RESEARCH ARTICLE

High Speed Data Processing for Imaging MS-Based Molecular Histology Using Graphical Processing Units

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Abstract

Imaging MS enables the distributions of hundreds of biomolecular ions to be determined directly from tissue samples. The application of multivariate methods, to identify pixels possessing correlated MS profiles, is referred to as molecular histology as tissues can be annotated on the basis of the MS profiles. The application of imaging MS-based molecular histology to larger tissue series, for clinical applications, requires significantly increased computational capacity in order to efficiently analyze the very large, highly dimensional datasets. Such datasets are highly suited to processing using graphical processor units, a very cost-effective solution for high speed processing. Here we demonstrate up to 13× speed improvements for imaging MS-based molecular histology using off-the-shelf components, and demonstrate equivalence with CPU based calculations. It is then discussed how imaging MS investigations may be designed to fully exploit the high speed of graphical processor units.

Key words: Imaging mass spectrometry, Molecular histology, Graphical processor units, GPU, Bioinformatics

Introduction

Matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) can generate MS profiles directly from tissue that contain hundreds of distinct biomolecular ions [1]. Spatially-correlated analysis, imaging MS, can simultaneously reveal how the intensity of each of these biomolecular ions varies across the tissue sample [2]. There is growing evidence that imaging MS is having an impact in disease detection, particularly cancer [3]. The differential MS profiles found in tumors can be used to identify candidate biomarkers [4], and when combined with clinical outcomes identify MS signatures associated with prognosis [5].

One of the advantages of imaging MS is that it can annotate tissues based on their MS profiles and thereby distinguish

biomolecularly different regions even if they are not distinct using established histologic and histochemical tools. Imaging MS-based molecular histology has been used to differentiate between histologically overlapping/identical tumors [6], found evidence of intratumor heterogeneity that could indicate clonal development [6–8] and revealed altered protein expression at tumor interface zones [9, 10]. These capabilities offer enormous potential to investigate biomolecular changes that occur prior to (or without) morphological change, or those for which a molecular specific stain is unavailable.

Imaging MS experiments are recorded as a list of discrete spectra, each associated with a specific pixel. MS images are created by displaying the intensities of an MS peak, from each spectrum, at their pixel locations as a false color image. When subjected to multivariate or cluster analysis, to identify regions with distinct MS profiles, the imaging MS data is analyzed as the list of discrete spectra. The image-outputs of the multivariate/cluster analysis are obtained by

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projecting each spectrum's score onto its pixel location as a false color image.

If multiple tissue sections, mounted on to the same MALDI target plate, are analyzed within a single data acquisition sequence the imaging MS data is still a list of pixel-associated spectra but the pixel coordinates reflect the different locations of the tissues. We have adapted this approach to enable the datasets of multiple tissue sections, recorded on different days (and on different MALDI target plates), to be combined [8]. Pixel offsets are used to place each tissue's MS data onto a common image plane. The imaging MS datasets are then unfolded into a collated list of pixel-associated-spectra (pixel coordinates within the common image plane).

The simultaneous multivariate analysis of a tissue series containing multiple patient tissue samples enables three distinct sources of variance to be distinguished. For example, for the differentiation of two tumors:

- intratumor variability within each patient tissue
- inter-patient variability within each tumor class (endogenous variability + measurement variability)
- variation between the two tumors

An estimate of the first two sources of variance will be essential in order to establish if the observed differences between the two tumors are statistically significant.

One of the challenges facing the clinical application of imaging MS based molecular histology, whether human samples or from animal model investigations (3D imaging MS, multiple time points, multiple MS and animal repeats), is the sheer size and dimensionality of the data. An imaging MS analysis of a single tissue section typically contains >10 k pixels, each with an associated mass spectrum. A 50-tissue patient series of 1 cm² tissue sections, imaged with 100 μ m spatial resolution, would contain half a million mass spectra. High performance computer processing is necessary to efficiently process such large, multidimensional datasets.

The increased processing power provided by General-Purpose computing on Graphics Processing Units (GPGPU) provides a cost-effective and low-energy solution for the data analysis capabilities required for imaging MS-based molecular histology of larger tissue series or higher spatial resolution data sets. GPUs excel at tackling large amounts of similar data if the problem can be split into hundreds or thousands of pieces and calculated simultaneously. When combined with CPUs, which are adept at serial-based tasks such as data organization, the end result is a tremendous speed-up in overall system performance. The increased processing speeds provided by GPUs have recently been exploited in mass spectrometry-based proteomics to increase the speed of comparing an MS/MS spectrum with all of the potential matches from a sequence database [11], and faster (200 $\times$) and improved feature detection using wavelet decomposition [12].

Off-the-shelf systems are available that combine high processing speeds (>1 TFLOPS) and abundant on-board

memory, with architectures (e.g., CUDA) that make GPUs increasingly more suitable for commercial programming and mathematical packages such as Matlab. Furthermore, it has been shown that the performance benefit of GPU processing increases with increasing matrix size (within memory limits); the large matrices generated by imaging MS of a series of tissue sections (>100k of pixels and mass spectra) are very well suited to GPU computing. Here we demonstrate the significant, >10 $\times$, improvements in processing speed that can be obtained by exploiting commercially available GPU processing boards. The greatly increased processing speeds and cost-effectiveness of GPU boards make them an excellent option for the further development of imaging MS as a novel molecular histology tool.

Methods

Computer Hardware and Software

A Dell Precision T7500 workstation equipped with a single 6-core Intel Xeon X5650 2.66 GHz CPU, 48 GB DDR3 RAM (1333 MHz), and an NVidia Tesla C2070 GPU processing board, running on 64-bit windows 7, was used. Calculations were performed using 64 bit MatLab v. 7.12.0 (R2011a), and the Matlab toolboxes Bioinformatics (v. 3.7), Image Processing (v. 7.2), Parallel Computing (v. 5.1), Signal Processing (v. 6.15), and Statistics (v. 7.5). A GPU engine for Matlab, Jacket (v.1.8.0, Accelerereyes), was used to convert the single threaded Matlab scripts to GPU-enabled code (www.accelereyes.com).

Imaging MS Datasets

A number of different imaging MS datasets were used to test the performance of the GPU processing board for imaging MS-based molecular histology:

- (1) Mouse brain tissue series: neuropeptide imaging MS datasets of five coronal mouse brain tissue sections, imaged with 200 μ m spatial resolution using a Ultraflex MALDI-TOF/TOF (Bruker Daltonics, Bremen, Germany) and 2,5-dihydroxy benzoic acid as the matrix. The project consists of 8976 pixels and a total raw data load of 2.1 GB.
- (2) Chondrosarcoma tissue series: protein imaging MS datasets of five central chondrosarcoma and five secondary peripheral chondrosarcoma, imaged with 200 μ m spatial resolution using a Bruker Autoflex II MALDI-TOF and α -cyano-4-hydroxycinnamic acid as the matrix [13] (for these bone tumors it was found that α -cyano-4-hydroxycinnamic acid provided better protein mass spectra than the more common protein matrix sinapinic acid). The project consists of 28201 pixels and a total raw data load of 9.8 GB.
- (3) Rat brain tissue section: neuropeptide imaging MS dataset of a coronal rat brain tissue section, imaged with 100 μ m spatial resolution using a Bruker UltrafleXtreme

MALDI-TOF/TOF and α -cyano-4-hydroxycinnamic acid as the matrix. The raw dataset consists of 19908 pixels and a total raw data load of 6.3 GB.

Mass Spectral Processing and Data Reduction

Each pixel's mass spectrum was processed using a smoothing and baseline subtraction routine using Flex-Analysis (Bruker Daltonics, Bremen, Germany). For protein imaging MS, a Gaussian algorithm was used for mass spectral smoothing (width $2m/z$, 4 cycles) and the ConvexHullV3 algorithm used for baseline subtraction. For neuropeptide imaging MS a SavitskyGolay algorithm was used for mass spectral smoothing (width $0.02m/z$, 2 cycles) and the TopHat algorithm used for baseline subtraction.

An automated mass-spectral feature detection algorithm was then applied to each imaging MS dataset [14]. For single tissue analyses the intensity of each peak was then extracted from each pixel's mass spectrum to reduce the large imaging MS datasets to much smaller image cubes that only contain the images of well-described MS peaks [14]. All GPU processing was performed on these reduced datasets.

For the analysis of projects containing imaging MS datasets of multiple tissues the mass-spectral feature detection routine was first applied to each individual dataset. The resulting dataset-specific peak lists were then collated into a final project-specific peak list (mass accuracy ± 500 ppm), which was then used to extract all features from each imaging MS dataset [8]. The reduced imaging MS datasets were then merged into a single project dataset using pixel offsets [8]. In this manner the multivariate techniques could be simultaneously applied to all imaging MS datasets in the project.

After data reduction with the automated feature detection and extraction routine the 2.1 GB imaging MS project of the mouse brain tissue series was reduced to a 33 MB image cube containing the images of 476 MS features; the 9.8 GB imaging MS project of the chondrosarcoma tissue series was reduced to a 92 MB image cube containing the images of 427 MS features; and the 6.3 GB imaging MS dataset of the rat brain tissue section was reduced to a 49 MB image cube containing the images of 325 MS features. Note: data reduction via automated peak identification and extraction has the disadvantage that peaks below the signal-to-noise (S/N) threshold, but which may contribute to the differentiation, are not included in the subsequent data analysis. While a lower S/N threshold may be used, this can lead to a rapid increase in the amount of chemical noise retained in the dataset, which can undermine the ability of the data analysis tools to differentiate between the different regions of the imaging MS datasets. The lower data loads provided by data reduction are essential to the practical application of imaging MS-based molecular histology [14].

Table 1. Data load, number of variables, and processing time as a function of series size and spatial resolution (before and after data reduction)

	Small, low res.		Large, low res.		Large, high res.	
	Raw	Proc.	Raw	Proc.	Raw	Proc.
No. of tissues	10		100		100	
Tissue size (cm ²)	2		2		2	
Resolution (μ m)	100		100		25	
Pixels (spectra)	200 k	200 k	2 M	2 M	32 M	32 M
spectral channels	70 k	150	70 k	150	70 k	150
Dataload (GB)	52.15	0.22	521.54	2.24	8344.65	35.76
FLOPs	1E+16	6E+10	1E+17	6E+11	2E+18	1E+13
Proc. time [#] (s)	201 k	0.9	2 M	9.0	31 M	144
Proc. time (d)	2.3		22.7		363	

[#]Using a3.8 GHz i7, 70 GFLOPs maximum processing speed.

^aNumber of floating point operations (FLOPs) given for a common PCA algorithm, where $FLOPs = 14 \cdot k \cdot N^2 + 8 \cdot N^3$, k is the number of pixels, N the number of channels [18].

Statistical Analysis Implementation

The reduced data is extracted as a 2D matrix with the extracted peak intensities from each pixel arranged in rows and normalized to each pixel's total-ion-count. A second matrix contains the coordinates of each pixel. Principal component analysis was performed using the *princomp* routine from the Matlab statistics toolbox without modification. Non-Negative Matrix Factorization is based on David Ross's (University of Toronto: <http://www.cs.toronto.edu/~dross/>) implementation of Lee and Seung's Non-Negative Matrix Factorization algorithm [15]. For determining the correlation between images, the images were unfolded into one-dimensional vectors and the Pearson correlation between the vectors calculated.

Figure 1. Relative processing speed of multivariate analysis using the Tesla C2070 GPU (grey bars) versus the Xeon X5650 CPU (white bars). The mouse brain tissue sections dataset contains the reduced imaging MS data of five coronal mouse brain tissues, and consists of 476 discrete MS peaks extracted from 8976 pixels. The larger chondrosarcoma dataset contains the reduced imaging MS data of 10 chondrosarcoma tissue sections, and consists of 427 peaks extracted from 28,201 pixels

Figure 2. Comparison of images and loading plot outputs obtained by NMF analysis of a rat brain tissue section using the Xeon X5650 CPU and the C2070 GPU. Reduced imaging MS data consisted of 325 peaks from 19,908 pixels

Table 2. Processing times for non-negative matrix factorization of model imaging MS datasets as a function of number of pixels and number of peaks retained in the reduced imaging MS dataset. Model data consists of random double precision matrices

'Pixels' × 'peaks'	Load (MB)	C2070 (s)	X5650 (s)	Improvement
2,000×100	1.6	0.17	0.49	2.88
10,000×100	8	0.57	2.44	4.28
50,000×100	40	2.56	11.80	4.61
100,000×100	80	10.33	46.33	4.48
500,000×100	400	25.81	115.61	4.48
1 500,000×100	1 200	79.16	362.50	4.58
2 000, 000×100	1 600	321.01	470.92	1.47
500,000×150	600	29.64	184.83	6.24
500,000×300	1 200	46.95	354.32	7.55
100,000×150	120	5.75	36.33	6.32
100,000×300	240	7.87	70.91	9.01
100,000×500	400	10.51	118.93	11.32
100,000×800	640	16.36	193.07	11.80
100,000×1200	960	21.71	284.54	13.11
100,000×1500	1 200	26.83	354.76	13.22
100,000×2000	1 600	251.40	462.52	1.84

Results and Discussion

The data load and estimated data processing times, using a single high performance processor for principal component analysis (PCA) of a tissue series are provided in Table 1 as a function of series size (number of tissues) and spatial resolution, for both raw data (containing all mass spectral channels) and data that has been reduced via an automated mass-spectral feature identification and extraction routine [14]. The estimated PCA data processing times are close to that obtained in reality. It was estimated that the reduced dataset from a single intermediate grade myxofibrosarcoma tissue section would require 0.1 s, in practice the analysis took 0.3 s, indicating the increase in processing times with an increasing number of tissue samples or spatial resolution will be substantial. This is especially true of the data analysis techniques that have been shown to provide improved data

analysis capabilities but which are computationally more demanding [8, 16]. The computation times for the analysis of the same reduced data with k-means clustering (3.1 s), maximum autocorrelation factor analysis (6.7 s), non-negative matrix factorization (18.3 s), hierarchical clustering (41.7 s), fuzzy c-means (34.3 s), and probabilistic latent semantic analysis (27.1 s) are all substantially longer than PCA. The application of these data analysis algorithms to tissue series containing many tissue samples or higher spatial resolution analyses (containing many more pixels) will require increased processing speeds.

Figure 1 shows the speed improvements that can be obtained for multivariate analysis of reduced imaging MS tissue series' using a single NVidia Tesla C2070 GPU processing board, which contains 448 CUDA cores and 6 GB of onboard memory. The mouse brain project contains the *reduced* (see the Methods section) imaging MS data from five coronal mouse brain tissue sections and consists of the intensities of 476 discrete peaks extracted from 8976 pixels and saved as double precision values, total data load 33 MB. Using the C2070 GPU board NMF and PLSA was performed in 1.11 s and 5.89 s, as opposed to 10.58 s and 59.50 s, using the Xeon X5650 processor (a high performance i7 6-core processor, using all six cores), a 10-fold increase in data processing speed. The chondrosarcoma project contains reduced imaging MS datasets of five central chondrosarcoma and five secondary peripheral chondrosarcoma patient tissues, and contains more pixels and a greater data load. The reduced dataset contains the intensities of 427 discrete peaks, saved as double precision values, extracted from 28,201 pixels, total data load 92 MB. Using the C2070 GPU board NMF and PLSA of the reduced chondrosarcoma dataset was performed in 11.52 s and 57.73 s, whereas with the Xeon X5650 CPU it required 109.59 s and 523.07 s; again a near 10-fold increase in processing speed.

Figure 3. (A) Comparison of processing times for NMF of model matrices using the X5650 CPU and the C2070 GPU board, (B) GPU processing speed improvement for the 100k and 500k pixel datasets, and (C) effect of matrix rectangularity on GPU processing speed improvement for the 150M datapoint datasets

PCA is a much faster data analysis algorithm, so the benefit of using GPU processing only became apparent with the larger chondrosarcoma dataset because of the time penalty of transferring the data to the GPU board. Using the GPU board PCA of the mouse brain and chondrosarcoma projects took 1.02 s and 4.99 s, whereas with the Xeon X5650 CPU it required 0.99 s and 10.8 s, respectively. For the smaller mouse brain dataset the time penalty required for transferring data to the GPU board caused the PCA algorithm to be faster on the Xeon X5650 processor.

The results of PCA analysis using the GPU processing board were identical to those obtained using the Xeon X5650 processor; for NNMF the results were largely identical. Figure 2 shows a comparison of the NNMF component outputs of a neuropeptide imaging MS dataset of a rat brain tissue section using the Xeon X5650 CPU and the C2070 GPU board. The Pearson correlation values indicate that the images and loading plots for each component output are very highly correlated (but sometimes not identical). Close examination of the images and loading plots reveals that any differences are small and characterized by different relative intensities. NNMF is an iterative

factorization process—the small differences in the results obtained using the CPU and GPU reflect the different starting points of the factorization process.

The NVidia Tesla C2070 contains 6 GB of onboard memory. Data transfer to the GPU processing board is slow, for example 0.638 s for transferring the reduced chondrosarcoma data to and from the GPU. Consequently optimum performance of GPU processing is maintained only if calculations are performed with minimal data transfer between the GPU board and the main system RAM.

To ascertain the data load that could be analyzed with the GPU board while maintaining high processing speed model datasets consisting of random double-precision matrices were constructed and the NNMF processing times determined as a function of length (number of pixels in an imaging MS experimental series) and width (number of peaks) using the GPU processing board and the Xeon X5650 CPU, Table 2. GPU processing provided significantly increased processing speeds for reduced imaging MS data sets up to and including 1.2 GB. The 1.2 GB is the memory required to store the reduced imaging MS datasets; additional memory is required to perform the multivariate

Table 3. Data load and rectangularity of reduced imaging MS datasets as function of spatial resolution and number of tissue sections in tissue series. Combinations highlighted in grey can be analyzed with high speed using the Tesla C2070 GPU board

A) Dataload of reduced imaging MS data

		Number of Tissue Samples in Experiment							
		1	2	4	8	16	32	64	128
Spatial Resolution / μm	5	11.92	23.84	47.68	95.37	190.73	381.47	762.94	1525.88
	10	2.98	5.96	11.92	23.84	47.68	95.37	190.73	381.47
	25	0.48	0.95	1.91	3.81	7.63	15.26	30.52	61.04
	50	0.12	0.24	0.48	0.95	1.91	3.81	7.63	15.26
	75	0.05	0.11	0.21	0.42	0.85	1.70	3.39	6.78
	100	0.03	0.06	0.12	0.24	0.48	0.95	1.91	3.81
	150	0.01	0.03	0.05	0.11	0.21	0.42	0.85	1.70
	200	0.01	0.01	0.03	0.06	0.12	0.24	0.48	0.95

B) $\text{Log}_{10}(\text{rectangularity})$ of reduced imaging MS data

		Number of Tissue Samples in Experiment							
		1	2	4	8	16	32	64	128
Spatial Resolution / μm	5	4.0	4.3	4.6	4.9	5.2	5.5	5.8	6.1
	10	3.4	3.7	4.0	4.3	4.6	4.9	5.2	5.5
	25	2.6	2.9	3.2	3.5	3.8	4.1	4.4	4.7
	50	2.0	2.3	2.6	2.9	3.2	3.5	3.8	4.1
	75	1.6	1.9	2.2	2.6	2.9	3.2	3.5	3.8
	100	1.4	1.7	2.0	2.3	2.6	2.9	3.2	3.5
	150	1.0	1.3	1.6	1.9	2.2	2.6	2.9	3.2
	200	0.8	1.1	1.4	1.7	2.0	2.3	2.6	2.9

analysis. The memory required to perform multivariate analysis of datasets greater than 1.2 GB leads to data transfer between the system RAM and the GPU board, impeding the apparent processing speed.

Figure 3A shows a graphical representation of Table 2, in which the abscissa is the total number of variables (number of pixels $\times$ number of peaks). It can be seen that the processing time using the Xeon CPU and the GPU increase approximately linearly with the total number of variables, but the rate of increase for the GPU is much less. The apparent scatter in the GPU processing times is related to the rectangularity of the 2D data matrices (number of pixels/number of peaks). Figure 3B shows the relative enhancement of GPU processing, with respect to the Xeon X5650 CPU, for the 100 and 500 pixel series of Table 2. It can be seen that the enhancement increases with an increasing total number of variables, which corresponds to an increasing number of MS peaks, until the memory limitations of the GPU card are reached. The enhancement of the 500 k pixel series is less because of the increased rectangularity of the data matrix. Figure 3C presents the relative enhancement of GPU processing for datasets containing 150 M data points (1.5 M pixels $\times$ 100 peaks, 500 k pixels $\times$ 300 peaks, and 100 k pixels $\times$ 1500 peaks) versus the rectangularity ratio of the matrix. Larger improvements in processing speeds are obtained with matrices of lower rectangularity.

It is instructive to consider how the above observations concern imaging MS based molecular histology of tissue series, specifically how one may design the experiment to maintain its compatibility with GPU processing. Table 3 shows the data load and rectangularity of the reduced imaging MS data as a function of spatial resolution and number of tissues (assuming tissue sections of 1 cm² and the images of 400 peaks extracted from the raw imaging MS data).

The data from the combinations of spatial resolution and number-of-tissue sections highlighted in grey can be analyzed on the C2070 GPU board without data exchange with system RAM and have rectangularities less than 1000, thus maintaining high speed data analysis capabilities. Using 100 μ m spatial resolution up to forty 1 cm² tissues can be analyzed (1.19 GB dataload). Such a scenario is expected from neuropeptide and protein imaging MS analyses (e.g., the mouse brain and chondrosarcoma imaging MS datasets used to construct Figure 1).

The detection of an increased number of distinct molecular ions, as may occur with a high mass resolution analysis of the proteolytic peptides produced via on-tissue digestion, decreases the rectangularity of the matrices but increases the data load. If 1000 distinct mass spectral features are retained in the reduced imaging MS datasets, only sixteen 100 μ m spatial resolution imaging MS datasets of 1 cm² tissues could be analyzed using the C2070 GPU board (before data exchange with the system RAM).

Table 3 also indicates one of the general challenges facing high spatial resolution imaging MS analysis—the large number of pixels lead to very large data loads and

highly rectangular data matrices. The data load from just four 1 cm² tissues, analyzed with 10 μ m spatial resolution and including the images of 400 ions, is 11.92 GB. Previous multivariate analysis of high spatial resolution imaging MS data has used mass spectral and spatial binning, as well as limiting the analysis to specific mass ranges, to reduce the data load and to focus the analysis on the more relevant molecular information [17]. In order to maintain high spatial resolution during data analysis it will be important to parse the data as much as possible, by focusing the analysis on specific regions of interest and MS features.

The highly encouraging performance metrics reported here for imaging MS based molecular histology using GPU processing were obtained using commercial systems, and so will be underestimates of the speed improvements that can be attained with codes written explicitly for such processing. For example, Hussong *et al.* [12] have reported 200 $\times$ speed improvements for mass spectral denoising and feature detection using wavelet based methods. Optimized programming, perennial advances in computational technology (hardware and software) and the possibility to develop cost-effective GPU clusters for super-computer processing capacity, at a fraction of the financial and environmental cost of a CPU based system (e.g. www.littlegreenmachine.org), means that there is widespread scope for further improving the data processing capacity of imaging MS datasets using GPU based systems.

Conclusion

There is increasing evidence that imaging MS based molecular histology can complement established histologic and histochemical methods by distinguishing between pathologic entities with overlapping/identical pathologies, revealing intratumor heterogeneity, and identifying tumor interface zones. The application of imaging MS based molecular histology to series of tissues, whether clinical cohorts or from animal models of disease, requires increased processing power to simultaneously analyze the large number of tissues. The high dimensionality of imaging MS data is particularly well suited to GPU processing; here it is demonstrated that improvements in processing speed up to 13 $\times$ can be obtained using relatively inexpensive, commercially available systems. GPU processing represents the most cost-effective method for obtaining the high processing capacity required to apply the multivariate data analysis techniques at the heart of imaging MS-based molecular histology to the larger tissue series necessary for its clinical application and cross-validation [8].

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