How to analyze CT scans of pellets

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*These are slight modifications to the methods used to write the paper about the AN-102 pellet run.*

Images called “projections” are collected in the CT scanner and stored on the computer along with data files describing the scan geometry and angles. The projections may need to have corrections made if they don’t have featureless backgrounds. Once corrected, the projections can be used to reconstruct a 3D volume of the object scanned. A program in ImageJ/Fiji can segment all the bubbles in a reconstruction, and those segmented bubbles can then be quantified in Avizo.

1. Use “no crop stipe” in ImageJ (or Fiji, Fiji is just ImageJ) to make correction to projections without cropping or compressing
	1. Make folder called “Original projections” in folder of raw data
	2. Move all projections into that folder
	3. Open all projections in imageJ
	4. Run “no crop stripe”
	5. Projections must be saved in the format CTPro expects. In Fiji, navigate to “Edit”, “options”, “Input/Output…”, and make sure the box is checked next to “save TIFF and raw in Intel byte order”.
	6. Take resultant image stack of corrected projections and save as image sequence, select TIFF, type the file name originally used minus numbers (If projections were called “Pellet 950 C\_0001.tif”, “Pellet 950 C\_0002.tif” etc, then save the tif sequence as “Pellet 950 C\_” say start at 1 (0 will be default), and say 4 digits).
2. Reconstruct the object
3. Open the vgl file made by the reconstruction using VGStudio. Export the volume as an image stack. A series of TIFF files is best. An image slice of a reconstruction is called a tomogram.
4. Drag and drop the folder of TIFF tomograms into Fiji. Open them as a stack.
5. Note the slice number of just below the bottom and just above the top of the pellet. (Life will be easier if every scan in one experiment has the exact same bottom, so that you can compare the height of features as the pellet changes shape.)
6. Crop the volume to the pellet.
	1. “Image”, “Stacks”, “Tools”, “Slice Keeper”, use bottom of pellet as “first slice”, top of pellet as “bottom slice”, “increment”=1.
	2. Use the rectangle tool to draw a rectangle around the pellet base. “Image”, “crop” (or use Ctrl+Shift+x).
	3. Navigate to approximately the center of the pellet. “Image”, “adjust”, “Brightness/Contrast” (Ctrl+Shift+c). “Auto”, “Apply”, answer ‘yes’ to the prompt asking “Apply LUT to all stack slices?”
	4. “Image”, “type”, 8-bit.
7. ~~Get ready to run the bubble segmentation~~
	1. ~~“Image”, “duplicate”, use default name but make sure “Duplicate stack” is checked.~~
	2. ~~“Image”, “rename”, make name of 8-bit cropped stack “bulk”. That name will be used by the macro.~~
8. Run the macro
	1. “Plugins”, “macros”, “run” navigate to and select “Take6”.
	2. The plugin takes about 2 minutes to run, for a 100 MB volume.
	3. Go to “Shanbhag bubbles” and crop the bottom slices to remove noise. Use the circle tool to circle the widest point of the pellet and “clear outside” and answering “yes” on the prompt about all slices to clear the outside of a circle you draw on all slices.
	4. Save “Shanbhag bubbles” as “[temp]ShanTake6”
	5. Try out other thresholding options on “bubbles no threshold”
	6. After cropping, save “mask” as “[temp]MaskTake6”
	7. Save “result of walls” as “[temp]MaskedTake6”
9. Open the “[temp]ShanTake6” bubble TIF stack in Avizo.
	1. Set the appropriate scale, referencing the data files made during the scan and reconstruction.
	2. Run auto thresholding on the bubble volume
	3. Run “separate particles”. Currently we use aggressive, neighborhood of 6, setting at 4, repeatable.
	4. Run label analysis, calling “Steven” settings
	5. Copy/Paste the data columns for volume, surface area, index and pasting into excel.
	6. Run “filter by measure range”, asking for Volume, taking top order of magnitude (eg, 1.0\*1011 through 1.0\*1012) in sequence and taking a snapshot of a volume rendering of the result. Could probably grab the data at that point to discriminate the bubbles based on size. You’re really looking for total volume, surface area, and counts for each size category.
	7. Do further analysis, maybe starting with “counts” sheet of “Bubble size distribution” to find how many bubbles are in each order of magnitude of size.

IJ1 Macro script for “Take6” ImageJ macro.

run("Duplicate...", "title=bulk duplicate");

run("Subtract Background...", "rolling=70 sliding stack");

run("Duplicate...", "title=walls duplicate");

run("Gaussian Blur 3D...", "x=1 y=1 z=4");

run("Unsharp Mask...", "radius=20 mask=0.80 stack");

selectWindow("bulk");

run("Duplicate...", "title=bulk-2 duplicate");

setAutoThreshold("Default dark no-reset stack");

//run("Threshold...");

setAutoThreshold("Yen dark no-reset");

setOption("BlackBackground", true);

run("Convert to Mask", "method=Yen background=Dark calculate black");

run("Gaussian Blur 3D...", "x=3 y=3 z=3");

setAutoThreshold("Huang dark no-reset stack");

setAutoThreshold("Huang dark no-reset");

run("Convert to Mask", "method=Huang background=Dark calculate black");

selectWindow("bulk");

run("Reslice [/]...", "output=0.001 start=Top avoid");

setAutoThreshold("Default dark no-reset stack");

//run("Threshold...");

setAutoThreshold("Yen dark no-reset");

setOption("BlackBackground", true);

run("Convert to Mask", "method=Yen background=Dark calculate black");

run("Gaussian Blur 3D...", "x=3 y=3 z=3");

setAutoThreshold("Huang dark no-reset stack");

setAutoThreshold("Huang dark no-reset");

run("Convert to Mask", "method=Huang background=Dark calculate black");

run("Reslice [/]...", "output=0.001 start=Top avoid");

rename("side thresholded");

selectWindow("bulk-2");

imageCalculator("AND create stack", "side thresholded","bulk-2");

selectWindow("bulk");

close();

selectWindow("Result of side thresholded");

rename("crop");

selectWindow("bulk-2");

close();

selectWindow("side thresholded");

close();

selectWindow("Reslice of bulk");

close();

selectWindow("crop");

imageCalculator("AND create stack", "walls","crop");

selectWindow("Result of walls");

selectWindow("crop");

run("Fill Holes", "stack");

run("Reslice [/]...", "output=0.002 start=Top avoid");

run("Fill Holes", "stack");

rename("Reslice1");

run("Reslice [/]...", "output=0.002 start=Top avoid");

run("Fill Holes", "stack");

rename("Reslice2");

selectWindow("crop");

close();

selectWindow("Reslice1");

close();

run("Rotate 90 Degrees Right");

run("Reslice [/]...", "output=0.002 start=Top avoid");

run("Fill Holes", "stack");

selectWindow("Reslice2");

close();

run("Reslice [/]...", "output=0.002 start=Top avoid");

run("Fill Holes", "stack");

run("Rotate 90 Degrees Left");

selectWindow("Reslice of Reslice2");

close();

rename("mask");

run("Options...", "iterations=3 count=1 black do=Erode stack");

imageCalculator("Subtract create stack", "mask","Result of walls");

selectWindow("Result of mask");

rename("bubbles");

run("Median...", "radius=2 stack");

run("Despeckle", "stack");

setAutoThreshold("Default dark no-reset");

selectWindow("bubbles");

run("Duplicate...", "title=bubbleNoThreshold duplicate");

selectWindow("bubbles");

setAutoThreshold("Shanbhag dark no-reset");

//run("Threshold...");

setAutoThreshold("Shanbhag dark no-reset stack");

//setThreshold(1, 255);

setOption("BlackBackground", true);

run("Convert to Mask", "method=Shanbhag background=Dark black");

run("Duplicate...", "title=bubbleHoles duplicate");

run("Erode (3D)", "iso=255");

run("Fill Holes", "stack");

imageCalculator("OR create stack", "bubbles","bubbleHoles");

selectWindow("Result of bubbles");

rename("Shanbhag bubbles");

selectWindow("bubbleHoles");

close();

selectWindow("bubbles");

close();