Real-time, image analysis of living cellular-biology measurements of intelligent chemistry

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ABSTRACT

This paper reports on the Pacific Northwest National Laboratory (PNNL) DOE Initiative in Image Science and Technology (ISAT) research, which is developing algorithms and software tool sets for remote sensing and biological applications. In particular, the PNNL ISAT work is applying these research results to the automated analysis of real-time cellular biology imagery to assist the biologist in determining the correct data collection region for the current state of a conglomerate of living cells in three-dimensional motion. The real-time computation of the typical 120 MB/sec multi-spectral data sets is executed in a Field Programmable Gate Array (FPGA) technology, which has very high processing rates due to large-scale parallelism. The outcome of this artificial vision work will allow the biologist to work with imagery as a creditable set of dye-tagged chemistry measurements in formats for individual cell tracking through regional feature extraction, and animation visualization through individual object isolation/characterization of the microscopy imagery.

Keywords: real-time, FPGA-hardware, image analysis, automated blob-analysis, cellular biology, confocal microscopy.

1. INTRODUCTION

The dramatic changes in Microbial and Cellular Biology over the last five years has provided a microscopic imagery analysis of living cells that is on the order of the image analysis performed in the National Technical Means (NTM) satellite imagery. Furthermore, the “datacube” created in multi-modal and multi-spectral imaging cellular biology systems is in three spatial dimensions (i.e., 3-D, in z-axis of depth-of-field slicing of full Mpixel-sized images), with time sequencing, and a number of spectral fluorescence emission bands (colors), forming a 5D hypercube for analysis. The ability to reliably extract quantitative information about cellular biology from this data stream is a current challenge to the research community. Contrary to NTM image analysis functions, such as automatically locating and characterizing “target” signals of interest in the environmental terrain clutter (e.g., remote sensing, RS), cellular image analysis requirements vary with instrumentation, sample type, and research objectives. The functions must clearly involve removing microscopy artifacts of optics, lighting, and camera pixel registration, including removal of camera skew due to the complex optical axes used in multispectral instruments. These *image regulation* artifacts can be dynamic, due to thermal fluctuations of the instrument. They also must deal with radiometric computations across the modalities of the data using ratio computations at the pixel level. More macro-level computations might involve *segmentation* of the image into individual cell regions and cell components, including the definition of cell membranes, *cell counting and tracking* of 3-D movement over time in living cell microscopy, and using *extracted feature values* for quantitative representation of the dynamic chemistry^{1,2,3,4}.

Many of these functions can utilize feature extraction algorithms in an *automated manner*, which must also be accurate when compared to a human analyst’s results. However, because these are living cells, one must make a rapid assessment of where the “action” of the experiment is occurring in 3-D, in order to have the imagery resolution in the region of interest before the laser light photobleaches the dyes and/or kills the cells. Hence, unlike in the NTM domain, local, *real-time image analysis* is required. Developing these algorithms and software running in efficient and low cost hardware can also have an impact on the overall cost of future optical cellular microscopy instrumentation. This paper presents work performed under the Pacific Northwest National Laboratory (PNNL) DOE initiative in Imaging Science and Technology (ISAT) to establish an approach for accomplishing these goals, and describes the similarity of NTM imagery with cellular biology (CB) imagery. Example imagery is presented with some example analysis, along with a

unique, real-time data processing implementation approach. The potential quality of this automated approach in *artificial vision measurements* can even be used in supporting the simulation and modeling of cellular biology for understanding the systemology of living cells.

2. BACKGROUND

Biological samples can be living or fixed, and span an increasing size range from individual microbial cells (~1 micron dia.), individual mammalian cells (~10 microns), microbial cellular communities, and cell agglomerates or tissues. The sample size and desired information dictate the imaging modality and sample handling required for study. There is a diverse range of biological imaging instrumentation available, which provides complementary anatomical, chemical, physical, and transport information. Present focus is on the development of image-processing capabilities for optical methods. This encompasses a large range of microscopy techniques, including various implementations of white-light microscopy and fluorescence microscopy, and provides anatomical and chemical information with ~1 micron spatial resolution over a ~1mm maximum field of view (FOV).

2.1 DOE Genomes to Life Program

The DOE Genomes to Life (GTL) Program has identified important *imaging requirements* to capture the complexity of microbial and cell-cell interactions and cell environmental response. In the November 2002 report⁵, a number of important image analysis functions are cited, which pertain to taxonomy of “the complex 3-D microbial communities and their environments and which characterize the physical, chemical, and biological interactions occurring in them,” and some are listed in Table 1.

Table 1. GTL declared image functions and image analysis techniques^{5,6}

Imaging Function	Image Analysis Technique
1) Determine structural and functional properties of multicellular systems (p 22)	regional segmentation with fluorescence functional tagging through spectral analysis
2) Determine how the microbial community and physical environment are arranged (p 26)	taxonomy of regional segmentation
3) Determine how microbes interact among themselves and with their community (p 26)	spatio-temporal analysis of regional taxonomy
4) Monitor the flow of energy and elements through the community and how that flow is regulated (26)	correlated, temporal-analysis of image sequence for spectral migration over regions
5) Identify the activation state of signaling networks triggered by environmental perturbation (p 26)	monitor regional spectrally tagged states with noted environmental changes
6) Determine cell culture stability and composition, and parametric monitoring (p 23)	track spatio-temporal region changes with spectral density and coloration
7) Combine wide-field and high resolution technologies for cell tracking in 2D and 3D space, and in time (p 23)	track regional structures inside membranes with extracted feature characterization
8) Determine spatial and temporal concentration measurements of signaling molecules and metabolites at the intra- and extra-cellular level (p 26)	combine function 6 and 7
9) Characterize the physical and chemical properties of interfaces (p 26)	characterize features from inside and outside membranes

Many of these goals apply equally well for the study of mammalian cells and cell systems, e.g., cell-cell interactions are believed to play an important roll in “bystander effects,” where cells react indirectly to their *irradiated* neighbors⁷. Preliminary ISAT biological imaging issues include automated 3-D image registration for multiple-camera instrumentation, using 3-D registration approaches from RS work. These instruments have several CCD cameras with separate optical paths, and thus it is necessary to account for differing camera alignment (e.g., rotation/scale orientation and skew), plus the effects of thermal expansion due to temperature fluctuation. Figure 1 shows current image co-registration data collected for a dual-camera confocal fluorescent microscope. Simultaneously acquired images for a few fluorescent-labeled beads provide the minimal amount of information to co-register the images. A more detailed fluorescent fiducial sample will be devised, which will allow for the correction of normal nonlinear optical distortions

(lens warp or pin-cushioning) and proper 3-D spatial co-registration. Subsequent monitoring of the image registration during experiments will employ the automated analysis of sample images.

Also, methods will be investigated for the rapid, coarse 3-D visualization of the entire sample. This will serve to rapidly identify features of interest for further detailed imaging, and minimize the image-degrading effects of photobleaching. Finally, a parallel instrument interface will be developed from which to visualize the 3-D image data in real time, and issues of improved hardware synchronization will be investigated.

Figure 1. Image co-registration for a dual-camera confocal fluorescent microscope. Top: Simultaneously acquired images (488 and 568 nm emission bands) for a few fluorescent-labeled 16-micron-diameter beads. Bottom: Overlay of pseudo-colored co-registered images.

2.2 Morphological characterization/classification

The morphological characterization of cells involves the determination and classification of cell size and shape. It is used for the recognition and classification of cells as normal, precancerous, and cancerous (e.g., for conventional Pap smear analysis of fixed cells for cervical cancer screening). Figure 2 shows a white-light microscopy image of a fixed tissue sample, stained to yield better feature contrast. The same stained regions of the original image regions are regrouped, after using an ISAT tool for performing an automated segmentation tiling and classification procedure. In vitro, morphological *change detection* can be monitored using another ISAT tool from RS applications; e.g., cells generally dissociate from the microscope slide and become round before undergoing cell division. This regrouping forms segmented image regions clustered together with a similar appearance.

Figure 2. Automated object recognition in white light cell imagery. Left: original image. Right: segmented image into similar structures with the arrows relating the clusters. Note that the text is automatically isolated.

2.3 Intercellular communication and interaction

Biological applications include the study of cell-cell interactions and intercellular signaling, such as the cue from neighboring cells to stop growing when there is no room for further growth. This is of direct relevance to cancer research, where a miscommunication of cells leads to continued cell replication. Another important intercellular interaction involves the cooperative interaction of cells to form tissues. Here, detection of one channel is optimized to detect the receptor emission or a volume-filling nonspecific dye, while a second detector is optimized to detect a contrast agent specific to a fluorescent-labeled ligand of a compound of interest. Figure 3 shows superimposed confocal

Figure 3. Superimposed confocal fluorescence images for Chinese hamster ovary (CHO) cells showing the chemical interaction between cell-receptors/growth-factors associated with intercellular communication for 3-D cell agglomeration.

fluorescence images for Chinese hamster ovary (CHO) cells beginning to form three-dimensional agglomerates. The image of Figure 3 was acquired from a 3-D cell sample on a microscope slide from the bottom-most layer of cells. The green pseudo-color component shown represents cells that have been exposed to an epidermal growth factor (EGF) and are beginning to organize into a 3-D cell cluster. The remaining (red) cells were not exposed to EGF and remain as individual cells. These green and red pseudo-color components represent fluorescent tracers detected at emission frequencies of 488 nm and 568 nm respectively. The FOV is approximately $250\text{ }\mu\text{m} \times 600\text{ }\mu\text{m}$ with an isotropic inplane resolution of $1\text{ }\mu\text{m}$. Red/green overlap is shown in yellow. These receptor-mediated cell-cell interactions trigger biochemical pathways that lead to an appropriate cell response, or improper response in the case of cell malfunction. The research challenge for ISAT is to develop an automatic representation of the cell structure and communication from 3-D image data sets, where the chemical dynamics of the living cell may be discerned.

2.4 Intracellular transport

Image analysis of intracellular transport processes such as intracellular calcium release and propagation in mammalian cells is even more challenging due to the short distances and, thus, short propagation times. A relevant biological example is calcium release and propagation within a cell. Here, multiple channel detection is useful, where each channel is optimized to detect the emission from a particular subcellular feature such as the cytoplasm, cytoskeleton or specific organelles like the mitochondria or nucleus, and another channel is dedicated to monitoring the trafficking of a particular contrast agent or a chemical process of interest. This type of study requires the highest attainable spatial and temporal resolution, e.g., 1-micron spatial resolution over a 100 micron FOV and 120 frames per second from each channel, resulting in a data stream rate of 1.2 Mpixels per second per channel. Figure 4 shows a representation of intracellular-extra cellular particle trafficking, in which an image of polydisperse, auto-fluorescent europium nanoparticles (color contour map) is superimposed onto the grayscale image of a 20-micron-diameter mammalian cell. This image represents a single time point in a high-temporal-resolution intracellular transport experiment. For the ISAT Initiative, the automated image analysis challenge in artificial vision is to track particle formation and migration over time.

Figure 4. Single time point for intracellular nano-particle transport (color) in a single mammalian cell (black/white). The contour levels indicate the particle concentrations. The FOV is approximately 20 microns (one cell diameter) per side.

3. APPROACH

3.1 CB image analysis algorithms

As an example of the use of the ISAT toolset useful for cellular analysis, the combined cellular image of Figure 3 is processed for each of its component images, in order to isolate cell regions. Figure 5 shows the two initial gray-scale cell images from each color component. These images are first analyzed for forming binary images by histogram-based thresholding.

Figure 5. Image components from composite image in fig. 3. Left: Red Component. Right: Green Component.

Figure 6 shows the histograms of this data used in automatically establishing the proper threshold for binarizing the image, as the first step in blob analysis (described in more detail in a later section).

Figure 6. Histograms of the respective images of fig. 5. Left: Red Component. Right: Green Component.

The histogram thresholding algorithm is adaptive, as each image will have a varying intensity and cell structure shown in the histograms of Figure 6. The thresholded images are then processed with run-length encoding (RLE), connectivity analysis (CA), blob analysis (BA) with area thresholding, morphological clean-up, and finally recombined as a replication of Figure 3, shown in Figure 7. Notice the common regions of color with those of Figure 3, only one must realize that this analysis represents a reduction in data of over a thousand-fold, and that each individual, connected-pixel blob region is absolutely located and capable of including feature measurements from the original imagery. The construction of these algorithms is in the form of open-source software that is transportable between various forms of computational engines.

Figure 7. The recreation of the fig. 3 “image” by blob analysis of the spectral components.

3.2 Software architectural issues

The ISAT algorithm development was conducted in the “C” language as an RS toolset, free of routines for input/output (I/O) or graphical user interface (GUI). This was to ensure the maximal portability of the core algorithms. Based on common data structures, the C cores were then adapted to run in different environments by either plugging them into proprietary GUIs, or wrapping them into the Java-based ImageJ from the National Institutes for Health (NIH). The computer-based processing can be enhanced using other programs written in C, such as used in the development of the ISAT toolset, and based on additional “tools” or algorithms contained in the Image Processing Library 98 (IPL98) routines available as an open-source freeware^{8,9}.

Algorithms were developed for seven RS functions, including nonlinear edge enhancement, auto-registration in 2D, change detection, spatial resolution extrapolation, spectral mixing from different resolutions, automated image object segmentation (Figure 2), and semi-automated registration fiducial location. Many of these algorithms prove to be also useful in CB microscopy. These routines can be used for the quick prototyping of an algorithm to evaluate its performance in extracting features of interest in the imagery.

3.3 Software

Software tasks also center on the need for high-dimensional image processing, implemented online whenever possible. Current ISAT 3-D object image fiducial registration tools will be modified for use in the cellular biology domain. For example, this will permit the co-registration of voxel spaces as well as stacks of two-dimensional imagery. Locations within individual images that correlate with prior and subsequent images will be stored as metadata to the archive imagery. Other metadata can be the result of feature extraction. The extracted features can then be submitted to an identification process, either automated or manual. In addition, the segmentation of 3-D volumes will be necessary in

order to be able to extract volumetric features such as those resulting from multiply sliced cells or MRI data. This type of processing will likely remain a post-processing step, due to the algorithmic complexity and possibly for the likely need for a human to interactively guide the segmentation process.

Fundamental artificial vision algorithms used in industry (e.g., machine vision, MV) include: thresholding, convolution, binary mapping, motion and spectral filtering, edge detection, run-length encoding, etc. These processing steps, when performed online, can dramatically reduce the time needed to manually post process images. Figures 8 and 9 below demonstrate this type of basic image processing for cell isolation that can be performed online, in real-time, which will be very useful to cellular biologists.

Figure 8. Cell Colony Unprocessed.

Figure 9. Cell Colony Processed.

3.3.1 FPGA real-time computing

As the algorithms developed using the C-based ISAT toolbox are tested and found to perform satisfactorily, the C code will be handed off to the migration into the real-time field programmable gate array (FPGA) hardware environment. The C code will be transported to Handel-C®, from which it will be compiled into an electronic data interchange file (EDIF) and placed-and-routed into a downloadable binary configuration (or .bit) file. Here a software “compiler” product, DK1®, is used for implementation in an FPGA-based hardware system^{10, 11}. The DK Design Suite® is a product from Celoxica® that compiles C code directly into FPGA compatible “load modules” in EDIF format. Handel-C® will be used for online analysis program (OLAP) FPGA development¹¹, so that stand-alone algorithms may be designed as combinations of load modules, which can be loaded into the FPGA system for execution. Performance of the hardware implementation will be carefully compared to the software results to verify that the hardware is correctly configured for the algorithm processing of data.

A critical issue in the development of MV algorithms for both software and hardware implementation is the use of floating-point versus fixed-point. For both implementation size and speed considerations, it is very desirable to perform all operations in fixed-point hardware. Therefore, it is essential that the co-development of the software and hardware algorithms be carefully coordinated to allow for as much fixed-point processing as possible.

3.3.2 Run length encoding and blob analysis

The algorithms will typically consist of the application of a series of fundamental processing steps contained in the toolbox, but with adaptively modified, algorithm parameter values. An example of such an application is given as follows for any one of the selected spectral bands showing tagged chemistry in the imagery:

1. Invert the image grayscale using a look-up table (LUT) so that all processing will work from the low pixel values (black) to the high pixel values (white).
2. Enhance the edges of the resulting image.
3. Create a histogram of the image.
4. Stretch the histogram using an LUT.
5. Search for peaks (modes) in the histogram. Select the largest modes which are closest to black and white.
6. Find the valleys in the histogram between the modes and sort by depth relative to the maximum mode value.
7. Find a threshold value, $d = F(x_1, x_1, \dots, x_i; x_1, x_1, \dots, x_j)$, which is a function of the valley and peak values and threshold the image.
8. Mask the original image using the thresholded image to isolate the cells from the background. The resulting image can then be enhanced to further isolate structure in the cells by repeated applications of steps 1-7.
9. Find the number of potential cells from the image produced in step 7. Erosion and dilation operators can be used to clean up the image and remove island pixels.
10. Begin blob analysis on the cells by applying run-length encoding (RLE) to the image to segment the blobs.
11. Perform connectivity analysis (CA) on the blobs.
12. Perform projection analysis on the blobs.
13. Extract binary features from the blobs such as mean, variance, eccentricity, area, etc.
14. Extract grayscale features from the blobs, using the projection, histogram, and BA/CA techniques.

From this example it is clear that a relatively small set of fundamental operations are used to perform the feature extraction process. These include:

1. Pixel value mappings (LUTs).
2. Histogram modification/equalization.
3. Edge enhancement.
4. Masking (image multiplication with a binary masking image).
5. Min/Max finding.
6. Erosion/dilation operators.
7. Run-length encoding.
8. Connectivity analysis.
9. Projection analysis.
10. Feature extraction.

3.3.3 MV functions

Figure 10 is a flow diagram of these simple ten functions, operating under a host computer's control, with continual changes in parameter settings for each function through "scratch memory" and intermediate image "framing." The general architecture for MV applied to blob processing is a set of enhancement, thresholding, frame multiplications, and other functions shown in the above example and using the architecture of Figure 10. It consists of seven major image-processing functions: pixel mapping, convolution operator (e.g., edge enhancement with a kernel, and a built in absolute value function), morphological operator (e.g., which has a built in min/max function), run-length encoding, profile projections, moment functions, and histogram processing. These processing functions are combined in many different ways to extract from the input image the desired information about the biological cells under investigation. It is therefore desirable to construct a flexible real-time implementation, which allows each of the functions to be used, possibly iteratively, in any order to achieve the desired result. Also note in Figure 10 the redundant single and dual frame "piping" to/from the frame memories, which in the CB application are on the order of 20 MB for single, two-color sets, and over 100 MB for sequences. This is a formidable research issue for efficient algorithm implementation.

Figure 10. Machine vision functions.

One approach to implement this architecture is to use an FPGA processor, which can implement, in parallel, each of the processing functions. As data is read into the FPGA processor from RAM storage, a central controller routes the data for processing by the desired function. After the data is processed, it is then routed to the next processing function. Since information extraction algorithms are a series of paths through these operators, each algorithm can be pipelined to take advantage of the parallelism of the FPGA and produce results at extremely high speed.

The intent is to preprocess the streaming data, incorporate the output as a metadata file, and send this information to the archive, along with the normal image data archiving. Then, using the offline image archive analysis tools developed under the ISAT program and available at the PNNL High Performance Computing (HPC) Center, the metadata can be analyzed as a *CB measurement* to use for analysis and simulation input, and for tagged searches to retrieve and post-process the archived imagery using the same existing and future ISAT tools operating in the HPC Parallel Computational Environment for Imaging Science (PiCEIS). Here, the biologist will use a set of visualization tools to determine if the parameters of the tool set are appropriate, and if necessary, new parameters can be determined, and a new OLAP/FPGA ISAT processing module can be formulated for download to the online processor. The post-processed imagery can also be reintroduced to the data stream for further analysis. Note that the advantage of the automated measurements is in the trade-off of having an exactness of analysis of maybe up to 95% by a human on only 100 images, vs. the automated analysis measurements that might have an 80% exactness on over 10,000 images. The latter will better support a statistical average for modeling, and provide a return to the original images for further analysis by the biologist, with the automated data as a guide. Figure 11 illustrates this process flow, where the dark gray shading indicates the ISAT work being integrated into the HPC activities (in light gray shading) and the Cellular Biology activities (in medium gray shading).

Figure 11. ISAT cellular biology process flow diagram.

4. DISCUSSION

While the individual base-functions of the algorithms are well known and readily available, the implementation of those algorithms in an FPGA environment is a new concept. Colorado State University's Cameron Project¹² has an ongoing project quite similar to this intended approach, as is used for image analysis to discover military targets from remotely sensed imagery. The excerpt below is from the final report of the research implementing some of the algorithms needed for blob analysis. They were impeded by the fact they were transferring the entire image data in and out of the FPGA system for each operation, in addition to reloading the appropriate algorithm into the FPGA when the operation changed. They also were not able to use parallel architecture in the implementation beyond the parallelism available in each processing operation, i.e., multiple processing steps were not pipelined in the same FPGA configuration. In the included table, SA-C refers to the FPGA-based computation.

"The SA-C programs were compiled using the November, 2000 version of the SA-C compiler and run on an Annapolis Microsystems StarFire with Xilinx XV-1000 FPGAs. The C programs were run on a 450MHz Pentium II. (The Xilinx XV-1000 is roughly contemporaneous with the 450MHz Pentium II.) The run-times are given in the table below. The erode and dilate times are for a single operation. (Times for the sequence of four operations are given later.)

Routine	C Exec.	SA-C Exec.	RCS Data Download	RCS Config Download	Frequency (MHz)	RCS Data Upload
Downsample	0.05	0.009761	0.033738	0.161508	29.963	0.012556
Erode (cross)	0.08	0.006102	0.008709	0.161871	28.279	0.012576
Erode (box)	0.08	0.006332	0.008712	0.161397	27.254	0.012562
Dilate (cross)	0.08	0.005499	0.008551	0.161516	31.383	0.012392
Dilate (box)	0.08	0.006198	0.008709	0.161615	27.842	0.012584
Bit wise AND	0.03	0.003136	0.016633	0.161510	38.877	0.012414
Pos. Diff.	0.03	0.003170	0.016405	0.161532	38.445	0.012370
Maj. Thresh	0.03	0.005396	0.008589	0.161505	31.977	0.012370

In general, execution times on the reconfigurable system are about ten times faster. The downside, however, is the cost of downloading the circuit configuration onto the FPGA, and of transporting the data back and forth between the RCS system and the host processor. In general, the time it takes to download a configuration swamps all other costs. As a result, it is only feasible to accelerate one operator per FPGA, since they cannot be quickly reconfigured and must therefore be pre-loaded. Given this, the gain in speed of the reconfigurable processor justifies the cost of downloading and uploading the image data for every routine except downsample (which gets the largest images), although the total improvement is reduced to a factor of four.”

Initial testing of the ISAT registration tool in FPGAs has shown a similar increase in processing speed. For example, multiple parallel convolutional operators were designed to process images at one pixel/clock at clock speeds exceeding 26 MHz on a previous generation FPGA with maximum clock rate of 80 MHz. This equates to almost a 25 (1k x 1k pixel image) frames/s processing speed. State-of-the-art processors can be clocked at speeds up to 420 MHz, providing the potential of real-time processing at the expected maximum frame rate of 30 frames/s.

The sheer volume of the data captured by the cellular biologists is daunting. A day’s worth of data can add up to gigabytes of imagery and metadata, which is on the order of NTM data sets in RS. To avoid the situation of sitting on a mountain of inaccessible data, as generated by the results of analysis of the image in Figure 3 *at a rate of 30 cells/sec*, procedural improvements are needed at each procedural stage in data acquisition and storage, with real-time analysis.

Figure 12 provides a projection of the expected improvements in real-time CB image analysis using this ISAT OLAP/FPGA technology, which potentially could exceed human analysis in FY04. These projected data are based on the processing times from the Cameron Project, modified by the procedures proven in the ISAT tool work and an assumption of a continuation of Moore’s Law growth in transistor density and clock speed. The increase in the processing throughput in the FPGA-based system beyond Moore’s Law growth is due to the ability to include more of the processing steps into a single reconfiguration of the FPGA system as FPGA gate count increases over time.

The ultimate goal should be to collect, analyze and perform an initial assessment of voluminous, multidimensional data—all during the same day or less! Clearly, this information needs to be processed at the highest rate possible, meaning that much of the more mundane processing can and should be moved upstream and performed online. Once the needed information has been extracted from the data stream, the image information can be moved in to the archive, where it can be called upon later for downstream post processing. The ISAT research agenda and available tools will be developed as necessary to be able to handle the 3-D and higher data set analysis.

Figure 12. Expected CB automated image analysis with OLAP/FPGA technology developments.

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- 12) The Cameron Project was founded in the Computer Science Department at Colorado State University in May 1999, and continues in collaboration with colleagues at the University of California at Riverside and Khoral Research, Inc. (KRI). In general, Colorado State is the primary site for language and compiler development, and for research integration. UCR is developing high-performance I/O interfaces, as well as targeting UCI's Morphosys chip. KRI has integrated the SA-C language and compiler into the Khoros image-processing environment. Additional information is contained in the related web sites: <http://www.cs.colostate.edu/cameron/related.html>, <http://www.cs.colostate.edu/cameron/>.