Chapter 14

A Micro-SPECT/CT System for Imaging of AA-Amyloidosis in Mice

Jens Gregor, Shaun Gleason, Stephen Kennel, Michael Paulus, Alan Solomon, Philip Hawkins, and Jonathan Wall*

1. Introduction

Amyloidosis refers to a diverse group of diseases characterized by the abnormal extracellular deposition of proteinaceous substances within the body's organs and tissues. The protein substances are known as amyloid and consist of insoluble fibrils. In general, amyloid deposits arise due to the overproduction of an amyloidogenic protein or as a result of a genetic mutation that occurs in a normally soluble, innocuous, protein that renders it amyloidogenic. Amyloid deposits have been found in virtually every organ and tissue and may be of a restricted localized form or systemic and involve multiple organ or tissue systems. More than 20 proteins have been identified as components of pathologic amyloid deposits, including the A(β) peptide in Alzheimer's disease; amylin in Type-2 diabetes; and the prion protein in the spongiform encephalopathies such as mad cow disease. Our focus is on AA- and AL-amyloidosis, which respectively are composed of apolipoprotein A synthesized during chronic inflammation and immunoglobulin light chains synthesized by abnormal plasma cells.

The understanding of amyloid, its structure and biology, has advanced with the available technology [Sipe and Cohen, 2000]. The term amyloid (meaning starch-like) was coined in 1854 when a macroscopic brain tissue abnormality was found to have staining characteristics similar to cellulose when treated with iodine. Many years later, studies revealed that amyloid-burdened organs and tissues exhibited birefringence when stained with the cotton dye Congo red; that is, when viewed microscopically through crossed polarizers, there is a striking change in color in the amyloid-bound dye from red to apple green. This test is still used to diagnose the disease. Subsequently, electron microscopic analysis showed that amyloid deposits contain rigid, non-branching fibrils (10 nm in diameter). Further chemical analysis

*J. Gregor, University of Tennessee, Department of Computer Science, Knoxville, Tennessee; S. Gleason, S. Kennel, M. Paulus, Oak Ridge National Laboratory, Engineering, Science and Technology Division, Oak Ridge, Tennessee; A. Solomon, J. Wall, University of Tennessee, Graduate School of Medicine, Knoxville, Tennessee; P. Hawkins, Royal Free and University College Medical School, National Amyloidosis Center, London, UK

revealed the presence of numerous non-fibrillar accessory proteins and carbohydrates. One such protein, called serum amyloid P-component (SAP), was found to be ubiquitously and avidly associated with amyloid deposits. In recent years, exploitation of these findings led to the advent of I-123-labeled SAP scintigraphic imaging for clinical monitoring of amyloid diseases [Hawkins, 1988].

In this paper, we give an overview of a microSPECT/CT imaging system that we are developing for the purpose of studying murine models of AA- and AL-amyloidosis *in vivo*. Preliminary experimental results are provided.

2. MicroCT instrumentation and reconstruction

We acquire CT data using a MicroCAT™ II (ImTek, Inc., TN), which is a circular orbit conebeam system is equipped with a 20-80 kVp microfocus x-ray source. The detector is a 2048×3072 CCD array, optically coupled to a minR phosphor screen. The data are downsampled on the CCD via 4×4 binning. The field of view is 57 mm transaxial by 85 mm axial. We acquire 360 projections of each animal over the course of 6 minutes. See e.g. [Paulus, 2000] for more details.

With respect to image reconstruction, we have developed a modified version of the Feldkamp algorithm [Gregor, 2003]. Modifications include focus of attention which is a data-driven preprocessing scheme that automatically precludes a large portion of the background voxels from being considered during backprojection thereby lowering the overall computational cost. We have also introduced support for multi-processor-based cluster computing. Using 12 dual-processor PCs, we can thus reconstruct a 100-μm resolution image volume in the same amount of time required to acquire the data in the first place.

Recently, we implemented a parallelized version of Fessler's mono-energetic weighted least-squares algorithm [Elbakri and Fessler, 2002]. Our conebeam system matrix is based on depth-weighted trilinear interpolation and exploits geometric symmetries to allow for pre-computation and storage to a disk for subsequent use. The iterative reconstructions are computationally intensive but of high quality.

3. MicroSPECT instrumentation and reconstruction

The microSPECT head is developed by the Detector Group at Jefferson Lab and consists of a crossed-wire, multi-anode photomultiplier tube coupled to a pixellated NaI(Tl) crystal array. See [Weisenberger, 2004] for more details. The detector has a 55×55-mm^2 active area which is discretized into a 64×64 array. The resolution is approximately 2 mm FWHM. For the experimental work presented in this paper, we configured the system with a parallel-hole collimator and acquired 60 projections over the course of 30 to 60 minutes. The microSPECT system is presently housed in a separate gantry, but will be merged with the microCT platform to yield a true dual-modality system.

We reconstruct SPECT images using a parallelized version of the EM-ML algorithm that employs a volume intersection-based system matrix. Each voxel is divided into a number of subvoxels. We compute the conic view of the image

volume for each detector pixel and count the number of subvoxels seen per voxel using an inner-product computation. Due to the symmetry that results from using parallel-hole collimation, we only model the intersection pattern for a single imaginary detector column, which is then used repeatedly. As a consequence, the system matrix contains a relatively small number of non-zero elements and computes in a few seconds. A $64 \times 64 \times 64$ SPECT image volume is typically reconstructed in less than a minute.

4. Preliminary experimental results

4.1 Study 1

The Solomon group at the University of Tennessee Graduate School of Medicine has developed a transgenic mouse model of systemic AA-amyloidosis [Solomon, 1999]. The so-called H2/huIL-6 mouse expresses the human interleukin 6 (IL-6) transgene under the control of the major histocompatibility complex class 1, H2, gene promoter. Rapid induction of systemic (splenic, hepatic, renal, and pancreatic) amyloid disease in eight week old mice is achieved by injection of an amyloid-enhancing factor (AEF). See Fig. 14.1 for more detail.

In our preliminary evaluation of the microSPECT/CT system, we injected four mice intravenously with 100 μg AEF and administered control mice an equivalent amount of saline solution. After seven weeks, the mice received 600 μCi of I-125 labeled SAP, having been provided two days prior with 1% Lugol's solution in their drinking water to prevent thyroid uptake of free radioiodine. Twenty-four hourst after SAP injection, the mice were sacrificed, and image data were acquired. Figure 14.1 shows the reconstructed microSPECT and CT images co-registered for simultaneous display, with the former being a color activity map overlaying a gray-scale version of the latter. To the best of our knowledge, the resulting images provide the first detailed 3D dual-modality view of systemic AA-amyloid disease in a mouse.

We computed the biodistribution of the amyloid and compared that with the equivalent I-125 SAP image counts. We obtained the former through organ biopsies and the latter by first segmenting out the relevant organs in the CT images and then summing up the activity in the corresponding regions of the SPECT images. The two data sets were in excellent agreement, clearly indicating that the SPECT images represent organ-specific amyloid deposits.

4.2 Study 2

Our second study deals with a novel mouse model of localized AL-amyloidosis. An amyloidoma composed of human AL-amyloid extract was introduced between the scapulae of Balb/c mice by administering a subcutaneous bolus of highly concentrated material (see Fig. 14.2). The Solomon group recently developed a therapeutic monoclonal antibody (mAb) designated 11-1F4, which binds to an amyloid fibril-related epitope [Hrncic, 2000]. Natural resolution of the amyloidoma in the mouse

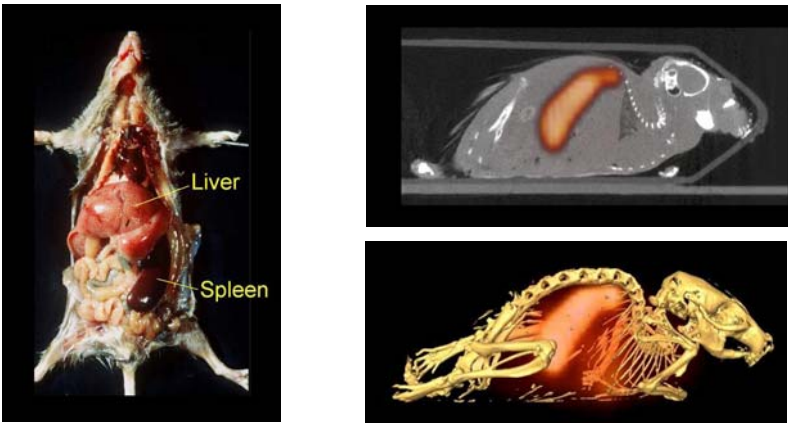

Figure 14.1. Mouse with AA-amyloidosis (left). Note the enlarged spleen and discoloration of the liver. Pseudo-colored SPECT image overlaid on top of co-registered CT image (top, right) and surface-rendered skeleton CT image (bottom, right). Bright object (high specific activity) is splenic amyloid, while cloudy object represents liver deposits.

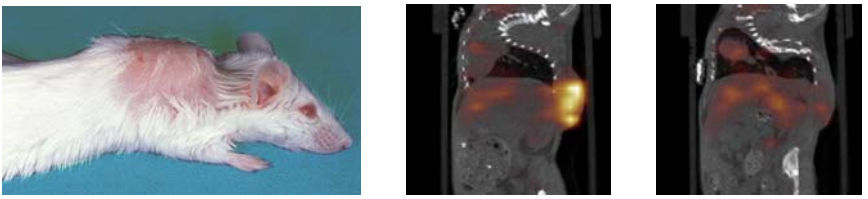

Figure 14.2. Mouse with AL-amyloidosis (left). Pseudo-colored SPECT images overlaid on top of co-registered CT images showing amyloidoma (middle) and non-specific blood pool background in the heart and liver (right).

occurs after two weeks, but can be accelerated to four days following 11-1F4 mAb treatment.

We again divided six mice into a test group of four and a control group of two. All mice received 50 mg of human-derived AL amyloid. After a week, the test mice were intravenously given I-125-labeled 11-1F4 mAb, while the control mice received an I-125 labeled isotype-matched control reagent. Following a three-day period to permit the clearance of unbound 11-1F4 mAb, the mice were sacrificed, and image data were acquired and processed as described above. The results shown in Fig. 14.2 represent the first 3D dual-modality view of localized AL-amyloidosis in a mouse model.

We again performed and compared biodistribution results with the imaging data and found the two to be correlated. In both cases, significantly higher concentrations of the 11-1F4 mAb were found in the amyloidoma compared with the heart, kidneys, and liver. The correlation indicates that radiolabeled 11-1F4 mAb is a good reagent for discerning AL amyloid deposits *in vivo*.

Our preliminary data demonstrate that the dual SPECT/CT imaging technology is capable of providing high-resolution detailed maps of amyloid deposits in mice using both SAP and amyloidophilic mAbs. Currently, a modified form of the 11-1F4 mAb is being produced in readiness for clinical trials of its efficacy as an imaging agent and therapeutic for patients with AL-amyloidosis, a devastating and invariably fatal disease.

Acknowledgments

This work was sponsored by the National Institutes of Health under grant number 1 R01 EB00789-01A2.

References

[Elbakri and Fessler, 2002] I.A. Elbakri and J.A. Fessler, "Statistical image reconstruction for polyenergetic x-ray computed tomography," *IEEE Trans. Medical Imaging*, vol. 21, pp. 89–99, 2002.

[Gregor, 2003] J. Gregor, S.S. Gleason, M.J. Paulus, and J. Cates, "Fast Feldkamp reconstruction based on focus of attention and distributed computing," *Intl. J. Imaging Systems and Technology*, vol. 12, pp. 229–234, 2003.

[Hawkins, 1988] P.N. Hawkins, M.J. Mayers, J.P. Lavender, and M.B. Pepys, "Diagnostic radionuclide imaging of amyloid: biological targeting by circulating human serum amyloid P component," *Lancet*, pp. 1413–1418, 1988.

[Hrncic, 2000] R. Hrncic, J. Wall, D. Wolfenbarger, C.L. Murphy, M. Schell, D. Weiss, and A. Solomon, "Antibody-mediated resolution of light chain-associated (AL) amyloid deposits," *Am. J. Pathol.*, vol. 157, pp. 1239–1246, 2000.

[Paulus, 2000] M.J. Paulus, S.S. Gleason, S.J. Kennel, P.R. Hunsicker, and D.K. Johnson, "High resolution x-ray computed tomography: An emerging tool for small animal cancer research," *Neoplasia*, vol. 2, pp. 62–70, 2000.

[Sipe and Cohen, 2000] J.D. Sipe and A.S. Cohen, "Review: History of the amoyloid fibril," *J. Structural Biol.*, vol. 130, pp. 88–98, 2000.

[Solomon, 1999] A. Solomon, D.T. Weiss, M. Schell, R. Hrncic, C.L. Murphy, J. Wall, M.D. McGavin, H.J. Pan, G.W. Kabalka, and M.J. Paulus, "Transgenic mouse model of AA-amyloidosis," *Am. J. Pathol.*, vol. 154, pp. 1267–1272, 1999.

[Weisenberger, 2004] A.G. Weisenberger, B. Kross, S. S. Gleason, J. Goddard S. Majewski, S.R. Meikle, M.J. Paulus, M. Pomper, V. Popov, M.F. Smith, B.L. Welch, and R. Wojcik, "Development and testing of a restraint free small animal SPECT imaging system with infrared based motion tracking," *IEEE Trans. Nuc. Sci.* To appear.