DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. **DO NOT EXCEED THE SPACE PROVIDED.**

It has been demonstrated that anthropometric data can accurately distinguish individuals with Fetal alcohol syndrome (FAS) from those who were alcohol exposed but do not manifest the full spectrum of clinical features, and those who were not alcohol exposed [2]. A more efficient means to collect such data may be through three-dimensional (3-D) digitizing instruments, which can capture a facial image that can then be used to collect a wide range of known and novel clinical variables. Through the collection of 3-D images from individuals of variable ethnicity, age and exposure histories, it should be possible to identify a series of variables that effectively discriminate individuals who prenatally exposed to alcohol and the degree to which they were exposed, from those who were not exposed.

The goal of this collaboration is to analyze three-dimensional (3-D) facial images from individuals of variable ethnicity, age and history of alcohol exposure. The analyses of 3-D facial imaging will be developed and utilized for more effective clinical diagnosis of FAS, as well as the more broadly defined FASD. In addition, we believe these studies will generate important insight regarding the changes that occur in the face both prenatally and postnatally that produce the clinical features associated with FAS and thereby provide improved understanding of the pathophysiological effects of ethanol on human development.

To accomplish these goals we propose the following specific aims: 1) Train and supervise personnel at each recruitment site to ensure collection of standardized data; 2) Analyze the 3-D facial imaging data to identify the measurements that most efficiently differentiate alcohol exposed from control subjects; 3) Utilize algorithms and methods derived from the emerging field of Automated Facial Recognition (AFR) to extract and identify the most discriminating higher order surface features from 3-D facial images, with the goal of developing an automated method of identifying facial features diagnostic of prenatal alcohol exposure; and 4) Combine the results from the direct and higher order measurements derived from the 3-D facial imaging with variables collected from other study domains to improve the power to accurately discriminate alcohol exposed from control subjects and to better understand the pathophysiological effects of ethanol on human development.

PERFORMANCE SITE(S) *(organization, city, state)*
Indiana University
Indianapolis, Indiana

KEY PERSONNEL. See instructions. *Use continuation pages as needed* to provide the required information in the format shown below. Start with Principal Investigator. List all other key personnel in alphabetical order, last name first.

Name	Organization	Role on Project
Foroud, Tatiana M.	Indiana University School of Medicine	Principal Investigator
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Disclosure Permission Statement. Applicable to SBIR/STTR Only. See instructions. ☐ **Yes** ☐ **No**

a. SPECIFIC AIMS

Jones and Smith first described fetal alcohol syndrome (FAS) in 1973 [1]. The basic features most frequently associated with prenatal exposure to alcohol include growth deficiencies and neurodevelopmental abnormalities of the central nervous system, as well as a pattern of minor facial anomalies including short palpebral fissures, thin upper lip, flattened philtrum and midface hypoplasia. The presence of these findings in an individual with documented prenatal exposure to alcohol typically results in a diagnosis of FAS. Posing a greater clinical challenge are those individuals who manifest some, but not all, of these clinical features and who were known to have prenatal alcohol exposure. Additional difficulties in diagnosing FAS arise from the reduced prominence of abnormal facial features as the child grows toward adulthood, and limited understanding of the effect of ethnic and racial variation on the associated facial features. The development of a series of categories, that attempt to encompass the presenting symptoms, has led to the terms fetal alcohol effects (FAE), partial FAS (PFAS), alcohol-related birth defects (ARBD) and alcohol-related neurodevelopmental disorder (ARND). The terms have been placed under the umbrella of fetal alcohol spectrum disorders (FASD).

It has been demonstrated that anthropometric data can accurately distinguish individuals with FAS from those who were alcohol exposed but do not manifest the full spectrum of clinical features, and those who were not alcohol exposed [2]. Collection of such data can be relatively tedious and requires specialized training however; the advent of new technology makes the collection of such data potentially easier and more cost-effective. Three-dimensional (3-D) digitizing instruments can capture a facial image that can then be used to collect a wide range of known and novel clinical variables. Through the collection of 3-D images from individuals of variable ethnicity, age and exposure histories, it should be possible to identify a series of variables that effectively discriminate individuals who were prenatally exposed to alcohol and the degree to which they were exposed, from those who were not exposed.

The goal of this collaboration is to analyze the images provided by each of the sites collecting data from individuals of variable ethnicity, age and history of alcohol exposure. The analyses of 3-D facial imaging will be developed and utilized for more effective clinical diagnosis of FAS, as well as the more broadly defined FASD. In addition, we believe these studies will generate important insight regarding the changes that occur in the face both prenatally and postnatally that produce the clinical features associated with FAS and thereby provide improved understanding of the pathophysiological effects of ethanol on human development. To accomplish these goals we propose the following specific aims:

1) Train and supervise personnel at each recruitment site to ensure collection of standardized data.

2) Analyze the 3-D facial imaging data to identify the measurements that most efficiently differentiate alcohol exposed from control subjects.

3) Utilize algorithms and methods derived from the emerging field of Automated Facial Recognition (AFR) to extract and identify the most discriminating higher order surface features from 3-D facial images, with the goal of developing an automated method of identifying facial features diagnostic of prenatal alcohol exposure.

4) Combine the results from the direct and higher order measurements derived from the 3-D facial imaging with variables collected from other study domains to improve the power to accurately discriminate alcohol exposed from control subjects and to better understand the pathophysiological effects of ethanol on human development.

b. BACKGROUND

Significance

Fetal alcohol syndrome is the most common nonhereditary cause of mental retardation. A number of studies have examined different populations both within the United States and throughout the world to estimate the incidence and prevalence of this devastating syndrome. It is estimated that the prevalence in the general population of FAS is likely to be between 0.5 and 2.0 per 1,000 births. Broadening the clinical definition, to also include ARBD with FAS, results in a combined prevalence estimate of at least 10 per 1,000 births, or 1% of all births [3]. Importantly, studies outside the U.S. have found even higher rates of prevalence in particular geographic regions. For example, in a town in the Western Cape Province of the Republic of South Africa the prevalence of FAS was estimated to be 46.4 per 1,000 in first grade school children [4]. It was estimated, over a decade ago, that the incremented annual cost of treating individuals with FAS is $74.6 million. About three-quarters of this economic burden is associated with care of FAS cases having mental retardation [5].

Clinical and Diagnostic Issues

When the term fetal alcohol syndrome was first coined, it was used to describe the phenotype of the most severely affected individuals. Since that time, it has become apparent that effects of prenatal alcohol exposure on the fetus can be broad-based and not limited to the original description of FAS [6], [7], [8], [9]. Studies have shown that the effects of prenatal alcohol exposure fall along a continuum from extreme (perinatal fetal demise) to more subtle anomalies (behavioral problems) and that FAS represents the severe end of the continuum in the phenotypic expression of prenatal alcohol exposure [10], [11], [7], [8]. However, the diagnostic guidelines that most clinicians and researchers currently use do not differ greatly from the original categories defined by Jones and Smith [7].

In the United States, most children affected by prenatal alcohol exposure are diagnosed later in life. Yet, the prevalence of FASD at birth is assessed through sequential evaluation of infants while they are still in newborn nurseries [12], [13], [14]. It is likely that the prevalence of FASD is underestimated because some of the primary diagnostic features of FAS may not be identifiable in the newborn period. Studies have found that the criterion used to diagnose FASD is not easily applied during the neonatal and infancy period. For example, many primary facial features associated with FAS are difficult to discriminate in newborns [7], [15], [16], [17], growth deficits associated with alcohol exposure are not specific to FAS [18], and neurodevelopmental outcomes are not easily measured during early infancy [19]. Improved detection of children with FASD is critical because research suggests that early identification of alcohol-exposed children fosters positive outcomes and reduces the likelihood of secondary disabilities [20].

Difficulties encountered in recognizing and diagnosing FASD are not limited to the neonatal period. Because many features associated with prenatal alcohol exposure are not discrete or unique to prenatal alcohol exposure and because the features have variable expression, its recognition can be elusive for all age groups. There are many factors that contribute to the variable expression of effects of prenatal alcohol exposure. These factors can include, but are not limited to: maternal characteristics (age, race, height, diet, genetics, etc.); the duration, timing, and quantity of alcohol exposure; infant characteristics (gestational age, race, genetics, etc.); and socioeconomic factors. Another confounding factor in diagnosing FASD is that standards have not been established for determining thresholds for the various subcategories of the disorder. In addition, “the ability to recognize this syndrome varies according to the physician’s skill and interest in dysmorphologic features; thus, the estimated prevalence rates for FAS among populations are directly affected by such ascertainment biases” [21]. Therefore, applying specific diagnostic criterion during the newborn period as well as at other ages could

dramatically increase the diagnostic capabilities, increase opportunities for services for both mothers and children, and improve the surveillance of FAS and other effects from prenatal alcohol exposure.

There have been several previous attempts to clarify and classify the variable patterns of expression seen in children prenatally exposed to alcohol. In 1978, an additional term was coined to further describe effects of prenatal alcohol exposure. The term fetal alcohol effects (FAE) was proposed by Clarren and Smith to be used as a diagnosis when a congenital anomaly could be proven to be related to alcohol exposure in utero. In 1980, the Fetal Alcohol Study Group of the Research Society on Alcoholism published new diagnostic criterion derived from the original guidelines published by Jones and Smith (1973) [22]. The modified criterion still required the presence of the three main features of FAS, but in order to give more standardization and objectivity to the diagnosis, the study group made the requirements more specific. For example pre- and post-natal growth retardation was defined as below the 10^{th} percentile and 2 of 3 facial features had to be present. The study group also proposed that if all three criteria could not be met, the diagnosis of FAE should be used. Later, documentation of maternal alcohol use during pregnancy was required for the diagnosis of FAS, which led to the FAE diagnosis being used in ways that the study group and others had not intended. Many clinicians would use the diagnosis of FAE whenever alcohol exposure was suspected, while others would use it when the clinical signs of FAS were present but lacked the documentation of prenatal alcohol exposure. To others, the term “effects” became synonymous with “less severe” and many patients were denied social services and early intervention because the diagnosis of FAE carried the stigma of the individual not being as seriously affected as those diagnosed with FAS. In 1996, in an attempt to address some problems with the diagnostic criterion and the use of the label FAE, the Institutes of Medicine (IOM) published a new diagnostic criterion for FAS and alcohol-related effects [8].

Many clinicians and researchers have reported on the pitfalls of the diagnostic criteria discussed above ([21], [23], [7], [24], [25], Khoury et al., 1996, [26], [8], [27]. These criteria lack sufficient specificity to assure diagnostic accuracy and precision. For example, the criterion for CNS dysfunction did not address how many areas of deficit must be present, how severe the deficits must be or what level of documentation must exist to substantiate the presence of the deficit (i.e. parental history, psychometric testing or structural imaging). The criterion for the facial phenotype was equally non-specific. It did not provide guidelines for how many facial features must be present, how to assess the severity of the facial features, and what standards should be used to assess their severity. Astley and Clarren [28] summarized the problems with previous diagnostic criteria, noting the “lack of objective quantitative scales to measure the magnitude of expression of key diagnostic features.” (p. 410). For example, no standards or quantitative measurement scales have been established to determine when features are “short,” “small,” “thin,” or “flat” enough to be considered a true case definition of FAS or FASD. Other problems include the likelihood that some of the characteristics change with time, and that many diagnostic traits fall along a continuum of expression. Finally, expression of key traits are likely to vary from individual to individual, and some may be influenced by racial and familial traits [7]. None of these problems were addressed in previous approaches.

Astley and Clarren [29] presented a more objective and comprehensive, case-defined method for diagnosing the full spectrum of outcomes in individuals prenatally exposed to alcohol [29], [30], [28]. The new method, called the 4-Digit Diagnostic Code, used a numerical scale that reflected the magnitude of expression of 4 key diagnostic features of FAS in the following order: 1) growth deficiency, 2) the FAS facial phenotype; 3) brain damage/dysfunction; and 4) gestational alcohol exposure. Each was ranked independently on a 4-point Likert scale, with 4 reflecting severe expression of the feature and 1 reflecting no expression of the feature. While earlier diagnostic methods required clinicians to subjectively identify the presence or absence of minor facial anomalies, the 4-Digit Diagnostic Code was based on empirical data gathered from previous studies that employed discriminant analysis. Discriminant analysis was used to identify the cluster of minor anomalies and their magnitude of expression that best differentiated individuals with FAS, from matched controls without FAS. Analysis identified 3 features: reduced palpebral fissure/inner canthal distance ratio; smooth philtrum; and

a thin upper lip. From discriminant analysis, a discriminant equation and score was created which could, with 100% sensitivity and specificity, discriminate FAS subjects from controls in the study sample. Later this method was refined to be used on photographs and to include only the measurement of the palpebral fissure instead of the ratio of palpebral fissure/inner canthal distance. Currently the 4-Digit Diagnostic Code facial rank is used to diagnose the facial component of the syndrome in all patients receiving an FAS diagnostic evaluation in the Washington State FAS Diagnostic and Prevention Network (FAS DPN) (n=1500) [31].

The 4-Digit Diagnostic Code is a vast improvement over previous subjective diagnostic criteria. It greatly increases the precision and accuracy of the diagnosis of outcomes of prenatal alcohol exposure, better characterizes disabilities of alcohol exposed individuals that do not have FAS, documents presence of alcohol exposure without judging its causal role, and utilizes a clinical nomenclature that separates a patient's functional disabilities from his or her exposure history [28]. There are, however, some difficulties presented by this approach. First, the diagnostic categories are very complex. There are 256 possible 4-Digit Diagnostic Codes ranging from 1111 to 4444, each having a corresponding clinical name. Each of the 4-Digit Diagnostic Codes falls into 1 of 22 unique Clinical Diagnostic Categories. Second, photographs vary tremendously in quality, making assessment difficult and dependent on an experienced observer. Third, the diagnostic criterion is best suited for detecting the full expression of FAS and may be less able to detect individuals with more subtle expressions of the disorder.

3-D Camera

Although as previously mentioned, photographs vary tremendously in quality, they do have several advantages over direct measurements. They provide a permanent record of the face, little specialized training is needed in their capture, and they are portable so they can be easily sent to specialists in other locations. However, two-dimensional photographs, even digital ones, contain much less detail than is available from direct assessment of a subject. We believe that a more effective imaging technology may overcome this problem while retaining the advantages of photographs. A variety of technologies exist for capturing 3-D craniofacial data. These include: computerized tomography; magnetic resonance imaging; ultrasonographic, stereophotographic, optoelectric and light projection systems; and laser-based digitized scanning, the method employed in our study.

Only a handful of previous studies have reviewed the efficacy of this laser-based technology for obtaining 3-D facial images and facial measurements. Aung et al. [32] compared the facial surface measurements obtained from images of 30 adults, generated from a variety of laser scanner, to those obtained from the same 30 individuals measured directly by anthropometry. They collected 83 facial measurements using 41 landmarks as defined in Farkas [33]. They found that: 1) the vast majority of necessary anthropometric landmarks could be easily identified on the scanned image (37/41); 2) those landmarks that were difficult to define, which were those requiring reference to the underlying bone or that tended to be blurred in the image, could be readily obtained if they were pre-marked; and 3) approximately one-half of the measurements (41) obtained on the scans were at least moderately similar (within +/- 2 mm) to the anthropometric results and one-half (42) were viewed as "unreliable" (difference greater than +/- 2 mm). This study, however, did not assess the repeatability of the laser measurements, or the correlation of the laser and anthropometric measurements. In a more recent study, Kusnoto and Evans [34] used a Minolta Vivid laser scanner, similar to the one we propose to use, to assess the reliability of measurements taken from the scans compared to those derived directly from an alginate facial mask. They utilized 12 standard facial landmarks (frontal perspective only) to generate 21 linear measurements. They found that differences between measurements derived from the scanned image and direct measurements of the face ranged from a low of "0" mm to a high of 2.5 mm. However, correlation between the 2 sources of measurement was not examined, nor were percentage errors (difference between 2 measurement techniques divided by magnitude of the measurement) calculated.

Summary

By utilizing new technologies and analytical approaches coupled with quantitative assessment of facial form as described below, we propose to build upon previous work to create a more efficient and broadly applicable approach to recognizing children of various ages and ethnicities who have been affected by prenatal exposure to alcohol. Greater understanding of the phenotypic correlates of prenatal alcohol exposure will allow for better understanding of the pathophysiology of alcohol exposure, especially when this information is combined with information collected on other systems affected by ethanol exposure such as neural and cognitive development. The integration of these research efforts will help to clarify the degree to which craniofacial variation reflects underlying disruptions in brain form and function. Further, we believe that this study will provide important data regarding facial changes that occur both prenatally and postnatally that produce the clinical features associated with FAS. This knowledge may greatly improve our understanding of the pathophysiological effects of alcohol on human development.

c. PRELIMINARY RESULTS

In creating this cooperative agreement, we have assembled a group of investigators who bring complementary expertise to the question of how best to consider traditional and novel methods of assessing facial variation in alcohol exposed individuals as compared with ethnically- and age-matched controls. Initially, we describe the expertise of our collaborative group. This is followed by a brief overview of previous work accomplished in the quantitative approaches to facial pattern recognition that we propose to use in this study. Subsequently, we provide details of two Pilot Studies that were completed in preparation for this application.

Tatiana Foroud, Ph.D. is a population geneticist whose research has focused on the study of complex genetic phenotypes, such as alcoholism and fetal alcohol syndrome. She has worked with a number of the researchers collecting samples for this study from throughout the world and will coordinate the transfer and analysis of all study data collected using the three-dimensional images. Richard Ward, Ph.D. is a biological anthropologist with extensive experience in medical genetics and the use of morphometrics in describing and analyzing variation in the human face, particularly as it relates to understanding genetic and teratogenic syndromes. He will bring this expertise to the construction and initial analysis of images collected from the various study sites and will oversee the quality control aspects of both image processing and subsequent anthropometric analysis. Elizabeth Moore, Ph.D. is a biological anthropologist with extensive experience on the use of anthropometric techniques to quantitatively define a Fetal Alcohol Syndrome phenotype. Her research has demonstrated the potential utility of these quantitative approaches in detecting children with both classic FAS and more subtle expressions of ethanol exposure (Partial FAS, etc.). Dr. Moore will be responsible for obtaining anthropometric measurements from the three-dimensional images obtained at the various study sites and reconstructed by Dr. Ward. Shiaofen Fang, Ph.D, is an associate professor of computer science. His main research expertise is on computer graphics, geometric modeling and biomedical visualization. He has extensive experience in applying computer science technologies in biomedical applications. He had previously worked with Dr. Joan Richtsmeier on a craniofacial modeling project using Euclidean Distance Matrix Analysis (EDMA), which will also be a key data analysis technique in this project. Dr. Huang is an assistant professor in computer science whose research areas include computer vision, pattern recognition, image processing, and machine learning on the applications of biometrics (human face and gesture recognition), Human Computer Intelligent Interaction, video surveillance, and multimedia. He participated in the DARPA FERET face recognition project between 1993 and 1998 and has worked on the missing children and criminal identification project funded by National Institute of Justice (NIJ) since 1999. He will bring his expertise in human face recognition to the analysis of FAS facial expression and the discovery of discriminating features for FAS classification.

Previous Work

Anthropometry

Craniofacial anthropometry has been used to assess and describe the facial phenotype and abnormal craniofacial variation in many syndromes such as Apert syndrome [35], Down syndrome [36], [37], hypohidrotic ectodermal dysplasia [38], Prader-Labhart-Willi syndrome [39], Treacher Collins syndrome [40], and Wiedemann-Beckwith syndrome [41], [42]. It has also been used in the clinical setting as a diagnostic aid [43] and as a means of objectifying clinical descriptions of individual patients [44], [45], [46], [43]. In previous studies, Moore and colleagues [42], [47] established that craniofacial anthropometry could be used to objectively identify individuals with FAS as well as those individuals prenatally exposed to alcohol who exhibited a subtler dysmorphia (partial FAS).

The purpose of the Moore et al study [42], [47] was to demonstrate that an objective, multivariate case definition of FAS and PFAS could be derived by means of craniofacial anthropometry. Craniofacial measurements (n = 21) were taken of 100 individuals who had been exposed to alcohol before birth (41 FAS, 59 PFAS) and 31 unrelated non-affected individuals (controls). A "pattern profile" was used to describe the craniofacial phenotype of FAS and PFAS individuals when compared to a reference population. Using stepwise discriminant analysis, a phenotypic discriminant function was developed from the anthropometric measurements. This statistical procedure was able to correctly classify all FAS and control individuals with just two measurements, resulting in 100% sensitivity and specificity. This is similar to the level of diagnostic sensitivity achieved by Astley and Clarren [25], [48] in their 4-digit approach. Stepwise discriminant analysis also identified 6 craniofacial measurements that could differentiate individuals with (FAS, PFAS) and without prenatal alcohol exposure (controls) with 96% accuracy, 98% sensitivity, and 90% specificity. These results demonstrate that individuals with FAS and PFAS have a distinctive phenotype that can be characterized anthropometrically. Results also suggest the phenotypic case definition has the potential to be used as a screening tool to identify individuals with FAS as well as those prenatally exposed to alcohol who exhibit subtle craniofacial dysmorphia. The study demonstrated that the anthropometric approach might be useful, even in relatively small samples.

Landmark-based craniofacial modeling

Analysis of craniofacial data, including that generated from anthropometry, has been primarily landmark-based, and dependent on a handful of predefined linear measurements between predetermined landmarks. However, higher dimensional features may also be defined and analyzed from the same landmark data. In a previous study, in collaboration with Dr. Joan Richtsmeier at the Johns Hopkins University, biological landmark feature points were used for craniofacial growth modeling and visualization. The goal was to develop growth patterns for growth prediction in craniofacial surgical planning [49], [50]. Landmark patterns were defined as a matrix of ratios of distance changes between landmarks over time. An inverse method was employed to derive the growth pattern using both the Euclidean Distance Matrix Analysis (EDMA) method [51] and a parameterized spring model [52]. Parameter estimation was carried out in a pseudo-energy minimization process. Other machine learning techniques such as neural networks were applied [53]. After the landmark growth patterns were derived, a scattered data interpolation technique was used to generate a continuous deformation function for the entire CT volume for visualization and simulation. As the landmarks were all selected on skull surfaces within the CT volume, this technique could be effectively modified for 3-D scanning data as well. This approach has clear implications for detection of discriminating features between alcohol and non-alcohol exposed samples as will be described later.

Automated Facial Recognition Analysis:

The ability to detect salient facial features is an important component of any face analysis system. The detection of facial landmarks underlies attention mechanisms similar to those used by the human visual system (HVS) to screen out the visual field and to focus its attention on salient input characteristics. Finding facial features allows one to focus attention on salient facial configurations, to filter out structural noise, and to achieve eventual face recognition.

Feature selection in pattern recognition involves the derivation of salient features from sensory input data or the feature vector. Feature selection is formed by appropriate geometry and statistic measurement in order to reduce the amount of data used for classification and simultaneously to provide enhanced discriminatory power. The selection of an appropriate set of features is one of the most difficult tasks in the design of pattern classification systems. At the lowest level, raw feature data is derived from noisy sensor data, the characteristics of which are complex and difficult to characterize. In addition, there is considerable interaction among features that must be identified and exploited. The typical number of possible features, however, is so large as to prohibit any systematic exploration of all but a few possible interaction types (e.g., pairwise interactions). In addition, any sort of performance-oriented evaluation of feature subsets involves building and testing the associated classifier, resulting in additional overhead costs.

Our prior work in the area of facial recognition and analysis involves many fundamental techniques related to feature detection and analysis. A variety of image processing and computer vision techniques, such as edge detection, active contour, curve fitting, and reflectance map, were used for feature identification. For analysis, identified features were placed in a multi-dimensional vector space called feature space. Pattern recognition techniques were then applied in the feature space for classification and recognition. The machine learning approach, using a hierarchy of pattern classification steps, was applied in the facial recognition application [54], [55].

Pilot Study To Evaluate and Optimize the Use of 3-D Camera Data for Anthropometric Measurements

We completed a pilot study designed to: 1) optimize study collection conditions to maximize the amount of data obtained from the camera image; 2) develop a protocol to be implemented at the study sites; 3) estimate the correlation of anthropometric measurements obtained directly from the subject with those obtained from the camera image; and 4) estimate the repeatability (intra-observer error) of the measurements from the camera image.

Forty-five subjects were recruited for this pilot work. Individuals were recruited regardless of age and ethnicity. The sample included 27 female and 18 male subjects who ranged in age from 3-77 (Mean: 32.2). They were primarily Caucasian, although the sample also included African Americans and Asians. Each study visit consisted of: 1) completion of the Informed Consent process; 2) collection of 2 sets of 26 anthropometric measurements using spreading and sliding calipers and a cloth tape (see Figure 1 and Table 1); 3) collection of four clinical measurements using a clear plastic ruler and cloth retractable tape; and 4) collection of 2 sets of 3 images (frontal and right and left angles). To reduce intra-measurer error, 2 sets of direct and indirect anthropometric measurements were taken. If the difference between 2 measurements was <2 mm, then a third measurement was taken. An average of the 2 closest measurements was used for data analysis.

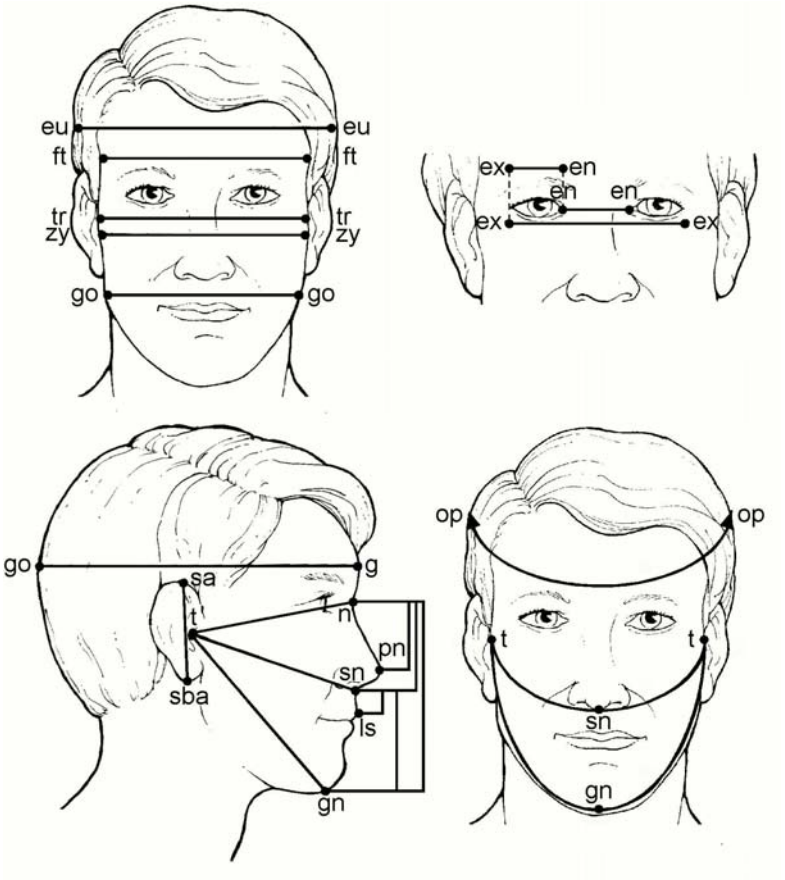


Figure 1: Measurements collected as part of the Study Visit. Head width, head length, head circumference and facial arcs were not obtained from the digital image.

Table 1: Craniofacial measurements used in analyses, their abbreviations and landmarks.

Breadths	**Landmarks**	**Lengths**	**Landmarks**
Head breadth (HB)	eu-eu	Head length (HL)	g-op
Minimal frontal breadth (MF)	ft-ft	Nasal bridge length (NB)	n-prn
Bitragal breadth (BT)	t-t	Nose length (NL)	n-sn
Bizygomatic breadth (BZ)	zy-zy	Philtrum length (PL)	sn-ls
Bigonial breadth (BG)	go-go	Lower facial height (LF)	sn-gn
Interocular breadth (IO)	en-en	Total facial height (TF)	n-gn
Biocular breadth (BO)	ex-ex	Ear length (EL)	sa-sba
Palpebral fissure length (PF)	en-ex		

Depths	**Landmarks**	**Circumferences**	**Landmarks**
Upper facial depth (UFD)	n-t	Maxillary arc (MX)	t-sn-t
Midfacial depth (MFD)	sn-t	Mandibular arc (MD)	t-gn-t
Lower facial depth (LFD)	gn-t	Head circumference (HC)	on-op

For the purposes of the present study, we required a portable, relatively inexpensive, non-invasive technique that necessitated only a brief period of cooperation from study subjects. Therefore, we elected to use the Minolta Vivid 700 Series Non-Contact 3-D laser scanner. This system uses a Class 1 (FDA), eye-safe laser to rapidly scan and digitize the face into a texture mapped 3-D model. Each scan takes 0.6 seconds to complete and has an accuracy of 0.35 mm on the X/Y axes and 0.25 mm on the Z-axis. The texture resolution is 400x400 pixels. For quality control, 2 scans each of the frontal, lateral right and left views of the face are captured. From these 6 captures, the highest quality model from each view is selected based on the continuity of facial expression between adjacent views. Then, stitching software will be used to merge the lateral and frontal images of the 3 selected models together to obtain a single 3-D model of the face.

Data from the pilot sessions were entered into an Access database for ease of manipulation. Initially, the 3-D model of the face was generated from the Minolta Vivid 300 hardware and software. From this image, all anthropometric measures were collected with the exception of head circumference, maxillary and mandibular arcs. These variables could not be estimated from the image because the reconstructed image did not allow for the measurement of these features (head circumference is distorted by the laser refraction from the hair, and the arcs were not a feature of our earlier measurement software package). However, we are currently in the process of developing software to measure maxillary and mandibular arcs.

We initially calculated the correlation of measurement values obtained from direct measurements to those obtained from camera images taken of the same individuals. Correlation assesses the degree to which the measurements in one medium predict those of another and as such, is a measure of reproducibility, but not of accuracy or deviation from the "true" measurement value [56]. It does not, for example, eliminate the possibility of a systematic difference (one medium always producing larger measurements than the other). Eleven variables had a correlation of 0.80 or greater while only 5 variables had a correlation lower than 0.80. Importantly, those variables with the lowest correlation were measurements with poorly defined landmarks (minimal frontal and bigonial breadth) or small size (palpebral fissure, inner canthal breadth and philtrum length).

Next, we calculated the repeatability or percent error between the first and second measurement in each technique, allowing the estimation of the average interobserver error [56], (Figure 2). These values are calculated after outliers greater than 2 mm were replaced by a third measurement. Measurement error was typically relatively small (0.06-0.13 mm) and was usually greater for camera data as compared with data collected directly from the subject. This difference may reflect our limited experience measuring these variables from the digitized image and may also indicate higher precision of the camera software (i.e. errors were reduced in anthropometric data through rounding). Similar to results from correlation studies, there was greater measurement (percent) error for variables of smaller physical size. This is a logical consequence of the fact that the same sized (millimeter) error in a variable with a small dimension will have a greater effect than in one with a large dimension. Ward and Jamison (1991) [57] noted the same relationship between error, measurement size and landmark definition in anthropometric studies. Similarly, the problem of localization of poorly defined landmark data was reported in other 3-D studies [32], [58]. Aung and colleagues [32] found that pre-marking less distinct landmarks helped localize them. This procedure could improve the correlation between direct (anthropometric) and indirect measurements. In addition, standardization of measurement methods for key small variables such as philtrum length, palpebral fissure and inner canthal distance should improve both accuracy and repeatability. However, it is important to point out that measurement error for all variables is below 2.5% of the measurement size, a value that is acceptable for most purposes.

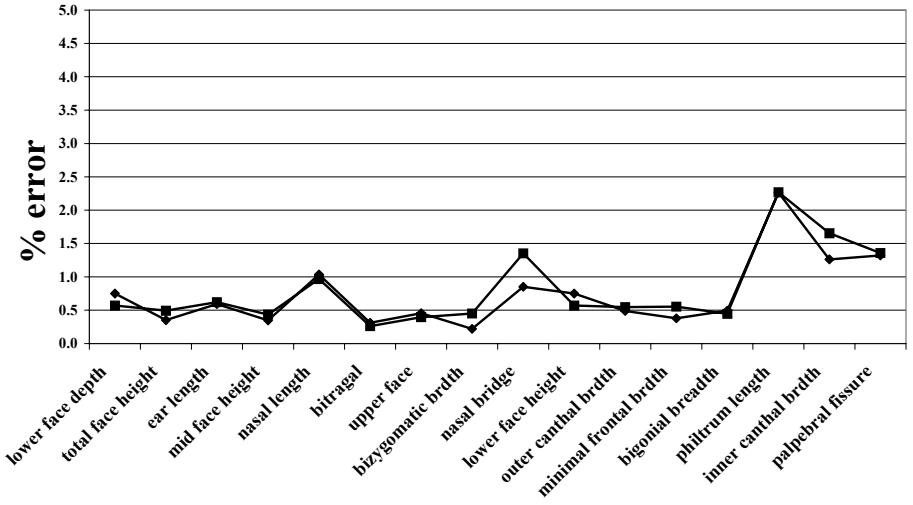


Figure 2: Average difference (error) between the first and second measurement obtained directly from the subject (♦) and the digitized image (■) taken as a percentage of the size of the measurement {(T1-T2)/ (T1+T2/2)}.

Through collection of extensive preliminary data, it was possible to optimize study collection conditions to maximize the amount of data obtained from the camera image. For example, improvements were made in technique that provided clearer views of significant landmarks. In addition, more accurate marking of ambiguous landmarks prior to photographs was found to improve reliability and repeatability. It is also clear that development of automated measurement software will significantly reduce error. Based on these results, a protocol has been developed that will lead to the uniform collection of digitizing images. This protocol can then be used in novel studies of facial changes and abnormalities, which are highly characteristic of intrauterine alcohol exposure.

Although we did not collect FAS or PFAS individuals in the anthropometric component of the pilot study, we can estimate the power of the proposed study. Because the direct anthropometric approach is clearly analogous to obtaining the same measurements from the 3-D images, the mean differences, observed by Moore et al [2], was employed to estimate the required sample size. The proposed studies would require 30 FAS and 30 controls in order to have 80% power with an alpha=0.05 to detect the previously observed [2] group differences. A larger sample of 60 subjects in each group would be required to have 80% power (alpha=0.05) to detect the previously observed mean differences in the PFAS vs. control groups.

Pilot Study of Automated Facial Recognition Technique for Classifying FAS and Non-FAS Images

Automated face recognition usually starts through the detection and boxing of a pattern, which represents the face. It proceeds by normalizing the face image to account for geometrical and illumination changes using information about the box surrounding the face and/or eyes location. Finally, it identifies the face using appropriate image representation and classification algorithms. Tools needed to detect and normalize face patterns [59] based on pose estimation [60] and eye detection [61], [55] were developed. Herein, we describe only tools developed to realize and implement stages of face recognition involved in classification tasks specifically targeted to recognizing and separating the FAS face from the normal face. Face analysis and classification, a difficult but fundamental task for intelligent systems, depends heavily on the particular choice of the features used by the (pattern) classifier.

Considering the approach for computer-aided FAS diagnosis as a pattern classification problem, we must deal with an issue called the "curse of dimensionality," which means more features do not necessarily imply a better classification success rate. Hence, data are first mapped into a lower dimensional space. Since the efficient selection of good features is very important, optimal linear projection is used to generate a tessellation of a space defined by the training images. This space can then be generated using different projections. Projections discussed and implemented to handle classification of FAS, FASD, and control groups

are Karhunen-Loève projection (*Eigenfaces Approach*) and Discriminating Analysis Projection (*Fisherfaces* or *Linear Discriminant Approach*).

Facial data for our Pilot Study consists of 30 FAS and 32 non-FAS facial images. The thirty FAS (FAS+) images were divided into 2 data sets, 20 images for training and 10 for testing. The 32 non-FAS (FAS-) were also randomly partitioned into 22 samples for training and 10 for testing. Facial images and data partitions are shown in Figure 3.

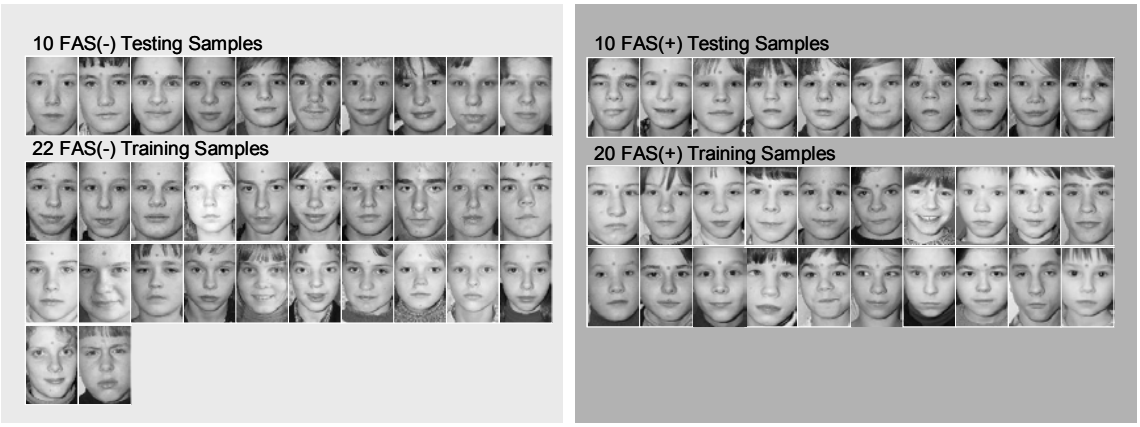


Figure 3: FAS(+) and FAS(-) sample images used for testing (validation) and training

Images were cropped and normalized into the region that contains only the face. The set of the most discriminating features (MDF) was generated for each image in the training set and stored in the recognition module. A simple Euclidean distance in this feature space was computed to find the exemplar image nearest to the query. Performance of classifiers for FAS prediction, using 25 components and Linear Discriminating Analysis (LDA) method, was 70.0%. Figure 4 shows that 2 classes, FAS(+) and FAS(-), could be well separated based on the training data in the MDF space of each sample. Six samples out of the total number of tested samples were misclassified. Four incorrect classifications were false positive results and two were false negative results. The confusion matrix and the misclassified images are shown in Figure 5.

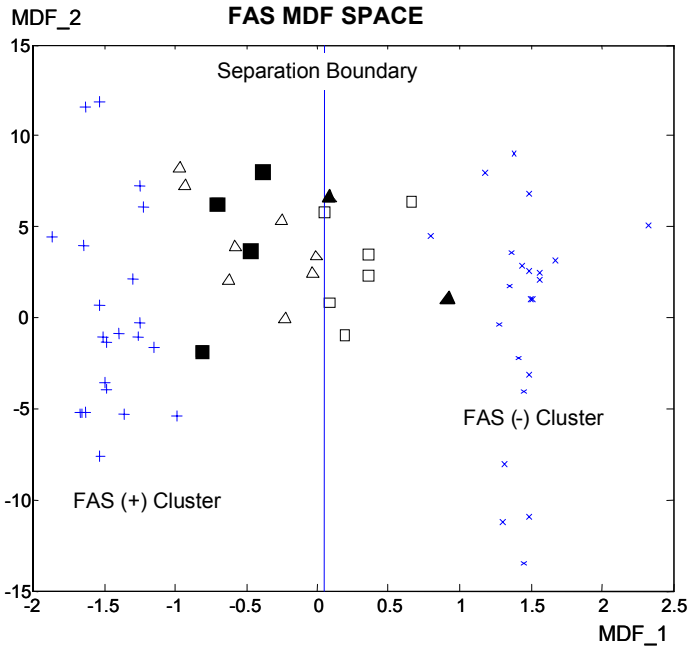
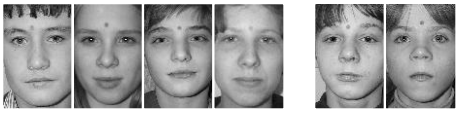


Figure 4: FAS and non-FAS samples on the most discriminating feature (MDF) space: The training samples of FAS positive (+) and FAS negative (✕) are well separated by the separation boundary found by the linear discriminating analysis (LDA). 10 FAS positive (△) and 10 FAS negative (□) samples were used for validation. 2 of the 10 FAS positive samples were misclassified (▲) while 4 of the10 FAS negative samples were misclassified (■).

Prediction	Ground Truth FAS(+)	FAS(-)
FAS(+)	8	4
FAS(-)	2	6



(a) (b)

Figure 5: The confusion map (left) and the misclassified images (right): (a) false positive and (b) false negative.

Two problems contributed to the misclassification. First, the automatically detected pixel features were too primitive for 3-D facial surface analysis. Handpicked landmark features and higher order surface features, as described earlier, will provide much more surface information for a more robust and accurate classification. Second, the lack of 3-D coordinates severely limits the scope of the feature analysis. The combination of the 3-D polygon mesh and the image's texture information should provide more insight to the feature analysis process.

Summary of Preliminary Data

To prepare for this grant submission, pilot data collection was performed to optimize the protocol for uniform data collection at multiple sites. A data collection protocol was developed that will insure collection of images from each site that are of uniform quality. An image processing protocol has also been developed to insure that images collected in the field are converted into high-quality 3-D images that will provide accurate data. Finally, analytical techniques have been developed both for acquiring anthropometric-like measures from the images, and for using the images for new analytical approaches, such as EDMA and facial recognition analysis.

Pilot data suggest that 3-D images will provide data comparable to that obtained from direct measurement of the face. We anticipate, therefore that observed group differences previously demonstrated for such a direct approach will be applicable to the proposed research. Each study site will collect sufficient sample sizes to independently test the hypotheses that FAS individuals as well as those with more subtle expressions of FASD (PFAS) can be distinguished on the basis of patterns of facial measurements from control and non-alcohol exposed subjects. Moreover, ethnic and age variability in the different sites will allow us to define possible differences in patterns of expression by ethnicity and/or age. Pilot data from the facial recognition approach were based on 2-D snapshots of individuals with FAS. We would anticipate better discriminating power for this technique when it is employed to samples of 3-D images gathered from the various research sites. It is also reasonable to assume that by combining various approaches described herein for defining an FASD phenotype, we will generate a set of discriminating features that has better clinical utility than currently available using existing methods.

The investigators in this core have extensive research experience that is directly applicable to the proposed study. Our study proposes to build on the work of previous researchers [25], [48], [30], [47], [2] by continuing to objectify the diagnosis of FAS and expand the resultant phenotypic criteria to improve detection of the more subtle expressions of FASD. We will incorporate recent technological and analytic advances such as a 3-D laser scanner, landmark analysis and automated facial recognition to identify a subset of measures and/or features that are important predictors of in utero alcohol exposure. These techniques will improve our ability to identify a wider range of individuals with fetal alcohol related birth defects and perhaps lay the groundwork for a more efficient application of the diagnostic process, through the creation of a better discriminant equation and/or machine based facial pattern recognition.

D. RESEARCH DESIGN AND METHODS

The main focus of this core is to acquire a greater understanding of the changes that occur in the face during development among individuals who have been exposed to alcohol in utero. Due to the significant differences in facial features among the various races as well as the notable changes that occur with age, all analyses will initially be site specific and race specific. Only after these data are analyzed to detect population and major age effects derived from initial site-specific analyses, will joint analyses be performed combining data and controlling for such factors across sites.

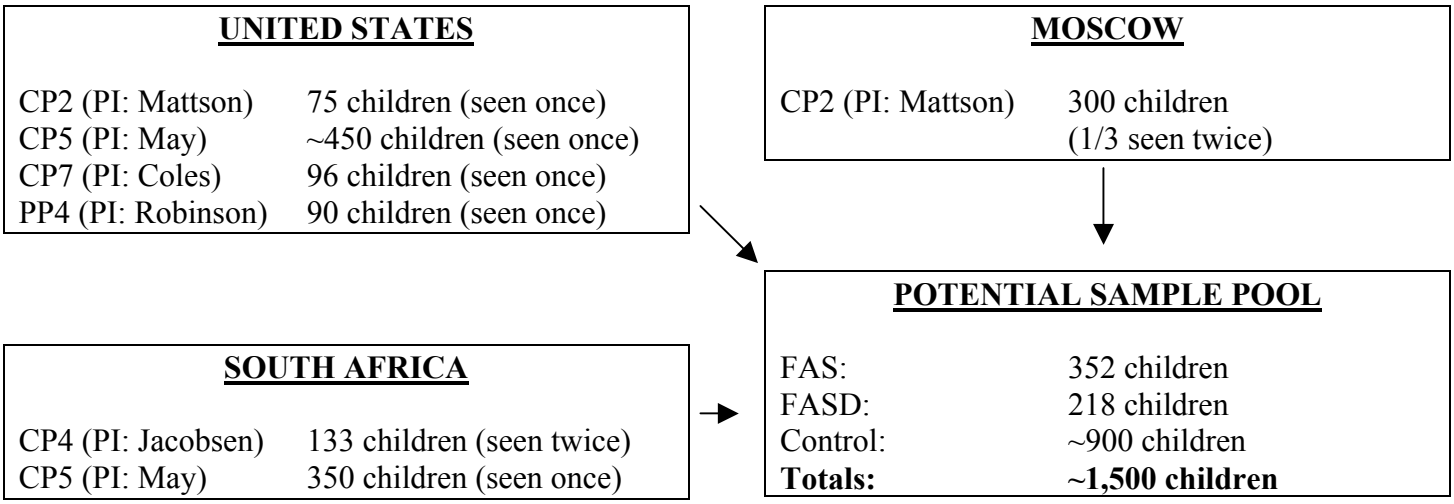
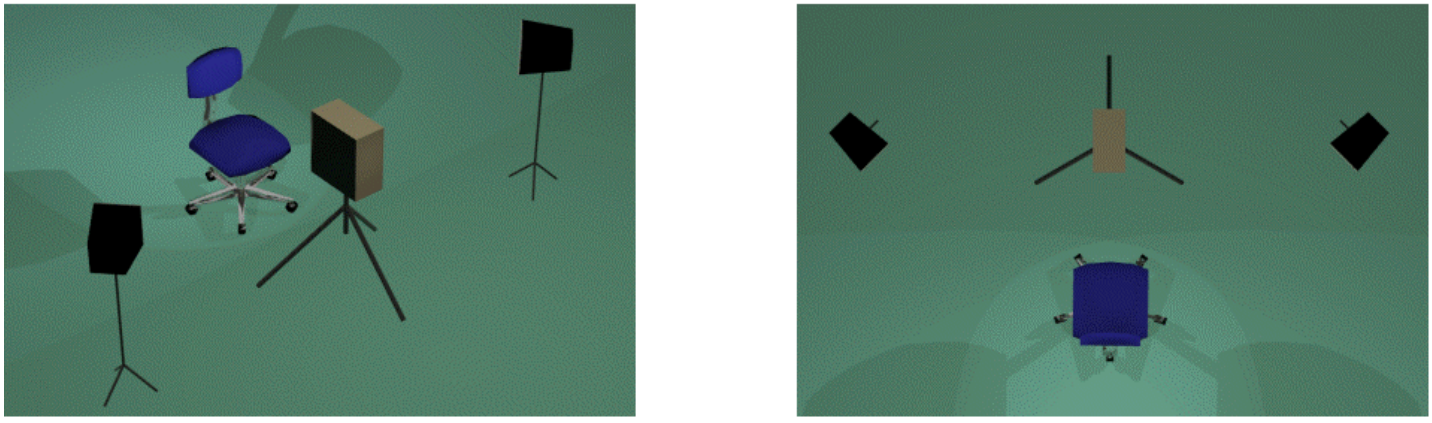


Figure 6: Proposed subjects for the 3-D camera protocol

Protocol for data acquisition at the sites

Camera Position and Location

Based on data from the Pilot Study, uniform camera position and location will be employed at each site. Figure 7 (A, B) illustrates the necessary hardware configuration. A Vivid 700 will be mounted to the tripod, and will face a swivel chair. The center of the tripod will be approximately 3 feet from the center of the swivel chair. Lights on tripods will be placed approximately 5 feet to the left and right of the Vivid 700. The left tripod will be rotated -45 degrees relative to the angle of the ray from the Vivid to the chair. The right tripod will be rotated 45 degrees relative to the angle of the ray from the Vivid to the chair.



(A) (B)

Figure 7: A. Isometric View of Tripod and chair layout. B. Top View of Tripod and Chair layout.

Subject Preparation

Subjects will be asked to remove eyewear and any jewelry that might interfere with the laser beam. All subjects will be fitted with a hair net because hair interferes with the laser beam and disrupts the image. Care will be taken to insure that the hair and cap do not occlude or distort important facial structures (e.g. ears or key landmarks on the forehead). The "stitching" software, that in latter processing will be used to merge the lateral and frontal images, requires each subject to be pre-marked with "registration points." The points are used by the software to interpolate the 3 images by overlapping homologous points on each image. The marks are placed with an eyeliner pencil of contrasting color to the subject's skin tone (black markers absorb too much of the laser light so are to be avoided). Two marks are placed on the forehead, 2 on the sides of the nose, 1 on each side of the face immediately below the malar (cheek) prominence, and 2 dots on the chin below the corners of the mouth and halfway to the bottom of the chin. The marks should be between 1 and 2 mm in diameter. In addition to the registration points, we know from previous studies and the pilot study, described above, that marking a few key anatomical landmarks improves subsequent measurement accuracy. Using the same contrasting eyeliner, the following anatomical points are to be marked: left and right tragion, defined as the point on the lateral surface of the face directly anterior to the tragal notch (Figure 8); left and right frontotemporale, defined as the point immediately superior to where the eyebrow crosses the temporal ridge; and left and right gonion, defined as the point immediately superior to the corner of the mandible (the outer corner where the ramus and body of the mandible meet.) As in the registration, these marks should be no larger that 2 mm in diameter.

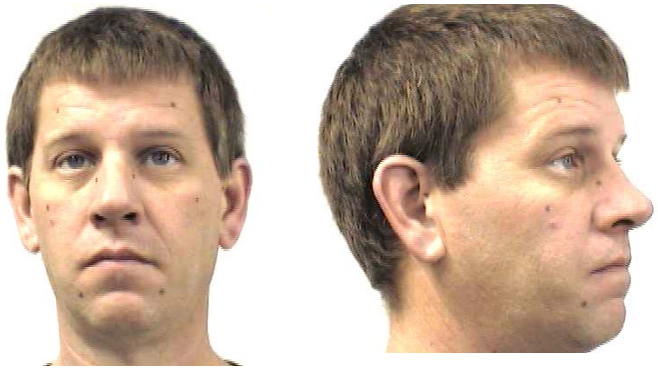


Figure 8: Illustration of placement of facial marks needed to facilitate interpolation of images from lateral and frontal views.

Procedure for Image Collection

The subject will be placed in the swivel chair facing the Minolta Vivid 700 scanner such that his/her face is approximately 26" from the front of the scanner. The scanner will target the image of the face centered in the ATI TV Tuner window. Using Minolta's PET software, two scans of the frontal view will be captured and reviewed for gross errors. The frontal view will be rescanned, as necessary, to obtain two quality captures. The subject will then be rotated on the swivel chair approximately 80 degrees to the right, with care taken to ensure that the subject's face is still approximately 26" from the scanner and centered in the ATI TV Tuner window. As with the frontal view, 2 quality scans will be acquired of the lateral left view using PET software. The subject will then be rotated on the chair 180 degrees to the left. After ensuring distance to scanner and targeting are maintained, 2 quality right lateral captures will be acquired. Before this process is repeated for the next subject, the resultant 6 captures must be saved.

Image Processing and Data Transfer

After capturing 6 scans, the images will be visually checked for gross mesh errors, including the following: 1) facial expressions; 2) exaggerated polygons on the mesh such as those caused by jewelry or hair over areas of interest; and 3) major holes in model. If error exists, the subject will be rescanned for the particular angle in question.

Once a week, data will be transferred from the collection site to the Informatics Core (PI: Stewart) via SFTP. The SFTP program uses the secure SSH protocol to physically transfer files between computers. Once data are submitted, the Informatics Core will process the files as explained in their core description. Image files will reside in an appropriate directory, whose location will be stored in an oracle database, designed to keep track of the location of each image using a full path name and to prevent images from entering the database twice by storing a checksum, which uniquely identifies each image, even if the filename has been changed. Members of the Informatics Core will work with collection sites to ensure efficient and rapid transfer of data. If any errors or problems arise in data transfer, the Informatics Core will work with the site to resolve any technical difficulties.

On a regular basis new data will be requested and transferred from the Informatics Core to this Core. As described in the Informatics Core, a specialized extraction and transfer routine will be developed between this Core and the Informatics Core to allow easy identification of new, released and/or updated images. Files will be transferred from the Informatics Core to a secure FTP site at Indiana University. Two scans each of the frontal, lateral right and left views of the face will be captured for redundancy by the site. From these 6 captures, the highest quality model from each view will be selected based on the continuity of facial expression between adjacent views. These 3 selected models will be stitched together to obtain a single 3-D model of the face. The lateral views will be registered with the frontal view within Raindrop Geomagic® software using the coinciding make-up reference points on each view. After registration of views, data points from the lateral views that reside 'more medial than the lateral corners of the eyes' will be discarded to eliminate as much area of overlap as possible. The 3 registered and trimmed models will be merged into a single model. Next, the texture of this model will be blended at the seam points using Geomagic's default value. The result will be a single texture mapping 3-D model.

The 3-D model for study subjects will be transferred to a SFTP web site on a weekly basis for permanent storage to the Informatics Core. As sufficient numbers of study subjects are accrued for analysis, 3-D images will be transferred back to this Core for completion of the work proposed in the specific aims.

Overview of Scientific Aims

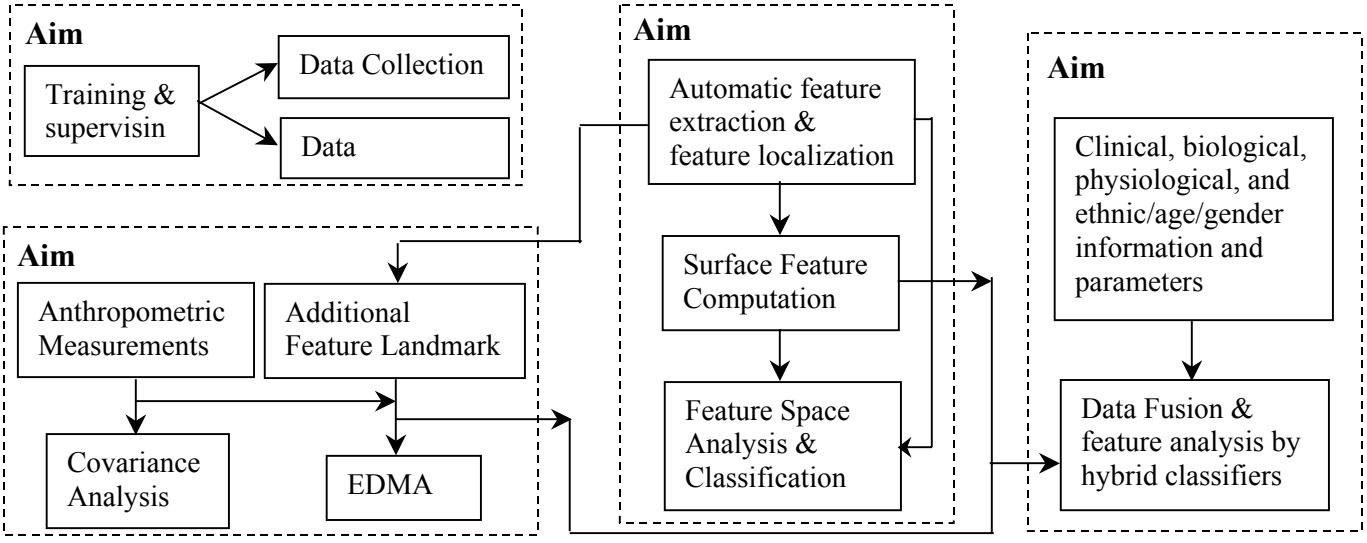


Figure 9: Flow Chart of the Research Plan and Design

The research plan is sequential with each step feeding data and results to subsequent steps (Figure 9). However, each aim will also produce independent results. Thus, it is anticipated that an outcome of Specific Aim 2 will be the production of a refined set of direct measures of the face that will have clinical utility in

diagnosing a wider range of individuals with alcohol related birth defects. The intent of Aim 3 is to produce a method by which (3-D) images of the face can be assessed by facial recognition software to produce a probability estimate that the individual has FASD. Ultimately, it is anticipated that data from multiple study sites can be combined (Aim 4) to produce both more efficient diagnostic criteria across diverse ethnic, age and sex groups as well as a better understanding of how the FASD phenotype reflects the underlying pathophysiology of ethanol exposure throughout intrauterine development.

SPECIFIC AIM 1: Train and supervise personnel at each collection site

Rationale: It is critical for a study of this scope, performed at numerous sites using a novel instrument, that site personnel be well trained to perform the study protocol. In addition, it is essential that sites be frequently evaluated to ensure that the study protocol is consistently applied and that new personnel are adequately trained. To perform regular quality assessment of international sites can be costly. Therefore, we propose to initially train all study personnel in the use of the camera during an orientation meeting scheduled in the first year, at the start of the study. Subsequently, to reduce costs, while still maintaining a frequent and high quality review of data collection procedures, an identical cast will be utilized at each site. Each collection site will send images obtained from the cast at regular intervals, which will then be used to ensure that the study protocol is correctly implemented.

Orientation Meeting and Initial Training

An initial orientation meeting attended by all critical study personnel will be held at the start of this collaborative study. This will be the venue for all site investigators and coordinators to meet in person with key project personnel. A manual with all study protocols for the collection of data using the Minolta camera will be prepared prior to the meeting and distributed to personnel from each site.

Two sessions will be held during the orientation meeting. The first session will be devoted to a review of the overall protocol and a demonstration of the study procedures, including data collection from subjects as well as data processing. During the second session, the investigators and coordinators responsible for collection of data at the sites will be trained in data acquisition and data transfer. Subjects will be prepared for imaging (hairnets, registration points, and landmark identification). Images will be collected from multiple volunteers, and feedback will be provided to the sites to improve the quality of their data collection. Following this second session, minor logistical protocol amendments will be developed, if necessary. All trainees will also be asked to mark and identify key landmarks on the study cast on 2 separate occasions and images will be prepared from each of these attempts. The 2 images will be used to generate baseline data on the likely interobserver and intraobserver measurement error caused by variation in landmark identification and marking.

Ongoing Training and Quality Assurance

To reduce costs associated with ongoing training and maintaining high quality, an identical cast will be made for each site. A single use alginate mold will be made from the head of an adolescent subject. From this alginate mold a dental stone casting will provide the master model of the adolescents subject's head and face. A durable, multi-use, silicon rubber mold will be made from the dental stone master model. Several identical hard polyurethane castings will be generated from the silicon rubber mold. The castings will be uniformly mounted on bases and used as photographic training models.

On a monthly basis, each site will be required to collect images using the cast and then transfer these data to Indiana University. Images for each of the 3 positions will be reviewed and if data quality issues are identified, the site will be contacted directly and procedures will be reviewed and the images recollected. Images sent from each site will also be processed rapidly, to allow for quick identification of any data

collection errors or protocol deviations. Sites will be contacted if any consistent data collection errors are identified. This will be particularly important when there is change in site personnel.

SPECIFIC AIM 2: Utilize the measurements from the 3-D facial imaging for analyses designed to identify those variables that most efficiently differentiate alcohol exposed from control subjects

Rationale: In previous work, Moore et al. [47], [2] demonstrated that individuals with documented FAS could be effectively separated from control subjects using anthropometric facial measurements and discriminant function analysis. Moreover, individuals with a more subtle expression of intrauterine alcohol exposure (PFAS) were also distinguishable from controls using this same methodology. We begin this aim with the assumption that the same ability can be demonstrated using measurements taken from 3-D images of the face (as opposed to direct measurement of the face). Thus, the null hypothesis we will test in this aim is that facial images of individuals with documented prenatal exposure to alcohol do not differ in facial form from individuals with no such exposure. This hypothesis will be tested using standard linear measurements taken from images of exposed and control individuals matched as nearly as possible for age ethnicity/race. In addition we will use EDMA to test the hypothesis. Euclidean distance matrix analysis has also been used in landmark-based craniofacial modeling and face recognition. We will apply 2 types of landmark matrices to study the morphological (surface and shape) differences between the exposed and control groups and to determine the ability of this method to correctly classify individuals in the FAS, PFAS, and control groups. We will test the following null hypothesis: there are no morphological differences between exposed and control groups, therefore, this method cannot correctly, with sufficient specificity and sensitivity, classify these individuals.

Anthropometric Variables

Methodologies used in previous studies will be replicated as closely as possible in this study [42], [47]. We will identify standard anthropometric landmarks on the images and utilize these to generate the same set of measurement variables that were used in our previous Pilot Study. Limitations of the laser imaging technique prevent assessment of head circumference from the image; therefore, this data will be collected separately by using a standard anthropometric measuring tape. Landmark identification on the 3-D images will follow the procedures for landmark identification outlined in Farkas [33] and Aung et al [32]. The latter study noted that certain key landmarks that are on bony or curved surfaces need to be pre-marked at the time the image is obtained to result in accurate and repeatable measurements. Similar findings were evident from our pilot study; therefore, the following points will be pre-marked at the time the image is obtained: right and left frontotemporale, bigonial, and tragion.

Since 2 images are taken of each individual, the measurer will select for measuring the image that has the clearest viewing of the needed landmarks.

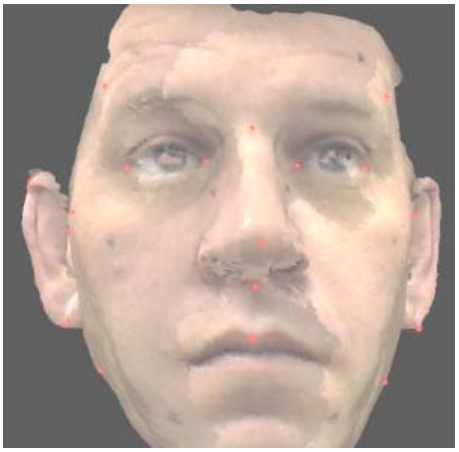


Figure 10: Final stitched image

Using the Raindrop Geomagic® software program, landmarks will be sequentially identified and marked on the previously stitched image. The software program automatically calculates the distances between the predetermined sets of points to produce the linear measurements. Following the same protocol used in Moore et al. [47], this procedure will be repeated at least twice on each individual. Thus, every image will have the landmarks identified on 2 separate occasions. The same set of linear measurements will be generated in each case and compared. In previous studies we used the rubric that any 2 measurements of the same variable that are more than 2 mm apart must be repeated a third time. This approach presented some problems of scale since a 2 mm error in a small measurement is of greater significance that the same error in a large measurement. In the present study the measuring software will allow a more precise approach. Thus, we will use the rule that any 2 measurements of the same variable that are more than 2% different between the first and second trials must be remarked and remeasured a third time. By convention, before data are analyzed we will average the 2 closest measurement values. All measurements will be taken by 1 individual (ESM) a trained anthropometrist, who will be blinded to the alcohol exposure status of the subjects. Linear measurements obtained from the average of the 2 closest trials in each individual will be used to test the null hypothesis of no difference between facial forms of exposed and non-exposed individuals.

Testing this null hypothesis will be complicated by several factors. First, the sample consists of individuals who will have a wide spectrum of craniofacial anomalies associated with differing levels of intrauterine alcohol exposure (FASD). Second, the sample consists of individuals of differing race/ethnicity (African, Russian, North American), different ages (infant to adult), and sex (Figure 8). Thus, it is necessary to control for effects of these factors in order to describe differences due to alcohol exposure. Analysis of covariance (ANCOVA), rather than the discriminate function analysis employed in earlier work, will be used in the present study because it can control for these factors and will identify those craniofacial variables that most significantly contribute to the differentiation of alcohol exposed and control groups. Analysis of covariance also allows us to determine which individual measurements are significantly influenced by race/ethnicity, sex and/or age. This information can lead to important insights on the pathophysiology of alcohol exposure as it reveals how patterns of expression vary according to exposure, developmental age, sex, and population group.

Analysis of covariance results will reveal those variables that are most significantly affected by intrauterine alcohol exposure. These variables will then be used in logistic regression analysis to develop a predictive model. This model will be tested or validated on the portion of the sample withheld for this purpose.

Landmark analysis using EDMA method

In landmark analysis, we limit ourselves to point features (landmarks) and linear distances between landmark points. There are a number of sophisticated morphometric techniques that use a higher dimension of analysis to assess biological form. These techniques purport to offer a better means of assessing form (either within a growing individual or between samples of individuals) than can be obtained by using simple linear measurements (which are generally just assessing size differences between forms). The approach we will use is based primarily on the EDMA (Euclidean Distance Matrix Analysis) method [51], because it provides a set of flexible and easy to use landmark analysis tools that allows the utilization of landmark data identified in the previous stage of the study, and because it has proven useful in the exploration of similar morphometric problems using 3-D landmark data (from computerized tomography scans). Our analysis will be carried out in three stages: 1) form matrix analysis; 2) optimal landmark set identification; and 3) distance ratio matrix analysis.

In the first stage, we will directly apply EDMA method and its software WinEDMA to analyze samples (FAS, PFAS and nonalcohol exposed, controls) of a fixed set of landmarks. For a given set of landmark points $[P_i]$ ($i = 1, 2, \ldots, k$), defined over a group of samples (e.g. FAS group or controlled group), the form matrix, *FM*, in EDMA is defined as

$$FM = [d_{ij}] \quad (i,j = 1, 2, \ldots k) \tag{1}$$

where d_{ij} is the distance between P_i and P_j. To allow comparisons between different sized face samples, the distances between landmarks are normalized by the mean value of this distance over a population [47]. The FORM procedure in WinEDMA provides a quantitative measure for comparing 2 populations of samples. For two face samples *A* and *B*, the Form Difference Matrix, *FDM*, is defined as

$$FDM(A,B) = [fdm_{ij}] = [d_{ij}(A) / d_{ij}(B)] \tag{2}$$

The Maximum Difference Ratio, T_{AB}, can then be computed to characterize the overall difference between 2 forms:

$$T_{AB} = max(fdm_{ij}) / min(fdm_{ij}) \tag{3}$$

In EDMA, the form *A* and *B* can be the statistical mean forms of two populations (computed within the FORM procedure). This process can be directly applied to measure the shape difference patterns between FAS, PFAS and control groups using a pre-determined set of landmark points (initially the same as used in the anthropometric analysis). The T_{AB} value computed for PFAS and control groups, or PFAS and FAS groups should be between 1.0 and the value computed for FAS and control groups. Large T_{AB} values indicate good discriminating power for the given landmark set. The mean form of each group will then represent the pattern of distance measures for each group. As WinEDMA also provides confidence interval testing for the Form Difference Matrix, the final pattern matrix for each case will have a confidence interval associated with each matrix element.

The second stage of analysis aims to compute an optimal subset of landmarks from a large set that can best discriminate given 2 groups (e.g. FAS and controlled). WinEDMA provides a procedure called INFLUENTIAL, which computes the most influential landmark for a given problem by ranking the off-diagonal elements in the *FDM* matrix [62]. This allows us to remove the least influential landmarks in an iterative fashion. Another way to measure the discriminating power of each landmark is to compute the Maximum Difference Ratio for each landmark individually. This gives a more cumulative measure of influence for each landmark. The Maximum Difference Ratio, T_i, of a landmark point P_i is computed by comparing only the *FDM* elements that are related to P_i, i.e.

$$T_i = max_j(fdm_{ij}) / min_j(fdm_{ij}) \tag{4}$$

This equation provides the maximum difference over all distances related to P_i. A large T_i within the FAS sample space indicates that P_i may not be a good discriminating point for the FAS problem. This allows us to iteratively move landmarks in and out of the target set (e.g. subtract the point with large T_i value within a sample group and small T_i values across two groups). This process will continue iteratively until the overall Maximum Difference Ratio T_{AB} is within a satisfactory level.

In the third stage of landmark analysis, we will examine the characteristics of FAS (and PFAS) faces that involve only a subset of the distances between landmarks and their relative ratios. Here elements of the form matrix are ratios of distances between landmarks. The goal is to extract the most useful distance measures instead of landmark points. Let $\{d_i\}$ be the set of all distance measures. The *Distance Ratio Matrix* (Form Matrix in EDMA) is defined as

$$FM = \{r_{ij}\}, \text{ where } r_{ij} = d_i / d_j \qquad (5)$$

The analysis process of this form matrix is very similar to the analysis for the form matrix based on normalized distances, except that the basic elements here are distance measures. For instance, the Maximum Difference Ration T_i is now defined for each distance measure, and we will be looking for the optimal set of distances instead of landmarks.

SPECIFIC AIM 3: Utilize algorithms and methods derived from the emerging field of Automated Facial Recognition (AFR) to extract and identify the most discriminating higher order surface features from 3-D facial images, with the goal of developing an automated method of identifying facial features diagnostic of prenatal alcohol exposure.

Rationale: Landmark based methods focus on point features and linear distances. However, we believe that facial features that best represent FAS characteristics are likely to be more complex and higher order than points and linear distances. In particular, features that are defined on a surface such as curvature, areas and arc length, generally cannot be defined by landmarks. In order to make use of higher order features in FASD diagnosis, automatic feature extraction algorithms need to be developed, as manual definition of such features are often difficult and inaccurate. Sophisticated feature analysis methods will also need to be applied to identify the optimal feature set that are most capable of separating FAS, PFAS and control groups. Our approach combines discrete differential geometry, pattern recognition, and facial recognition techniques, and will provide a comprehensive and automatic process for 3-D feature extraction, identification, classification and validation.

Surface Feature Computation

Features are information extracted from the input data that represents certain characteristics of the original object. As a general concept, features can include simple elements such as points and pixels, or more complex information such as areas, curvatures, flatness, etc. The feature analysis technique presented in the next section is a general technique that can, in principle, be applied to any type of features. However, the appropriate selection of features is often critically important to the data analysis process. As discussed earlier, higher order 3-D facial surface features will be necessary to produce robust and accurate FASD classifications. A main problem in surface feature extraction is the discrete nature of the scanning data. Most surface features are defined on a mathematical surface. With only 3-D scanning data available, we will be limited to computing surface features using discrete differential geometry techniques [63]. Two types of features will be computed: local curvature features and surface measurement features.

Curvatures are local properties of a point on the surface. There are 3 types of curvatures that are of interest to us:

1. Principle curvatures: k_1, k_2. They are the maximum and minimum curvatures in the 2 principle directions (the directions with maximum and minimum curvatures).
2. Gaussian curvature: K. It represents the local curvature change at a point. A positive value represents an ellipsoid-like local shape, a zero value represents a locally flat tendency or a parabola-like local shape, and a negative value represents a change of curvature direction, with a hyperbolic local surface.
3. Mean curvature: H. It represents the average curvature over all directions around a point. It provides a quantitative measure for the "curving" level of the surface at a point.

Surface measurements include arc-distances and area-related measures. On a continuous surface, arc-distance can be defined as the geodesic distance, which is theoretically the shortest distance on a surface between 2 points. A discrete algorithm for geodesic distance can be derived using discrete differential geometry operators. A simple application of the arc-distance is to replace the linear distance in landmark analysis. It should provide better descriptive power than linear distance. The path used to compute the arc-distance can also be used to define the boundaries of regions by picking only landmark points. Many properties and features can be computed for such regions. The obvious measure is the area of the region. Certain integrated curvatures over the region may also be properly defined to represent the overall curving level of the region.

Feature Extraction, Selection, and Classification – A Discriminate Features Space for FAS/FASD Classification

The non-accidental spatiotemporal properties of the world surrounding us have much to do with the design of visual systems. This viewpoint, as formulated by Barlow [64] (adaptation and decorrelation in the cortex called sensory coding), and more recently by Ruderman [65] amongst others, has led to a growing interest in: (1) how the statistical properties of natural images (signals) enter into the optimization of the visual system, and (2) the statistical characterization of the natural images themselves. The regularities of the surrounding world have been encoded mostly in terms of 2nd order statistics or corresponding spectral information, even though most recently there is a growing and justified interest in using higher order statistics as well. Methods based on decorrelating 2nd order statistics belong to the class of PCA (Principal Component Analysis) methods, while those concerned with independent higher order statistics belong to the class of ICA (Independent Component Analysis) methods. Optimization of the visual system would include design criteria, such as: 1) redundancy minimization - decorrelation and independent component analysis (ICA); 2) minimization of the reconstruction error (rms); 3) maximization of information transmission (infomax); and 4) sparseness of the neural code [66]. While there has been a growing interest concerning natural scene statistics and building the neural code to capture them [67], the range of imagery which could be of interest goes much beyond natural scenes.

Feature selection in pattern recognition involves the derivation of salient features from the sensory input data or the feature vector formed by appropriate geometry and statistic measurement in order to reduce the amount of data used for classification and simultaneously to provide enhanced discriminatory power. The selection of an appropriate set of features is one of the most difficult tasks in the design of pattern classification systems. At the lowest level, the raw feature data is derived from noisy sensor data, the characteristics of which are complex and difficult to characterize. In addition, there is considerable interaction among features, which must be identified and exploited. The typical number of possible features, however, is so large as to prohibit any systematic exploration of all but a few possible interaction types (e.g., pairwise interactions). In addition, any sort of performance-oriented evaluation of feature subsets involves building and testing the associated classifier, resulting in additional overhead costs.

Data will first be mapped into a lower dimensional space. Optimal linear projection will be used to generate a tessellation of a space defined by the training images. This space will then be generated using different projections. The Karhunen-Loève projection (*Eigenfaces Approach*) and Discriminating Analysis Projection (*Fisherfaces* or *Linear Discriminant Approach* will be employed.

FAS Facial Space based on Karhunen-Loève Projection (Eigenfaces)

Karhunen-Loève Transformation (KLT) is an information theory approach of getting insight into the information content of facial images [68]. In mathematical terms, the principal components (PC) of the distribution of faces are calculated. These are the eigenvectors of the covariance matrix of the set of facial images, treating an image as a point or vector in a very high dimensional space. The eigenvectors are ordered, each one accounting for a different amount of the variation among the facial images. A scatter matrix formed by training images defines the principal components analysis (PCA) subspace. PCA generates a set of orthonormal bases known as principal components. Let $\mathbf{X}=[X_1, X_2, ..., X_n]$ be the sample set of the original FAS images. After normalizing the images to unity norm and subtracting the ground mean a new image set $\mathbf{Y}=[Y_1, Y_2, ..., Y_n]$ is obtained. Each Y_i represents a normalized image with dimensionality N, $Y_i=(y_{i1}, y_{i2}, \ldots, y_{iN})^t$, where $i=1,2,...,n$. The eigenvector and eigenvalue matrices $\mathbf{\Phi}$, $\mathbf{\Lambda}$ are computed as

$$(\mathbf{Y}\,{}^t\mathbf{Y})\mathbf{\Phi}=\mathbf{\Phi}\mathbf{\Lambda} \quad (6)$$

Note that $\mathbf{Y}^t\mathbf{Y}$ is an n-by-n covariance matrix where $\mathbf{\Lambda}=diag(\lambda_1, \lambda_2, \ldots, \lambda_n)$, and $\mathbf{\Phi}=[\Phi_1, \Phi_2, \ldots, \Phi_n]$. If one assumes that the eigenvalues are sorted in decreasing order, $\lambda_1 \geq \lambda_2 \geq \ldots \geq \lambda_n$, then the first m leading eigenvectors define matrix $\boldsymbol{P}$

$$\boldsymbol{P}=[\Phi_1, \Phi_2, \ldots, \Phi_m]. \quad (7)$$

The basis vectors in $\boldsymbol{P}$ are also known as *eigenfaces*.

The new feature set $\mathbf{Z}$ with lower dimensionality m ($m<<N$) is then computed as

$$\mathbf{Z} = \boldsymbol{P}\,{}^t\mathbf{Y} \quad (8)$$

The KL projection produces a set of *Most Expressive Features* (MEFs) and reduces the feature dimensionality from N to m. Fig. 11 shows the sample FAS and non-FAS data points represented as linear combination of eigenfaces with different projection (coefficients).

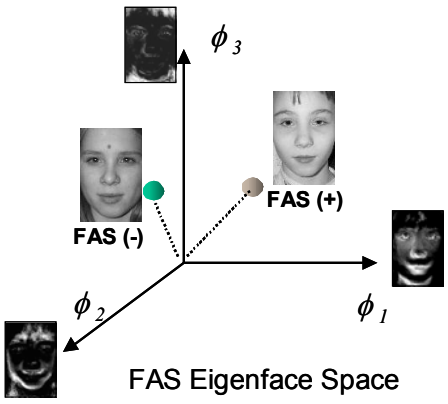


Figure 11: Sample FAS and non-FAS face image in an eigen space (illustrated by the first three components).

Discriminant Karhunen-Loève (DKL) Projection for FAS Classification

The Linear Discriminant Analysis (LDA) projection is performed in the space of the KL projection. Thus, the Fisher Linear Discriminants are defined in the m dimensional subspace using the first m principal components. Fisher's method defines c basis vectors where $c=k-1$ or $c= k$, where k is the number of classes. The aim is to find a projection matrix $\boldsymbol{W}$ that maximizes the ratio of distances between classes and distances within each class in order to find the best separation boundary for different classes (see Fig.12) [69].

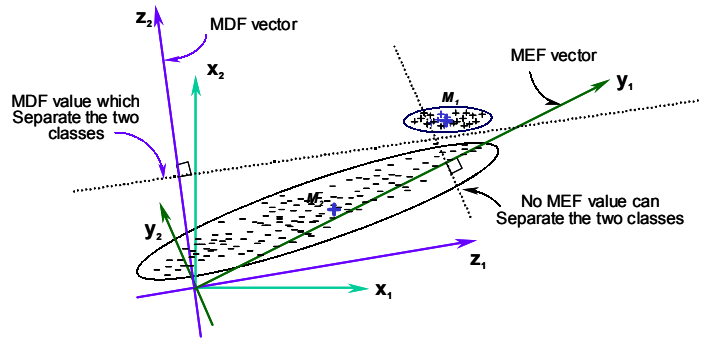


Figure 12: **Most discriminating features (MDFs) space vs. Most expressive features (MEFs) space**

Let the class means be M_i, *i=1,2,.., c.* The *within-class* scatter matrix S_w for n_i samples from class *i* can be defined as

$$S_w = \sum_{i=1}^{c} \sum_{j=1}^{n_i} (X_j - M_i)(X_j - M_i)^T \quad (9)$$

where X_j is the projection of the sample point *j* in MDF space. For a grand mean vector M for all samples from all classes, the *between-class* scatter matrix S_b is defined as

$$S_b = \sum_{i=1}^{c} (M_i - M)(M_i - M)^T \quad (10)$$

Then the column vectors of projection matrix $\boldsymbol{W}$ are the eigenvectors of $S_w^{-1}S_b$ associated with the largest eigenvalues [70]. Training images must be partitioned into classes and are used to determine $\boldsymbol{W}$. During testing, the LDA algorithm performs the classification in LDA space in exactly the same manner that the PCA algorithm performs classification in the PCA subspace and then projected on the LDA space for classification.

SPECIFIC AIM 4: Combine the results from the direct and higher order measurements derived from the 3-D facial imaging with variables collected from other study domains to improve the power to accurately discriminate alcohol exposed from control subjects and to better understand the pathophysiological effects of ethanol on human development.

Rationale: One of the major goals of this research is to develop tools to assist in the definitive diagnosis for FAS and, more broadly FASD, as well as to differentiate the core deficits that may be expressed in different age and ethnic/racial categories. To accomplish this goal, information from the various cores, collecting clinical, morphometric, behavioral, and neurological data, will be used in a combined analysis to: 1) determine the most effective set of criteria for differentiating controls and alcohol exposed individuals; and 2) define key differences in the pattern of expression that segregate by ethnicity/race and/or age; 3) analyze correlations between data types and between subject groupings to generate a better understanding of the action of ethanol on human development . To meet these objectives, quantitative morphometric data (both landmark based and feature-space based) from 3-D images will be combined with significant variables collected from other domains to define the most efficient grand set of variables or algorithms for differentiating alcohol exposed individuals from controls. In addition, data from various domains will be compared across sex, age, and ethnic groups to identify patterns of variation that correlate with these subject categories. Examination of relationship between data sets can reveal, for example the degree to which facial form in alcohol exposed individuals reflects specific underlying neural anatomical disruptions. Such information should improve our

understanding of both the broad effects of ethanol exposure on development as well as improve our understanding of the factors contributing to the wide variation in the expression of these effects.

Data Fusion Through Hybrid Classifier

The definitive diagnosis for FAS (or more broadly FASD) as well as the ability to differentiate the core deficits associated with prenatal alcohol exposure, will require correlation of data from multiple sources, (dysmorphology, morphometric, neurological, behavioral) as well as of different sub-categories such as age, ethnicity and sex. Data fusion is a term used to describe the process of correlating data acquired by different means. The use of discriminating feature space discussed earlier and the method of intelligent hybrid learning systems, complements analysis of FASD by fusing data from multiple modalities. It is also particularly important to combine and derive a set of key discriminating features that can be highly correlated to FAS/FASD.

Intelligent hybrid learning systems involve specific (hierarchical) levels of knowledge defined in terms of concept granularity and corresponding interfaces. This knowledge is usually represented by different data input from various measurements. Fusing multiple data, the hierarchy is designed to include connectionist and symbolic levels, with each level possibly consisting of ensemble architecture by itself, and with proper interfaces between levels. As one moves upward in the hierarchical structure, we witness a corresponding degree of data compression allowing more powerful ('reasoning') methods to be employed on reduced amounts of data. The advantages provided by each level consist of:

- Connectionism can handle the whole range of sensory inputs and their variability ('noise'). Its distributed nature provides for fault tolerance to missing and incomplete data. The output of such modules can be combined across ensemble of such networks. Last but not least, the output of such modules yields the sought after symbolic units needed for later stages of processing.
- Symbolic methods are compact and can fuse data from different sensory modalities and cognitive modes. As a consequence one can interpret the sensory input and explain it using meaningful coding units.

An early example of homogeneous ensembles is the Meta-Pi architecture suggested by Hampshire and Waibel [71] for speech interpretation. Homogeneous ensembles of symbolic modules are usually referred to as multistrategy learning methods. As an example of heterogeneous ensembles, Greenspan [72] has proposed architecture for the integration of neural networks and rule-based methods using unsupervised and supervised learning for pattern recognition tasks.

The hybrid classifiers consist of an ensemble of connectionist networks - radial basis functions (RBF) (Fig. 13.a) - and inductive decision trees (DT). The reason behind using RBF is its ability for clustering similar features before classifying them. Decision trees (DT) implement the symbolic stage using the RBF outputs. We propose a hybrid learning system (Fig. 13.b) using the ensembles of RBF (ERBF) and the hybrids consisting of ERBF and DT [54].

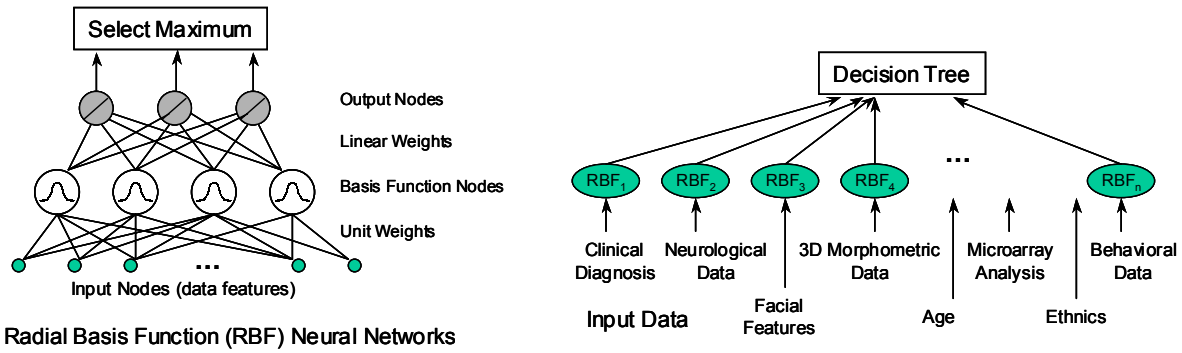


Figure 13: **(a) Radial Basis Function (RBF) and (b) the hybrids consisting of**

ensembles of RBF (ERBF) and decision Tree (DT).

SUMMARY

Alcohol exposure results in a variety of clinical sequellae, with significant implications for the individual's future potential. We have created an interdisciplinary group of investigators who will employ novel applications of 3-D facial imaging to improve our ability to delineate the effect of prenatal alcohol exposure on facial features and lead to more effective clinical diagnosis of FAS, as well as the more broadly defined FASD. . These studies will inform basic science research by identifying common pathways affected following alcohol exposure thereby provide improved understanding of the pathophysiological effects of ethanol on human development.. Completion of the proposed specific aims also has the potential to improve our ability to identify and diagnose individuals who have had alcohol exposure in utero. Through this collaborative research effort organized under the "Collaborative Initiative on Fetal Alcohol Spectrum Disorders" (CIFASD), large numbers of diverse subjects will be recruited providing the optimum research environment to complete these studies.

e. HUMAN SUBJECTS

1. Risks to Subjects

Human Subjects Involvement and Characteristics

The human subjects for this core will all be recruited and evaluated by the individual projects comprising this Collaborative Study. We anticipate a total of 352 FAS, 218 FASD and ~900 non-alcohol exposed individuals will have 3-D images collected through their participation in the primary project and these data will then be analyzed by this core. The subjects will vary in age, but typically will be under the age of 18 years. There will be no exclusion by race, gender or ethnic characteristics. Importantly, this core will not recruit any subjects. The recruiting site will assign a unique study identifier to the subject and will not provide our core with the study subject's name.

Sources of materials

All 3-D images will be collected for research purposes only, from consenting study subjects.

Potential Risks

The physical, social and legal risks of this project are minimal. This system uses a Class 1 (FDA), eye-safe laser to rapidly scan and digitize the face into a texture mapped 3-D model. While completing the protocol to obtain a 3-D image, the subject might experience a small amount of discomfort when required to remain still for short periods of time. Computer files will be permanently stored as part of the Informatics Core (PI: Stewart) which has extensive security measures in place to protect subject data. During data processing and analysis, the files will be stored on SUN microcomputers located in the Department of Medical and Molecular Genetics. Security of data is protected by the need for access to the SUN microcomputer, specific account numbers and passwords and specific commands allowing the user to reach critical data. Every precaution has been taken to assure that computer confidentiality is maintained.

2. Adequacy of Protection Against Risks

Recruitment and Informed Consent

All study subjects for whom data is generated as part of this project will be recruited through the projects included in this collaborative effort. Importantly, this core will not recruit any subjects. The recruiting

site will assign a unique study identifier to the subject. This core will not have the study subject's name and will identify subject's only through their unique study identifier.

Protection Against Risk

To reduce the risk of eye damage due to the use of laser to capture the 3-D image, a system will be used which employs a Class 1 (FDA), eye-safe laser. Extensive security measures have been taken to ensure the privacy and confidentiality of all data that will be analyzed at Indiana University. Security of data is protected by the need for access to the SUN microcomputer, specific account numbers and passwords and specific commands allowing the user to reach critical data. Use of a microcomputer for data storage has many advantages over the use of mainframe computers, the greatest of which is heightened security. Every precaution has been taken to assure that computer confidentiality is maintained.

3. Potential Benefits of the Proposed Research to the Subjects and Others

There are no medical interventions or direct benefits gained by the subject from completing the 3-D image protocol to generate data for this core. The indirect benefits of participation include an opportunity to be proactive in the search for causes and/or contributing factors associated with FAS and the satisfaction of having the opportunity to contribute to the general knowledge of FAS.

4. Importance of the Knowledge to Be Gained

Alcohol exposure results in a variety of clinical sequellae, with significant implications for the individual's future potential. We have created an interdisciplinary group of investigators who will employ novel applications of 3-D facial imaging to improve our ability to delineate the effect of prenatal alcohol exposure on facial features. These studies will inform basic science research by identifying common pathways affected following alcohol exposure. Completion of the proposed specific aims also has the potential to improve our ability to identify and diagnose individuals who have had alcohol exposure in utero. Through this collaborative research effort organized under the "Collaborative Initiative on Fetal Alcohol Spectrum Disorders" (CIFASD), large numbers of diverse subjects will be recruited providing the optimum research environment to complete these studies.

Women and Minority Inclusion in Clinical Research

Inclusion of Women

FAS affects both men and women. It is the aim of the Collaborating investigators to recruit subjects irrespective of gender.

Inclusion of Minorities

FAS affects individuals of all races. It is the aim of the Collaborating investigators to recruit subjects irrespective of race. In addition, through the recruitment of subjects worldwide, there will be broad representation of many minority subjects including Native Americans, African Americans, and Mixed Race individuals from South Africa.

Inclusion of Children

The focus of this Collaborative study is the recruitment of children, particularly those under the age of 18 years. Therefore, there will be almost exclusive inclusion of children. Importantly, this site will not recruit or image any subjects. We will solely receive and analyze data collected by Collaborating sites.

f. VERTEBRATE ANIMALS

Not Applicable.

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h. CONSORTIUM/CONTRACTUAL ARRANGEMENTS

i. CONSULTANTS

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Joan Richtsmeier, Ph.D. (see letter)