Enhanced material contrast by dual-energy microCT imaging

Method note
1. **Introduction**

1.1. *The basis for dual energy imaging*

Micro-computed tomography (microCT) generates a 3D map of x-ray attenuation coefficient of materials within an object, corresponding to the x-ray “opacity” of the material. So the reconstructed image stack from microCT can be considered as essentially a 3D map of x-ray opacity.

There are certain occasions where different materials in a sample have very similar or overlapping ranges of x-ray opacity. In such cases it is difficult or impossible to separate these materials from each other by density thresholding.

Dual energy CT means scanning an object or animal at two different x-ray energy settings, and it can sometimes enhance the segmentation of materials on the basis of x-ray absorption. It is based on difference between materials in absorption of x-rays at different x-ray photon energy, i.e. difference in the energy dependence of materials’ x-ray absorption.

Figure 1 shows that absorption of x-rays by materials such as air, water and bone increases with decreasing x-ray photon energy, and also that relative difference in absorption, such as between bone and water, also decreases with less x-ray energy. This is the basis for optimizing contrast in standard, single energy microCT imaging of tissues such as bone, by choosing a suitably low x-ray photon energy. (In practice this means a suitable choice of x-ray filter and applied voltage.)

Thus in general x-ray absorption decreases as x-ray energy increases; this is intuitive, one can imagine how higher energy photons will be “harder” for a material to absorb and will travel further through a material. However the phenomenon of the “absorption edge” opposes this trend, as shown in figure 2, for two elements aluminium and silicon. There is a “saw tooth” edge in these graphs indicating a certain x-ray energy at which x-ray absorption abruptly increases, opposite to the general trend of declining absorption. Above the absorption edge, absorption continues to decline again with increasing photon energy, but from a higher value.

The absorption edge is a consequence of electron shell quantum energy levels that are specific to each element. Each element has its “K, L and M” electron shells. An x-ray photon needs to have energy at or above the “binding energy” associated with each electron shell in order to undergo photoelectric absorption in that energy shell. The absorption edge is an x-ray photon energy equal to the binding energy of one of these shells. Figure 2 shows that the absorption edges of Al and Si are in the range 1-2 keV.

Absorption edges provide a basis for separating certain elements in CT reconstructions by comparing x-ray absorption at different energies. Dual-energy discrimination of microfil contrast agent from bone has been demonstrated by Depypere et al. [1] using the SkyScan1172 microCT scanner in *ex-vivo* scans of infiltrated bone.
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Figure 1. The mass-energy absorption coefficient of water, air and bone is shown to increase with decreasing x-ray photon energy (upper). In addition to the falling absolute absorption, the relative difference in absorption between bone and water (lower) also increases to a peak at low photon energy (around 30 keV).
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Figure 2. The energy-attenuation curves for aluminium and silicon [2], neighboring elements in the periodic table with atomic numbers of 13 and 14 respectively. The absorption edge characteristic of each element is evident as the up-spike in each curve. Differences in the energy-absorption curve and in the energy value of the absorption edge between elements provides the basis for dual-energy discrimination of materials in microCT.

2. Method and Results

The method for dual energy microCT acquisition is relatively simple: just perform two scans of your object or animal, at a higher and a lower x-ray energy. This is achieved by using different x-ray filters and applied voltages. The object should not be moved between scans and image magnification and pixel size should be the same. Try to make the energy difference as large as possible, while keeping sufficient transmission and absorption for reasonable image reconstruction.

Here, two examples will be given. First, a sample containing embedded silicon and aluminium particles. Second, a mouse thorax scanned to look at lung and segment the lung from the adipose (fat) tissue.
2.1. Sample containing silicon and aluminium particles

A resin embedded mixture of aluminium and silicon particles was scanned in the SkyScan1272 at applied voltages of 50 (no filter) and 100 kV (Cu 0.11mm filter). These and other combinations of filter and voltage, and the x-ray photon energy spectrum of each combination, are shown in figure 3.

Figure 3. The range of x-ray filter and voltage combinations, and their x-ray photon energy spectra.

The application program DEhist allows opening of two reconstructed datasets of the same object at different filter and voltage settings. The program window of DEhist is shown in figure 4. In the upper part of the program window, crossections are displayed for the low energy (left) and high energy (right) scans. These datasets are loaded from the “…” path buttons just above each image window.

Note – before loading each dataset, you should choose which individual crossection image level you wish to compare, and load this same crossection level for both datasets. DEhist does not allow scrolling up and down through the datasets. So do some testing (in DataViewer) to find a crossection level which contains suitable examples of the materials you wish to separate by dual-energy.

At the bottom left of the program window is the dual-energy grey histogram display, where dual-energy segmentation is performed. In this plot, the horizontal x axis is the 0-255 grey scale range from the low energy scan; on the vertical y axis, the corresponding 0-255 grey scale range from the high energy scan. Thus the grey scale histogram is extended into a 2D “phase space” representing the reconstruction results of the high and low energy scan results simultaneously.
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Figure 4. The DEhist program window. Upper left and right – the low and high energy datasets – opened at the same crosssection level. Lower left – the dual energy phase space plot of grey level, with the lines for dual-energy segmentation shown, and lower right – the red-green image display of the material segmentation of the two materials by dual energy.

Note further, when doing the high and low x-ray energy scans and reconstructions, the following:

- Keep the sample in exactly the same position for the different energy scans – do not re-position between scans;
- The scans should all have the same magnification and pixel size;
- When reconstructing the datasets, either use exactly the same ROI selection for limiting the reconstructed part of the image, or use no
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ROI: the reconstructed area, and number and range of slices, must be exactly the same;

- However your choice of reconstruction parameters such as beam hardening correction, smoothing and contrast limits etc., do not have to be the same. In fact it would be impractical to use the same parameters over a wide range of different x-ray energies. It is OK to normalize the contrast range for each scan in the normal way, i.e. lower contrast limit = 0, upper contrast limit about 10-20% above the maximum of the grey histogram as viewed in histogram log display mode.

Note – you might obtain improved dual-energy discrimination when using low values of beam hardening correction.

Applying the dual-energy segmentation

Once the two datasets are loaded, both at the same crossection level, segmentation lines should be applied to the dual-energy grey histogram plot – the image window lower left. Segmentation is applied by left mouse click on this image.

Note that the segmentation lines are in a “T” shape. Refer to the segmentation plot from figure 4, the lower left image. The long top bar of the T separates the segmented from the non-segmented domains of the dual-energy phase space plot. In this image, all pixels below this long line, which extends all the way across the image, are set to black in the segmented image, i.e. excluded as space.

On the other side of the long T-bar, the segmented domain is separated into two parts by the upright bar of the T shape. This implements the separation of the segmented part into two phases or materials.

![Figure 5. “T-bar” segmentation in DEhist. The short T-bar separates the two materials, which are displayed as red and green. The long T-bar separates material (red/green) from space. It results in a “trinary” image – three grey scales.](image)
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In the dual energy grey histogram plot, different materials are sometimes separated into different spatial regions. In figure 5, copied from figure 4 and annotated, the upright line of the T-bar divides two distinct regions of purple colored clouds. This represents the dual-energy separation of the aluminium and silicon particles. Placing the T-bar between appropriate regions in the dual energy plot will achieve the dual energy segmentation.

Control of the T-bar: click and spin

Clicking with left mouse button on the dual-energy histogram plot will activate the T-bar and set the location of its central intersection. Rolling the wheel of your mouse will rotate the T-bar. The T-bar is free to rotate without limit around 360 degrees, so the long top line of the T can be either horizontal or vertical or at any angle. To move the intersection of the T-bar to another position, just left-click at the new location.

If possible find the separation between the materials of interest in the dual energy phase space and position the short bar of the T to divide these two regions. They might be visible as patches or clouds, as in the example with Al and Si (figure 4, or the image just above).

2.2. Separating adipose tissue (fat) from lung in a mouse thorax in-vivo

Another application of dual-energy is in enhancing separation of fat from lung in in-vivo scans of a mouse thorax.

The result of the dual energy fat-lung separation is shown in figure 6 with low and high x-ray energy scans of a mouse thorax loaded in the upper dataset panes. The lung, segmented to green in the lower right image, is separated from fat which has a very similar range of x-ray opacity.

Looking at the upper images, one can see that the subcutaneous fat tissue around the periphery of the thorax is more clearly contrasted in the low than the high energy image. (The reasons for this are explained in the adipose tissue method note.)

In this case the two scans were done at 50 kV and 0.5mm Al filter, and 90 kV with 0.11mm Cu filter, respectively, in the SkyScan1176.

The dual energy phase-space plot (figure 6 lower left) again shows the short T-bar separating regions representing lung (below the short bar) and other tissues such as fat (above the short bar).
Figure 6. The DEhist program window showing dual energy scans for adipose tissue in a mouse thorax. Dual energy enhances segmentation of the lung by excluding fat regions.
2.3. Command buttons on the DEhist window

1. Load the low x-ray energy dataset
2. Load the high x-ray energy dataset
3. Save the histogram XY plot as an image
4. Save the T-bar parameters: the low energy scan threshold (on the x axis), the high energy scan threshold (on the y axis) and the angle of the long T-bar
5. Toggle between colour and greyscale view
6. Toggle image magnification between fit to screen and real display resolution
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7. Save the 3-colour dual energy segmented image as a single image for the current crosssection only.

8. Save the 3-colour dual energy segmented image for all crosssections of the dataset.

9. Displays the three T-bar dual energy segmentation parameters: the low energy scan threshold (on the x axis), the high energy scan threshold (on the y axis) and the angle of the long T-bar.

When you click on the checkmark button then an “ini” text file is saved, with filename following the dataset name. The contents of this text file look like this:

```
[D:IN-VIVO\dual energy datasets\mouse_dual_energy\mouse Cu 90kV\mouse Cu 90kV_Rec\mouse cu 90kv_Rec]
Threshold Lo=89
Threshold Hi=69
Slope=180.137931
```

The three T-bar dual energy segmentation parameters are recorded:

- “Threshold Lo”: the low energy scan threshold (on the x axis)
- “Threshold Hi”: the high energy scan threshold (on the y axis)
- “Slope”: the angle of the long T-bar

The ini file is saved in the folder of the low energy scan, and records the directory path of the corresponding high energy scan.

With the ini file saved in the low energy dataset folder, the next time the same dataset is re-opened in DEhist program is the 3 parameters of the segmentation T-bar will be automatically set and displayed.

If you wish to analyse several combinations of datasets using the same 3 T-bar parameters, then copy the same parameters into the ini file of the low energy scan dataset from each low-high energy pair of scans.
3. Conclusion

Drawing out the reconstructed grey-scale of voxels from scans at two different energies into a dual-energy XY phase-space provides an added dimension and capability in differentiating and segmenting materials. This method allows visualisation of the separation of materials using the different energy-dependence of x-ray absorption of those materials.

The dual energy capability in desktop microCT systems is limited by the broad polychromatic energy spectrum emitted by laboratory x-ray sources.

None-the-less, it can help practically in difficult cases of segmenting materials of differing elemental composition but similar and overlapping x-ray opacity at one given energy setting.

References
