.
   button2 = questdlg('Plot reference plane?','','Yes','No','Yes');
   if button2
       planetest=button2(1) == 'Y';
   end
end
% Define equation for reference plane. Reference plane is defined, even if
% not being plotted, because it is necessary for rotation of the volume,
% and potentially for analysis of density as a distance from the plane, or
% for determination for path angle with respect to the plane. Coefficients
% are for the following equation of the plane: Z = aX + bY + c
% The vector orthogonal to the plane must have a non-zero z-component.
% Set default plane to be Z = 0. When using this default, the data set will
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% information should be exported using ImarisXT and should include

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% not be rotated at all.
XCoeff = 0; % a = X coefficient
YCoeff = 0; % b = Y coefficient
CCoeff = 0; % c = constant term
% If plotting the plane, analyzing density, or analyzing angle, discards
% default plane and loads user-defined plane.
if planetest
% Determines whether to define plane manually, or by finding plane of
% best-fit through saved filament coordinates. Default is manual.
   button3 = questdlg('Define equation of the plane manually, or by finding plane of best \checkmark
fit from saved filament coordinates?','','Manual','Plane of Best Fit','Manual');
    if button3
        fitplanetest=button3(1) == 'P';
    end
% If defining the plane using the plane of best fit through saved filament
% coordinates, loads filament coordinates from given directory
    if fitplanetest
        directory name = uigetdir('','Select directory containing the filaments that ¥
define the reference plane (exported from Imaris)')
        vFilamentXYZ=csvread(strcat(directory name, '\vFilamentXYZ.txt'));
        Xcolv = vFilamentXYZ(:,1);
        Ycolv = vFilamentXYZ(:,2);
        Zcolv = vFilamentXYZ(:,3);
        Const = ones(size(Xcolv));
% Use regression to determine plane of best fit
        Coefficients = [Xcolv Ycolv Const]\Zcolv;
        XCoeff = Coefficients(1);
        YCoeff = Coefficients(2);
        CCoeff = Coefficients(3);
% If defining the plane manually, queries the coefficients. Default is
% a=b=c=0
        prompt2 = \{'Z = aX + bY + c \checkmark
a =','b =','c ='};
        dlg title2 = 'Define plane coefficients';
        num lines2 = 1;
        def2 = \{ '0', '0', '0' \};
        answer2 = inputdlg(prompt,dlg_title,num_lines,def);
        if ~isempty(answer2)
            XCoeff = str2num(cell2mat(answer2(1)));
            YCoeff = str2num(cell2mat(answer2(2)));
            CCoeff = str2num(cell2mat(answer2(3)));
        end
    end
end
% Gets directory containing the coordinates of the vessel network. This
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% vFilamentXYZ.txt, tracks.txt, and vFilamentRadius.txt
directory name2 = uigetdir('','Select directory containing the filaments that define the ✓
vessels (exported from Imaris)');
vFilamentXYZ=csvread(strcat(directory name2, '\vFilamentXYZ.txt'));
tracks=csvread(strcat(directory name2, '\tracks.txt'));
vFilamentRadius=csvread(strcat(directory name2, '\vFilamentRadius.txt'));
% Guesses the x-, y-, and z- limits for the volume that is loaded. This is
% important for determining vessel density with respect to reference plane,
% because after rotation, the total area of each slice will not be equal at
% different distances from the reference plane. Also sets the default slice
% interval to be 1 um.
xmax=max(vFilamentXYZ(:,1));
xmin=min(vFilamentXYZ(:,1));
ymax=max(vFilamentXYZ(:,2));
ymin=min(vFilamentXYZ(:,2));
zmax=max(vFilamentXYZ(:,3));
zmin=min(vFilamentXYZ(:,3));
sliceint=1;
if modetest
% Sets the directory for saving files - only used when analyzing density or angle
    savedir = uigetdir('','Select directory where files will be saved');
    if savedir==0
        'Error - Must select directory where files will be saved'
        return
    end
% Queries the x-, y-, and z- limits for the volume that is loaded. The
% default values will only be close if the entire volume is filled with
% vessels, so use them with care. Sampling interval is also important - a
% smaller value will improve resolution, but requires more memory and
% runtime.
% This step is only necessary if in density analysis mode.
    if ~analysismode
        prompt = {'Xmin (um)','Xmax (um)','Ymin (um)','Ymax (um)','Zmin (um)','Zmax ✔
(um)','Sampling Interval (um)'};
        dlg title = 'Data Limits';
        num lines = 1;
        def = {'0', num2str(xmax), '0', num2str(ymax), '0', num2str(zmax), '1'};
        answer = inputdlg(prompt,dlg title,num lines,def);
        if ~isempty(answer)
            xmin=str2num(cell2mat(answer(1)));
            xmax=str2num(cell2mat(answer(2)));
            ymin=str2num(cell2mat(answer(3)));
            ymax=str2num(cell2mat(answer(4)));
            zmin=str2num(cell2mat(answer(5)));
            zmax=str2num(cell2mat(answer(6)));
            sliceint=str2num(cell2mat(answer(7)));
        end
    end
end
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% Part 2 - Rotate and translate entire data set so that the reference plane
% = 10^{-2} is parallel with the plane Z = 0. This transforms the z-coordinates so
% that z= perpendicular distance from the reference plane.
% To do this, we must first determine what rotations are necessary to
% rotate the normal vector to the reference plane so that it transforms to
% [0\ 0\ 1] . Then we must rotate all of the vessel coordinates by these same
% angles. Because 3D rotations are NOT commutative, order is important
% here. Finally, the rotated coordinates must be shifted in z so that the
% transformed reference plane is located at z=0.
% We start by defining the normal vector to the plane.
vector=[-XCoeff -YCoeff 1];
arclength=sqrt(dot(vector, vector));
% Next, we determine what angle this vector must be rotated by in order for
% it to lie in the x-y plane. After determining this angle (-phi), we
% rotate the vector by -phi around the x-axis by multiplying by the
% appropriate rotation matrix.
phi=atan2(vector(1,3), vector(1,2));
vector2=[1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*vector';
vector2=vector2';
% Then, we determine what angle this transformed vector must be rotated by
% in order for it to lie parallel with the x-axis. After determining this
% angle (-theta), we are ready to rotate the coordinates of the vessels.
theta=atan2(vector2(1,2), vector2(1,1));
% Now, we rotate the coordinates of the vessels. We start by rotating
% around the x-axis by -phi.
vFilamentXYZ=[1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*vFilamentXYZ';
% Next, we rotate around the z-axis by -theta.
vFilamentXYZ=[cos(-theta),(-sin(-theta)),0;sin(-theta),cos(-theta),0;0,0,1]*vFilamentXYZ;
% Finally, we rotate around the y-axis by -90 degrees. This is required so
% that the vector normal to the plane is parallel with the z-axis instead
% of the x-axis. This is a special case of the corresponding rotation
% matrix.
vFilamentXYZ=vFilamentXYZ';
xb=vFilamentXYZ(:,1);
yb=vFilamentXYZ(:,2);
zb=vFilamentXYZ(:,3);
vFilamentXYZ(:,3)=xb;
vFilamentXYZ(:,1)=-zb;
% To zero our transformed coordinates such that the reference plane is
% located at z=0, we determine at what z-location the plane sits after the
% rotations above. Because z is constant after the rotations, all we need
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% to do is calculate z for any point on the plane. We do this with the
% point (x,y,z)=(0,0,c).
PlaneCoord=CCoeff*sin(phi)*sin(theta);
% Then, we subtract the z-values of our rotated data set by this constant.
vFilamentXYZ(:,3)=vFilamentXYZ(:,3)-PlaneCoord;
% In order to calculate density with respect to the distance from the
% reference plane, a surface that bounds the volume must be defined, and
% then these same transformations must be applied.
% The surface is defined by the vertices of the rectangular prism bounding
% the data set, and by the centroids of the upper and lower faces of this
% rectangular prism. These faces are required in order to close the
% surface, so that contours can be drawn.
x=[xmin xmax xmax xmin];
y=[ymin ymin ymax ymax];
x = [x; x];
y=[y;y];
z=[zmin zmin zmin; zmax zmax zmax zmax];
z=[z(1,:);z;z(2,:)];
xdel=(xmin+((xmax-xmin)/2));
ydel=(ymin+((ymax-ymin)/2));
x=[xdel xdel xdel xdel;x;xdel xdel xdel xdel];
y=[ydel ydel ydel;y;ydel ydel ydel ydel];
x(:,5)=x(:,1);
y(:,5) = y(:,1);
z(:,5)=z(:,1);
coord1=[x(1,:);y(1,:);z(1,:)];
coord2=[x(2,:);y(2,:);z(2,:)];
coord3=[x(3,:);y(3,:);z(3,:)];
coord4 = [x(4,:);y(4,:);z(4,:)];
% Then, the same transformations are applied to the coordinates defining
% this surface.
coord1t = [1, 0, 0; 0, cos(-phi), (-sin(-phi)); 0, sin(-phi), cos(-phi)]*coord1;
coord2t = [1, 0, 0; 0, cos(-phi), (-sin(-phi)); 0, sin(-phi), cos(-phi)]*coord2;
coord3t = [1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*coord3;
coord4t = [1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*coord4;
coord1tt = [cos(-theta), (-sin(-theta)), 0; sin(-theta), cos(-theta), 0; 0, 0, 1]*coord1t;
coord2tt = [cos(-theta), (-sin(-theta)), 0; sin(-theta), cos(-theta), 0; 0, 0, 1]*coord2t;
coord3tt = [cos(-theta), (-sin(-theta)), 0; sin(-theta), cos(-theta), 0; 0, 0, 1]*coord3t;
coord4tt = [cos(-theta), (-sin(-theta)), 0; sin(-theta), cos(-theta), 0; 0, 0, 1]*coord4t;
\verb|xdiamond=[coord1tt(1,:);coord2tt(1,:);coord3tt(1,:);coord4tt(1,:);]|;
ydiamond = [coord1tt(2,:); coord2tt(2,:); coord3tt(2,:); coord4tt(2,:);];
zdiamond=[coord1tt(3,:);coord2tt(3,:);coord3tt(3,:);coord4tt(3,:);];
xdiamondb=xdiamond;
ydiamondb=ydiamond;
zdiamondb=zdiamond;
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zdiamond=xdiamondb;
xdiamond=-zdiamondb;
zdiamond=zdiamond-PlaneCoord;
% Finally, determine the x-, y-, and z-limits, and use this and the slice
% interval to determine the number of slices required
xmax=max(max(xdiamond));
xmin=min(min(xdiamond));
ymax=max(max(ydiamond));
ymin=min(min(ydiamond));
zmax=max(max(zdiamond));
zmin=min(min(zdiamond));
zdelta=zmax-zmin;
numslices=floor(zdelta/sliceint);
v=zeros(1, numslices);
for i=1:numslices
   v(1,i)=i;
end
v=v-1;
v=v.*sliceint*(-1);
v=v+zmax;
v=rot90(v);
% Part 3 - Plot the entire vessel network, and use it to either display the
% vessel network, analyze the density, or analyze the angle (as selected in
% Part 1 above).
% Use the list of tracks to determine how many tracks there are, and how
% many points in each track.
trklength=sum(tracks>0);
testsize=size(trklength);
tracknum=testsize(2);
% Open a figure, and set defaults.
figure;
[x y z] = cylinder([1 2]);
xold=x(1,:).*0;
yold=xold;
zold=xold;
if analysismode
   planarity=[];
end
% Draw each track segment by segment. i=track number, and j=point number
% within the current track
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for i=1:tracknum
    for j=1:(trklength(1,i)-1)
% Determine the location of points j and j+1 along this track, as well as
% the radius of the filament at each of these points. Define a vector using
% these two points.
        xyz2=[vFilamentXYZ(tracks(j+1,i),1),vFilamentXYZ(tracks(j+1,i),2),vFilamentXYZ 
(tracks(j+1,i),3)];
        xyz1=[vFilamentXYZ(tracks(j,i),1),vFilamentXYZ(tracks(j,i),2),vFilamentXYZ(tracks ⊌
(j,i),3)];
       r2=vFilamentRadius(tracks(j+1,i),1);
        r1=vFilamentRadius(tracks(j,i),1);
       vector=xyz2-xyz1;
        arclength=norm(vector);
% If run-mode is for determining angle, use the dot product of this vector
% with the dot product of the vector normal to the reference plane [0 0 1]
% to determine the angle. Add this to the list and go to the next track
% point.
        if analysismode
            wallangle=((acos(vector(1,3)/arclength))*180/pi)-90;
            planarity = [planarity; ((xyz1(1,3)+xyz2(1,3))/2), abs(wallangle)];
            continue
        end
% To plot each vessel segment, we will use the MATLAB cylinder function,
% which plots a cylinder with an axis of (x,y,z) = (0,0,0) to (0,0,1), and
% a user-defined radius at each end of the axis. This cylinder then needs
% to be rotated +90 degrees around the y-axis, and then rotated again by an
% angle of theta around the z-axis, and then one last time by an angle of
% phi around the x-axis. Finally, it will have to be translated from the
% origin to the appropriate location in 3-space.
% To determine phi and theta, we perform the inverse by determining what
% angle the vector of the segment must be rotated by in order to move it to
% the x-y plane, and then from there to be parallel with the x-axis.
% Remember that 3D rotation is not commutative, so these rotations must be
% done in reverse when moving the cylinder from the z-axis to its
% appropriate angle and location with respect to the origin.
        phi=atan2(vector(1,3), vector(1,2));
       vector2=[1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*vector';
        vector2=vector2';
        theta=atan2 (vector2 (1,2), vector2 (1,1));
% Here, we use the cylinder function to determine the coordinates of our
% cylinder with the appropriate length and radii, albeit at the wrong angle
% and location.
        [x y z] = cylinder([r1 r2]);
        z(2,:) = arclength;
% If this is not the first segment in this track, then it must be axially
% rotated so that it will be axially aligned with the previous segment.
% Otherwise, the internal volume of the cylindrical segment will be
% artificially reduced because the walls will be twisted together.
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% For example, from
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% Determine the angle psi that the new cylinder must be axially rotated by,
% and then perform the rotation.
        if j>1
            \texttt{testvec=[(xold(1,1)-xyz1(1,1)),(yold(1,1)-xyz1(1,2)),(zold(1,1)-xyz1(1,3))];}
            testvecA=[1,0,0;0,cos(-phi),(-sin(-phi));0,sin(-phi),cos(-phi)]*testvec';
            testvecB=[\cos(-theta), (-\sin(-theta)), 0; \sin(-theta), \cos(-theta), 0; 0, 0, 1] \checkmark
*testvecA;
            testvecC=[-testvecB(3,1);testvecB(2,1);testvecB(1,1)];
            psi=atan2(testvecC(2,1),testvecC(1,1));
            coord1=[x(1,:);y(1,:);z(1,:)];
            coord2=[x(2,:);y(2,:);z(2,:)];
            coord1tt = [cos(psi), (-sin(psi)), 0; sin(psi), cos(psi), 0; 0, 0, 1]*coord1;
            coord2tt=[cos(psi),(-sin(psi)),0;sin(psi),cos(psi),0;0,0,1]*coord2;
            xtt=[coord1tt(1,:);coord2tt(1,:)];
            ytt=[coord1tt(2,:);coord2tt(2,:)];
            ztt=[coord1tt(3,:);coord2tt(3,:)];
            x=xtt;y=ytt;z=ztt;
        end
% Now, we use the angles we determined above to transform the coordinates
% of our cylinder so that it is rotated at the appropriate angle.
% We start by rotating by +90 degrees around the y-axis.
        xb=x;
        yb=y;
        zb=z:
        z=-xb;
        x=zb;
        coord1=[x(1,:);y(1,:);z(1,:)];
        coord2=[x(2,:);y(2,:);z(2,:)];
% Next, we rotate by theta around the z-axis
        coord1t = [cos(theta), (-sin(theta)), 0; sin(theta), cos(theta), 0; 0, 0, 1]*coord1;
        coord2t = [cos(theta), (-sin(theta)), 0; sin(theta), cos(theta), 0; 0, 0, 1]*coord2;
% Then, we rotate by phi around the x-axis
        coord1tt=[1,0,0;0,cos(phi),(-sin(phi));0,sin(phi),cos(phi)]*coord1t;
        coord2tt=[1,0,0;0,cos(phi),(-sin(phi));0,sin(phi),cos(phi)]*coord2t;
        xtt=[coord1tt(1,:);coord2tt(1,:)];
        ytt=[coord1tt(2,:);coord2tt(2,:)];
        ztt=[coord1tt(3,:);coord2tt(3,:)];
% Finally, we translate the cylinder so that it is located at the right
% location in 3-space.
        xfinal=xtt+xyz1(1,1);
        yfinal=ytt+xyz1(1,2);
        zfinal=ztt+xyz1(1,3);
% If this is the second (or later) segment within this track, we must
% replace the coordinates of the beginning edge of the cylinder with the
% coordinates used previously for the ending edge of the cylinder. This
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% ensures continuity of vessel filament from segment to segment, with no
% gap in between segments.
        if j>1
            xfinal(1,:)=xold;
            vfinal(1,:) = vold;
            zfinal(1,:)=zold;
        end
% The coordinates for both faces of the cylinder must be added to the list
% of cylinder coordinates. This ensures a closed surface, and makes sure that
% contours can be drawn.
        xfinal = [((xfinal(1,:).*0) + xyz1(1,1)); xfinal; ((xfinal(1,:).*0) + xyz2(1,1))];
        yfinal = [((yfinal(1,:).*0) + xyz1(1,2)); yfinal; ((yfinal(1,:).*0) + xyz2(1,2))];
        zfinal = [((zfinal(1,:).*0) + xyz1(1,3)); zfinal; ((zfinal(1,:).*0) + xyz2(1,3))];
% The coordinates for the ending edge of the cylinder are saved, so that
% they can be used as the beginning coordinates of the cylinder in the next
% iteration.
        xold=xfinal(3,:);
        yold=yfinal(3,:);
        zold=zfinal(3,:);
% If run-mode is for displaying a plot of the vessels, use the surf
% function to draw this filament segment, and then go on to the next segment.
        if ~modetest
            surf(xfinal,yfinal,zfinal,'FaceColor',[1 0 0],'EdgeColor',[0 0 0]);
            hold on
            continue
        end
% Otherwise, run-mode must be set for analyzing density. Use the contour3
% mode instead in order to draw contours, which can be used for determining
% cross-sectional area of the vessel network at each z-plane of interest,
% as was defined above.
        [c h]=contour3(xfinal, yfinal, zfinal, v, 'k');
       hold on
% Each of these contours is saved as a graphics object with handle h. For
% each of these unique handles, save tags marking these contours as
% belonging to a vessel, and with tags that note the z-coordinate where the
% contour was taken. These tags will facilitate analysis of density as a
% function of z (which after our transformations, represents distance from
% the z-plane).
        sizeh1=size(h);
        sizeh2=sizeh1(1,1);
        for iii=1:sizeh2
            a=get(h(iii),'ZData');
            height=a(1,1);
            set(h(iii), 'UserData', height);
            set(h(iii), 'Tag', 'Vessel');
        end
    end
% Track progress as vessel tracks are plotted.
VesselPlotProgress=i/tracknum
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end
% If run-mode is for displaying a plot of the vessel network, then plot the
% reference plane if desired, and then end program.
if ~modetest
    if planetest
        [xxp, yyp]=meshgrid(xmin:((xmax-xmin)/10):xmax,ymin:((ymax-ymin)/10):ymax);
        zzp = 0 * xxp;
        surf(xxp,yyp,zzp,'FaceColor',[0 0 1],'EdgeColor',[0 0 0])
        hold on
    end
    daspect([1 1 1]);
    output='Finished Plotting Vessels'
    return
end
% If run-mode is for analyzing angle, plot a scatterplot of vessel angle
% with respect to distance from reference plane. Then, plot the cumulative
% probability distribution of vessel angle. Finally, save these figures,
% and the data that make up these tables as comma separated value text
% files, and end program.
if analysismode
    planarity(:,1)=planarity(:,1);
    x\lim([\min(planarity(:,1))-5\max(planarity(:,1))+5]);y\lim([-5 95]);plot(planarity(:,1), \checkmark)
planarity(:,2),'kd');hold on;
    title('Vessel Angle from Plane as a Function of Depth');
    xlabel('Depth (um)');
    ylabel('Angle (degrees)');
    hgsave(strcat(savedir,'\AngleProfile.fig'));
    csvwrite(strcat(savedir,'\AngleData.txt'),planarity);
    cumangle=[10 20 30 40 50 60 70 80 90]';
   plnsize=size(planarity);
   plnsize2=plnsize(1,1);
    for i=1:9
        cumangle(i,2)=sum(planarity(:,2)<cumangle(i,1));</pre>
    end
    cumangle(:,2) = cumangle(:,2) /plnsize2;
    cumangle=[0 0;cumangle];
    figure; plot (cumangle(:,1), cumangle(:,2));
    title('Cumulative Probability Distribution of Vessel Angle');
   xlabel('Angle (degrees)');
    ylabel('Cumulative Probability');
    hgsave(strcat(savedir,'\CumAngle.fig'));
    csvwrite(strcat(savedir,'\CumAngle.txt'),cumangle);
    output='Finished Angle Measurements'
    return
end
% If program has gotten to this line, then run-mode must be for analyzing
% density. Countours must now be drawn for the surface that bounds the
% volume.
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[c h]=contour3(xdiamond, ydiamond, zdiamond, v, 'b');
hold on
% Again, each contour is saved as a graphics object with handle h. For
% each of these unique handles, save tags marking these contours as
% belonging to the frame, and with tags that note the z-coordinate where
% contour was taken. These tags will facilitate analysis of density as a
% function of z (which after our transformations, represents distance from
% the z-plane).
sizeh1=size(h);
sizeh2=sizeh1(1,1);
for iii=1:sizeh2
    a=get(h(iii), 'ZData');
    height=a(1,1);
    set(h(iii), 'UserData', height);
    set(h(iii), 'Tag', 'Frame');
end
% Make sure data aspect ratio is correct.
set(gca, 'DataAspectRatio', [1 1 1]);
% Find every unique value of 'UserData' -- this should be the list of
% z-locations chosen for measurements of vessel density, as defined earlier
% with slice interval. These values are saved into the first column of areasav.
h=findobj;
a=get(h,'UserData');
a=cell2mat(a);
a=unique(a);
sizeal=size(a);
sizea2=sizea1(1,1);
areasav=a;
areasav(:,2)=0.*a;
% Measure the cross-sectional area of the frame and of the vessel network
% at each z-location
for ii=1:sizea2
% Zero the running-area counts, and open a new figure.
    cumarea=0;
    framearea=0;
    figure; hold on;
% Find all the contour objects for the frame at the current z-location. Use
% the x- and y- coordinates of these contours to determine cross-sectional
% area of the frame at this z-location.
    b=findobj('UserData',a(ii),'-and','Tag','Frame');
    sizeb1=size(b);
    sizeb2=sizeb1(1,1);
    for jj=1:sizeb2
        xtest=get(b(jj),'XData');
        ytest=get(b(jj), 'YData');
        index=find(~(isnan(xtest)));
        xtest=xtest(index);
```

```
ytest=ytest(index);
        framearea=framearea+polyarea(xtest, ytest);
% Plot cross-section of the frame in blue.
        fill(xtest, ytest, 'b');
        hold on
    end
% Find all the contour objects for the vessels at the current z-location. Use
% the x- and y- coordinates of these contours to determine cross-sectional
% area of the vessel network at this z-location.
   b=findobj('UserData',a(ii),'-and','Tag','Vessel');
   sizeb1=size(b);
   sizeb2=sizeb1(1,1);
    for jj=1:sizeb2
        xtest=get(b(jj), 'XData');
        ytest=get(b(jj), 'YData');
        index=find(~(isnan(xtest)));
        xtest=xtest(index);
        ytest=ytest(index);
% Plot cross-section of the vessel network in black.
        fill(xtest, vtest, 'k');
        hold on
        cumarea=cumarea+polyarea(xtest, ytest);
    end
% Save an image of the cross-section of the frame and vessels at this
% z-location to enable verification of correct sectioning.
    axis([xmin xmax ymin ymax]);
    hgsave(strcat(savedir, '\Fig', int2str(ii)));
    close;
% Save the cross-sectional area of the frame and vessel network at this
% location into areasav.
    areasav(ii,2)=cumarea;
    areasav(ii,3)=framearea;
% Display the progress of the density calculation process.
    DensCalcProgress=ii/sizea2
end
% Save measurements of vessel area and frame area as a function of distance
% from the reference plane in comma separated value formatted text files.
areasav(:,1) = areasav(:,1);
csvwrite(strcat(savedir,'\VesselArea.txt'), areasav(:,1:2));
csvwrite(strcat(savedir,'\TotalArea.txt'),[areasav(:,1),areasav(:,3)]);
close
% Calculate the ratio of the cross-sectional area of the vessel network to
% the cross-sectional area of the frame at each z-location. When small
% slice interval is used, this is a good estimate of the fraction of space
% taken up by the vessel network (in other words, the vessel density) as a
% function of distance from the reference plane. This is saved into
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% areasavfin as both a fraction and a percentage, and the entire table is
% saved as a comma separated value formatted text file.
index=find(areasav(:,3)>0);
areasavfin=areasav(index,:);
areasavfin(:,4) = areasavfin(:,2)./areasavfin(:,3);
areasavfin(:,5) = areasavfin(:,4) *100;
csvwrite(strcat(savedir,'\AllAreaData.txt'),areasavfin);
% Finally, this vessel density as a function of depth is plotted as a line
% graph, and saved to file.
figure;plot(areasavfin(:,1), areasavfin(:,5));
title('Vessel Density as a Function of Depth');
xlabel('Depth (um)');
ylabel('Vessel Density % (v/v)');
hgsave(strcat(savedir,'\DensityProfile.fig'));
% With that, the program is complete.
output='Finished Density Measurements'
```