8-2011

Image registration and visualization of in situ gene expression images.

Ernur Saka
University of Louisville

IMAGE REGISTRATION AND VISUALIZATION OF IN SITU GENE EXPRESSION IMAGES

By

Ernur Saka
B.S., University of Dokuz Eylül, Turkey, 2008

A Thesis
Submitted to the Faculty of the
University of Louisville J.B. Speed School of Engineering
In Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Department of Computer Engineering and Computer Science
University of Louisville
Louisville, Kentucky

August 2011

IMAGE REGISTRATION AND VISUALIZATION OF IN SITU GENE EXPRESSION IMAGES

By

Ernur Saka
B.S., University of Dokuz Eylül, Turkey, 2008

A thesis Approved on

August 1, 2011

by the following Thesis Committee:

Dr. Eric C. Rouchka, Thesis Director

Dr. John R. Pani

Dr. Ming Ouyang

DEDICATION

Dedicated to my parents

Nuran Saka and Erol Saka

and to my sister

Esin Saka

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Eric C. Rouchka for his direction, assistance and guidance. I would also like to thank the members of my thesis committee, Dr. Pani and Dr. Ouyang for their time. Additional thanks to Bioinformatics lab members Fahim Mohammad, Dazhuo Li, Abdallah Eteleeb, John Kirtley and Dr. Flight. Most of all, I am so grateful to my family, in particular my mother Nuran, my father Erol, and my sister Esin for supporting me in every possible way unconditionally and selflessly.

ABSTRACT

IMAGE REGISTRATION AND VISUALIZATION OF IN SITU GENE EXPRESSION IMAGES

August 1, 2011

In the age of high-throughput molecular biology techniques, scientists have incorporated the methodology of in-situ hybridization to map spatial patterns of gene expression. In order to compare expression patterns within a common tissue structure, these images need to be "registered" or organized into a common coordinate system for alignment to a reference or atlas images. We use three different image registration methodologies (manual; correlation based; mutual information based) to determine the common coordinate system for the reference and in-situ hybridization images. All three methodologies are incorporated into a Matlab tool to visualize the results in a user friendly way and save them for future work. Our results suggest that the user-defined landmark method is best when considering images from different modalities; automated landmark detection is best when the images are expected to have a high degree of consistency; and the mutual information methodology is useful when the images are from the same modality.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Rapid technology development for high-throughput data generation in the fields of molecular and cell biology in recent years have caused an explosive growth in the amount of biological data available, particularly in the -omics (proteomics, metabolomics, lipidomics, transcriptomics, genomics, etc.) fields. Analyzing, interpreting and integrating this data has become a big challenge. A relatively new interdisciplinary field, bioinformatics, has evolved to address the resulting challenges. Our particular research interest has been in developing methodologies for interpreting results visually representing localized gene expression through in situ hybridization experiments so these images can later be seamlessly integrated with other -omics data.

Bioinformatics is the application of mathematical sciences (most traditionally computer science, mathematics and statistics) for the management and analysis of biological and medical data. It includes development and implementation of computational tools, algorithms, mathematical modeling, and statistical analysis to manage, analyze and manipulate large sets of data. Bioinformatics plays an important role in biological and medical research. Some of the major research topics in bioinformatics are sequence alignment, gene finding, genome assembly, drug design, drug discovery, protein structure prediction, protein-protein interaction, gene expression analyzing and genome-wide association studies.

Gene expression or transcriptome analysis examines the entire process that takes gene coding information contained in DNA and turns that information into a particular worker molecule (usually a protein or a small RNA molecule). Gene expression of many genes can be determined by measuring their respective mRNA levels within a cell. Multiple techniques exist to measure mRNA levels, including microarrays, RNA-Seq, massively parallel signature sequencing and in situ hybridization.

This thesis mainly focuses on the alignment and visualization of 2D gene expression images obtained via in-situ hybridization. The alignment involves image registration with two images. The first image is used as a reference image (ideally an atlas with known features) and the second is a sample image to be aligned to the reference. In our particular case, the sample image is an in-situ hybridization image, and the reference is either in situ hybridization or atlas image. An in-situ hybridization image is a high resolution image that shows the location of a specific DNA or RNA sequence in the entire or portion of a tissue (covered in more detail in section 2.8). An atlas image is constructed from one or more representations of a sample image and shows the structure in terms of known features or structures. The alignment is achieved via image registration, one of the fundamental tasks in image processing which involves determining the optimal geometric transformation between a set of related images (covered in more detail in section 3). We use three different registration methodologies. The first methodology is based on landmark-based image registration. It uses user defined corresponding landmark pairs on both images to estimate optimal geometric transformation. Estimation can be performed by one of the six different algorithms. They are affine, linear conformal, projective, polynomial, piecewise linear and local weighted

mean. The second methodology automatically detects corresponding landmark pairs. First it detects corner points from both images via the Harris-Stephens corner detector. Later, it matches points that are maximally correlated with each other. The rest of the technique is the same as the first. The last technique uses mutual information to register two images. Unlike the others, it is an area based registration technique. It works on whole image intensity values and searches for the best transformation equation that maximizes the mutual information between two images.

1.1 Motivation

Our research has been motivated by the following facts:

- In-situ hybridization images contain information about how genes control cell type identity, cell differentiation, and cell-cell signaling.
- In-situ hybridization can produce a large number of high resolution 2D or 3D images. Analyses and visualization of ISH images is a difficult yet necessary process.
- To be able to analyze ISH images via valuable queries, comparisons and associations, images needs to be organized into a common coordinate system. This can be challenging due to the high resolution and distortions contained within the images.

1.2 Main Contributions

A preprocessing tool was created for image separation and character recognition of retina in-situ hybridization images. Three different methodologies developed for registration. A Matlab based user interactive registration tool was created. It lets the user

apply different methodologies with different parameters. Additionally it saves all the work in order to let the user continue working on the same data or see old results.

1.3 Outline of the Document

This thesis is organized in five sections. Section 2 presents the background information for molecular biology. Section 3 presents the background information for image registration and some literature review. Section 4 presents the data information and developed methodologies. Finally section 5 presents the conclusion and future work.

2 OVERVIEW OF MOLECULAR BIOLOGY

Molecular biology is the field of study that involves studying cell structure and function down to the level of the individual molecules within those cells that contain the programming for life functions [1]. Cells are the smallest living things and the basic unit of any living organisms. They have all the properties of life such as reproduction, response to environment signals, a need for energy, and release of waste products. All cells are built out of similar materials (including an organism's DNA) and function in similar ways. Prokaryotic cells, such as bacteria and some single celled organisms, have simple organization. They do not have a true membrane-bound nucleus and organelles. Eukaryotic cells, such as plants, animals, and fungi are structurally complex. They contain a membrane-bound nucleus and organelles.

The nucleus is a small spherical, dense body in a eukaryotic cell. It is called the control center of the cell since it controls many of the activities of the cell including cell reproduction. Contained within the nucleus are chromosomes which are microscopic, threadlike strands composed of DNA. Regions of the DNA are gene coding segments used by a cell to create cellular workers like proteins that control the function of a cell. The proteins are coded by the sequence of DNA which is written in the chemical letters A, T, C, and G. When proteins are needed, the information contained in the DNA is transcribed into RNA. The RNA is first processed and then transported out of the nucleus. Outside the nucleus, the proteins are built based upon the code in the RNA.

Figure 1 shows the relationship between the cell, its nucleus, chromosomes in the nucleus, genes, and DNA.

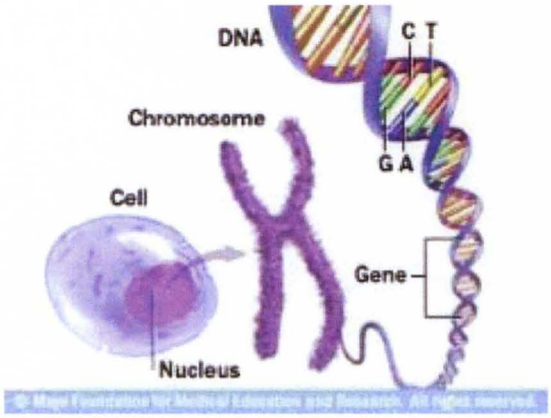


Figure 1. The relationship among the cell, the nucleus, the chromosome, the gene and DNA [2]

2.1 DNA

Deoxyribonucleic acid (DNA) is a long term storage device of organisms to store genetic information. It enables the transmission of genetic material from one generation to the next by passing copies of DNA to the offspring. Stored information is read by working cells to build molecules, such as protein and RNA. These molecules are used to control how an organism looks, behaves and reproduces.

DNA may be single or double stranded. A single stranded DNA molecule, called a polynucleotide, is a chain of small molecule nucleotides. Each nucleotide is made with three separate parts: a phosphate, sugar, and nitrogenous base (Figure 2).

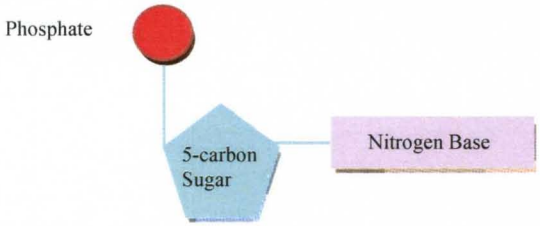


Figure 2. The basic unit of polynucleotide chain is the nucleotide

I. Phosphate component: The phosphate group is a phosphorus atom surrounded by four oxygen atoms. When nucleotides are joined together to form a polynucleotide, a phosphate group is attached to the sugar molecule to form the sugar phosphate backbone.

II. Sugar component: There are two different kinds of sugars found in a nucleotide: deoxyribose and ribose. In DNA the sugar component of the nucleotide is deoxyribose.

III. Nitrogenous base: There are five different canonical nucleotide bases. These bases are Adenine (A), Guanine (G), Cytosine (C), Thymine (T) and Uracil (U) (Figure 3). DNA is composed of the four bases adenine, guanine, cytosine and thymine.

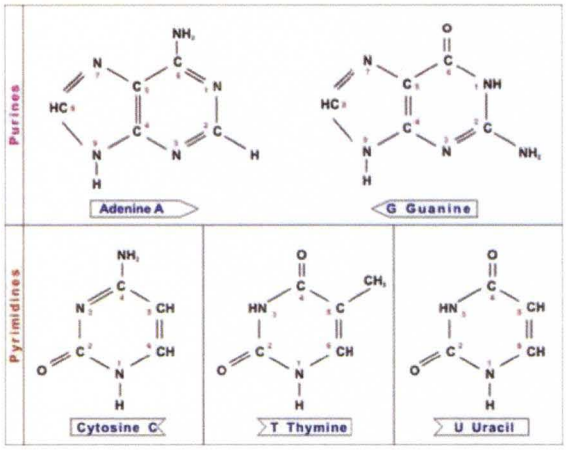


Figure 3. Types of nitrogenous bases [3]

Adenine and guanine are both called purines and have the same structure. Cytosine, thymine and uracil are pyrimidines and have a smaller structure than the purines.

In joining nucleotides together, the sugar part of one nucleotide connects up to the phosphate part of the next nucleotide to produce a polynucleotide.

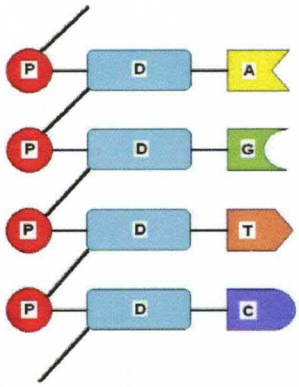


Figure 4. Adjacent nucleotides connect to form a DNA polynucleotide [4]

A polynucleotide can be any length and have any order. The end of the polynucleotide is marked either 5' or 3' representing the location of the hydrogen bond. DNA is usually written with 5' left and 3' right. The following figure shows the semantic representation and sequence of a single strand DNA.

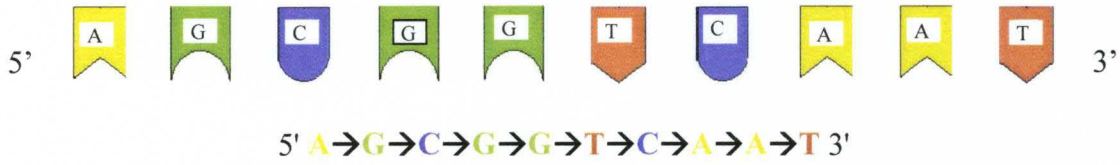


Figure 5. Semantic representation and sequence of a single strand DNA

DNA is typically a double-strand molecule, consisting of two complementary strands running in opposite directions. One chain runs 5'-3' and the other runs 3'- 5'. The two strands are connected to each other by a chemical pairing of each base on one strand to a specific partner on the other strand. These complementary base pairs are adenine (A) - thymine (T), and guanine (G) - cytosine (C) (Figure 6). Referring to the complementary base pairing mechanism, the second strand can be called the reverse complement of the first strand (Figure 7). The complementary base pairing helps to achieve direct synthesis

of a complementary strand by using one strand of a DNA as a template to copy and pass on to the next generation of cells.

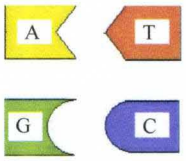


Figure 6. Complementary base pairs

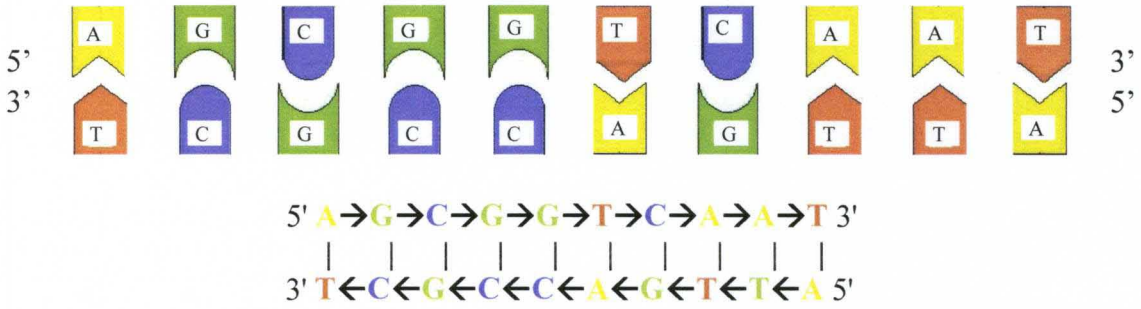


Figure 7. Semantic representation and sequence of complementary strands

Two complementary DNA strands are twisted into a helix like a spiral staircase to make a double helix structure (first discovered by Watson and Crick[5]). In the helix structure, the four nucleotides make up the stairs and strands still run in opposite directions.

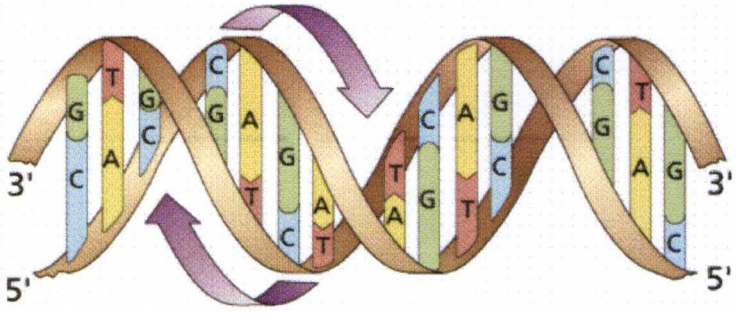


Figure 8. DNA double helix [6]

2.2 RNA

Ribonucleic acid (RNA) is a polynucleotide made up of one or more nucleotides. RNA is very similar to DNA; however a few important structural details are different. RNA is usually a single stranded molecule, while DNA is usually double stranded. RNA nucleotides contain ribose as a sugar component while DNA nucleotides contain deoxyribose. Adenine, guanine, and cytosine are common bases for both RNA and DNA. But RNA uses the nucleotide uracil, instead of the thymine.

RNA plays a key role in the Central Dogma of Molecular Biology [7] which is a pathway from DNA to proteins. Three kinds of RNA molecules perform different but cooperative functions in protein synthesis. These are:

I. Messenger RNA (mRNA): mRNA is a single strand RNA molecule. DNA is first encoded into mRNA by the process of transcription, because the information in DNA cannot be decoded directly into proteins.

Transcription of DNA to mRNA is a two-step process. First, pre-mRNA is synthesized from one strand of a DNA using a complementary mechanism.

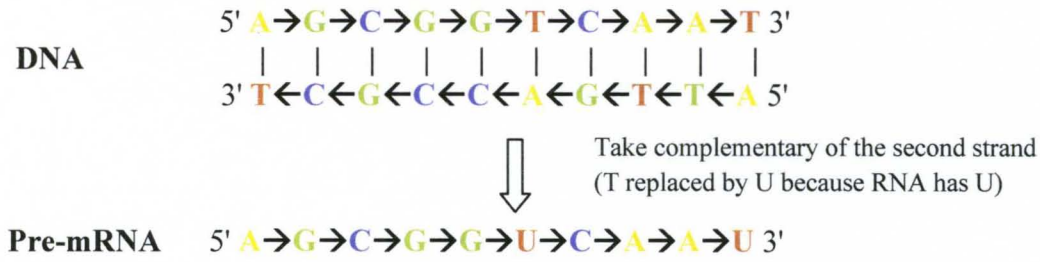


Figure 9. DNA to pre-mRNA

Pre-mRNA contains both non-coding regions (introns) and coding regions (exons). Only exons are used to build proteins. Therefore, in the second step the introns

are removed and the exons are spliced together to form mRNA. Later the formed mRNA is carried from the nucleus to the cytoplasm to be translated into a protein sequence.

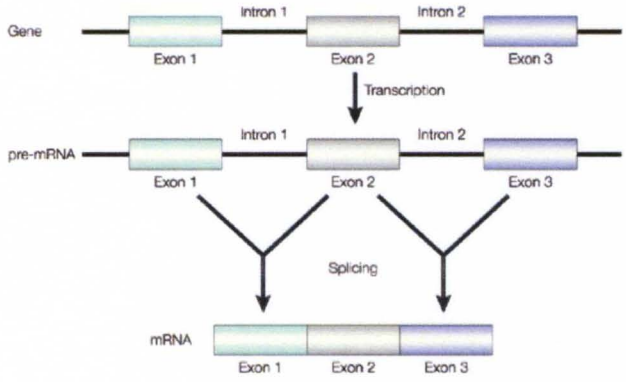


Figure 10. DNA to mRNA [8]

The amount of mRNA produced from DNA gives a measurement of the activity of individual genes in a cell, since only active genes are translated to mRNA.

II. Ribosomal RNA (rRNA): Ribosomal RNA (rRNA) is a non-coding ribonucleic acid that is an essential and functional component of ribosomes. Ribosomes are small particles located in the cytoplasm (jelly like material that fills the cell).

As non-coding RNA, rRNA itself is not translated into a protein, but provides a mechanism for translating mRNA into protein by interacting with the transfer RNAs during translation.

III. Transfer RNA (tRNA): Transfer RNA (tRNA) is a specialized RNA that is produced by transcription like mRNA. tRNA's function in carrying amino acids to the ribosome to form proteins. tRNA has a unique three dimensional structure that helps to perform their function (Figure 11).

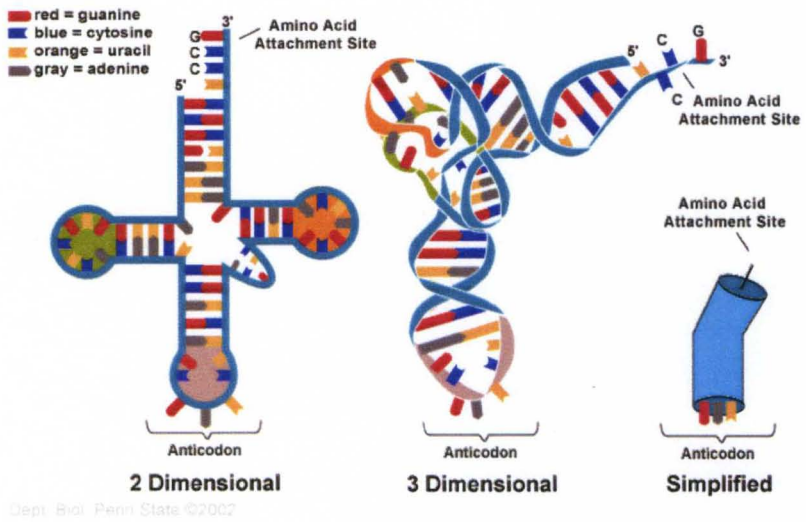


Figure 11. tRNA structure [8]

Each tRNA molecule has an anticodon (three nucleotide sequence) and amino acid attachment site. The anticodon is unique for each amino acid which means that each amino acid binds only a specific type of tRNA. During the translation of mRNA sequence to amino acid chain (protein), the anticodon temporally pairs with a complementary consecutive triplet, the codon, in mRNA. At the same time the amino acid binds to tRNA by the help of enzymes. Subsequently the bound amino acid is transferred to the ribosome, where proteins are assembled according to the information carried by mRNA, and attached to growing amino acid chain.

2.3 Chromosome

In a single human cell there are 46 chromosomes in DNA that makes 6 million base pairs of DNA per cell. Because each base pair is around 0.34 nanometers long, each diploid cell contains about 2 meters of DNA. Moreover an adult human body has about 50 trillion cells, which means there is 100 trillion meters of DNA per human. Since the sun is 150 billion meters away from earth, each human has enough DNA for more than 300 trips from earth to the sun and back [9]. In order to fit long DNA strands into the

microscopic space of a cell nucleus, DNA is packed into structures called chromosomes. The packing of DNA into a chromosome is done in several steps, starting with the double helix of DNA. Then, DNA is wrapped around proteins called histones. The resulting DNA protein complex is called a chromatin. The fundamental packing unit of chromatin is nucleosome. The nucleosome must be stable and tightly bound to compact DNA but at the same time must allow access to the DNA for the regulatory control to ensure correct gene expression. Eventually nucleosomes are folded and form chromosomes.

2.4 Gene

A gene is a continuous subpart of a single stranded DNA molecule. One strand of DNA contains many genes. All of these genes are needed to give instructions for how to build all of the proteins for an organism. Figure 12 shows the relationship between a gene and DNA.

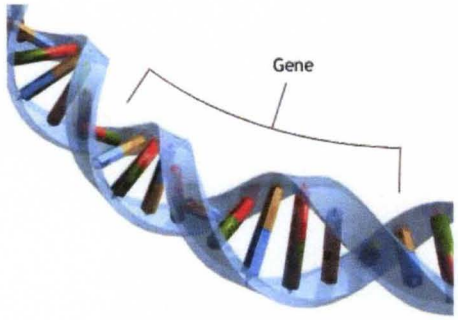


Figure 12. Gene is a short segment of a DNA coding for a protein [10]

2.5 Gene Expression

Almost every cell in an organism contains a complete set of genes but each gene in the set is not used by a specific cell due to the effect of cell type, cell development and environmental changes. This important mechanism of cells relates the importance of differential gene expression.

The separation of in use and out of use genes is carried out by a process known as gene regulation. When a gene is turned on, the molecular product of this gene can be synthesized, and subsequently identified as expressed. Oppositely when a gene is turned off, the molecular product of this gene cannot be synthesized and the gene is identified as unexpressed.

Gene expression analyses reveals the function of genes, cell-cell differences, cell interactions and where, when and in which conditions a gene expressed. The expression analyses of many genes can be determined by measuring mRNA levels with multiple techniques includes in situ hybridization.

2.6 Central Dogma of Molecular Biology

In 1958, Francis Crick used the term "Central Dogma" for the idea of one-way genetic information flow between macromolecules and he explained it as "once 'information' has passed into protein it cannot get out again. In more detail, the transfer of information from nucleic acid to nucleic acid or from nucleic acid to protein may be possible, but transfer from protein to protein, or from protein to nucleic acid is impossible"[7].

In the Central Dogma there are three major classes of macromolecules: DNA, RNA (both nucleic acids) and proteins [11]. By using the three macromolecules, nine information transforms are defined and classified into three groups: general, special and unknown transfers. The general transfers are: DNA→DNA, DNA→RNA and RNA→Protein transforms. They usually occur in most cells. The special transfers are: DNA→Protein, RNA→DNA and RNA→RNA. They only occur in a laboratory or in the case of some viruses under specific conditions. The unknown transfers are:

Protein→DNA, Protein→RNA and Protein→Protein. Figure 13 is taken from original work of Francis Crick [12] which shows the diagrams for the transfer of information.

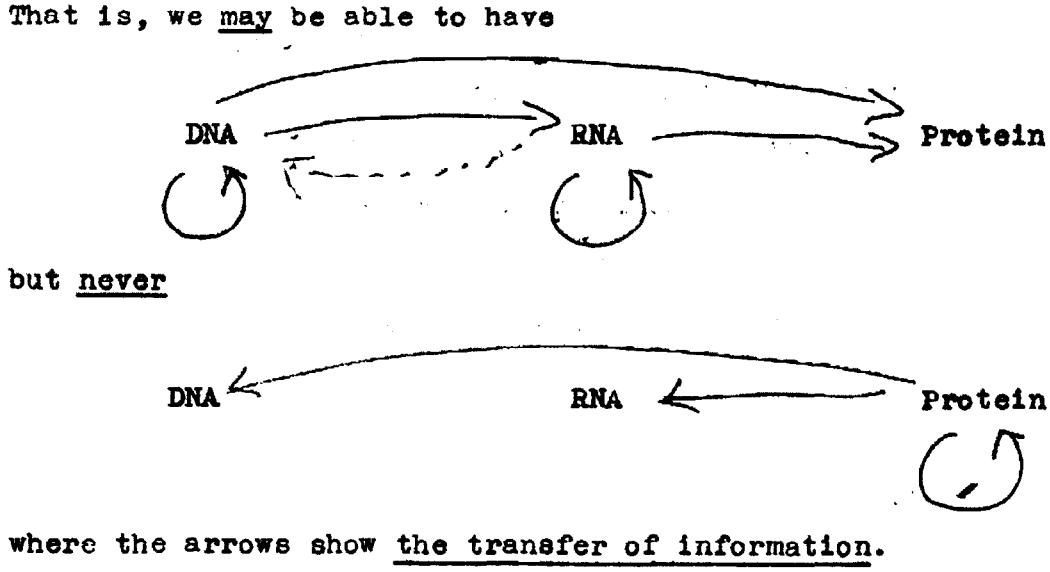


Figure 13. Central dogma [12]

2.6.1 General Transfers

a) DNA replication

One of the most important mechanisms for all cells is transmitting genetic information to offspring. DNA replication provides the base of this mechanism. DNA replication is the process that a cell uses to copy DNA with the help of enzymes and proteins. In order to start DNA replication, the double stranded DNA helix must be opened. Helicases and single strand DNA binding proteins unwind the DNA into two single strands. After that, DNA polymerase III, I, ligase and primase proteins work together and build the copy strands.

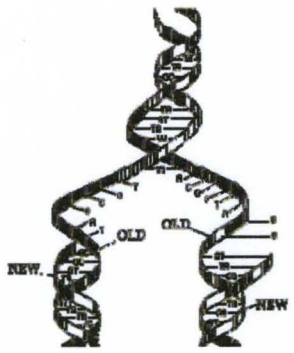


Figure 14. DNA replication [13]

b) Transcription

The process of creating a complementary RNA from a DNA template is called transcription. The resulting complementary RNA copy is called mRNA. mRNA naturally exists in single stranded forms and acts as a template for protein synthesis. The enzyme called RNA polymerase transcribes DNA to mRNA. After transcription, mRNA is moved to the ribosome where it is translated into protein.

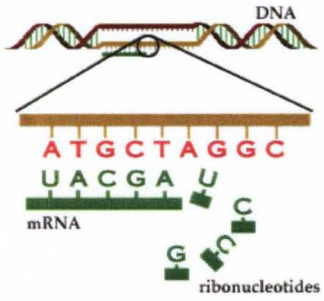


Figure 15: Transcription [14]

Transcription has main three steps:

I. Initiation: In this step RNA polymerase attaches to the DNA at a specific area called the promoter region by using specific nucleotides sequences which shows begin and end points on DNA.

II Chain elongation: The proteins called transcription factors unwind the double stranded DNA to allow RNA polymerase for single strand mRNA transcription. After the

unwinding process, RNA polymerase moves along the single strand DNA and creates mRNA.

III. Termination: When the RNA polymerase reaches the termination sequence, it releases the mRNA and detaches from the DNA.

c) Translation

Translation is the part of protein synthesis that produces a specific amino acid chain by decoding the mRNA generated by transcription. It is performed by the ribosome which is a component of a cell.

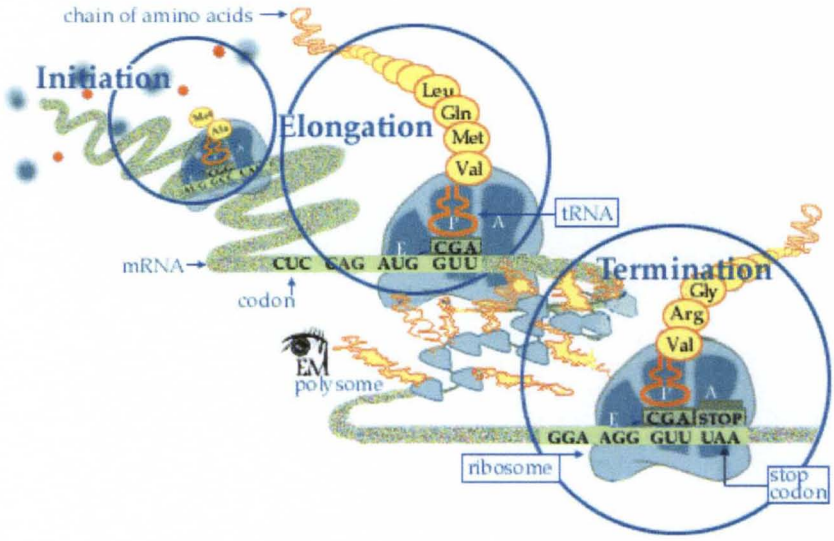


Figure 16. Translation [15]

Translation has main four steps:

I. Activation: Amino acids are attached to the tRNA (covered in more detail in tRNA section).

II. Initiation: mRNA binds to the small subunit of the ribosome and the ribosome moves along the mRNA until it reads the start codon AUG. At that point in time, the large subunit of the ribosome attaches to allow starting of translation and start codon AUG binds with the tRNA that has anticodon UAC and the bound amino acid methionine.

III. Elongation: In elongation another tRNA attaches to the ribosome next to the start codon and binds a new amino acid to the first one to form polypeptide chain. The binding process repeats until a full polypeptide chain is formed according to the sequence of bases in the mRNA.

IV. Termination: When the ribosome reads a stop codon (UAA, UAG, UGA) on the mRNA, the completed protein is released from the final tRNA then the last tRNA and the mRNA are detached from the ribosome.

2.6.2 Special Transfers

a) Reverse transcription

The transfer of information from RNA to DNA is reverse transcription. It occurs in retroviruses (RNA virus), such as HIV or retrotransposons (amplifying the genetic elements) and telomere (a region of repetitive DNA sequence at the end of a chromosome) synthesis.

b) RNA replication

Producing a new RNA from RNA is RNA replication. It is used to reproduce some viruses. These viruses can be double stranded or single stranded RNA [16]. Double stranded RNA viruses make single-stranded RNA molecules from the double stranded RNA molecules. Single stranded RNA viruses are divided into two groups: negative-sense single stranded RNA viruses and positive-sensed single-stranded RNA viruses. The RNA molecule of negative-sense viruses cannot be read directly to create proteins. First, complementary RNA is created and used to produce viral proteins. RNA molecules of positive-sensed single stranded RNA viruses can be read directly for the synthesis of viral proteins.

c) Direct translation from DNA to Protein

Translation of proteins directly from DNA has been shown in cell-free systems. DNA is obtained from mammalian cells and added into an *Escherichia coli* cell-free system. Later antibiotics are added to the system and protein is produced directly from single stranded DNA [17].

2.7 Genetic Code

When the information is needed to make a protein, one strand of a DNA is transcribed to mRNA. Later the mRNA is translated to a protein composed of amino acid molecules. Since there are only four bases in mRNA to code the 20 amino acids, more than one base must be used to specify an amino acid. Even using two bases to code all 20 amino acids is not enough (4x4 = 16). Therefore three bases are required to decode one amino acid. A single set of these three bases is called a codon and the set of all possible combinations of three bases called the genetic code first discovered by Marshall Warren Nirenberg [18] in 1968 (Table 1). There are 64 (4x4x4) different combinations or codons. Three of them are stop codons which gives a signal to terminate the amino acid chain being synthesized on the ribosome. The start codon is AUG. It also encodes the amino acid methionine. The rest of the amino acids are encoded by the each of the remaining sixty codons.

TABLE 1. Genetic code

	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	STOP	STOP	A
	Leu	Ser	STOP	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

The following is an example to show how codons decoded from mRNA to amino acid chain. "*" denotes stop codons and the sequence is partial, where it assumes that the start codon already passed.

DNA: UUA ACA UGA AAG AUG ACA UAC GAU AGC GAU GAU CGA CGC

Leu	Thr	*	Lys	Met	Thr	Tyr	Asp	Ser	Asp	Asp	Arg	Arg
L	T	*	K	M	T	Y	D	S	D	D	R	R

2.8 In Situ Hybridization

In situ hybridization (ISH) is a technique that visualizes nucleic acid sequences such as DNA or mRNA in a tissue sample, where a tissue is a group of similar cells that together perform specific functions[19]. ISH is utilized by biologists to determine where a specific gene is being expressed in a tissue. The underlying basis of ISH is that if mRNAs are preserved adequately within a tissue, they can be detected through the

application of a label attached to the complementary strand of a nucleic acid. The ISH process involves the following critical steps.

Tissue preparation: In order to detect nucleic acids of interest, one has to store and fix a tissue to increase the permeability of the cells and the visibility of the nucleotide sequences[20]. Some of the techniques are freezing the tissue, embedding the tissue into paraffin wax (chemical preservative) and suspension of the cells onto glass slides.

Probe preparation: Probes are strands of nucleic acids complementary to the genes which are being investigated. The length of probes can be as small as 20-40 base pairs or up to 1000 base pairs. Four different types of probes can be used for in situ hybridization. These probe types are double strand DNA (dsDNA), single strand DNA (ssDNA), single stranded complimentary RNA (sscRNA) and synthetic oligonucleotides.

Labeling probe: At the labeling stage, an easily detectible substance or label is attached to the probe to make the probe which has bound to the expressed gene in a tissue visible.

Hybridization: Hybridization includes placing the properly labeled probes into a solution and flooding the solution onto the slide containing the tissue sections. In hybridization, labeled probes and tissue come together and complementary probes bind to the expressed gene of the tissue.

Washing: Following hybridization the slide is washed to remove unbound or loosely bound probes.

Microscopy and image collection: To get the results of ISH, hybridized and washed tissue slides are digitally imaged using specially developed optical microscopes. One of the important specialties of those microscopes is being able to detect labels bound to probes.

Optical microscopes have different detection techniques associated with the type of the label including autoradiographic emulsion, enzyme-based amplification detection, histochemical chromogen development.

Optical microscopes cannot capture the entire tissue section at one time. For this reason captured images are stitched together to produce a single mosaic image that represents the entire tissue. Figure 18 shows an ISH image obtained for a sagittal section of a postnatal day 7 (P7) mouse brain. The cells expressing a probed gene of interest are marked by ISH. Dark blue indicates greater amounts of the specified mRNA in the cell [21].

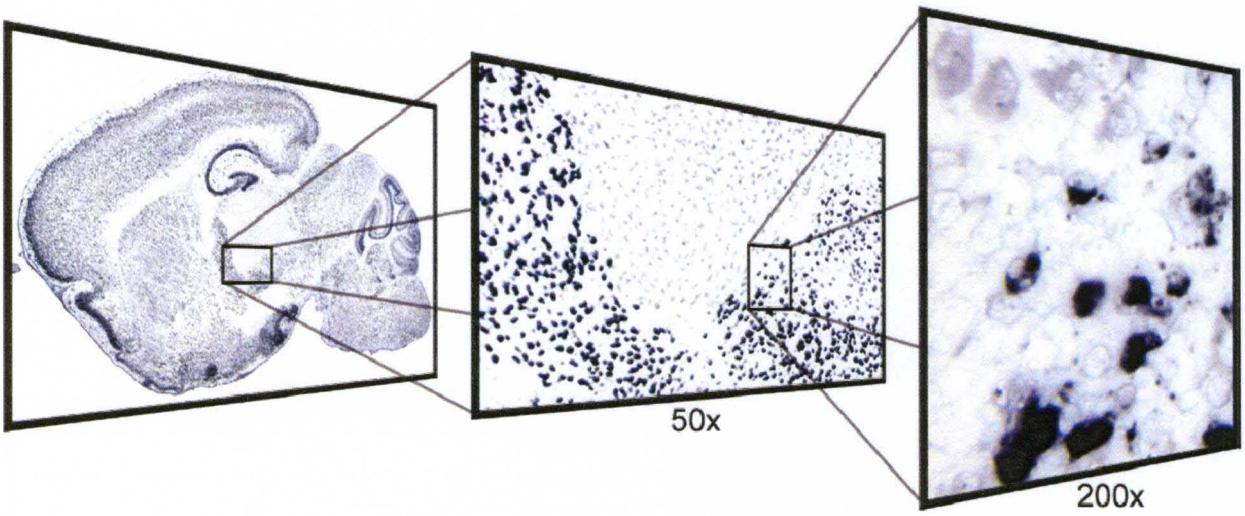


Figure 17. Gene expression display using in situ hybridization. [21]

3. IMAGE REGISTRATION

In image processing, comparing or combining information contained within a set of images is as attractive as analyzing one image. Therefore image registration is one of the essential tasks within image processing. The task of image registration is to take a set of related images and transform them to have the same geometry as a reference or atlas image. This can be accomplished by aligning images with a computer model or aligning features in an image with locations in physical space [22]. Images can be taken at different times, from different viewpoints or from different imaging devices.

3.1 Usage of Image Registration

Image registration is widely used in astro- and geophysics, remote sensing, medical, cartography, and computer vision [23]. Image registration techniques can be divided into one of four different classes. They are multimodal registration, template matching, viewpoint registration and temporal registration [24]. The typical goal of registration with multimodal, viewpoint and temporal registration is to align images so that the respective changes in viewpoint, imaging devices and time can be detected. In template matching, the goal is to find the optimal location and orientation of a template image to another image. Template matching techniques typically are used for object recognition.

3.1.1 Multimodal Registration

Multimodality refers to acquisition of the same scene by different imaging devices. The goal in multimodal registration is integration of the information obtained from different sources to gain a more complex and detailed scene representation.

Examples of multimodal image registration include medical imaging and remote sensing. In medical imaging structural information can be combined from magnetic resonance imaging (MRI), computed tomography (CT) or ultrasound with functional and metabolic body activities such as positron emission tomography (PET), single photon emission computed tomography (SPECT) or magnetic resonance spectroscopy (MRS). For medical imaging, multimodal registration can be used in radiotherapy and nuclear medicine. In radiotherapy planning, a CT image is used to calculate dose distribution and an MRI image is used to outline the contours of the target lesion [25]. In nuclear medicine, studies to understand functional-structural relationships and anatomical location detection of dysfunctional areas are facilitated by multimodal registration. Figure 18 shows multimodal registration of PET and CT images in the thorax [26]. The information obtained from PET and CT images are quite different. PET provides functional information while CT provides detailed anatomical structure. They both have complementary information so the conjunction of PET and CT is very useful.

Figure 18. (A) CT image, (B) PET image, (C) Merged image [26]

In Figure 18, images from a 60-yr-old woman with a known right lung mass. Using only the CT image, it is hard to determine the extent of tumor invasion. Using only the PET image, it is difficult to decide whether there is an invasion into the mediastinum,. After combining the two images it can be determined "the tumor does not invade the mediastinum, but it is intimately involved with the superior vena cava and density encroaching the chest wall in the CF image to more likely be a postobstructive pneumonia secondary to occlusion of the airway by the primary tumor"[26].

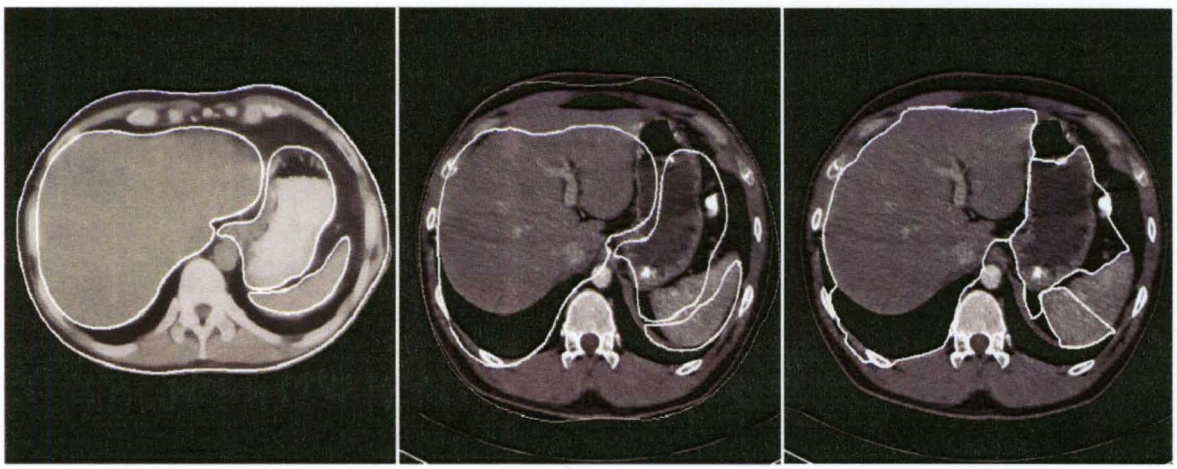
In remote sensing, combining images from different characteristics like panchromatic images offers better spatial resolution, and registration of radar images enables obtaining images without cloud cover. Figure 19 shows the registration of ASTER (Advanced Spaceborne Thermal Emission and Reflection Radiometer) sensor optical image with PALSAR (Phased Array type L-band Synthetic Aperture Radar) sensor image of Tokyo Bay, Japan [27]. The optical satellite images are high resolution and can be influenced by clouds and weather. PALSAR images are not influenced by climate, but have a speckle noise problem. Hence the combination of these two different kinds of images is very useful for geological problems associated with remote sensing.

Figure 19. Registration of radar and optical satellite images ASTER(left), PALSAR (middle) and registration result (right) images [27]

3.1.2 Template Matching

Template matching localizes or compares images of a scene in the pattern of the scene. The pattern can be a computer representation of the scene such as maps or digital elevation models (DEM) in geographic information systems (GIS) or digital anatomical atlases.

Examples of multimodal image registration include medical imaging, remote sensing, and computer vision. In medical imaging, the patient's image can be compared with digital anatomical atlases to detect tumors or complex CT/MR images can be segmented to compute the size of organs. Figure 20 shows the segmentation of CT images that represent liver, stomach and spleen of a human body [28]. The segmentation is performed with a 2D atlas based registration technique.



(a) (b) (c)

Figure 20. (a) Manually segmented CT image, (b) Registered atlas onto an target image after global transformation, (c) Result after contour refinement [28]

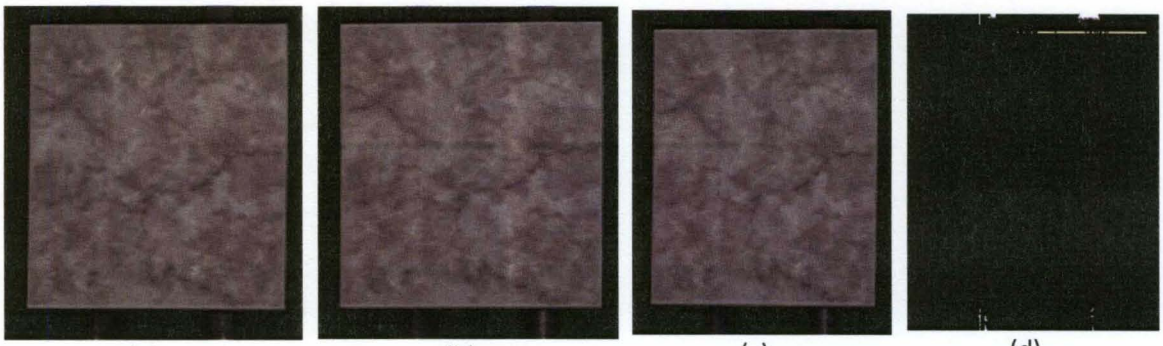
In remote sensing, aerial or satellite data can be registered into maps and well-defined scenes such as airports, runways, terminals, and parking lots can be located. Figure 21 shows an example for registration of aerial images with a cadastral map [29]. Aerial images are taken from an inclined angle and provide information on building

heights, appearance of facades, and terrain elevation. They enable 3D city modeling, texturing, dense stereo matching, and photo augmentation. To improve 3D city reconstruction and simplified cadastral application, combination of aerial images with cadastral maps is performed.

(a) (b) (c)

Figure 21. (a) Aerial image, (b) Cadastral maps, (c) Initial registration result [29]

In computer vision, target template matching with real-time images is performed for automatic quality test. For example, in the ceramic tile manufacturing industry, automated quality control test is applied during the intermediate stages and on final product to detect surface faults of tiles by direct comparison of a tile image with a reference template pattern [30]. Figure 22 shows an example of tile registration.



(a) (b) (c) (d)

Figure 22. (a) Reference tile, (b) Test tile, (c) Test tile registered, (d) Error map [30]

3.1.3 Viewpoint Registration

Viewpoint registration refers to combination of images taken from different viewpoints to reconstruct depth or shape like gaining larger 2D view or 3D representation of the scanned scene.

Examples of multimodal image registration include computer vision and remote sensing. In computer vision, shape recovery, tracking object motion, and stereo mapping to recover depth or shape from inequality are known applications. Figure 23 shows an example of 3D model computation from video frames [31].

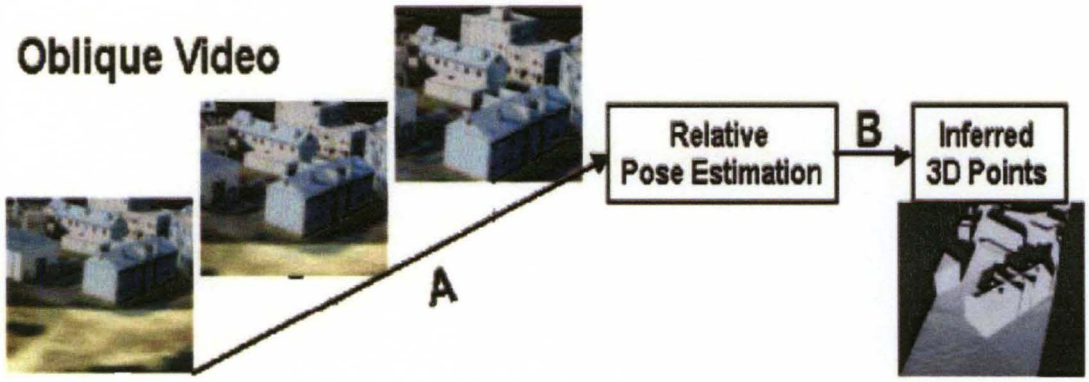
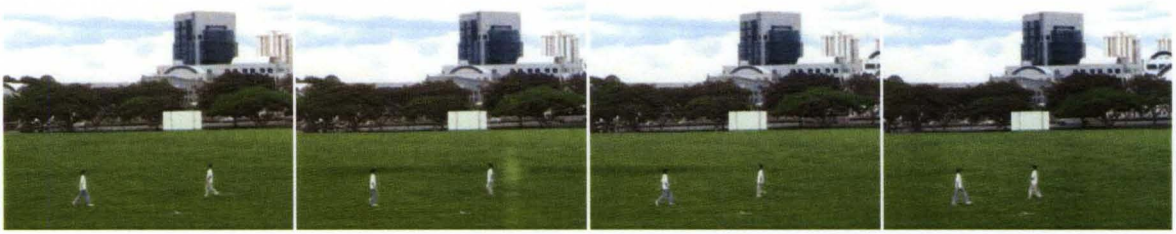
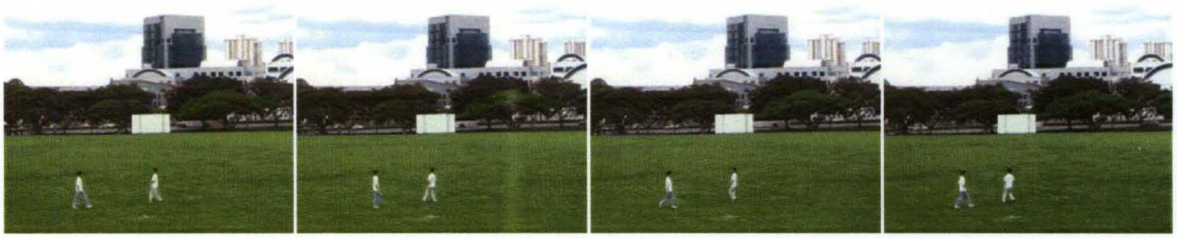


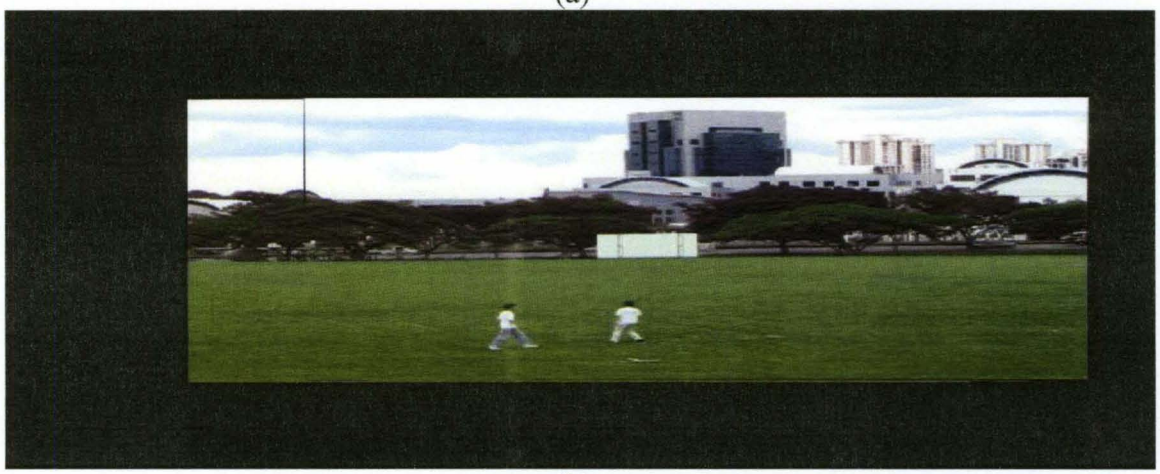
Figure 23. Recovering 3D geometry from video frames [31]

In remote sensing image mosaicking of the surveyed area is a well known application of viewpoint registration. Figure 24 shows the results of an image registration used to create mosaics.





(a)



(b)

Figure 24. (a) Eight frames of two people moving in a field captured by a moving camera, (b) Resulting mosaic using image registration [32]

3.1.4 Temporal Registration

Temporal registration aligns images of the same scene acquired at different times or under different conditions to detect and monitor chances and growths. Examples of temporal image registration include medical imaging, remote sensing and computer vision. In medical imaging, tumor evolution can be monitored by temporal image registration. Z. Quksili et al proposed an indirect registration method to determine lung tumor evolution [33]. They use two pairs of CT and PET images acquired at two different stages of a therapeutic process. The method has three stages. First, pairs of CT and PET images are registered among themselves. Second, registration of CT images is performed to estimate anatomical deformations. Third, the PET image of the second pair is reconstructed using a transformation obtained from stage two. As a result they get

comparable PET images that can be analyzed to determine the change of the tumor. To validate their algorithm they add synthetic tumors to real patient images. Figure 25 shows the results of indirect registration method applied to these synthetic data. Figure 25(a) illustrates the difference between PET images after first step and Figure 25(b) illustrates the difference between PET images after third step.

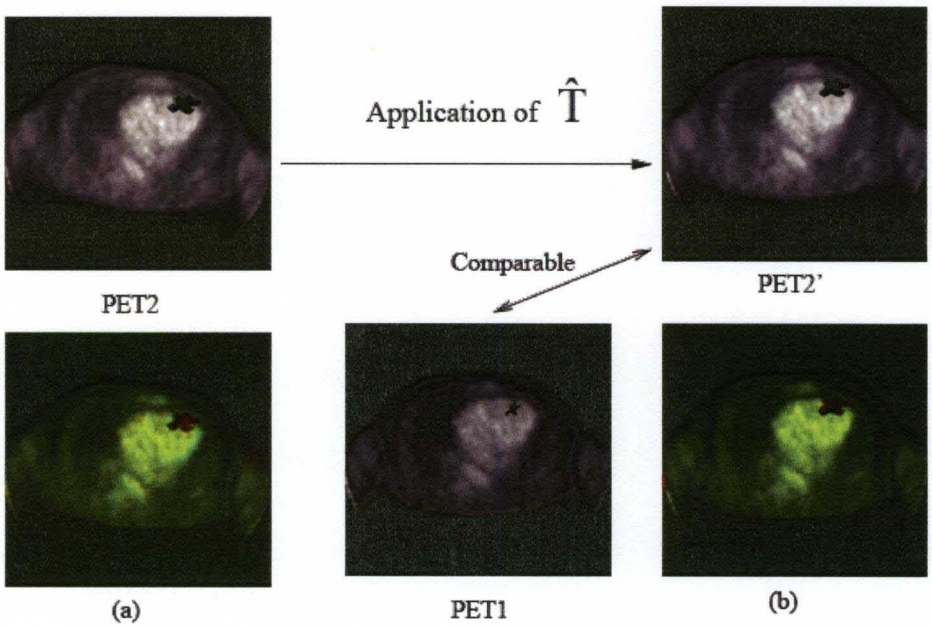


Figure 25. Reconstruct PET images with obtained transformation [33]

In remote sensing, temporal image registration is used for the inspection of nuclear plants, monitoring of national resources or urban growth. Schneider et al used registration to monitor urban growth and the traffic patterns of urban expansion in Chengdu, China [34]. Eight images of the study area were acquired between 1978 and 2002 registered to a master image. Figure 26 illustrates the explicit growth in the southeast, east and northeast regions of the city.

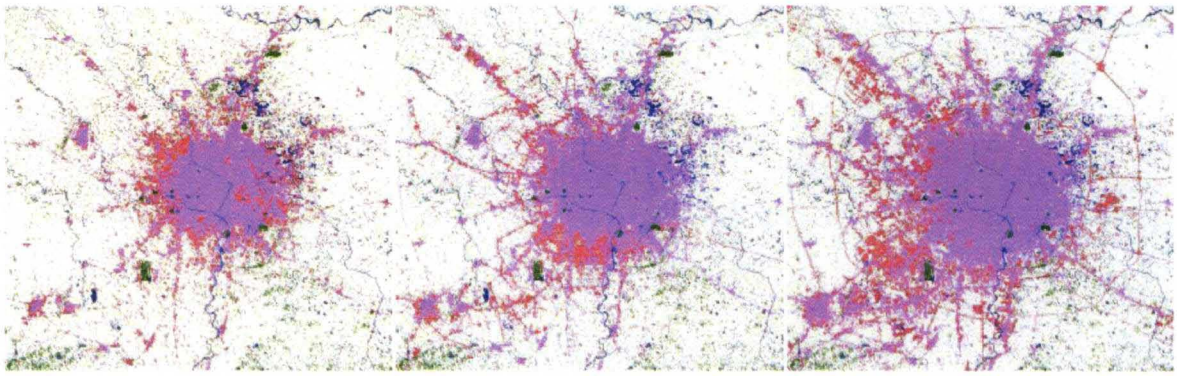


Figure 26. Maps of land cover change in Chengdu for (a) 1978 to 1988, (b) 1988 to 1995, and (c) 1995 to 2002. Urban areas are shown in purple, urban expansion is shown in red, agriculture is white, and natural vegetation is green [34]

In computer vision, automatic change detection for security monitoring and motion tracking are known applications. Figure 27 shows an example for motion tracking. The images of moving person was captured by a nonstationary camera then these two images were aligned and detected region of motion was bounded with a red box [32].

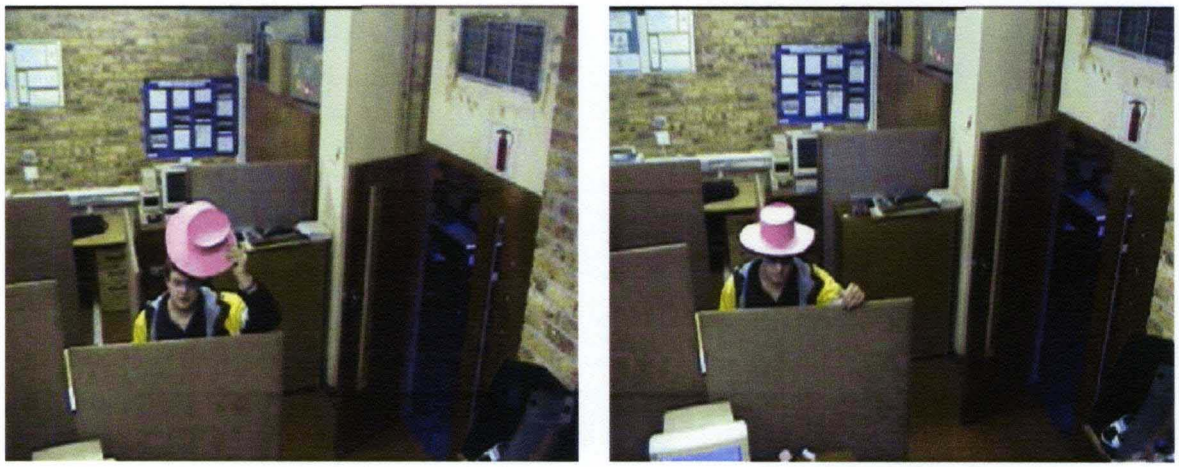


Figure 27. (Top) 2 images of one moving people captured by a moving camera, (Bottom left) the frame difference between the registered, (Bottom right) the resulting bounding box around the detected motion [32]

3.2 Basic Steps for Registration

As a result of the diversity of images to be registered and various types of degradations, designing a universal method applicable to all registration tasks is impossible. Nevertheless, the majority of the registration methods consist of four main steps. These four steps are 1) Feature detection; 2) Feature matching; 3) Transformation model estimation; 4) Image resampling and transformation.

3.2.1 Feature Detection

Feature detection is an automatic or manual detection of remarkable and individual objects from an image. When the images contain enough distinctive and easily detectible objects, using feature based methods is recommended. Feature detection is performed as the first operation of feature based registration methods. Feature based registration methods are usually used by remote sensing and computer vision. In contrast, area based registration methods skip this step and put an emphasis on feature matching

because they work directly with image intensity. This is usually the case with medical imaging that does not have distinctive objects.

Features that are obtained at the end of the feature detection step should satisfy some conditions. They should be distinctive objects and features obtained from different images that have enough common elements regardless of the changes in the covered scene and image geometry. They can be edges, lines, closed-boundary regions and points. Region features represent items such as reservations, forests, buildings, urban areas, and fields. They are detected by segmentation methods. Line features represent general line segments such as object contours, coastal lines, region boundaries, roads, rivers, and anatomical structures in medical imaging. They are detected by edge detection methods. Point features represent region corners, road crossings, oil and gas pads, line intersections, and points on curves with high curvature. Local extreme of wavelet transforms and similarity measures can be used to detect distinctive points. The thing to notice for all detection methods used for point, line and region detection is that they should be able to detect same features from all images even in situations when the images have additional noise or deformations.

After detection, these features can be described by their point representatives called control points (CPs). CPs can be distinctive points, end points or centers of line features, and centers of gravity of regions.

3.2.2 Feature Matching

The process of feature matching establishes which point on one image corresponds to a particular point on the second image. Feature matching methods can be group into two general classes: area based and feature based methods. Area based

methods are appropriate when the distinctive information is provided by gray levels or colors rather than remarkable objects like structures and local shapes. Therefore, in order to use area based methods, reference and sensed images must have identical or statistically dependent intensity values. To estimate the correspondence between references and sensed images, different kinds of similarity measures are used for entire images or predefined window sizes. Known area based methods are cross correlation, Fourier transform and mutual information (covered in more detail in section 4.4). Area based methods have two disadvantages. First, predefined rectangular windows can match images that locally differ only by a translation. If the images have more complex transformations, the same parts of the scene in the reference and sensed image cannot be corresponded by using only rectangular windows. To overcome this problem, usage of circular shape windows is proposed but still more complicated geometric deformations like perspective transforms causes problems. Secondly, a window containing a smooth area without any remarkable objects might be matched incorrectly with other smooth areas in the reference image due to a lack of locally complex globally discriminative objects.

Feature based methods are preferably applied when the structural information is more descriptive than the intensity information of the images. The aim of these methods is finding pairwise correspondence between features of reference and sensed images. They enable registration of different images such as registration of a patient image with an anatomical atlas. In feature based methods, detected features must handle all differences between images. For matching, spatial relations or invariant descriptions of features can be used and the selected feature type affects the choice of the technique to

find the best matches. Graph matching [35], clustering [36], and iterative closest point (ICP) [37] are some of the techniques used by previous studies to match spatial relations. The minimum distance rule with thresholding, matching likelihood coefficients [38], correlation coefficients or mutual information is usually applied to match invariant descriptive of features. The disadvantage of feature based methods is that detection of corresponding features may be difficult and unstable. The description of the features should satisfy the conditions of invariance, uniqueness, and stability. Usually these conditions cannot be satisfied together but it is necessary to find an appropriate trade off. Invariance is needed to make sure the corresponding features form the reference and sensed images are the same. Uniqueness means that different descriptions are used for different features. Through stability, descriptions of features are still close to the descriptions of the original features even though the images have some distortions.

3.2.3 Transformation Model Estimation

In transformation model estimation, the type and parameters of the mapping function are estimated to correlate the position of features in one image or coordinate space with the position of the corresponding feature in the second image or coordinate space. With transformation, the image can be shifted, rotated or stretched in different directions.

Notation and terminology of transformation

The transformation is T{.} called a forward transformation, or forward mapping if it maps input space points to output space points [39].

$$(x,y) = T\{(w,z)\}$$

Where (w,z) input space and (x,y) output space. If T{.} has an inverse, the output space points can be mapped to the input space points and is called the inverse transform or inverse mapping.

$$(w,z) = T^{-1}\{(x,y)\}$$

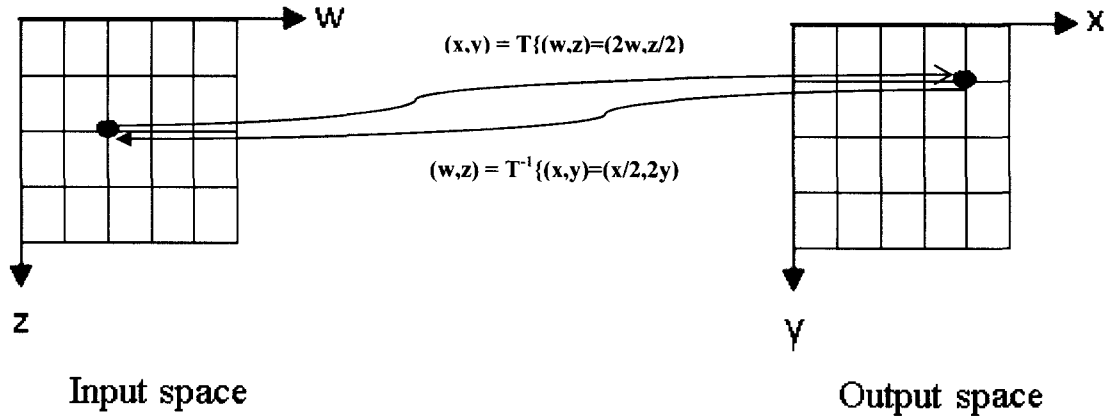


Figure 28. Forward and inverse transformation of a point for $T\{(w,z)\} = (2w,z/2)$

Application of a geometric transformation to images requires a different equation than the geometric transformation of points. The equation is:

$$g(x,y) = f(T^{-1}\{(x,y)\})$$

The procedure that is supplied by the equation can be expand into three steps to compute the output pixel at location (x_k,y_k). These steps are:

1) Evaluation of $(w_k,z_k) = T^{-1}\{(x_k,y_k)\}$.

2) Evaluation of $f(w_k,z_k)$.

3) $g(x_k,y_k) = f(w_k,z_k)$.

After reverse transformation, w_k and z_k usually are not integers but for digital images the values of f are known only at integer value locations. Therefore non-integer values must be converted. This operation is performed at steps 2 and 3 is an example of image interpolation. Image interpolation is construction of a continuously defined

function from discrete data. In signal processing, interpolation is explained as a two step resampling procedure. They are:

1) Constructing the function $\hat{f}$ defined on a continuous domain from the function f defined on discrete domain.

2) Assessment of $\hat{f}$ at the desired locations.

The first step can be formulated as a sum of scaled and shifted functions called interpolation kernels. The most commonly used interpolation kernels are box, triangle and cubic kernels.

The box kernel equation is:

$$h_B(x) = \begin{cases} 1, & -0.5 \le x < 0.5 \\ 0, & otherwise \end{cases}$$

The triangle kernel equation is:

$$h_T(x) = \begin{cases} 1 - |x|, & -0.5 \le x < 0.5 \\ 0, & otherwise \end{cases}$$

The cubic kernel equation is:

$$\begin{cases} 1.5|x|^3 - 2.5|x|^2 + 1 & |x| \le 1 \\ -0.5|x|^3 + 2.5|x|^2 - 4|x| & 1 < |x| \le 1 \\ 0 & otherwise \end{cases}$$

One dimensional interpolation is illustrated in figure 29. Figure 29(b) shows the method for computing $\hat{f}(6.3)$ using a triangular kernel. $\hat{f}(6.3)$ is computed as the sum of two shifted kernels: $f(3)h_T(0.4) + f(4)h_T(0.6)$. Figure 29(c) shows $\hat{f}(x)$ computed by using the box kernels and figure 29(d) shows $\hat{f}(x)$ computed by using the cubic kernels.

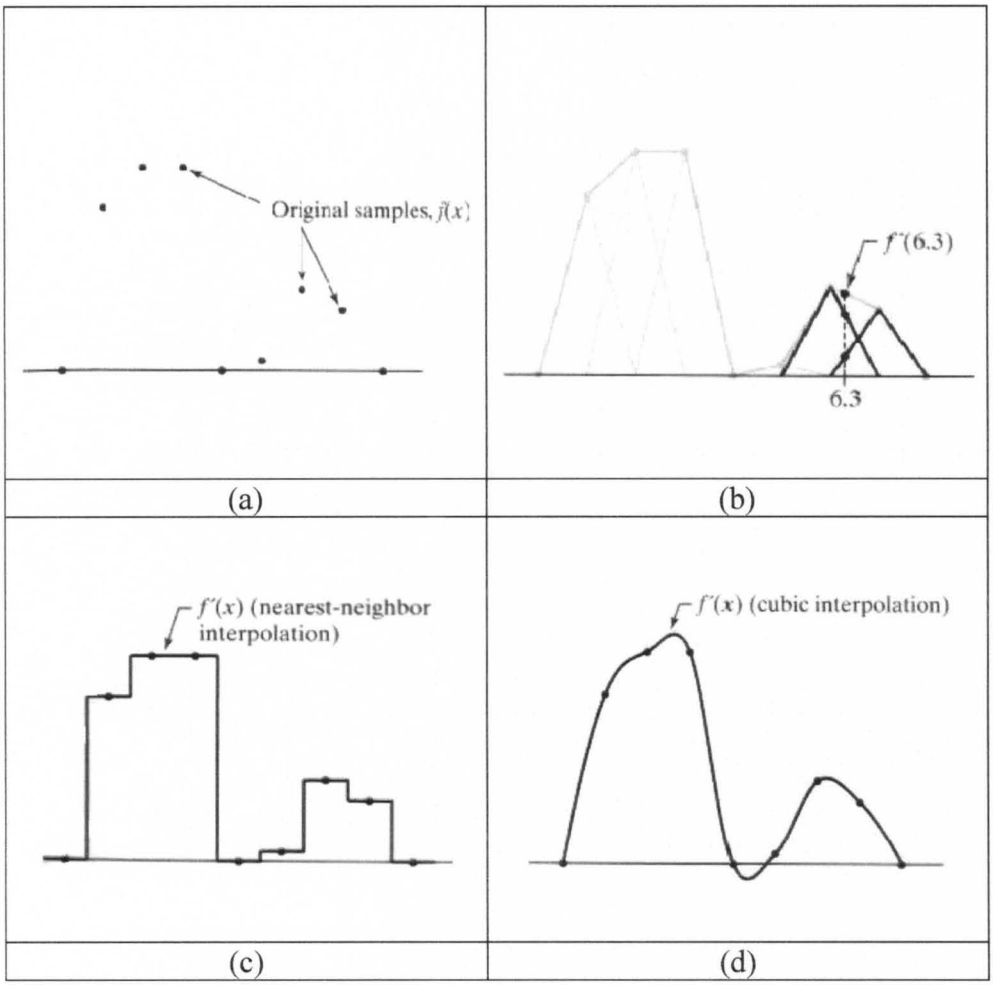


Figure 29. Signal interpolation (a) Original signal, (b) Triangle interpolation, (c) Linear interpolation, (d) Cubic interpolation [39]

In order to apply a one dimensional interpolation approach to two dimensional data, the problem is decomposed into a sequence of several one dimensional interpolation tasks called bilinear interpolation. For example $\hat{f}(2.6,1.0)$ can be calculated by linearly interpolating between f(2,1) and f(3,1) or $\hat{f}(2.6,1.4)$ can be calculated by linearly interpolating between $\hat{f}(2.6,1)$ and $\hat{f}(2.6,2)$. Bilinear interpolation is most commonly used in image processing.

Dimensionality of transformation

Dimensionality has two main subdivisions: spatial dimensions and time series. Spatial dimensions is divided into three parts I) 2-D to 2-D; II) 2-D to 3-D; III) 3-D to 3-D.

2-D to 2-D registration is the simplest, due to the limited number of parameters and volume of the data. 2D images can be registered using rotation, orthogonal translations, and scaling.

2-D to 3-D registration is needed to establish correspondence between 3D volumes and projection images. Image guided interventions are one of the most important uses. In image guided interventions, detailed 3D description of patient anatomy and preoperative images are provided. It provides accurate alignment of the preoperative images to physical space for the clinician in order to ensure that the given treatment is compatible with the preoperative plan. The work of K. Herring et al provides a technique which is the combination of the iso-surface creation and the Bed-McKay point-to-surface registration algorithms for image guided surgery of the spine [40].

The major focus for registration is 3-D to 3-D registration. 3-D methods consider the data set as a volumetric data that can be registered to another volumetric data set. Compared with 2-D to 2-D registration, 3-D to 3-D registration is far more complex. The number of parameters and the volume of the data are mean that obtaining a registration is in many cases difficult and slower than in the 2-D to 2-D case. Many of the 2D techniques can be generalized to higher dimensional aspects as long as computational cost and amount of data are considered. In medical imaging, registration of multiple 3D images such as magnetic resonance (MR) and computed tomography (CT) volumes has

wide applicability. That kind of work usually assumes the internal anatomy of the patient has not distorted or changed in spatial relationships between organs, so that registration involves only three rotations and three translations along the X, Y and Z axes.

All registration methods produce a set of equations to transform each point in one image into the coordinates of the corresponding point in the other image. The transformation can be either global or local. If the transformation applies to the entire image, it is called global transformation. If each subsection of the image has their own transformations, it is called local transformation.

Registration of time series is used when two time series images need to be compared. In registration methods, time can be included as an extra dimension. As a result, comparing time series of 2-D images becomes a 3-D registration, and comparing of a time series of 3-D images becomes a 4-D registration. In medical imaging, time series data is needed for various reasons such as monitoring bone growth in children, monitoring tumor growth or evolution of drug.

Domain and nature of the transformation

All registration methods produce a set of equations to transform each point in one image into the coordinates of the corresponding point in the second. The transformation can be either global or local.

No matter if the domain is local or global, there are four main different transformation types. The first type of transformation is rigid. While any two points are mapped onto the second image, it conserves the distance between any two points in the first image. Rigid transformation allows translation and rotation. In a 2-D coordinate system the following formula is used to transform point (x, y) into (x', y')

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} cos\theta \pm sin\theta \\ sin\theta \pm cos\theta \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} + \begin{pmatrix} t_x \\ t_y \end{pmatrix}$$

where θ denotes the rotation angle, and $\begin{pmatrix} t_x \\ t_y \end{pmatrix}$ the translation vector.

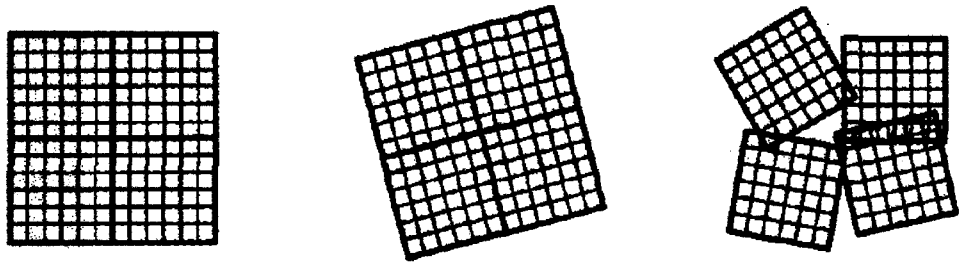


Figure 30. Rigid transformation [25]

The second type of transformation is affine. In an affine transformation, any straight line in the first image is mapped onto a straight line in the second image without losing parallelism of these lines. Uniform and non-uniform scaling and shearing are examples of an affine transformation. Affine transformation can be formulated with a matrix transformation and a translation. In a 2-D coordinate system, the following formula is used to transform point (x, y) into (x', y')

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix} \begin{pmatrix} x \\ y \end{pmatrix} + \begin{pmatrix} t_x \\ t_y \end{pmatrix}$$

where $\begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}$ denotes any real value matrix, and $\begin{pmatrix} t_x \\ t_y \end{pmatrix}$ the translation vector.

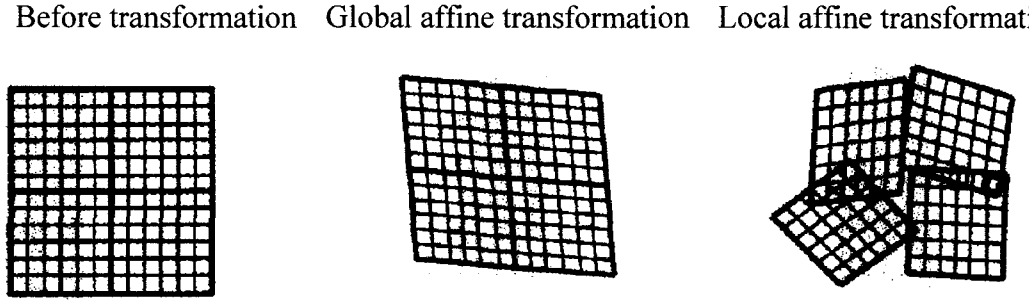


Figure 31. Affine transformation [25]

The third type of translation is projective. In a projective transformation, any straight line in the first image is mapped onto a straight line in the second image without the need for preserving parallelism between straight lines. Projective transformation can be formulated with a matrix transformation. In a 2-D coordinate system, the following formula is used to transform point (x, y) into (x', y'):

$$\begin{pmatrix} x' \\ y' \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ a_{31} & a_{32} & a_{33} \end{pmatrix} \begin{pmatrix} x \\ y \\ 1 \end{pmatrix}$$

Before transformation | Global projective transformation | Local projective transformation

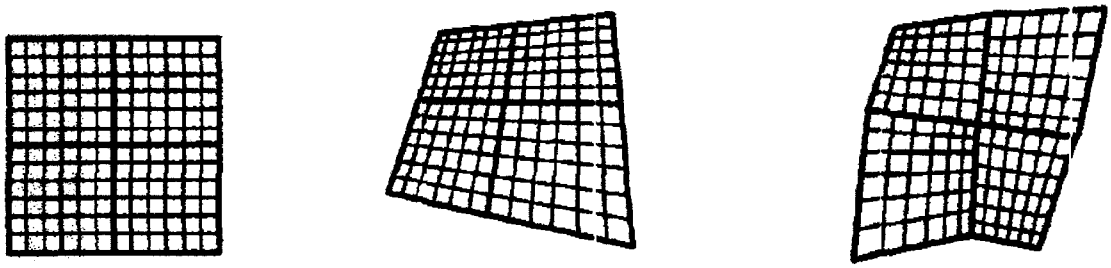


Figure 32. Projective transformation [25]

The final type of the transformation is curved or elastic. It maps a straight line onto a curve. In 2-D dimensions it is formulated as the following formula.

$$(x', y') = \mathrm{F}\,(x, y)$$

Where F denotes any mapping function. One common curved transformation involves polynomial functions. In a 2-D coordinate system, a polynomial function can be written as:

$$x' = a_{00} + a_{10}\mathrm{x} + a_{01}\mathrm{y} + a_{20}\mathrm{x}^2 + a_{11}\mathrm{xy} + a_{02}\mathrm{y}^2 + \ldots..$$

$$y' = b_{00} + b_{10}\mathrm{x} + b_{01}\mathrm{y} + b_{20}\mathrm{x}^2 + b_{11}\mathrm{xy} + b_{02}\mathrm{y}^2 + \ldots..$$

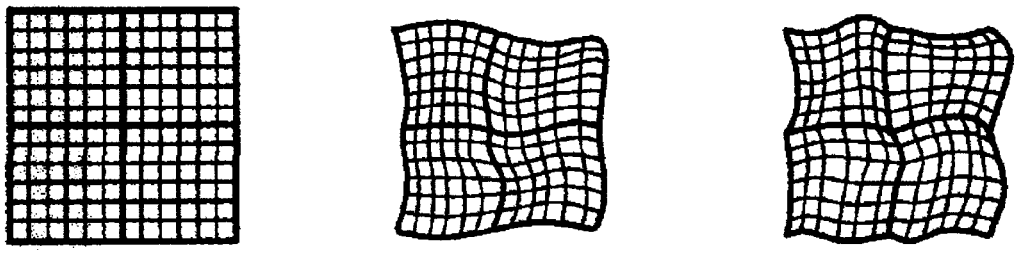


Figure 33. Curved transformation [25]

3.2.4 Image Resampling and Transformation

In image resampling and transformation, the image is transformed through the mapping functions constructed during the previous steps. The transformation approach can be forward or backward. If each pixel from the sensed image can be directly transformed using the estimated mapping functions, it is called the forward method. If the registered image data from the sensed image are determined using the coordinates of the reference image's pixel and the inverse of the estimated mapping function, it is called the backward method.

3.3 Registration Methods

2.3.1 Landmark-based Registration

Landmark-based registration methods are based on the identification of corresponding point landmarks in two images. A landmark is an outstanding feature location of an image. Hard landmarks or prospective landmarks which are positioned before imaging are called fiducial markers [41]. Soft landmarks or retrospective landmarks are positioned after imaging. Landmarks are usually identified by the user. In theory, landmark-based registration methods can be applied to any image no matter the object or subject. They are typically used for rigid and affine transformations.

Additionally, if the identified landmarks are large enough, they can be used for more complex transformations. These methods typically have a fast optimization procedure since the set of identified points is sparse compared to the original image content.

The algorithm for calculation of the transformation for landmark-based registration is rather straightforward [42]. First, the centroid or average for each set of points is calculated. Later, the translation that must be applied to one set of points is resolved by checking the difference between centroids. Later, these points are rotated to their new centroid until the sum of square distance between each corresponding point pair is minimized. The square root mean is also called as the root mean square (RMS) error, residual error, or fiducial registration error and it is often recorded by the registration algorithm. Although RMS error is useful, it is not meaningful enough since RMS error is not a direct measure of the accuracy for which features of interest in the images are aligned. Sometimes changing the positions of the landmarks can reduce RMS error but increase the error in relevance between other structures of the images. To overcome this problem, the target registration error (TRE) can be used [43]. TRE is position dependent and gives the accuracy of aligned interest points in two images.

The disadvantage of landmark-based methods is that user interaction is typically needed to identify initial landmarks, and the accuracy of the landmarks is based on the skill of the user. The advantages of the methods are computational speed and ease of implementation.

2.3.4 Surface Based Registration Methods

In surface based registration, indentified corresponding surfaces are used for registration. These can be rigid based or deformable model based. In rigid based

segmentation, the same structures are extracted from both images. Later the structures are registered and aligned. Head and hat is one such algorithm for rigid based registration [44]. In the head and hat algorithm, two corresponding surfaces are identified in the images. One surface is referred to as a head, the other as a hat. The head is fixed in space and represents the contours of the surface drawn from one image. The hat is a set of points from the second image that corresponds to the same surface of head. The hat surface is iteratively transformed according to the head surface until the best fit of the hat points onto the head counter is found. The sum of the squares of the distances between each hat point and the head counters is calculated at each iteration and ends when the sum of squares is minimized. When surfaces have symmetries to rotation, the algorithm fails. Figure 34 illustrates a multiple solution for the contour that has symmetry axes. Even though the results have different rotations, both alignments of the thick dotted line and thin continuous line will produce very similar mean distances between contours.

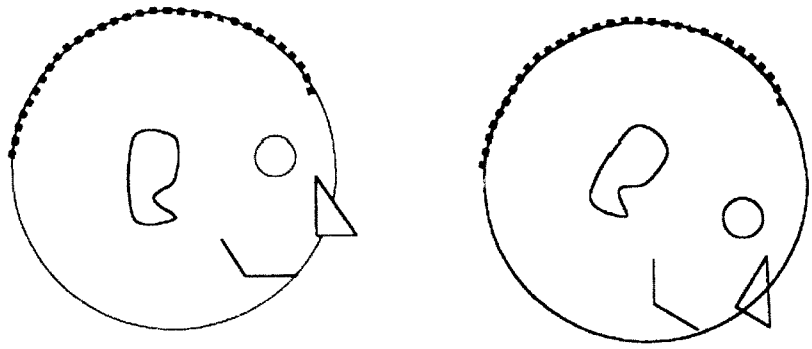


Figure 34. Contour based algorithm produced multiple alignments due to symmetric axes [22]

Another widely applied algorithm for surface based registration is iterative closest point algorithm [37]. In most applications of this algorithm, triangular patches are used to represent one surface while a set of points represent the second. The algorithm finds the

closest point on the appropriate triangle patch to each of the points. Later these points are registered using landmark based registration with RMS error. These two processes are repeated until the RMS error minimized.

In the deformable model based, a structure is extracted from one image and is elastically deformed to fit the second image using deformable curves. Deformable curves are known as snake or active contours, or sometimes nets for 3-D deformable models. The general idea of active contours is creating computer generated curves that move within the images to find object boundaries. For registration, a template model defined in one image is then deformed to match a segmented structure in the second image. The deformation process iteratively proceeds using small deformations at a time. The first drawback of the deformable model is it needs good initial template modeling. Secondly, if the target structure is adequately different than the template, it can be inconsistent. Deformation models are good for finding local curve transformations between images. These models are usually automated and can be used on any modality.

2.3.5 Intensity Based Registration Methods

The intensity based registration methods directly work on the image grey values. It does not require any segmentation or corresponding structures. There are two different approaches for voxel based registration. The first is the reductive registration methods which reduce the image gray level to representative scalars. The main examples for reductive registration methods are principal-axes [45] and moment-based [46] methods. The second one is the full image content base methods. Because of computational cost, their usage for 3-D – 3-D applications is limited. Examples for full image content based methods are cross-correlation, Fourier-domain based cross-correlation, minimization of

variance of intensity ratios, minimization of variance of grey values within segments, minimization of the histogram entropy of difference images, histogram clustering and minimization of histogram dispersion, maximization of mutual information, maximization of zero crossing indifference images, cepstral echo filtering, determination of the optic flow field, minimization of the absolute or squared intensity differences, matching local low-order Taylor expansions determined by the image grey values, implicitly using surface registration by interpreting a 3-D image as an instance of a surface in 4-D space.

4. APPLIED METHODOLOGIES

4.1 Datasets

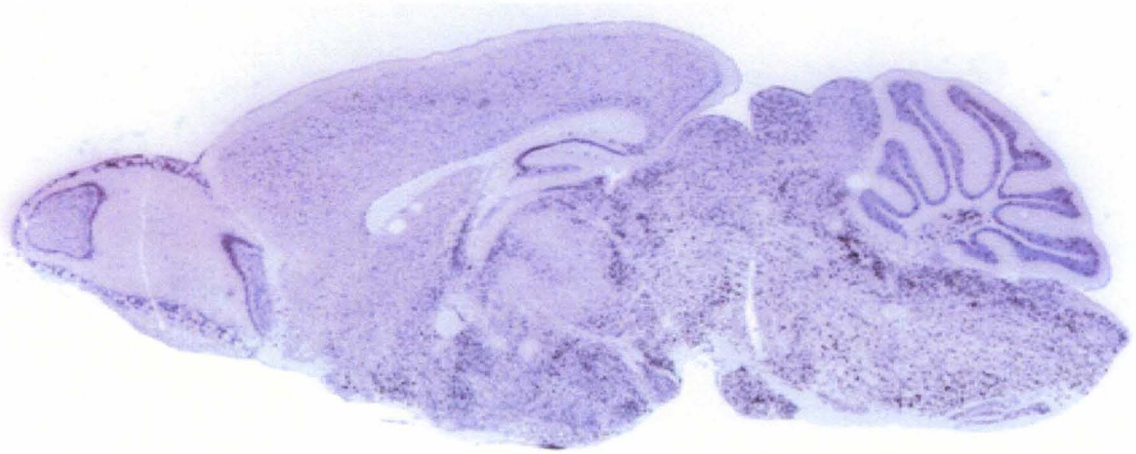
In this research in order to test our methodologies, we used two different datasets: the Allen mouse brain and the retina gene expression images.

4.1.1 Allen Mouse Brain

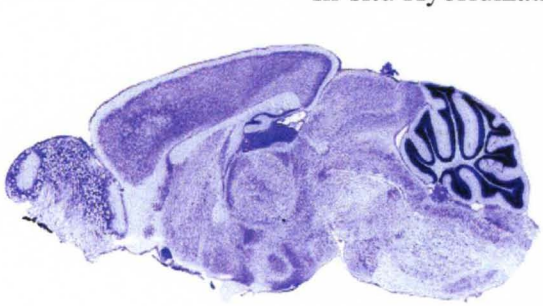
The Allen Institute for Brain Science is a non-profit medical research organization [47]. Initiated in 2003 with a $100 million seed donation from philanthropist, founder and former Microsoft executive Paul Allen in order to accelerate the understanding of how the human brain works. It combines genomics with neuroanatomy by creating gene expression maps for the mouse brain and supplying viewing tools and data search that enables users to short data according to the gene, age, and expression level. The data is searchable and sortable by gene, age, and expression. The Allen brain resources are accessible from the Allen Brain Atlas data portal. The portal includes the Allen Mouse Brain Atlas, Allen Spinal Cord Atlas, Allen Developing Mouse Brain Atlas and Allen Human Brain Atlas.

To test our methodologies, we collected sagittal mid brain in-situ hybridization images with their corresponding atlas and nissl images. The reference atlases are full-color, high resolution and annotated. They can be used to compare different gene expression data or taken as a reference to create hand drawn structures on nissl images in

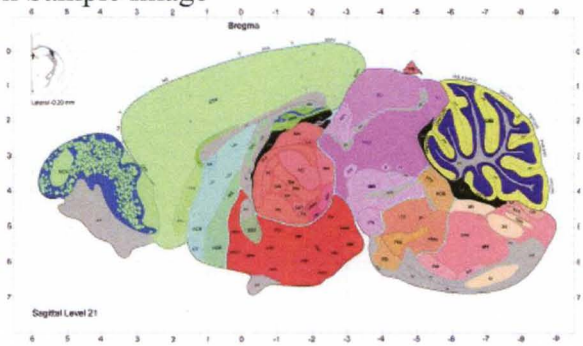
order to get information from interest areas [48]. Nissl images are obtained with nissl staining technique that labels nissl substance (The ribosomal RNA associated with rough endoplasmic reticulum) [49]. Nissl staining is used as a cytoarchitectural (body tissues cellular composition study [50]) reference to identify specific cell populations in the brain. In this research, we take advantage of the gray level similarity between in-situ and nissl images to perform registration. We convert images to gray scale which means each pixel is defined by a single numerical value representing its brightness. Figure 35 shows an example set of images. The size of the in-situ hybridization sample image is 14545x7345 pixels, the nissl image is 7632x4242 pixels, and the atlas image is 7561x4418 pixels.



In-situ Hybridization Sample Image



Nissl Image



Atlas Image

Figure 35. Sagittal mouse mid brain data

4.1.2 Retina Gene Expression Images

In addition to mouse brain gene expression images, we have mouse retina gene expression images obtained via in-situ hybridization. They were produced by Dr. Nigel Cooper's lab from University of Louisville. In contrast to the Allen Brain data, this dataset has neither an atlas nor a nissl data. One of the original in-situ hybridization image from the Cooper Lab results for several different postnatal days. For our purpose, we are interested in registering the images acquired from different samples on the same postnatal day. In order to do registration, first we preprocessed the data to separate and save each subimage according to their postnatal day. Figure 36 shows an example in-situ hybridization image that has seven different postnatal days (P1, P7, P12, P14, P16, P30, P60). The size of the image is 13200x2400 pixels. After separation each image is approximately 1600x1300 pixels.

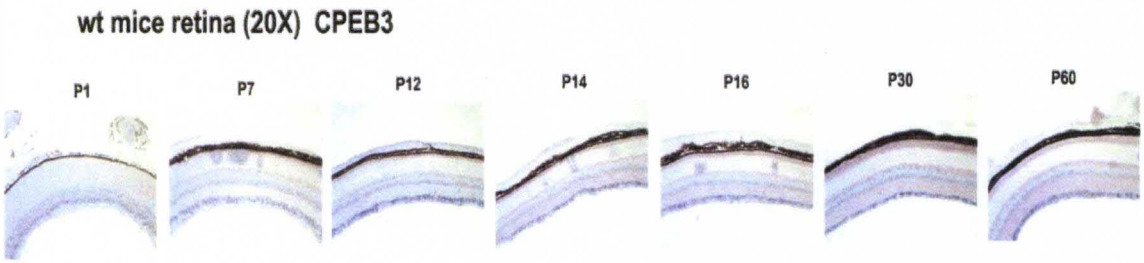


Figure 36. A retina in-situ hybridization images for different postnatal days

4.2 Preprocessing of Retina In-Situ Hybridization Images

A tool is developed to preprocess data in Matlab. Preprocessing of the data has two main steps: character recognition and image separation.

Character recognition provides the postnatal day of each separated image and annotation information for the sample (such as the source information, resolution and gene being studied. The results are used by image separation to save the data without mixing. To recognize characters, the algorithm divides the image into two parts. The first part includes characters; the second part includes in-situ hybridization images. The image division finds columns that do not have color patterns such as empty areas or text information and removes these from the original image. After division, each character is extracted and compared with previously prepared templates. The template with the higest correlation is chosen as the current character. For some characters such as "i" and ".", correlation does not yield correct results. In order to recognize these characters, index information of pixels is used. For example, if the consecutive characters start from the same column, these two characters represent "i'.

Algorithm Character Recognition

Input: Image file, templates

Output: Postnatal days, information of the sample, words lengths

```
1:  Divide image into two parts
2:  Convert image to binary image
3:  While 1
4:      If image is not empty
5:          Get first line from the binary image
6:          Remove first line from the binary image
7:          Find the number of words and length of the each word in the line
8:          Label each letter in the line
9:          For every labeled letter
10:             Check special characters. If character is found exit
11:             Calculate correlations between the letter and the templates
12:             Find the most similar one and send as a result
13:         End
14:     Else
15:         Exit while
16: End
```

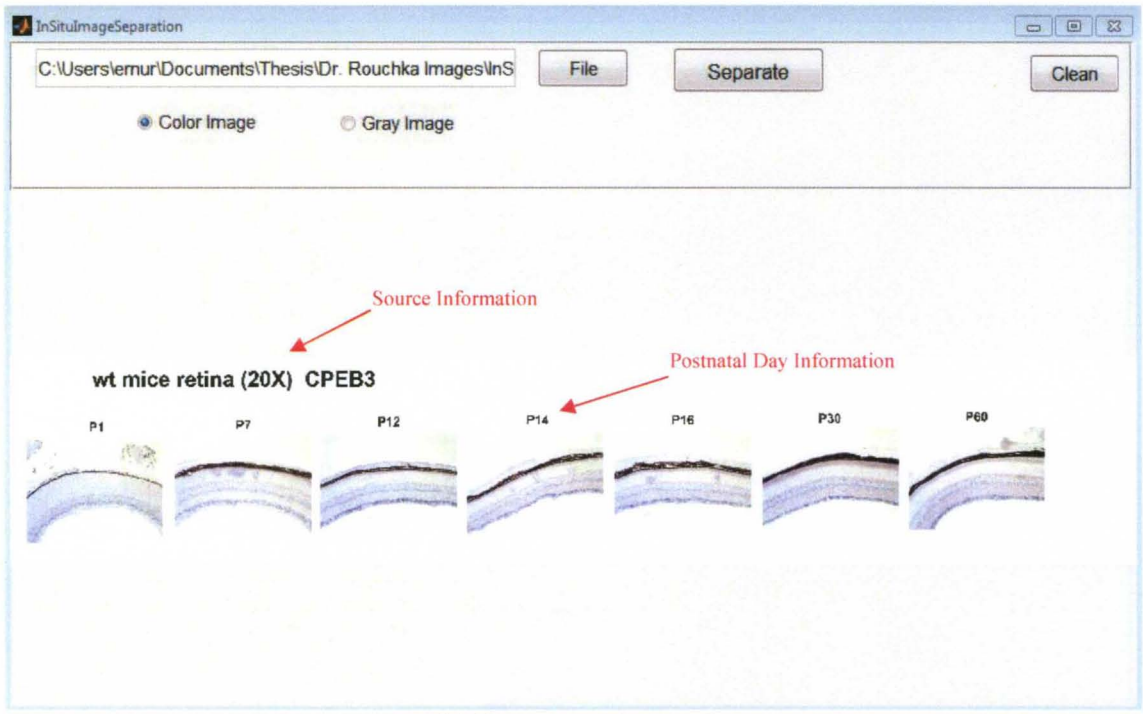
In order to find separation points between different postnatal data, the algorithm checks intensity of each column to find the white areas between two images. Images can be saved as a gray or color images.

Algorithm Image Separation

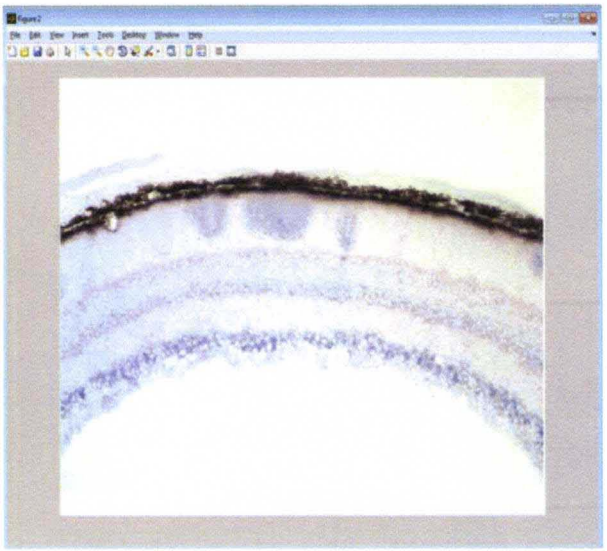
Input: Image file

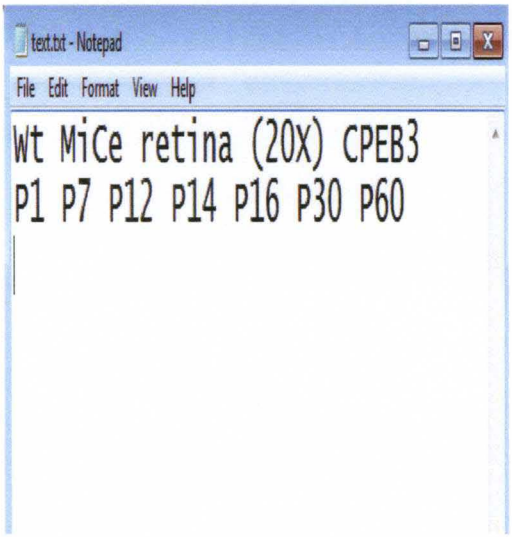
Output: Separated images

```
1: For each column of the image
2:     If column is not white
3:         Insert this column to new sub image
4:     Else
5:         If it is first white column
6:             Save the sub image
7:     End
8: End
```



(a) Preprocessing tool





(b) Separated Image (c) Obtained Data Information From Image

Figure 37. Data preprocessing

4.3 Registration Tool

The registration tool is implemented in Matlab as a graphical user interface. It allows the user to select a methodology from three options. These options are manual, correlation and simple registration. At the end of registration, the original images, corresponding image without transformation and corresponding images after registration are saved as a PNG images. The transformation matrix results, methodology name and parameters are saved in a text file. Additionally these results can be loaded. There are five tabs on the visualization area. The first tab allows, the user can see four images at the same time in four different boxes. These images are sample image, reference image, corresponding sample image without registration, and corresponding sample image after registration. When one of the tabs, except the first is selected, the user can see a specific image according to the selected tab on the whole visualization area. For example, if tab

four is selected, only the corresponding sample image after registration is shown on the whole visualization area. Figures 38 and 39 show screen shots from user interface.

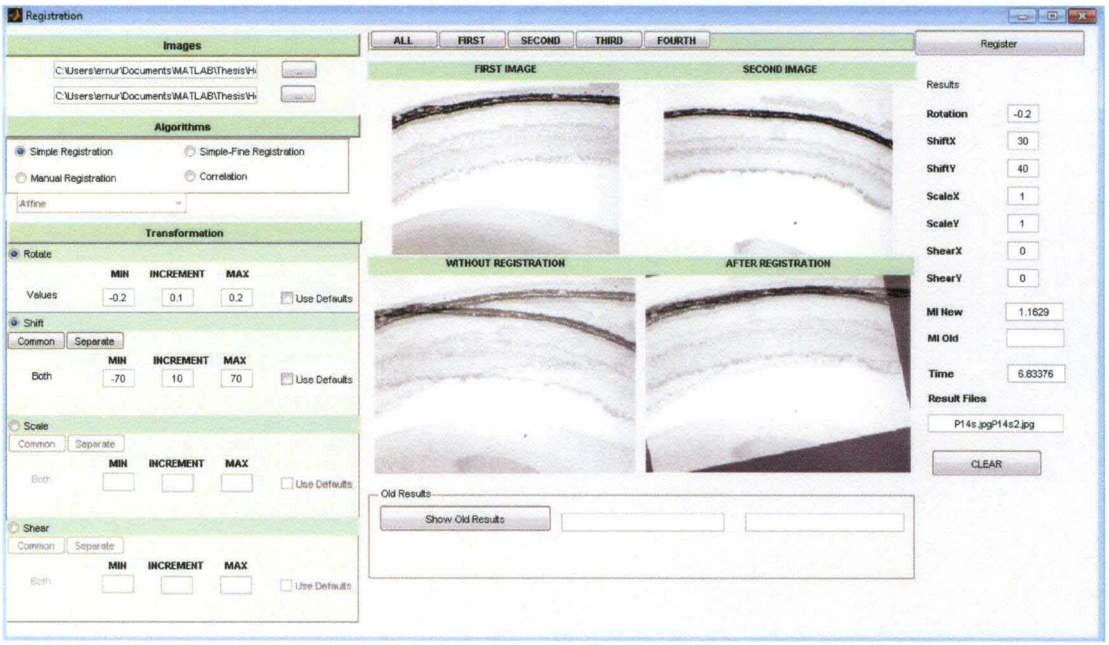


Figure 38. Application of simple registration

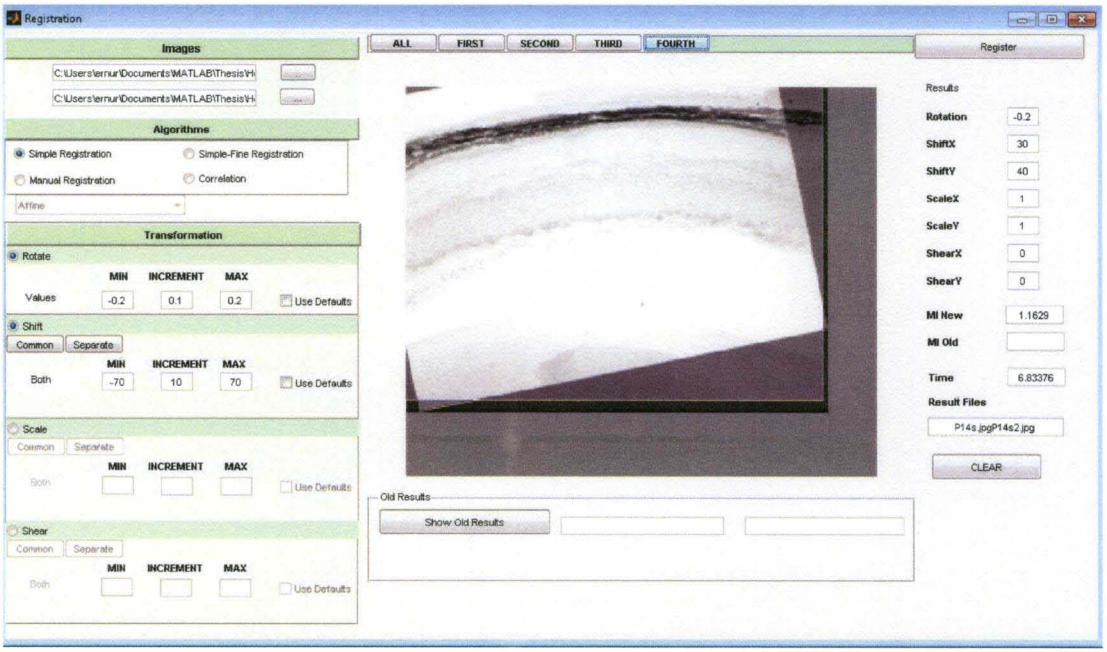


Figure 39. Application of simple registration.

4.4 Manual Registration

The first methodology applied is manual registration. It is similar to landmark based registration methods. Landmarks are identified by the user. After landmark identification, an equation is produced according to the selected algorithm to transform each point in one image into the coordinates of the corresponding point in the other image. There are six different algorithms: affine, linear conformal, projective, polynomial, piecewise linear and local weighted mean. Table 2 shows the minimum number of landmark pairs required for each of these algorithms. Figure 40 shows the user interface for manual registration.

TABLE 2. Minimum landmark pair number for each algorithm

Algorithm	**The Least Minimum Landmark Pair Number**	
Affine	3	
Linear Conformal	2	
Projective	4	
Polynomial	Order 2	6
	Order 3	10
	Order 4	15
Piecewise Linear	4	
Local Weighted Mean	6	

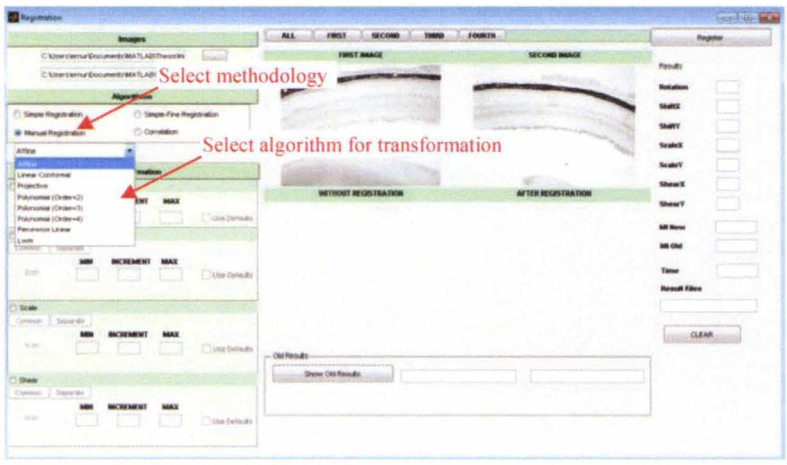


Figure 40. Manual registration

4.4.1 Affine Transformation

Affine transformation consists of a matrix transformation, and a translation. For two dimensional spaces, it can be written as:

$$[x \quad y] = [w \quad z] \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} + [b_1 \quad b_2]$$

In order to simplify for computational purposes, the affine transformation can be written as a single matrix multiplication by adding a third coordinate.

$$[x \quad y \quad 1] = [w \quad z \quad 1] \begin{bmatrix} a_{11} & a_{12} & 0 \\ a_{21} & a_{22} & 0 \\ b_1 & b_2 & 1 \end{bmatrix}$$

This equation can be written also as

$$[x \quad y \quad 1] = [w \quad z \quad 1]\, T$$

where T is called the affine matrix. Table 3 shows how to choose values for the affine matrix, T, to achieve scaling, rotation, translation, shearing and vertical reflection.

TABLE 3. Affine Matrixes

Type	Affine Matrix T	Coordinate Equations
Scaling	$\begin{bmatrix} s_x & 0 & 0 \\ 0 & s_y & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$x = s_x w$ $y = s_y z$
Rotation	$\begin{bmatrix} cos\theta & sin\theta & 0 \\ -sin\theta & cos\theta & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$x = w \cos\theta - z \sin\theta$ $y = w \sin\theta + z \cos\theta$
Shear (horizontal)	$\begin{bmatrix} 1 & 0 & 0 \\ \alpha & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$x = w + \alpha z$ $y = z$
Shear(vertical)	$\begin{bmatrix} 1 & \beta & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$x = w$ $y = \beta w + z$

Translation	$\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ \delta_x & \delta_y & 1 \end{bmatrix}$	x = w y = - z
Vertical Reflection	$\begin{bmatrix} 1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & 1 \end{bmatrix}$	$x = w + \delta_x$ $y = z + \delta_y$

For calculation of the affine matrix T, six unknowns: a_{11}, a_{12}, a_{21}, a_{22}, b_1, b_2 can be solved with at least three landmark pairs. Another way to write this is:

$$[x\ y] = [w\ z\ 1] * \begin{bmatrix} a_{11} & a_{22} \\ a_{12} & b_1 \\ a_{21} & b_2 \end{bmatrix}$$

Rewriting the above matrix equation:

$$X = W * T$$

T can be solved with three or more correspondence points.

$$T = W \backslash X$$

which gives us the first two columns of T, and the third column must be $\begin{bmatrix} 0 \\ 0 \\ 1 \end{bmatrix}$.

4.4.2 Linear Conformal

Linear conformal transformations can include a rotation, a scaling, and a translation. It is a subset of the affine transformation. It can be written as:

$$[x\ y] = [w\ z\ 1] * \begin{bmatrix} sc & -ss \\ ss & sc \\ tx & ty \end{bmatrix}$$

where sc = s*cos(θ) and ss = s*sin(θ). Four unknowns: sc, ss, tx, ty can be solved with at least two landmark pairs. Another way to write this is:

$$x = [w\ z\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix} \quad y = [z\ -w\ 0\ 1] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}$$

With two or more correspondence points the x equations and y equations can be combined to solve unknowns.

$$[x_1] = [w_1\ z_1\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}, [x_2] = [w_2\ z_2\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}$$

$$[\ldots] = [\ldots\quad\quad] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}, [x_n] = [w_n\ z_n\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}$$

$$[y_1] = [z_1\ w_1\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}, [y_2] = [z_2\ w_2\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}$$

$$[\ldots] = [\ldots\quad\quad] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}, [y_n] = [z_n\ w_n\ 1\ 0] * \begin{bmatrix} sc \\ ss \\ tx \\ ty \end{bmatrix}$$

or rewriting the above matrix equations:

$$X = W * r, \text{ where } r = [sc\ ss\ tx\ ty]' \text{ so } r = W \backslash X.$$

4.4.3 Projective

Projective transformations are useful for reversing perspective distortion in an image. It can be written as:

$$[x' \quad y' \quad h] = [w \quad z \quad 1] \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ a_{21} & a_{22} & a_{23} \\ b_1 & b_2 & 1 \end{bmatrix}$$

Where a_{13} and a_{23} are non-zero and where x = x'/h and y = y'/h. Eight unknowns can be solved with at least four landmark pairs.

$$x = (a_{11}w + a_{21}z + b_1) / (a_{12}w + a_{23}z + 1)$$

$$y = (a_{12}w + a_{22}z + b_2) / (a_{13}w + a_{23}z + 1)$$

Multiply both equations, by denominator:

$$x = [w\ z\ 1\ 0\ 0\ 0\ -xw\ -xz] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y = [0\ 0\ 0\ w\ z\ 1\ -yw\ -yz] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

With four or more correspondence points the x equations and y equations can be combined to solve unknowns.

$$x_1 = [w_1\ z_1\ 1\ 0\ 0\ 0\ -x_1w_1\ -x_1z_1] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$x_2 = [w_2\ z_2\ 1\ 0\ 0\ 0\ -x_2w_2\ -x_2z_2] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$x_3 = [w_3\ z_3\ 1\ 0\ 0\ 0\ -x_3w_3\ -x_3z_3] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$x_4 = [w_4\ z_4\ 1\ 0\ 0\ 0\ -x_4w_4\ -x_4z_4] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$\ldots = [\ldots\ ] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$x_n = [w_n\ z_n\ 1\ 0\ 0\ 0\ -x_nw_n\ -x_nz_n] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y_1 = [0\ 0\ 0\ w_1\ z_1\ 1\ -y_1w_1\ -y_1z_1] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y_2 = [0\ 0\ 0\ w_2\ z_2\ 1\ -y_2w_2\ -y_2z_2] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y_3 = [0\ 0\ 0\ w_3\ z_3\ 1\ -y_3w_3\ -y_3z_3] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y_4 = [0\ 0\ 0\ w_4\ z_4\ 1\ -y_4w_4\ -y_4z_4] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$\ldots = [\ldots\ ] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

$$y_n = [0\ 0\ 0\ w_n\ z_n\ 1\ -y_nw_n\ -y_nz_n] * [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]'$$

or rewriting the above matrix equations:

$$X = W*T \text{ where } T= [a_{11}, a_{21}, b_1, a_{12}, a_{22}, b_2, a_{13}, a_{23}]' \text{ so } T = W/T$$

4.4.4 Polynomial

In polynomial transformation, polynomial functions of w and z determine the mapping. It can be written as:

$$x = W*A,\ y = W*B, \text{ solve for A and B: } A = W\backslash x,\ B = W\backslash y$$

The matrix W depends on the order of the polynomial. W will be M-by-K, where K = (order+1)*(order+2)/2; so A and B will be vectors of length K.

I. Second order polynomials

The second order polynomial transformation can be written as:

$$[x\ y] = [1\ \ w\ \ z\ \ w*z\ \ w^2\ \ z^2] * T'$$

x and y are second-order polynomials of w and z. Each second order polynomial has six terms. T' has size 6-by-2. At least six landmark pairs are needed to solve for the twelve unknowns.

II. Third order polynomials

The third order polynomial transformation can be written as:

$$[x\ y] = [1\ \ w\ \ z\ \ w*z\ \ w^2\ \ z^2\ \ z*w^2\ \ w*z^2\ \ w^3\ \ z^3] * T'$$

x and y are third order polynomials of w and z. Each third order polynomial has ten terms. T' has size 10-by-2. At least ten landmark pairs are needed to solve for the twenty unknowns.

III. Fourth order polynomials

Fourth order polynomial transformation can be written as:

$$[x\ y] = [1\ \ w\ \ z\ \ w*z\ \ w^2\ \ z^2\ \ z*w^2\ \ w*z^2\ \ w^3\ \ z^3\ \ w^3*z\ \ w^2*z^2\ \ w*z^3\ \ w^4\ \ z^4] * T'$$

x and y are third order polynomials of w and z. Each third order polynomial has 15 terms. T' has size 15-by-2. At least 15 landmark pairs are needed to solve for the 30 unknowns.

4.4.5 Piecewise Linear

In piecewise linear transformation, landmarks are used to find Delaunay triangles and transformation is applied separately to each triangle [51]. Transformation is performed by applying an affine mapping to each vertices of a triangle. At least four landmark pairs are needed.

4.4.6 Local Weighted Mean

In local weighted mean transformation, a mapping is created by inferring a polynomial at each landmark using neighboring control points. In this case, N closest control point pairs to the each of the landmark pairs are found. Later the distance from the center control point to the farthest control point used to infer the second order polynomial [52]. At least four landmark pairs are needed and the number of closest points

can be defined by user but it needs to be bigger than six. The default value for N is twelve.

4.5 Registration with Correlation

Registration with correlation is similar to manual registration except landmark pairs are determined using a fully automatic procedure. First it detects corners from both images with a Harris corner detector [53]. Later correlation is used to match the detected corners. Finally the transformation equation is calculated.

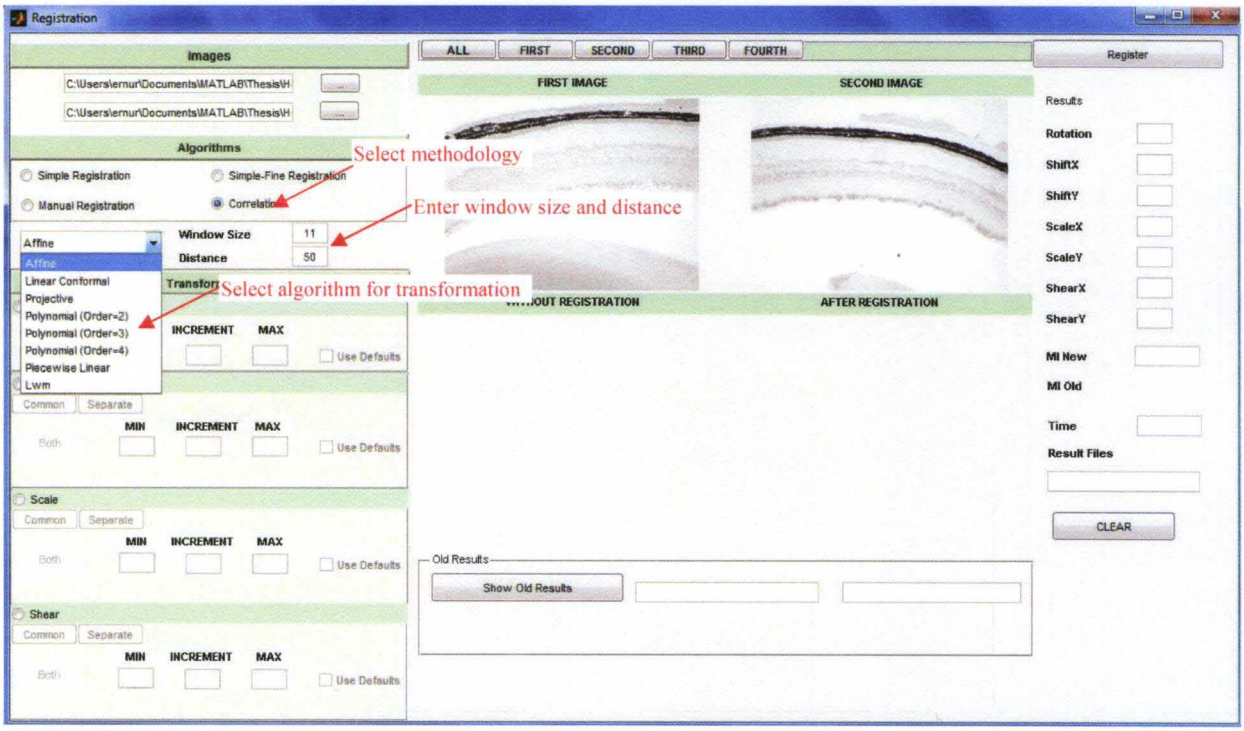


Figure 41. Registration with correlation

4.5.1 Harris-Stephens Corner Detector

Applications that try to extract information from images require relating two or more images by matching only interest points. Many different interest point detectors are

used in computer vision such as finding points of high local symmetry and areas of highly varying texture or corner points. Corner detectors find the corner points that are formed from two or more edges and have large intensity changes in more than one direction. The Harris corner detector is a popular interest point detector proposed Harris and Stephens [53]. Rotation, scale, illumination variation and image noise do not affect Harris corner detection [54]. It is based on Morevec's approach [55] which measures the local changes in image intensity that results from shifting a local window by a small amount in various directions. There are three cases to be considered:

- If the intensity of area covered by the window is approximately constant, then small average intensity change appears after shifts.
- If a shift along the edge will result in a small change but a shift perpendicular to the edge will result in a large change, this indicates an edge.
- If all shifts will result in a large change, this indicates a corner.

These concepts can be expressed mathematically as follows.

$$c(x,y) = \sum_{w} [I(x_i, y_i) - I(x_i + \Delta x, y_i + \Delta y)]^2$$

where c denotes an auto-correlation function that measures the local changes with small amount of shift in different directions, (Δx, Δy) denotes a shift, (x,y) denotes a point, I(.,.) is the image function, and (x_i, y_i) are the points in the window that is centered on (x,y). Taylor expansion truncated to the first order terms approximates the shifted image.

$$I(x_i + \Delta x, y_i + \Delta y) \approx \mathrm{I}(x_i, y_i) + [\, I_x(x_i, y_i)\; I_y(x_i, y_i)] \begin{bmatrix} \Delta x \\ \Delta y \end{bmatrix}$$

where $I_x(.,.)$ and $I_y(.,.)$ denote the partial derivatives in x and y. The results of substituting approximation into auto-correlation function:

$$[\Delta x, \Delta y] * C(x,y) * \begin{bmatrix} \Delta x \\ \Delta y \end{bmatrix}$$

where matrix C(x,y) captures the intensity structure of the local neighborhood.

4.5.2 Matching by Correlation

Previously detected feature points in reference and sample image are matched by looking for points that maximally correlated with each other within windows surrounding each point. The window size and search distance are given by user. The similarity measure for normalized cross correlation is defined as:

$$C(x,y) = \frac{\sum_{s,t}[w(s,t) - \bar{w}][f(x+s,y+t) - \overline{f_{xy}}]}{\sqrt{\sum_{s,t}[w(s,t) - \bar{w}]^2[f(x+s,y+t) - \overline{f_{xy}}]^2}}$$

Where w is the window in the first image, $\bar{w}$ is the average value of the window, f is the window in the second image, and $\overline{f_{xy}}$ is the average value of the window in the region. A high value for C(x,y) indicates a good match between windows.

4.6 Registration with Mutual Information

The last applied methodology is based on mutual information. It is an area based registration method. The basic idea of this method is maximization of the mutual information of the images with respect to the transformation. It is a search oriented algorithm. It calculates mutual information for every parameter in search space and finds the best parameter set that maximizes mutual information. The transformation type for this methodology is affine which includes scaling, rotation, translation and shearing. According to the user preferences, it can be fully automated or semi-automatic. User can select type of affine transformation via user interface. Moreover, the user can define the

search space for each affine transformation. If the user does not define search space, default values are used.

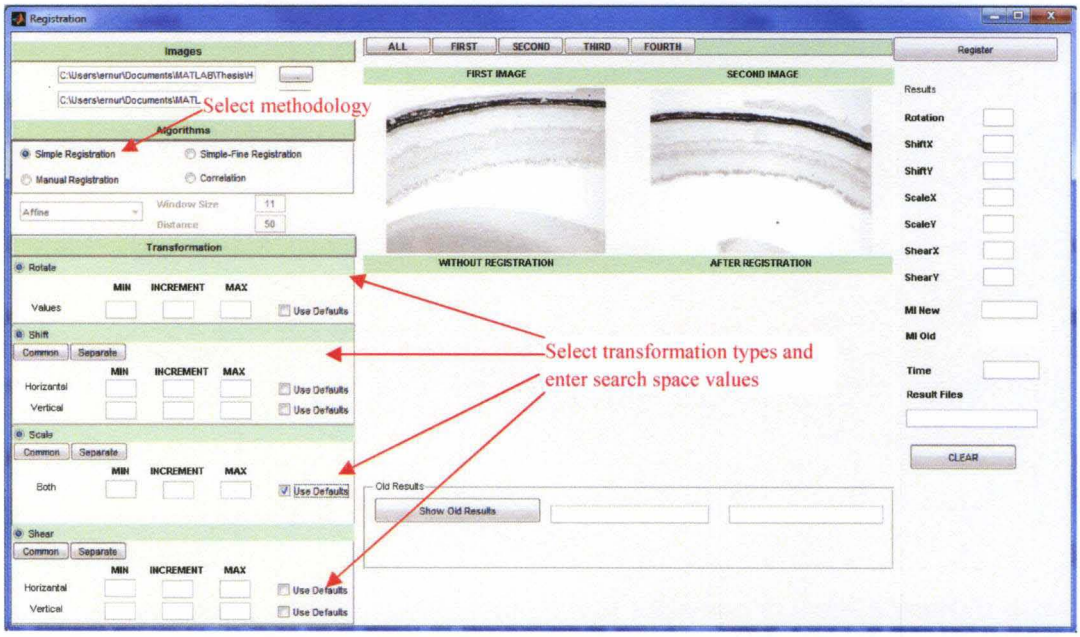


Figure 42. Registration with mutual information

4.6.1 Mutual Information

Mutual information (MI) is the measure of statistical dependency between two data sets. Mutual information between two random variables X and Y is given by

$$MI(x,y) = H(Y) = H(Y/X) = H(X) + H(Y) - H(X,Y)$$

where $H(X) = -E_X(\log(P(x)))$ represents entropy of random variable and P(X) is the probability distribution of X.

4.7 Results

The section includes results from experiments we made in order to test our methodologies. To overcome long running time problem due to the high resolution

characteristic of data, we use minimized data for the Allen brain. Retina gene expression images have their original size.

Manual registration

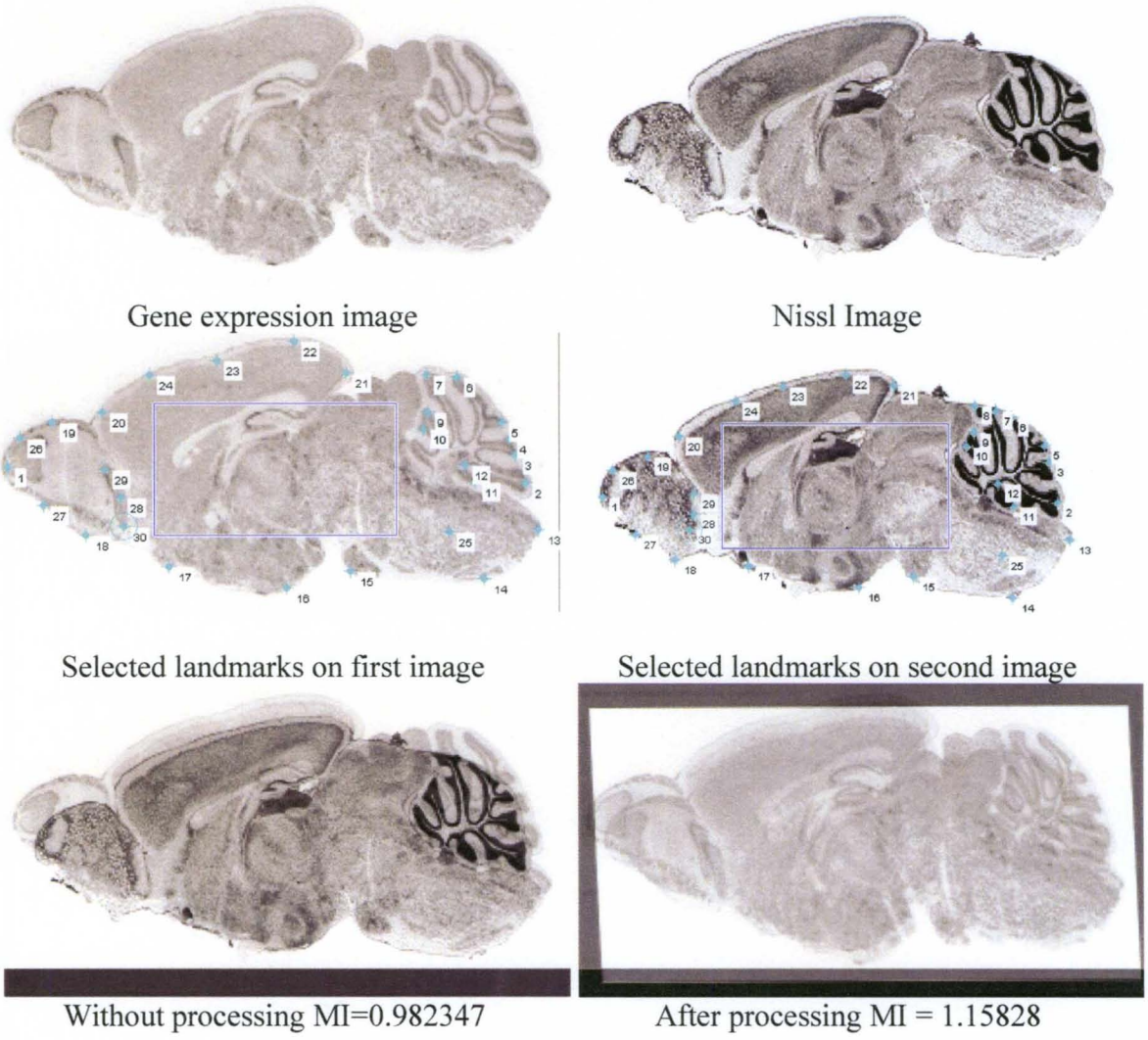
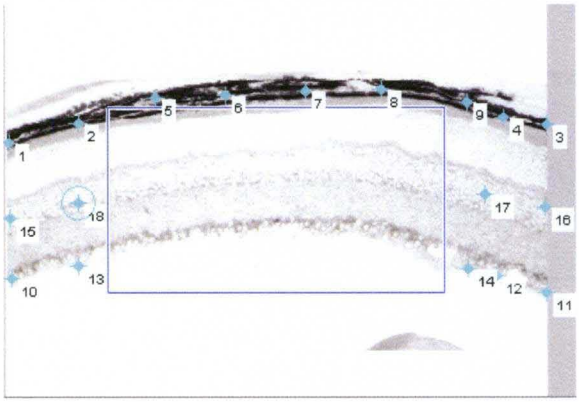


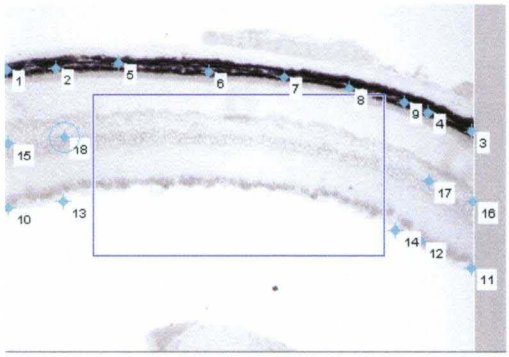
Figure 43. Results for manual registration of Allen brain data with affine algorithm

Gene expression image

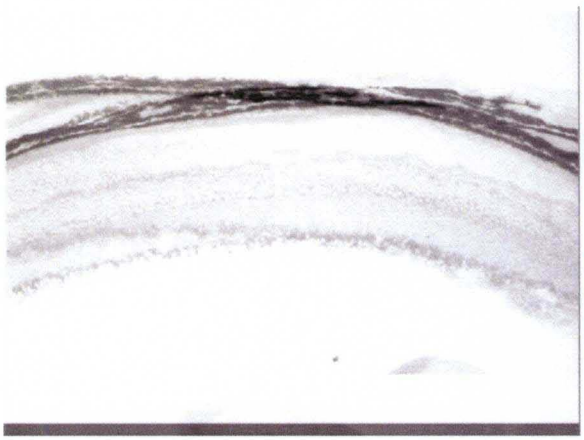
Gene expression image



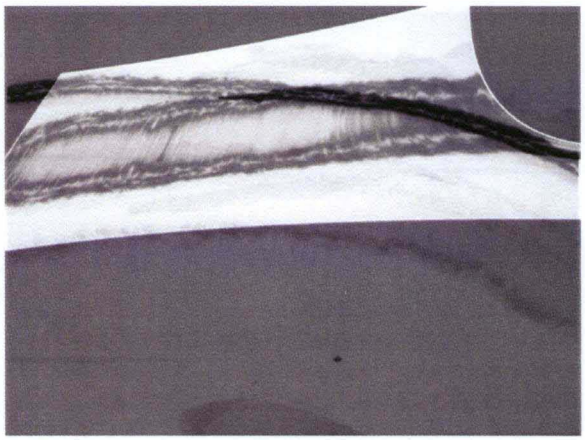
Selected landmarks on first image



Selected landmarks on second image



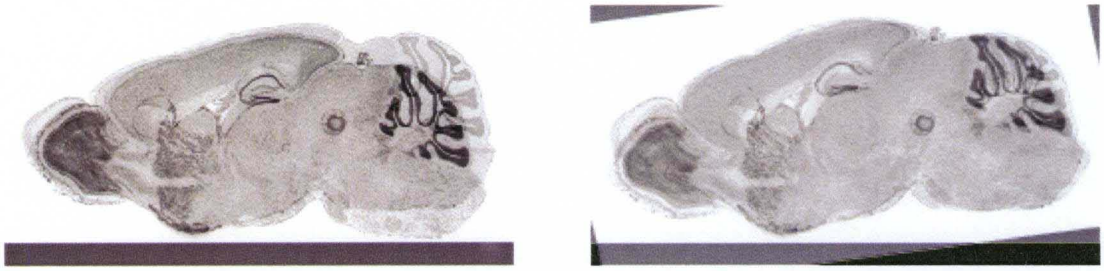
Without processing MI = -122.472



After processing MI = -125.946

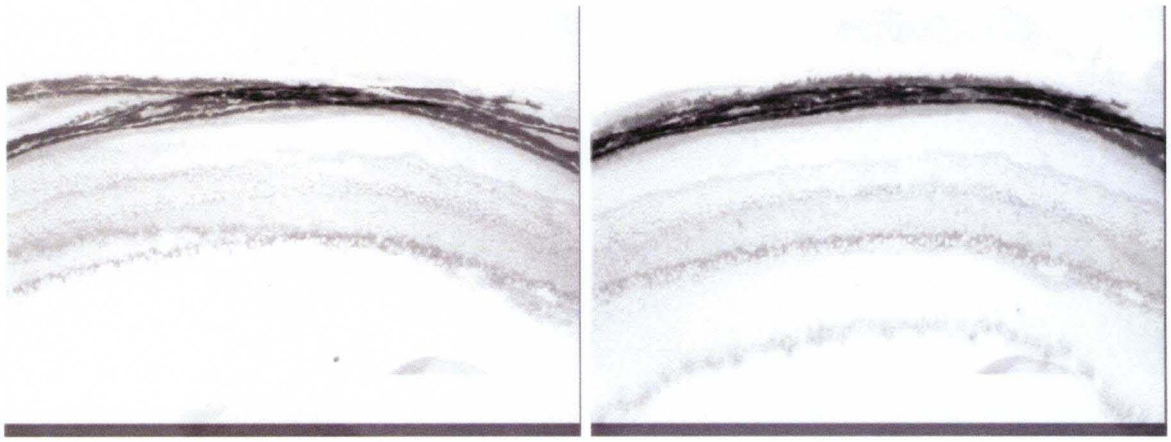
Figure 44. Results for manual registration of postnatal day 14 retina data with polynomial order two

Registration with correlation



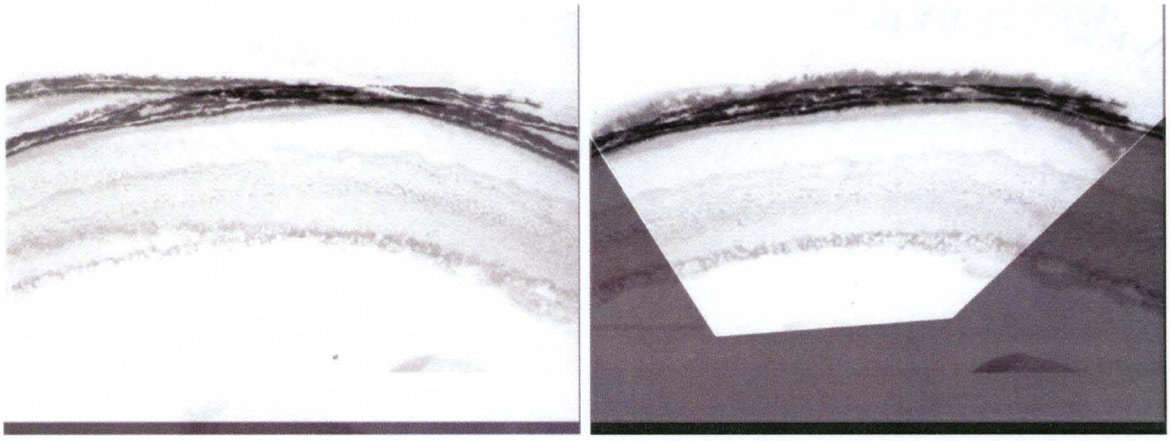
Without processing MI = 1.05314 affine

After processing MI = 1.07281 affine



Without processing MI = -122.472 affine

After processing MI = -123.67 affine

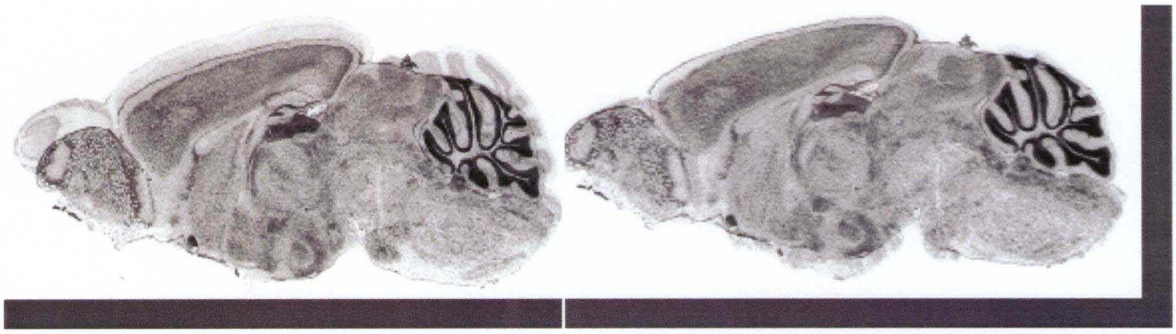


Without processing MI = -122.4 projective

After processing MI = -123.7 projective

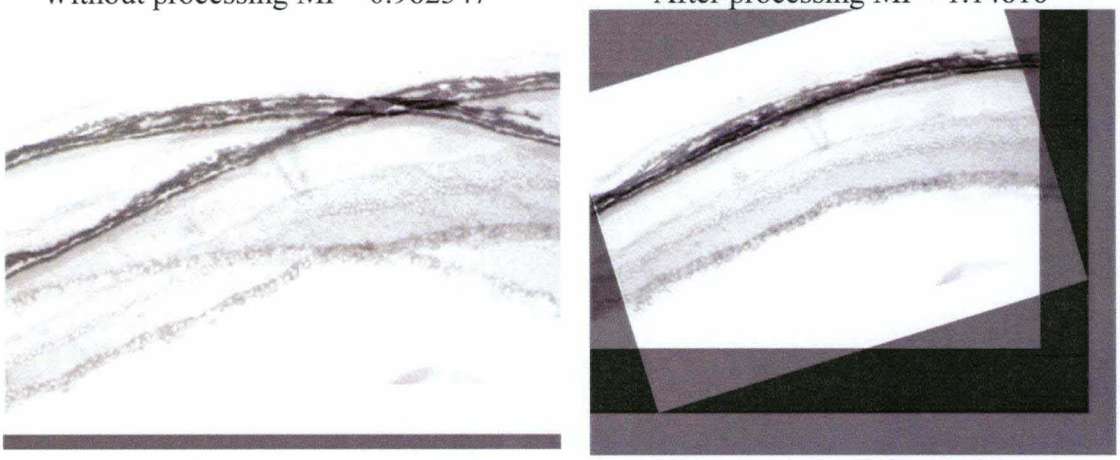
Figure 45. Results for correlation based registration of postnatal day 14 retina data with polynomial order two

Registration with mutual information



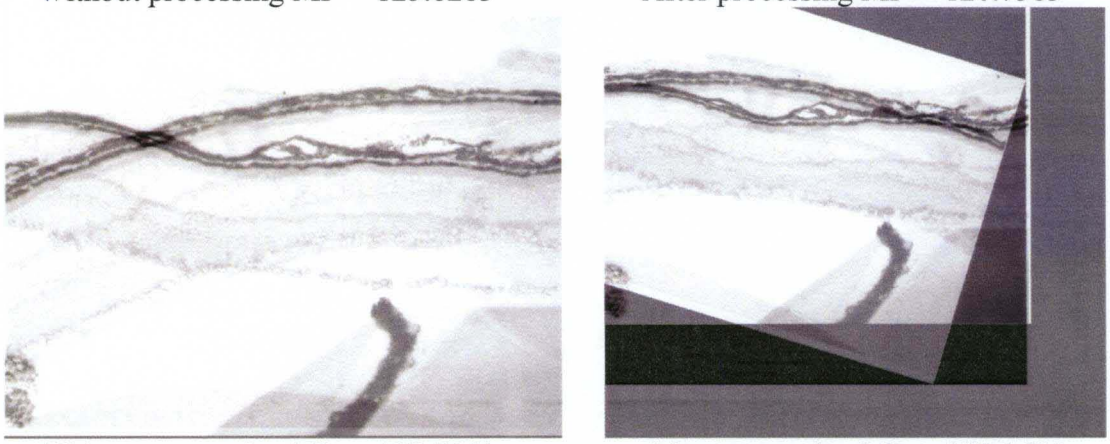
Without processing MI = 0.982347

After processing MI = 1.14816



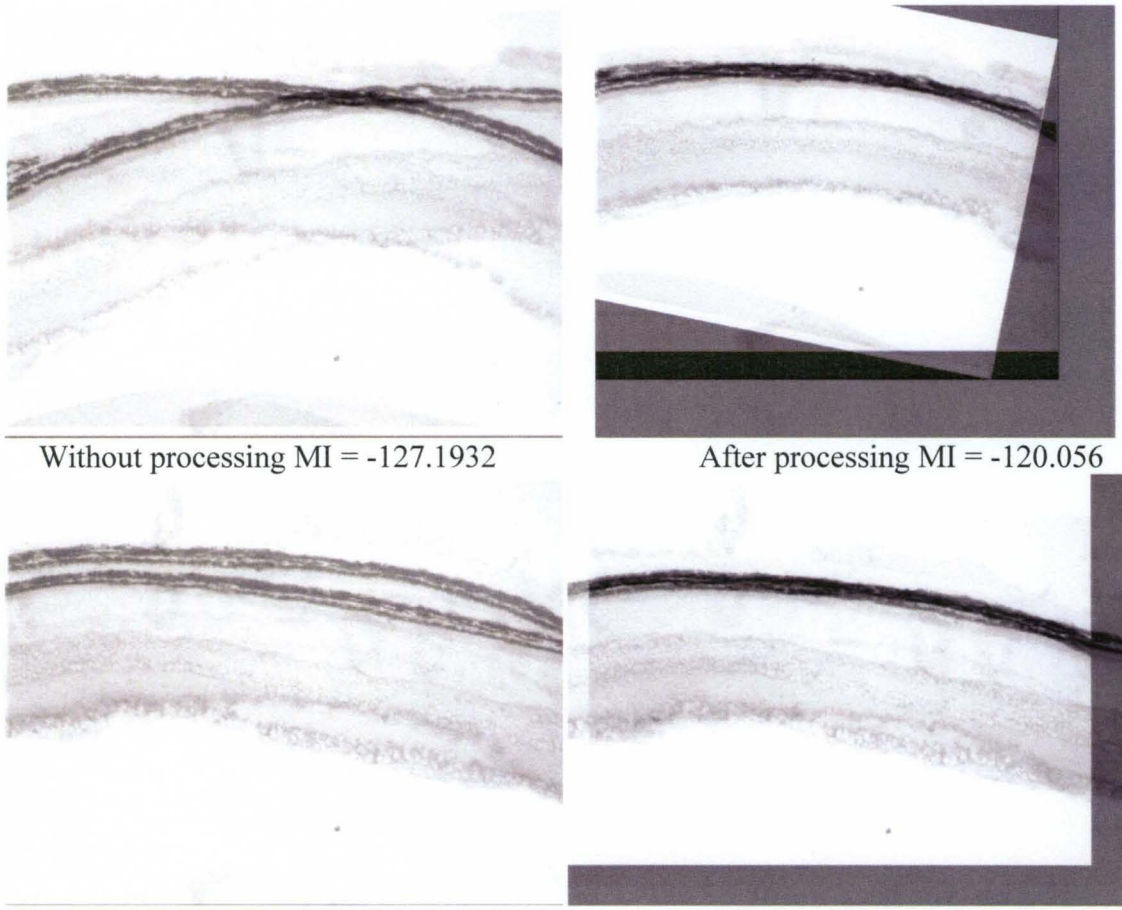
Without processing MI = -125.8283

After processing MI = -120.7383



Without processing MI = -132.5541

After processing MI = -122.2686



Without processing MI = -127.1932

After processing MI = -120.056

Without processing MI = -122.4 affine

After processing MI = -122.26

Figure 46. Results for mutual based registration

5. CONCLUSIONS AND FUTURE DIRECTIONS

In-situ hybridization is one of the most important techniques for gene expression or transcriptome analysis. It produces high resolution 2D and 3D gene expression images that contain information about how genes control cell type identity, cell differentiation, and cell-cell signaling. In order to get relative information, images need to be organized into a common coordinate system. In this thesis, we concentrated on developing methodologies to put a gene expression image and a reference image into a common coordinate system and creating a tool with a user friendly interface.

In registration, there are three methodologies. First two methods are feature based; the final one is an area based method. The first two methods are similar to landmark based registration algorithms. They both try to predict transformation equations to put the reference image into the same coordinate system of the sample image. The first method takes corresponding landmark pairs from the user. If the user has enough talent to define correct landmarks, registration algorithm results are successful. The second method finds corresponding landmark pairs automatically. First it detects distinctive corner features via the Harris-Stephens corner detector. Then it matches every corner point in the first image with a point in the second image. In order to perform this matching, it calculates a correlation matrix and finds the pairs that give highest correlation value in both directions. After corresponding landmark pairs are defined, the transformation matrix is predicted via the selected algorithm. The algorithms are affine,

linear conformal, projective, polynomial, piecewise linear and local weighted mean. We mainly focus on affine transformation because it lets translation, rotation, scaling and shearing. Additionally we calculate the mutual information before registration and after registration in order to see the success of methods and compare results. The last method tries to optimize parameters to find the best affine transformation that maximizes mutual information between two images. It can be fully or semi automated. A user can select type of translations and the intervals of parameters or the user can use the default values. In addition to the supplied methods, the tool visualizes and saves the results. A user can see the input images, the aligned images before registration and the registration result together. The estimated parameters are presented at the end of the registration. This feature helps users to analyze results and define new parameter intervals for more successful registration. At the end of the registration, all results are saved in a folder. One can reload the old results and continue working on them.

The results demonstrate that some improvements to be performed as future work. They are:

- All three methods use global image registration. However global image registration is not enough when images have substructures like mouse brain has. To overcome this problem, local registrations can be made as a second step after global registration.
- For the correlation based method, corner detection is not successful for images that do not have intensity value changes. Other feature detectors can be used for that type of images.

- Mutual feature based method checks every parameter set in the search space to find best one and it makes the method too slow. Optimization algorithms can be applied to find the best result without trying every possible parameter.

In conclusion, the improved tool with three different methodologies can register different kind of data pairs, visualize and save the results to allow analyzing gene expression images.

REFERENCES

1. Kratz, R.F., *Exploring the World of the Cell*, in *Molecular&Cell Biology for Dummies*2009, Wiley Publishing, Inc: Indianapolis. p. 9-14.

2. *Of Mice and Man.* WHAT A YEAR; Available from: http://www.whatayear.org/04_11.php.

3. *Nitrogen Bases And Its Types.* Molecules Of Living Organisms May 2011]; Available from: http://www.transtutors.com/homework-help/Biology/Molecules+Of+Living+Organisms/nitrogen-bases-and-its-types.aspx.

4. *Genetics in 10 Minutes*. Available from: http://www.freewebs.com/genetics37/genetics101.htm.

5. Watson JD, C.F. *Molecular structure of nucleic acids; a structure for deoxyribose nucleic acid.* 1953. Nature.

6. *DNA and molecular genetics*. Available from: http://www2.estrellamountain.edu/faculty/farabee/biobk/BioBookDNAMOLGEN.html.

7. CRICK, F.H.C. *ON PROTEIN SYNTHESIS.* 1958.

8. *The splice of life*. Nature.com; Available from: http://www.nature.com/horizon/rna/background/figs/splice_f1.html.

9. *DNA Packaging: Nucleosomes and Chromatin.* Scitable by nature education [cited 2011 July]; Available from: http://www.nature.com/scitable/topicpage/dna-packaging-nucleosomes-and-chromatin-310.

10. *Prostate Cancer*. Fitsugar; Available from: http://www.fitsugar.com/Prostate-cancer-2331417.

11. *Central dogma of molecular biology*. Wikipedia; Available from: http://en.wikipedia.org/wiki/Central_dogma_of_molecular_biology.

12. Crick, F. *Ideas on Protein Synthesis*. 1956.

13. *DNA Replication & Synthesis*, in *Think Quest*.

14. *Molecular Genetic* Available from: http://mahnaz-moleculargenetic.blogspot.com/2011/01/transcription.html.

15. *Translation*. Nobelprize.org [cited 2011 May]; Available from: http://nobelprize.org/educational/medicine/dna/a/translation/index.html.

16. Kratz, R.F., *Dead or Alive: Viruses*, in *Molecular & Cell Biology*2009, Wiley Publishing.

17. McCarthy, B.J. and J.J. Holland, *Denatured DNA as a direct template for in vitro protein synthesis.* Proceedings of the National Academy of Sciences of the United States of America, 1965. **54**(3): p. 880-6.

18. Nirenberg, M.W., *The genetic code, Nobel Lecture*, 1968.

19. Lloyd, L.J.R.V., *In Situ Hybridization: Methods and Applications.* 1997.

20. *IN SITU HYBRIDIZATION*. GeneDetect.com 2011 April; Available from: http://www.genedetect.com/insitu.html.

21. J. P. Carson, T.J., C. Thaller, J. Warrren, M. Bello, I. Kakadiaris, W. Chiu, G. Eichele. *Automated Characterization of Gene Expression Patterns with an Atlas of the Mouse Brain*. 2004. San Francisco, CA: IEEE.

22. Joseph V. Hajnal, D.L.G.H., David J. Hawkes, *Medical Image Registration*. The biomedical Engineering Series, ed. M.R. Neuman2001: CRC Press.

23. Zitova, B. and J. Flusser, *Image registration methods: a survey*, in *Image and Vision Computing 21*2003. p. 977-1000.

24. Brown, L.G., *A survey of image registration Techniques*, in *ACM Computing Surveys*1992.

25. van den Elsen, P.A., E.J.D. Pol, and M.A. Viergever, *Medical image matching-a review with classification.* Engineering in Medicine and Biology Magazine, IEEE, 1993. 12(1): p. 26-39.

26. Yu, J.N., et al., *Intermodality, retrospective image registration in the thorax.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1995. 36(12): p. 2333-8.

27. Li, Q., B. Fu, and Y. Dong. *Registration of Radar and Optical Satellite Images Using Multiscale Filter Technique and Information Measure*. in *Geoscience and Remote Sensing New Achievements*. 2010.

28. Ding, F., W.K. Leow, and S.-c. Wang. *Segmentation of 3D CT volume images using a single 2D atlas*. in *Proc. ICCV Workshop on Computer Vision for Biomedical Image Applications*. 2005.

29. Habbecke, M. and L. Kobbelt, *Automatic Registration of Oblique Aerial Images with Cadastral Maps*, in *ECCV Workshop on Reconstruction and Modeling of Large Scale 3D Virtual Environments*2010.

30. López, F., et al., *A Study of Registration Methods for Ceramic Tile Inspection Purposes.*

31. Wenyi, Z., D. Nister, and S. Hsu, *Alignment of continuous video onto 3D point clouds.* Pattern Analysis and Machine Intelligence, IEEE Transactions on, 2005. 27(8): p. 1305-1318.

32. Nkanza, N., *Image Registration and its Application to Computer Vision: Mosaicing and Independent Motion detection*, in *Department of Electrical Engineering*2005, University of Cape Town.

33. Ouksili, Z., et al. *ACCURATE PET-PET REGISTRATION TO ASSESS LUNG TUMOR EVOLUTION*. in *Biomedical Imaging: From Nano to Macro, 2007. ISBI 2007. 4th IEEE International Symposium on*. 2007.

34. Schneider, A., K.C. Seto, and C.E. Woodcock. *Spatial and temporal patterns of land cover change in Chengdu, China, 1978-2002.* in *Geoscience and Remote Sensing Symposium, 2003. IGARSS '03. Proceedings. 2003 IEEE International.* 2003.

35. Goshtasby, A. and G.C. Stockman, *Point Pattern Matching Using Convex Hull Edges*, 1985, IEEE Transactions on Systems, Man and Cybernetics. p. 631-637.

36. Stockman, G., S. Kopstein, and S. Benett, *Matching Images to Models for Registration and Object Detection via Clustering.* Pattern Analysis and Machine Intelligence, IEEE Transactions on, 1982. PAMI-4(3): p. 229-241.

37. Besl, P.J. and H.D. McKay, *A method for registration of 3-D shapes.* Pattern Analysis and Machine Intelligence, IEEE Transactions on, 1992. **14**(2): p. 239-256.

38. Flusser, J., *Object matching by means of matching likelihood coefficients.* Pattern Recogn. Lett., 1995. 16(9): p. 893-900.

39. Gonzalez, R.C., R.E. Woods, and S. L.Eddins, *Geometric Transformation and Image Registration*, in *Digital Image Processing Using MATLAB*2009, Gatesmark Publishing.

40. Herring, J.L., et al., *Surface-based registration of CT images to physical space for image-guided surgery of the spine: a sensitivity study.* IEEE transactions on medical imaging, 1998. 17(5): p. 743-52.

41. Modersitzki, J., *Numerical Methods for Image Registration*2004: Oxford Science Publications.

42. Arun, K.S., T.S. Huang, and S.D. Blostein, *Least-Squares Fitting of Two 3-D Point Sets.* Pattern Analysis and Machine Intelligence, IEEE Transactions on, 1987. PAMI-9(5): p. 698-700.

43. Fitzpatrick, J.M., J.B. West, and C.R. Maurer, Jr., *Predicting error in rigid-body point-based registration.* Medical Imaging, IEEE Transactions on, 1998. **17**(5): p. 694-702.

44. CA, P., et al., *Accurate three-dimensional registration of CT, PET, and/or MR images of the brain.* 1989. **13**(1): p. 20-26.

45. Alpert, N.M., et al., *The principal axes transformation--a method for image registration.* Journal of nuclear medicine : official publication, Society of Nuclear Medicine, 1990. 31(10): p. 1717-22.

46. Dong, L. and A.L. Boyer, *A portal image alignment and patient setup verification procedure using moments and correlation techniques.* 1996. **41**(4).

47. *Allen Institute for Brain Science*. Wikipedia [cited 2011 May]; Available from: http://en.wikipedia.org/wiki/Allen_Institute_for_Brain_Science.

48. *Allen Mouse Brain Atlas*. Allen Institute for Brain Science; Available from: http://mouse.brain-map.org/about/index.html.

49. Jagalur, M., et al., *Analyzing in situ gene expression in the mouse brain with image registration, feature extraction and block clustering.* BMC bioinformatics, 2007. 8 Suppl 10: p. S5.

50. Science, A.I.f.B., *Technical White Paper: Allen Developing Mouse Brain Reference Atlas*.

51. *Cytoarchitecture*. [cited 2011 July]; Available from: http://en.wikipedia.org/wiki/Cytoarchitecture.

52. Goshtasby, A., *Piecewise linear mapping functions for image registration.* Pattern Recognition, 1986. **19**(6): p. 459-466.

53. Goshtasby, A., *Image registration by local approximation methods.* Image Vision Comput., 1988. **6**(4): p. 255-261.

54. Harris, C. and M. Stephens, *A COMBINED CORNER AND EDGE DETECTOR.* 1988.

55. Derpanis, K.G., *The Harris Corner Detector*, 2004.

56. Moravec, H.P., *Obstacle avoidance and navigation in the real world by a seeing robot rover*, 1980, Stanford University p. 177.

57. Viola, P.A., *Alignment by maximization of mutual information*, 1995, Massachusetts Institute of Technolog

CURRICULUM VITAE

NAME	Ernur SAKA
ADDRESS	Department of Computer Science and Computer Engineering University of Louisville Louisville, KY 40292
EDUCATION	B.S. in Computer Engineering University of Dokuz Eylül 2008
EXPERIENCE	University of Louisville Research Assistant
	University of Louisville Teaching Assistant
	Birim Information Operation Consultancy and Commerce Limited Company Turkey Engineering Intern
	Turkey Telecommunications Authority Telecommunication Board Engineering Intern
	University Of Dokuz Eylül Hardware Intern
AWARDS	University of Louisville Sponsored Research Tuition Award for PhD 2011
	Acceptance to the SIGGRAPH as a Student Volunteer from a worldwide application pool and representing UofL 2010
	Scholarship for Master's Degree from University of Louisville 2009