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Three-dimensional digital image processing and reconstruction of granular particles

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Three-Dimensional Digital Image Processing And Reconstruction Of Granular Particles

by

Jorge A. Rivas

A thesis submitted in partial fulfillment
of the requirements for the degree of

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College of Engineering
University of South Florida

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Keywords: sands, photomicroscopy, edge detection, grain morphology, measurements

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THREE-DIMENSIONAL DIGITAL IMAGE PROCESSING AND
RECONSTRUCTION OF GRANULAR PARTICLES

Jorge A. Rivas

ABSTRACT

This thesis presents a method for digitization of the two-dimensional shape of granular particles by means of photo microscopy and image processing techniques implemented using a software package from Media Cybernetics, Inc: Image-Pro Plus 5.1 and the add-ins Scope-Pro 5.0, SharpStack 5.0 and 3D Constructor 5.0. With the use of these tools, it was possible to implement an efficient semi-automated routine that allows the digitization of large numbers of two-dimensional silhouettes of particles in minimum time, without endangering the quality and reliability of the shapes obtained. Different sample preparation techniques, illumination systems, deconvolution algorithms, mathematical functions, filtering techniques and programming commands are brought into play in order to transform the shape of the two-dimensional projection of particles (captured as a set of successive images acquired at different planes of focus) into a binary format (black and white). At the same time, measurements and statistical information such as grain size distribution can be analyzed from the shapes obtained for a particular granular soil. This information also includes but it is not limited to perimeter, area, diameter (minimum, maximum and mean), caliper (longest, smallest and mean),
roundness, aspect ratio and fractal dimension. Results are presented for several sands collected from different places around the world. In addition, some alternatives for three-dimensional shape reconstruction such as X-ray nano tomography and serial sectioning are discussed.
CHAPTER ONE: INTRODUCTION

Conditions at which Earth has evolved through ages, has made soils everywhere in the world have their own “fingerprint”. Soils have been formed and transformed through different environmental conditions in time and have their own characteristics.

Sands are granular materials composed of small particles of rock and minerals (produced by mechanical disintegration), shells and fossils (biogenic particles), and chemical precipitates. Sands can be described in terms of particle size, composition, morphology (angularity and shape), color and texture. Grain size results from factors such as composition, durability, weathering conditions, physical sorting and transportation conditions. Mechanical and chemical processes determine grain shape once it is released from the original mineral matrix (Margolis and Krinsley, 1974, Raham, 1995).

From an engineering perspective, sand is everything that has grains, no matter the composition, ranging from a diameter of 74 μm (particles retained on U.S. Standard Sieve No. 200) till 4.76 mm (passing U.S. Standard Sieve No.4).
Table 1. ASTM Particle Size Definition

<table>
<thead>
<tr>
<th>Definition</th>
<th>Metric Size (mm)</th>
<th>ASTM Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine Gravel</td>
<td>4.76 to 10.0</td>
<td>4 to 3/4”</td>
</tr>
<tr>
<td>Coarse Sand</td>
<td>2.0 to 4.76</td>
<td>10 to 4</td>
</tr>
<tr>
<td>Medium Sand</td>
<td>0.42 to 2.00</td>
<td>40 to 10</td>
</tr>
<tr>
<td>Fine Sand</td>
<td>0.074 to 0.42</td>
<td>200 to 40</td>
</tr>
<tr>
<td>Silt &amp; Clay</td>
<td>&lt; 0.074</td>
<td>&lt; 200</td>
</tr>
</tbody>
</table>

Size and shape of particles are part of the history of the formation of a soil. Cho et al. (2005) explored the effects of particle shape on packing as well as small and large-strain properties of sandy soils. Correlations between index properties and mechanical parameters that are justified by particle shape were also explored. The macro scale behavior of soils results from particle interactions which are affected by particle shape. Therefore, it is a significant soil index property that has emerged as a parameter that needs to be properly characterized and documented (Santamarina et al., 2004). Digital image analysis facilitates the evaluation of mathematical descriptors of particle shape including Fourier analysis, fractal analysis and other hybrid techniques (e.g., Meloy 1977, Clark 1987, Hyslip and Vallejo 1997, Bowman et al. 2001, Sukumaran and Ashmawy 2001). In the study of the influence of particle shape on liquefaction behavior using Discrete Element Modeling for granular particles (Ashmawy et al., 2003), the use of Overlapping Rigid Clusters (ORC) method was proposed. This technique simulates the two-dimensional shape of granular particles by overlapping disks of different diameters. The resulting outline resembles the original particle outline. Later on, Sallam and Ashmawy (2005) proposed a operator-independent modified ORC technique. The new modification ensures that the mass, center of gravity and mass moment of inertia of the simulated particle matches with the shape of the original grain.
The use of digital image processing techniques has been found to be a useful tool for shape recognition and digital imaging of objects in two and three dimensions. Maerz and Zhou (1999) remark the potential impact of using image analysis for characterizing aggregate size and shape in terms of quality, time and cost savings. Chandan et al (2004) used photo microscopy techniques to characterize the geometry and the analysis of aggregate particles used in highway and geotechnical applications. In their work, texture was analyzed by multi-scale wavelet decomposition representation on grayscale images. Their review emphasized the need for a method to capture particle texture. Angularity was described employing a gradient-based method, plus form analyses of particles are done using binary images and computing shape factors and sphericity indexes. As
mentioned in their publication, several studies addressed the relationship between particle shape and performance of asphalt mixes, concretes and unbound layers. In the geotechnical field, material classification and mechanical response of soil particles are the main focus of similar studies. Advances in hardware technology permit an increase in speed and accuracy for such tasks. The implementation of advanced image processing techniques is imperative for image noise removal and a successful analysis of particle shapes.

This research uses photo microscopy techniques and the software package Image-Pro Plus 5.1 (including the add-ins Scope-Pro 5.0, SharpStack 5.0 and 3D Constructor 5.0) from Media Cybernetics, Inc. to digitalize the two-dimensional shapes obtained from silhouettes projected by grains of different types of sand collected from different regions of the world.

It is of fundamental importance to detect and accurately define the edges that define the contour of particles. For this research, several types of illumination systems and optical filters were tested to get the best result possible in photo microscopy. The images obtained were improved with software tools available on the Image-Pro software family.

There are different methods for image enhancement and edge detection available as built-in functions on the Image-Pro software family. But the best results were obtained by combining and applying several algorithms, functions, commands and arithmetic operations that can be called by the user as individual semi-automated routines.

Thanks to the versatility of the Macro Programming Language (Auto-Pro) of Image-Pro Plus, these routines offer certain degree of standardization for the process of
acquisition and improvement of images, edge recognition, particle shape imaging, counting and measurements of images. They are intended to save a significant amount of time and minimize user error, thus providing a higher level of efficiency and facilitating the whole process to the user.

The recollection of a large number of two-dimensional grain shapes can be also used to estimate the grain size distribution for a specific type of granular soil (between 5,000 to 10,000 particles). Pirard et al (2004) reported that an adequate image analysis is capable of predicting the sieve size distribution with a fraction of the quantity needed for a sieve analysis. This research shows a way on how to easily recollect measurements from digitalized shapes. They can later be used to estimate a grain size distribution and other statistical data from the sands analyzed.

In the case of 3D reconstruction, alternatives such as X-Ray Nano Tomography Systems and 3D Reconstruction from monoscopic images acquired at incremental focal planes are examples of non destructive methods.

X-ray Computed Tomography is a branch of X-ray microscopy in which a series of projection images are used to calculate a 3D reconstruction of an object. The equipment is expensive but it is a high-resolution technique with the ability to resolve details as small as 0.4 um in size for visualizing the 3D microstructure and geometry of materials. Garboczi (2002) described a mathematical procedure using spherical harmonic functions that can completely characterize concrete aggregate particles and other particles of the same nature form 3D particle images obtained via X-ray Tomography. These new systems usually come fully equipped and include their own 3D reconstruction software.
They are being implemented by several science fields because of its simplicity of use, reliability and none destructiveness of the samples analyzed.

Shape-from-focus uses different focus levels to obtain a sequence of object images and relies on surface texture for the computation of depth (Noguchi and Nayar, 1994; Niederoest et al., 2003). Algorithms and techniques exist that perform both the calculation of a sharp image and the recovery of the three dimensional structure of the specimen. But it was not until recently that more robust, fast and user-friendly systems have been implemented due to advances in computing technology. Shape-from-focus has found great use in light microscopy visual inspection tasks, mainly to obtain accurate depth estimates on several materials.

Serial Sectioning is a destructive method which relies on the use of high precision equipment to finely remove layers of material from the sample. It does not only consume a considerable amount of time but also requires special care in comparison with other alternatives.

Serial sectioning techniques have been used several times (Bower et al., 1966; DeHoff et al., 1972; Mangan et al., 1997; Wolfsdorf et al., 1997; Li et al., 1998 and 1999). But the processes were tedious and provided awkward quality. Alkemper and Voorthees (2001) describe a technique for serial sectioning analysis in which alignment and rotation errors as well as sample tilting are negligible. The technique was illustrated through the reconstruction of the microstructure of a cast standard alloy specimen. Approximately 20 cross-sections per hour with spacings in the range of 1 and 20 μm can be acquired. Chawla and Wunsch (2004) performed a serial sectioning process on
aluminum composites reinforced with Silicon Carbide (SiC) particles for 3D visualization and Finite Element modeling. The main objective was to understand the microstructure morphology and the connection between material structure and performance. Modeling of the composite correlated very well with their experimental results.

The serial sectioning process is being extended to other materials, as in this case, granular particles in soils. The exploration of the morphological characteristics and numerical modeling now days can be performed with a greater degree of accuracy thanks to advances in technology.

There are several software packages available for three-dimensional reconstruction and visualization of serial sections. Fiala and Harris (2002) developed techniques for practical and cost-effective serial sectioning analysis and 3D reconstruction. Fiala continued refining the technique and created Reconstruct, a Windows application for montaging, aligning, tracing, measuring, and reconstructing objects from serial section images. This freeware software is available at http://synapses.bu.edu/. A common limitation found in some programs available is the need of a constant thickness or slice interval between cross sections for 3D reconstruction. In serial sectioning, it is not an easy task to remove material at equal intervals, so programs with this limitation can not be used.

Each method mentioned before has its advantages and disadvantages and their applicability may have a greater level of success if applied to certain type of materials.
1.1 Purpose

The main objective of this research is to establish a reliable method for acquisition, processing, measuring and storage of 2D images of particles of different types of sands collected. Other goal was to evaluate alternatives for 3D reconstruction. The process for such task has to be practical, systematic and should be capable of a quantitative and qualitative reconstruction. The techniques established and proposed for two dimensional characterizations are designed to offer good results with less operator dependency and systematically increasing efficiency.

The shapes obtained with the application of this research will be used to: a) Characterize mathematically different types of sands using shape descriptors, b) Use those shapes for numerical modeling. The development of an operator-independent Modified Overlapping Rigid Clusters method, proposed by Sallam and Ashmawy in 2004 to simulate 2D shapes, is in progress and will be used for the numerical modeling and c) Shape analysis from sand particles can be used to check the influence of size/size distribution versus shape. Particle size distribution from a sieve analysis and an image analysis could be compared and their correlation verified.
1.2 Organization of Thesis

This thesis is organized as follows:

A brief description of the equipment, tools and software utilized for this research is summarized in Chapter Two.

Chapter Three explains an initial procedure developed to assist the operator in the following areas:

1) Sample preparation for photomicrography
2) Digital image acquisition of granular particles
3) Image processing
4) Edge detection and delineation of the boundary defined by the 2D shape projection of particle silhouettes
5) Creation of binary images (Black and White) that represent that shape.

Basic routines assembled for Image-Pro Plus (IPP) are used to explain the whole process. These routines are intended for detailed inspection and evaluation purposes only. Basic theoretical aspects about all the steps and equipment involved are also included throughout the chapter. They help the user to assimilate and understand the whole process.

Chapter Four explains a group of semi-automated macros that provide a fast way for the acquisition and processing of images, thus simplifying the whole operation of shape imaging and the obtaining of measurements and statistical data of the samples.

Three-dimensional reconstruction methods are introduced to the reader and discussed in Chapter Five.
Results, conclusions and recommendations are summarized in Chapter Six.

The appendices of this work contain the programming codes for all the scripts assembled for Image-Pro Plus. Some images that show some results obtained under different conditions, some measurement comparisons made and standard sieve analysis for some types of sands are also included.
CHAPTER TWO: EQUIPMENT, TOOLS AND SOFTWARE

This chapter describes the hardware and software used for this research. The main components for image acquisition and processing are basically an inverted optical microscope equipped with a monochromatic digital camera, optical filters, illumination systems and a motorized focus control. All these interfaced with a desktop computer. This powerful computer is Microsoft Windows compatible and has commercial imaging software installed that also controls the automated hardware. The purpose of the implementation of these tools is to set a semi-automated system that allows the digitization of particle shapes in a qualitative and quantitative manner. Specialized macro routines have been implemented to automate as much as possible all steps involved in the process and digital image processing has been used to facilitate particle edge detection and identification. Finally, binary shapes representing the silhouettes of particles are created and several measurements can also automatically be performed by the imaging software.

If needed, more detailed information on any of the items described in this section may be found in their respective manufacturer’s documentation.
2.1 Hardware

The types of microscopes used are the SWZ-168TL Trinocular Microscope and the AE31 Inverted Microscope manufactured by Motic Instruments, Inc.

The SWZ-168TL (Figure 7) was used as an inspection and sample preparation microscope. It comes with zoom objectives ranging from 0.75x to 5x. Incident halogen and transmitted halogen are two types of illumination available by default on this model. It is digital camera ready.

![Figure 2. Motic SWZ-168TL Trinocular Microscope](image)

The AE31 Inverted Microscope (Figure 3) was selected because it provides longer working distance objectives with higher numerical apertures than upright microscopes. It comes with the following magnifications: 4x, 10x, 20x, 40x and 60x. Also, there is more working space available for handling and placing samples. It is also digital camera ready. A mechanical stage with low positioned coaxial controls was attached and used for X and Y horizontal displacement of the samples. The focusing of the microscope was controlled via hardware manufactured by Prior Scientific, Inc. The Focus Control System is
composed of a *Motorized Focus Control* (MFC), which offers step sizes as small as 0.002\(\mu\)m in the Z-axis. A *Digipot Focus Only Control* for the MFC, which has buttons for fast up and down movements (for coarse focusing) and fine focus adjustments by means of a tactile feel. These two pieces are attached to a Prior *ProScan II Advanced Controller*, which is connected to a PC computer and is capable of controlling a motorized stage, motorized focus control, three filter wheels and three shutters (See figure 3).

With respect to optical filters used for the Motic AE31 Inverted Microscope, please go to section 3.4.2 for more detailed information.

The Evolution VF Monochromatic digital camera employed has a maximum resolution of 1.4 million pixels in a 12-bit digital output. It can capture digital data at frame rates of up to 110 fps in region of interest with binning. It comes with an IEEE 1394 FireWire digital interface. If necessary, it can be converted to a color camera through an optional kit.

The focus control system and the camera are connected to a Dell OptiPlex GX280 Desktop Computer. This PC has comes with an Intel® Pentium® IV processor running at 3.2 GHz, 2 GB of RAM DDR, 160 GB Hard Drive, a DVD Burner, and a 19” Flat Screen Monitor. An ATI Radeon XT700 Pro Video Accelerated Card with 256MB of memory was added for better video performance.
Figure 3. Illustration of Hardware: a) Motic AE31 Inverted Microscope, b) Evolution VF Monochromatic Digital Camera, c) Attachable Mechanical Stage, d) Prior Focus Control System and e) Dell OptiPlex GX280 PC.
2.1.1 Illumination

Regarding to illumination, it has been found to be a key factor for successful imaging. Depending on the application, extensive trials have been made in order to achieve the best illumination technique possible. The purpose of good illumination is to provide the best contrast for easier edge detection. Depending on the type of particles being analyzed some may work better than others, but a baseline is provided for future reference. Results for the same type of particles observed under the microscope could differ if the particles are illuminated by different systems. Light reflection and haze in very angular particles may distort the light, thus making it difficult to detect a well defined shape. Quartz particles are the most challenging particles to digitize because of the problem explained. The illumination systems explained on this section were found to be very useful to minimize image artifacts and to get better quality images.

2.1.1.1 Koehler Illumination

The Motic AE31 comes with a Koehler Illumination System with a 6V-30W quartz halogen lamp (Figure 4). It requires proper set up of the microscope for obtaining the best possible specimen image. It has an externally operated mechanism for control of all facets of illumination, and is adjusted with rack and pinion mounted on the illumination pillar. The Extra Long Working Distance (ELWD) condenser with an aperture diaphragm and a Numerical Aperture (N.A.) of 0.30 provides a working distance of 7.2 cm and is suitable for 4X to 40X objectives. Koehler illumination provides very even specimen illumination from the back for both visual and photo microscopy. The way and type of
light radiation that illuminates the sample(s) can be altered by using the filters. The following came with the system: 45mm Blue, Green interface (546nm) and Ground glass (used as a light diffuser).

Figure 4. Koehler Illumination System on Motic AE31 Microscope

2.1.1.2 Ring Light Illumination System

Fiber optic ring light provides 360º of uniform, high intensity, on-axis illumination (Figure 5). It is effective in minimizing shadows because light reflects off the object in a 360º arc. When used with a polarizer set, ring lights are also effective solutions for minimizing specular reflection and glare.
2.1.1.3 Double-Pipe Light Guide System

This system offers a high degree of adjustable, interference free and uniform illumination of focused white light (Figure 6). Standard stainless steel obedient sheathing provides light where it is needed.
2.1.1.4 Brightfield Incident Illumination System

This type of illumination has been adapted to the Motic AE31 microscope due to the need of a homogenous source of illumination from beneath the sample(s). It uses one of the fiber optic pipe light guides described above, which is inserted on a lamp adapter located on the back of the microscope (See figure 7). Light is directed to a chroma brightfield filter, which reflects light to sand particles or any other specimen through the microscope objective lens. Then it is returned by either specular or diffused reflectance. The light diffuser screen, placed on the lamp adapter, is used to alter light distribution. It can be removed from the optical pathway to project a more concentrated light beam. Sometimes, when the diffusion screen is utilized to spread light from the light source, the overall intensity of light reaching the specimen is often not enough and could lead to a significant amount of image noise. This source of illumination has the advantage of providing a constant, even and homogeneous form of luminosity. If the specimen is opaque and cannot transfer light, this is the only choice of light that will provide good quality images.
Figure 7. Schematic of the Brightfield Incident Illumination System
[Based on Motic AE30/31 Brochure]

Ring Light Illumination System and the Double-Pipe Light Guide System are easily connected to the TechniQuip FOI-150 Fiber Optic Illuminator. It provides up to 150 watts of uniform illumination of white light. The front light output also permits adding a holder for Schott glass filters and the on/off switch allows fine adjustment of light intensity when the illuminator is activated.

The illumination alternatives suitable for 2D imaging are Ring Light, Koehler and Brightfield Incident Illumination. The ways these options illuminate the particle enhance the contrast between background and particle edge. It is recommended to try and compare results before starting a massive acquisition of images. Depending on the nature and type of sand being analyzed, one illumination may work better than others.
2.2 Software

The Operative System installed in the computer is a Microsoft Windows XP Professional Edition. Image-Pro Plus is a powerful image analysis software package for materials imaging, quality assurance, and various other scientific, medical, and industrial applications. It includes extensive enhancement and measurement tools and allows the user to write application-specific macros and special add-ins.

Three add-in packages from Media Cybernetics have been used in conjunction with Image-Pro Plus version 5.1: Scope-Pro 5.0, SharpStack 5.0 and 3D Constructor 5.0. Scope-Pro automates the use of a digital camera and the movement of a microscope and/or stage. It can control complex microscopy equipment in a simple, repeatable manner for reproducible results with minimum intervention from the operator. It reduces the need to place one’s eye to the ocular during image acquisition and virtually eliminates the need to manually adjust microscope controls. The automated repetitive routines result in better quality control and increased efficiency. SharpStack 5.0 helps remove haze and improve resolution in two- and three-dimensional image stacks using deconvolution and deblurring algorithms. 3D Constructor 5.0 helps the user to perform extensive 3D visualization, measurements and three-dimensional relationships within and among objects. All this software is fully compatible with the hardware mentioned before.
CHAPTER THREE: GENERAL BASE MODEL FOR TWO-DIMENSIONAL DIGITAL IMAGING OF GRANULAR PARTICLES USING IMAGE-PRO PLUS VERSION 5.1

3.1 Introduction

The material in this section is based upon tools, functions and routines found on Image-Pro Plus (IPP) plus add-ins Scope-Pro5.0, SharpStack 5.0 and 3D Constructor 5.0, from Media Cybernetics, Inc. and various microscope hardware devices available for this research. Although IPP does not carry out all the tools needed for image processing and 3D reconstruction, it does offer a comprehensive list of features that were helpful for this work.

Convenient characteristics of IPP include the possibility to adapt its interface to specific needs plus the capability for the user to automate routine procedures, add variable definitions as well as flow control statements which can interact with the operator.

Thanks to the Auto-Pro scripting facilities and some programming code publicly available and borrowed from the Solutions Zone at the Media Cybernetics website, several macros were implemented to facilitate edge recognition and to speed up the process of 2D image characterization of angular particles. They were stored as script files and can be called individually within IPP whenever needed. The script file explained in
this chapter is named “GET2DSHAPE.SCR”. The source code for this file is located on Appendix A.

3.2 Description of the Process for Two-Dimensional Characterization of Angular Particles Using Image-Pro Plus Version 5.1

As mentioned before, IPP Auto-Pro scripting facility allows the user to automate routine procedures, customize its interface, and perform other programming manipulation. IPBasic is the language in which IPP macros are written and read. Commands on the IPBasic component of Auto-Pro are a subset of the BASIC language, and conform to Visual Basic™ syntax.

When a macro is recorded, Auto-Pro functions transform a sequence of actions and write those instructions to a script file. A script file is a collection of macros. When needed, macros can be called from the selected script file, and at that time, the coded instructions are interpreted and executed. This is of great help when a series of repetitive steps for image manipulation and analysis have to be performed for each particle. In order to create more robust and powerful routines, some commands that add variable definitions, manipulate strings, branch under certain conditions or loop when required, have to be added and edited into the macro.

A script file can be loaded on IPP by selecting from the Macro menu, the Macro command. When the Macro dialog box is presented, the button Change is used to select the script file from any folder on the system. The OK button will load the script into
memory and the macro(s) within the script will be available at the end of the *Macro*
menu.

The script explained here has three macros denominated as:

1) *Acquisition*

2) *EdgeDetec*

3) *MakeBIN*.

The first macro, *Acquisition*, guides the operator in a successful generation and storage of series of successive digital images of sand particles, acquired at different focal planes. For each particle, the stack of images obtained is saved as one sequence file. It is recommended that, immediately after the sample is placed on the microscope, this routine is executed for the number of particles that the operator intends to acquire. Long time exposure of the glass slide holding the samples has the risk of dust particles suspended in the air to get attached to the surface. These particles may create image noise and that could affect the quality of the images.

The *EdgeDetec* macro is intended to improve efficiency and minimize errors by automating most of the image processing for 2D characterization. The operator has the choice to set several parameters according to the settings and conditions. This macro comprises a chain of functions and algorithms that can deblur and deconvolve a sequence, process the sequence for edge enhancement, and generate several alternatives for evaluation. In that way, the operator can see which optimized image(s) are best suited for the creation of a binary file that represents that specific particle shape.
*MakeBIN* is the macro that converts the area defined by the trace of the boundary detected, into a binary image (Black and White).

### 3.3 Sample Preparation

The user has to place few particles of the same type of sand on a very clean microscope glass slide. Ideally, most of the particles should not be in touch with each other. After carefully placing the slide on the microscope X-Y mechanical stage, the operator may observe the particles through the eye pieces of the microscope and use micro tweezers to carefully isolate particles touching each other on the surface of the slide.

Note that glass slides, microscope and camera lenses should be cleaned using compressed gas duster, an optical cleaning spray and optical lens wipes formulated for optical instruments.

Samples can be observed through the eye pieces of the microscope. But the *Video/digital capture* command (located on the *Acquire* menu), has a *Preview* screen that lets the user monitor the video as the mechanical stage is manually adjusted to place a sand particle into full view.
3.4 Microscope Settings

3.4.1 Microscope Objectives

The objectives used for this research are denominated as *Plan Achromats*. *Achromatic Objectives* are the least expensive and most commonly used. They are corrected for axial chromatic aberration in blue (about 486nm) and red (about 656nm) wavelengths, which are brought into a single common focal point. At the same time, they are corrected for spherical aberration in the color green (546 nm). Better images are obtained when the light passes through a green filter (or interference filter) and using black and white photo microscopy. In addition, Plan Achromat objective lenses provide corrections for field curvatures and give low distortion. But in general, they sacrifice a considerable amount of free working distance. Issues about spherical aberration are discussed on section 3.5.2.2.

<table>
<thead>
<tr>
<th>Description</th>
<th>Type</th>
<th>N.A.</th>
<th>W. D. (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long Working Distance Plan Achromat</td>
<td>4X ∞</td>
<td>0.1</td>
<td>23.5</td>
</tr>
<tr>
<td></td>
<td>10X</td>
<td>0.25</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>LWD 20X ∞</td>
<td>0.4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>LWD 40X ∞</td>
<td>0.6</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Figure 8. Characteristics of Motic Objective Lenses Used for the Motic AE-31 Inverted Microscope
[Source: Motic AE30/31 Brochure]
The revolving side facing nosepiece on the Motic AE31 can hold up to five objective lenses. It is ball bearing mounted and provides effortless objective changes.

For sand particles tested so far, with mean diameters between 75 μm and 2.0 mm, only the 4X, 10X and 20X objectives are commonly used.

Microscope magnification objectives should be selected according to particle size. Each time a particle is selected individually for recognition, it has to be shown entirely on the screen and should fit nicely on the available capture area of the camera. The maximum area possible on the Evolution VF camera is 1392x1040 pixels, which is an adequate resolution for this application.

In order to increase the particle size that can be captured by the camera, a C-Mount Adapter 0.65X was placed between the microscope and the digital camera. Sizes of objects seen through this adapter are decreased by 35%. This is greatly useful in case of large sand particles, which normally may not fit in the camera screen area, even with a low power (4X) objective.

### 3.4.2 Electromagnetic Spectrum, Optical Filters and Digital Photo Microscopy

Sources of natural and artificial light emit wavelengths that cover the visible light spectrum. Sometimes they go beyond and cover the ultraviolet (UV) and infrared (IR) regions as well. Note from figure 9, that visible light ranges from around 400 nanometers (violet) to 700nm (red). Near Infrared (NIR) light consists of light just beyond visible red light (wavelengths 750 to 2500nm).
Some objects reflect infrared radiation (they look bright on IR photography) and some absorb it (they look dark). For example, water absorbs infrared radiation and human flesh and foliage reflect it.

![The Electromagnetic Spectrum](http://acept.la.asu.edu/PiN/rdg/color/color.shtml)

Figure 9. The Electromagnetic Spectrum
[Source: http://acept.la.asu.edu/PiN/rdg/color/color.shtml]

Filters are constructed in a wide variety of shapes and physical dimensions. They are employed to reduce, limit, reflect, refract, diffract or absorb wavelengths bands of the electromagnetic spectrum at which they are exposed.

In Black and White (B&W) photomicrography, filters are used to control contrast in the final image captured on a Charge Coupled Device (CCD) digital camera system. Filtration techniques for B&W are significantly different from those employed in color.

CCDs are light-sensitive devices used on digital photographic and video cameras, scanners and other optical devices. A CCD chip is a solid-state electronic component made from a silicon wafer which has been micro-manufactured and segmented into an array of individual light-sensitive cells called "photosites". The number of photosites in the horizontal and vertical direction gives the resolution of the camera.
Photosite cells sense incoming light and are capable of producing various amounts of electronic charge in response to the amount of light they receive. The CCD photosites accomplish their task of sensing incoming light through the photoelectric effect, which is a characterization of the action of certain materials to release an electron when hit with a photon of light.

In a B&W digital camera, the hardware converts the information from analog to digital, and records the image electronically by producing a file of encoded digital information in which bits represent tonal values on the gray scale.

The Evolution VF camera has a Sony CCD sensor chip model ICX205AL, which can “see” from about 280nm (estimated) to 1000nm according to the Relative Response versus Wave Length (nm) graph shown in figure 10.

Figure 10. Spectral Sensitivity Characteristics for the Sony ICX205AL Chip
[Source: Sony Technical Paper “Diagonal 8mm (type ½) Progressive Scan CCD Image Sensor with Square Pixel for B/W Cameras]
There are a series of filters called opaque filters that allow only infrared light to pass through them. The 87 series Wratten filters from Kodak are one of the most popular on the market. These filters are excellent for scientific work and in pictorial photography. They give the most infrared look and stop all other light from reaching the CCD on the camera.

The term Wratten is derived from and English inventor and manufacturer of filters in the 1870s, Frederick C. Luther Wratten. Kodak bought the Wratten Company in the 1920s. Photographers and filter manufacturers now use the Wratten ratings as a very accurate way of specifying the spectral characteristics of filters, regardless of the manufacturer.

Filters obtained and tested in this research were the Kodak #87 Wratten IR Pass Gel filter and the Schott BG40 1mm Glass IR/UV cut filter. The BG40 glass filter allows only visible light to reach the CCD. Additionally, a gelatin polarized filter can be used in combination of the BG40. When a polarized filter is used in front of the camera, it reduces reflected glare off of the surface of the particles. In color cameras, Wratten 87 filters deliver a grayscale because the 87's 50% cutoff at around 800nm blocks both visible light and most of the color-generating shortwave NIR below 770 nm.
SharpStack 5.0, an image-stack deblurring and deconvolution software add-in for IPP is used for image improvement. It requires the user to input a value for the peak wavelength emission illuminating the sample. The peak wavelengths that reach the Evolution VF CCD chip using the Kodak Wratten 87 filter and the Schott BG40 were estimated by superimposing the spectral sensitivity graph for the Sony ICX205AL chipset (figure 10) and the transmission spectra for both filters (figure 11). The peak wavelength that reaches the CCD chip using the Kodak Wratten 87 filter is estimated at 775 nm (figure 12). The peak wavelength that reaches the CCD chip using the Schott BG40 filter is estimated at 510 nm (figure 13).
Figure 12. Estimation of the Peak Wavelength Captured by the Evolution VF Camera Using the Kodak #87 Wratten IR Pass Gel Filter

Figure 13. Estimation of the Peak Wavelength Captured by the Evolution VF Camera Using the Schott BG40 IR/UV Cut Filter
3.5 Exploratory Routine Implemented for 2D Characterization of Angular Particle Using Image-Pro Plus 5.1

The script created is named GET2DSHAPE.SCR. The source code is available on Appendix A. Information on how to load a script file is mentioned in section 3.2.

This script has three macros: Acquisition, EdgeDetec and MakeBIN. In this section, a brief description of the chain of steps contained in each macro is explained. Step by step, basic theoretical background for functions and commands from IPP is also included. The intention is to illustrate to the reader the method proposed for two-dimensional imaging of granular particles.

3.5.1 Acquisition of Image Stacks

The Acquisition macro is a simple routine which opens and sets the Digital Capture Dialog and the Scope Pro add-in. The operator is asked to locate sand particles by repositioning the mechanical X-Y stage so they appear on the Live Preview screen (Figure 14). Also, the operator must make proper adjustments such as focusing and setting the proper illumination. By clicking on the Auto-Set button, the software will reset the driver to the factory results and then an auto exposure time will be calculated considering actual illumination conditions. By default, the live preview and capture areas are set to full frame, which are 1392x1040 pixels. The auto-exposure time is set to be calculated on every frame. These settings have been stored as a text file named Camsettings.vpf in the default folder C:\IpWin5\Documents and Settings. Then, Scope-
Pro is called and the user is prompted to choose a setting from recorded calibrations. Calibrations are available for 4x, 10x and 20x magnifications under the names 4x_0.65.scp, 10x_0.65.scp and 20x_0.65.scp respectively. The *.SCP files contain Scope-Pro settings in text format and are saved by default in the folder C:\IpWin5\CamCalibration. The operator must load the settings according to the objective lens used.

![Figure 14. Illustration of the Acquisition Process of Image Stacks on Image-Pro Plus](image)

The operator has to adjust the Top and Bottom limits using the Prior ProScan II controller before acquiring a set of images at different focal planes. The top limit is the upper plane of the particle which can be seen from the bottom. The lower limit is the very
bottom plane of the particle in contact with the glass slide. The default number of frames for each sequence was set to be 25. This means that Scope-Pro will divide that distance (on the Z axis) between the limits in 25 constant intervals. Human error and operator dependency is inherent when the user is trying to determine which plane is exactly top or bottom. That is why it is recommended to go a little bit beyond focus for both top and bottom limits of the particle(s). Although this does not save time, it is more accurate since the Extended Depth of Field (EDF) process applies algorithms to obtain a composite image showing all parts in focus (refer to section 3.5.2.3 for more information about the EDF process).

![Figure 15. Tile Images for a Rhode Island Sand Particle Obtained at Different Focal Planes Using Scope Pro 5.0. Scale Not Shown](image.png)
The *.TIF and *.SEQ are the two formats in which an image set can be saved after a series of images have been obtained. The *.TIF file extension, Tag Image File format is a flexible and very popular format for archiving high quality images. The standard TIFF is the most universal and most widely supported format across all platforms including Windows, Mac and UNIX. TIFF supports most color spaces (RGB, CMYK, YCbCr, etc), multiple images, thumbnails, and image directories or tags with information about the image. TIFF can support data up to 48 bits.

Image-Pro supports single-frame or multi-frame TIFF image documents (or workspaces) with the *.SEQ or *.TIF file extension. Image-Pro supports all known varieties of the TIFF file format, including most multiple-image varieties. Products from Media Cybernetics may write one or more proprietary TIFF tags along with the images(s). However, some other software packages may not recognize TIFF files or may not read all the information within images.

Files with the extension *.SEQ are handled by Image Pro and some functions require image sequences to use this exclusive format. It is recommended to use this format during the image processing and to save the final binary images with the TIFF or BMP extension.

3.5.2 Imaging Processing for Edge Enhancement

The *EdgeDetec* macro prompts the user to open a sequence file. The file extension used on a sequence of images could be “*.TIF” or “*.SEQ” format. This macro will be divided and explained according to the following steps:
3.5.2.1 Selection of an Area of Interest from an Image Sequence

IPP includes an Area-Of-Interest (AOI) tool which is used to isolate designated areas from the rest the picture or to define a portion of an image. An AOI can be defined in any shape (available tool buttons can be used to define a rectangular, elliptical or freeform shape). When an AOI is active in an image, many commands on IPP will affect only the pixels constrained by the AOI. Some other commands, for example *Save* and *Color Segmentation*, will always operate upon the entire image.

It is recommended to reduce the entire area of the sequence to a smaller region of interest where the particle can be isolated. The macro will activate a rectangular AOI where the user can adjust its size to confine the particle. It is necessary that the user leaves enough space in the top left corner of the rectangular AOI. That space is reserved for a text box that will be displayed after the Extended Depth of Field (EDF) function is applied to the sequence. After defining the rectangle, that area will be extracted and will be the default workspace for future processing.
3.5.2.2 Process of Deconvolution Using SharpStack 5.0

Media Cybernetics SharpStack version 5.0 is the first image processing package applied to the image-stack. SharpStack employs routines involving haze removal and restoration techniques used to sharpen and clear image sets by applying deblurring and deconvolution algorithms.

Deconvolution algorithms are mathematical procedures, specifically high pass filtering, used for a systematically removal of noise and out-of-focus haze and blur for three dimensional data obtained from a set of image planes. It is very important to have consistent illumination and low noise conditions. External variations can dominate the results and hide or distort the reconstruction and errors may get amplified.

Given a specific application and depending on the image set selected for improvement, SharpStack offers one deconvolution technique based upon blind deconvolution concept (Inverse Filter), and two deblurring techniques (No Neighbor, Nearest Neighbor). SharpStack must be applied to monochrome images and image sets. Table 2 guides the user to determine which alternative is most appropriate and shows some characteristics for each method:
Table 2. Characteristics of Methods Available In Media Cybernetics’ Sharpstack 5.0 for Image Deconvolution

[Source: SharpStack Version 5.0 User Guide]

<table>
<thead>
<tr>
<th>Brightfield/Phase</th>
<th>Nearest Neighbor</th>
<th>Inverse Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td>May be used to physically remove haze from a single image plane.</td>
<td>Best method to remove approximately haze from image stacks containing fewer than five image planes. Useful for thinner volumes as there may not be enough information to construct a proper point spread function.</td>
</tr>
<tr>
<td>Single Plane</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>3-5 Planes</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>&gt;5 Planes</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quick Inspection</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

The easiest type of algorithm to use would be the Nearest Neighbor algorithm, which can handle a minimum of three slices. This algorithm uses images above and below the image plane of interest to estimate the amount of blurring in the central frame. It has the advantage of being very fast and yielding good results but it is sensitive to illumination variations. It can be used with all magnifications.

The Inverse Filter will yield more accurate results, but requires many more slices and more computational time (Agard, 1989). This algorithm uses a Point Spread Function (PSF) to determine the amount of blur in the image volume. Then it deconvolves the entire PSF to deblur the center object. Several trial images enhanced with this technique presented edges that were easier and faster to trace. Although the Inverse Filter method requires more computational time and memory as it performs Fourier Transforms in all three dimensions, it is the default option for this macro. On the
other hand, there is always the flexibility to change the options according the type of application and judgment of the operator. This algorithm works with 10x magnifications and beyond.

Figure 16 shows an example of the different options and settings available for SharpStack v 5.0. Each option is described below.

Figure 16. SharpStack 5.0 Window

Type: Drop-down with three options available: No Neighbor, Nearest Neighbor and Inverse Filter (default option).

NA: Type the Numerical Aperture for the objective lens used to acquire the image. This number indicates the numerical aperture of the lens used in capturing the images.
Numerical Aperture is an index of the ability of an objective lens to resolve fine detail at a fixed object distance. It is defined as a measure of the light gathering capacity of the lens system and determines its resolving power and depth of field. It can be mathematically expressed as the sine of the refractive index of the medium between objective and specimen (value that ranges from 1.00 for air to 1.51 for specialized immersion oils) times one-half the angular aperture (Equation 1). As the numerical aperture is varied, the size and shape of the illumination cone entering the objective front lens is altered, as shown in figure 17.

\[ NA = RI \sin\left(\frac{1}{2} \alpha\right) \]  
(Equation 1)

Where:
- \( NA \) = Numerical Aperture
- \( RI \) = Refractive Index
- \( \alpha \) = Angular aperture

\[
\begin{align*}
NA &= RI \sin\left(\frac{1}{2} \alpha\right) \\
RI &= 1 \text{ (for Air)} \\
\alpha &= 30^\circ \\
NA &= 1.0 \sin (15)^\circ = 0.25 \\
\text{Magnification} &= 10x \\
\end{align*}
\]

\[
\begin{align*}
NA &= RI \sin\left(\frac{1}{2} \alpha\right) \\
RI &= 1 \text{ (for Air)} \\
\alpha &= 47.40^\circ \\
NA &= 1.0 \sin 23.6^\circ = 0.40 \\
\text{Magnification} &= 20x \\
\end{align*}
\]

Figure 17. Demonstration of Change in Numerical Aperture for Two Objective Lenses in an Inverted Microscope with Magnifications: a) 10x and, b) 20x
**Refractive Index:** Indicates the refractive index of the immersion media used to couple the objective lens to the specimen. Five choices are available: Air (1.0), Water (1.33), Glycerin (1.47), Oil (1.515) and Custom. When any of these options is selected, the adjacent refractive index value will be automatically updated. Air is the default option since “dry” microscope objectives were used.

**X Spacing** (horizontal) and **Y Spacing** (vertical): These values are automatically displayed and represent distances in pixels per microns. They were obtained from information stored in the image sequence. It is noted that the image has been previously calibrated according to the objective lens used, and all spatial calibrations were done in micrometers.

**Z spacing:** This value is automatically displayed and obtained from the directory information of the image sequence. When acquiring different Z planes of a particle, Scope-Pro calculates this Z distance measured in microns between images.

**Process in Montage:** The check in this box allows SharpStack to break the image volume into smaller sub regions to be deconvolved. Then it is reassembled into the complete volume. This option may speed up the algorithm and processing time. Inverse Filter requires up to five times the amount of memory used for the image set because virtual volumes are created during the processing. This is helpful to overcome limitations in Microsoft Windows ®, which does not allow the use of large contiguous blocks of RAM memory. But some images may produce discernible discrepancies between those
smaller sub regions. The user must pay attention and inspect deconvolved image sets for any defects that may distort the representation of any particle shape.

Overlap: If Process in Montage is applied, the resulting blocks must be reassembled into the original image volume. The potential of appearance of image artifacts is reduced when the blocks are allowed to be positioned by assigning an overlap value, from 1 to 100 pixels. This option may be ignored if the image is small enough and Process in Montage is not necessary.

Bright field and Phase Objects: None of these options are selected. The user may try and compare these options and see if there is any improvement on image quality for certain types of sand particles.

Convert results to floating point: Converts the resulting image into the floating point format. This option creates a better quality image.

Spherical Aberration: Spherical aberration causes the specimen image to appear hazy or blurred and slightly out of focus. Its effects are evident when the center of the image is more in focus than the edges, and the intensity of the edges falls relative to that of the center. The button “Calculate SA” calculates and displays results for the “Spherical Aberration” constant. When the checkbox “Use accelerated spherical aberration calculation” is activated, the calculation speed increases but some accuracy is sacrificed. For several images of particles tested, this option does not show discernible effects.

Spherical aberration occurs in a lens with hyperbolic curvature on its face(s), because light rays passing on the periphery are greater refracted. The lens focuses to different points, depending on the angle with the central axis, instead of focusing all rays
to a specific point. Therefore, peripheral rays are brought to a focus closer to the lens than those passing closer to the axis. Illustrations a, b and c on figure 18 are an exaggerated view of hypothetical light rays passing through a biconvex lens. Refraction of the peripheral rays is greatest followed by those in the middle, and then shows little effect on the rays in the center. Note how spherical aberration varies with lens shape.

Table 3 shows the corrections for optical aberrations for different type of objectives.

Table 3. Corrections for Optical Aberration of Different Types of Microscope Objectives
[Source: http://www.microscopyu.com/articles/optics/objectiveintro.html]

<table>
<thead>
<tr>
<th>Objective Type</th>
<th>Spherical Aberration</th>
<th>Chromatic Aberration</th>
<th>Field Curvature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achromat</td>
<td>1 Color</td>
<td>2 Colors</td>
<td>No</td>
</tr>
<tr>
<td>Plan Achromat</td>
<td>1 Color</td>
<td>2 Colors</td>
<td>Yes</td>
</tr>
<tr>
<td>Fluorite</td>
<td>2-3 Colors</td>
<td>2-3 Colors</td>
<td>No</td>
</tr>
<tr>
<td>Plan Fluorite</td>
<td>3-4 Colors</td>
<td>2-4 Colors</td>
<td>Yes</td>
</tr>
<tr>
<td>Plan Apochromat</td>
<td>3-4 Colors</td>
<td>4-5 Colors</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Deconvolve:* This button applies the Inverse Filter algorithm to the whole image stack.

*Save Settings:* Saves the current settings for use with similar images in the future. These settings are saved with as a file with extension *.DCS.*

*Open Settings:* Click this button to open deconvolution parameter settings.
Figure 18. Effect of Spherical Aberration on a Diatome Frustule According to Lens Shape: a) Minor Effect, b) Medium Effect and c) Mayor Effect
[Source: http://micro.magnet.fsu.edu/primer/java/aberrations/spherical/]
3.5.2.3 Obtaining an Extended Depth of Field Test Strip for a Sand Particle

One limitation of the microscope hardware available is that the viewer can not observe an actual shape of the particle at high resolutions. Parts above and below the focus plane fall outside the depth of field of the objective lens and do not look sharp. To overcome this limitation, the use of an extended depth of field or projection must be employed to show the overall shape of particles.

In this particular case of shape characterization of angular particles, the actual two-dimensional shape obtained is really a projection of three-dimensional particles displayed in a two-dimensional format.

The Extended Depth of Field (EDF) method requires several images of an object at different focal planes. This is done by adjusting the height on the focal axis at which each image is captured. Later on, those images are combined and merged into a single, in-focus, composite image that shows in a well defined manner, all zones of the particle that would normally lie outside a single plane of focus. The EDF tool on IPP provides speed and a high level of accuracy that facilitates the entire process of image characterization.

Immediately after the image sequence has been deconvoluted, the EdgeDetec macro calls the EDF feature and generates a Test Strip. Due to the nature of shapes, mineralogy and topography of sands, it is necessary to test various combinations of the parameters on the EDF algorithms to see which results are best suited for edge recognition on specific particles.
Brief descriptions, borrowed from the IPP 5.0 Reference Manual, for different EDF settings which were tested and implemented in this chapter are presented next.

1) Order Options:

   *Stack Order, Top Down*: The first image at the top of the list will be the first image in the attack and the bottom image the last.

   *Stack Order, Bottom Up*: The image at the top of the list will be the last in the stack, and the image at the bottom for the list will be the first image in the stack.

   The order of the images may affect which pixel is selected for some of the focus analysis operations.

2) Output Options:

   *Composite Best-Focus Image*: This output option generates a composite image using the best pixels analyzed from a series of images. This is the default option.

   *Return Best-Focus Frame*: this option creates a new work space containing a copy of the selected frame with the most in focus material.

   This option is not suitable for objects such as sand particles.

3) Focus Analysis Options:

   *Normalize Illumination*: This option corrects uneven illumination or light intensity emitted by the source as the Z planes changes.

   *Maximum Local Contrast*: Areas with high/maximum contrast are placed in focus. Through out the series of images, pixels around a
current location are analyzed and the one with largest variance or local contrast is selected.

Maximum Through-Depth Contrast: The mean values of the pixels are calculated. Pixels from planes with the largest variance are selected. This option is intended for transparent objects.

Maximum Intensity: It is applicable for objects that are more intense than the background. Pixels from different planes with highest intensities are selected.

Minimum Intensity: It is used for regions that are darker than the surroundings are placed in focus. Pixels from planes with lowest intensities are selected.

A Test Strip automatically generates a series of images representing different settings, output options and various focus analysis for the (EDF) function. The total number of combinations shown in a Test Strip sequence is of 16 images. Table 4 shows the categories combined on a Test Strip. Figure 19 shows individual frames of a Test Strip for a Rhode Island sand particle. As mentioned before, the EDF function generates a label on the upper left corner for each frame of the sequence. Note that not all images on the EDF test strip result in a nice composite image.
Table 4. Settings and Combinations Generated in an EDF Test Strip Sequence. In Total, 16 Frames Represent all Possible Combinations of Settings for Each of the Output Options (Best-Focus Image or Best-Focus Frame)

<table>
<thead>
<tr>
<th>Stack Order</th>
<th>FOCUS SETTINGS</th>
<th>Focus Analysis Options</th>
<th>Normalized Illumination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top Down</td>
<td>Maximum Local Contrast</td>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Bottom Up</td>
<td>Maximum through-depth contrast</td>
<td>Maximum Intensity</td>
<td>No</td>
</tr>
</tbody>
</table>

Figure 19. Tile Images of an Extended Depth of Field Test Strip Sequence for a Rhode Island Sand Particle. Scale Not Shown
3.5.2.4 Application of Contrast Improvement Techniques for Better Particle Edge Detection

It was intended at the beginning of this research to implement the best illumination possible for sand particles to differentiate the grains from the background. By doing this, edge detection would have been achieved with a minimum amount of digital processing. Standard contrast techniques are good for overall contrast enhancement, but showed no great effect for revealing a detailed contour of sand particles. After several trials, the need for a more complex processing and enhancing technique became evident. Immediately after the EDF function is applied, a copy of the sequence is created. This duplicate will be subjected to further processing and enhancement.

The macro “Local Best Fit Contrast Enhancement” is publicly available from the Solutions Zone website of Media Cybernetics, Inc. It has been successfully implemented in the script after being adapted to the structure of the routine.

This macro applies a combination of morphological (Erosion and Dilation) and enhancement (Gauss) filters to various intensity ranges within the image. According to the description provided by Media Cybernetics: “Local Best Fit Contrast Enhancement uses a combination of the Erosion, Dilation, and Gaussian filters found in Image-Pro, and applies them to the various intensity ranges found within the image to find local contrast. The macro also provides a means of controlling the degree of local contrast that the filter will apply to the image. The macro operates in the following fashion: In the first step an ‘iteration’ value is selected. This value determines the size of the local area to which the
filter will be applied. Based on this information, the second step is performed. Within each local area, the minimum and maximum intensity values are determined using Erosion, Dilation and Gaussian filters. A Best Fit contrast stretch is performed relative to the local maxima and minima - subtracting the minima and scaling by the difference between the minima and maxima. A new image is generated, showing local contrast effects. The resulting image is optionally averaged with the original to provide partial enhancement”.

The Erode filter dilates bright objects, and enlarges dark ones. The Dilation filter dilates bright objects and erodes dark ones. The Gauss filter is used to soften the image by eliminating high-frequency information. It has an effect of blurring sharp edges. To “Average” an image with its original is the arithmetic operation of replacing each pixel with the mean value obtained from the two images.

In order to accentuate the contour of the particle, a Variance Edge Filter is then applied. This filter helps emphasize edges and textures by substituting the pixels with the calculation of the statistical variance of their neighborhood about the mean intensity within a local region. Then it is averaged again with the sequence.

Once image improvement has finished, the operator can evaluate on which image the tracing of particle edge is satisfactory. A simple EDF Test Strip and an improved EDF Test Strip, each hold 16 frames where to choose from. When a frame is tested and elected, the macro MakeBIN is used to convert the image from grayscale to binary (black and white).
Figure 20 shows the frame 1/16 on both simple and improved EDF Test Strip. In this case, the improved frame shows a better trace of particle edge.

3.5.3 Creation of Binary Images of Particle Shapes

The macro MakeBIN is the next step for converting a gray scale image of a sand particle into a binary image that defines a sharp contour of its 2D projection.

Once the user identifies on what frame a nice contour can be traced, the macro converts the active EDF Test Strip from a floating point format to an 8 bit gray scale. The 8 bits gray scale, which can show 256 shades of gray, is a fully compatible format and requires less memory per image. Besides, not all software packages support images on a floating point format. Then, the macro prompts the user to input a frame number to extract from the sequence. After that, the Magic Wand option on the irregular AOI tool
appears, and the operator traces again an outline on the boundary. Now, the macro announces the creation of a binary image. When the operator clicks on the Continue button, the macro uses the Fill command to fill the active AOI with a solid white color. Then, the Threshold/Segmentation tool is automatically called and all the areas of the image with a different shade other than white (0) will be converted to black (255). See figure 21 for illustration. This process is used for separating the particle shape from the background. Now, the operator must save the binary image on a specified folder. Any of the following formats is recommended for a binary image: TIFF (TIF, TIFF) and Windows Bitmap (BMP).

Figure 21. Binary Image of a Rhode Island Sand Particle Created Using the Macro MakeBIN

Figure 22 summarizes the whole operation of the macrons contained on the script GET2DSHAPE.SCR
Figure 22. Diagram of Operation of the Macros Contained on the Script GET2DSHAPE.SCR
CHAPTER FOUR: QUANTITATIVE IMAGE DIGITIZATION OF GRANULAR PARTICLES

One of the goals of the entire project is the creation of a database of image shapes for different types of sands. And for that, it is necessary to process large number of particles. Now that the procedure presented on chapter 3 has shown effective results for edge detection and shape digitization, it is necessary to adjust those techniques to a new level of automation for a faster image processing.

This chapter explains new scripts implemented for 4x, 10x and 20 magnifications. Their source codes are on appendices B, C and D under the names Mult2DShape4x, Mult2DShape10x and Mult2DShape20x respectively. The level of automation in these scripts is planned to dramatically increase the number of particles processed in less time, without sacrificing quality. The macros contained on the scripts require less intervention from the operator because they prompt the user to input certain parameters and apply the same parameters to all the images within a folder. At every stage of the script, the macros ask the user to select folders where to store and open images, specify parameters such as the number of frame to extract from an EDF test strip and the intensity range from an image prior converting to binary. The key to obtain good quality images and reproducible results is to take all the images under the same conditions (type, intensity and position of the illumination system). One of the macros allows the editing of shapes on each image. Here, the user fills with background color all those defective particle shapes before
converting to binary. Once the procedures create binary images, important statistical data can also be obtained from those shapes.

The main differences between the scripts are some of the parameters used according to each magnification but the structure remains the same. All the scripts use the Nearest Neighbor algorithm for the deconvolution process. Logically, with a higher magnification, more resolution can be obtained, but the number of particles captured per image is reduced. The magnification settings are set to be used with the 0.65x C-mount adapter attached to the camera. A comparison of measurements for a sand particle was made using the three magnifications. Results are presented on section 4.1 and they show that the difference in measurements is not significant. This means that the 4x magnification offers satisfying images plus the advantage of acquiring more particle shapes per image.

A brief explanation of the macros contained in the scripts, as well as some sample images are presented next:

_Acquisition._ This macro opens the Digital Capture Dialog and the Scope Pro window. The settings for 4x, 10x and 20x magnifications are set to acquire 15 frames per sequence. The operator spreads several particles on a clean glass slide and then places the sample on the microscope X-Y mechanical stage. The process of acquisition of image sequences should be done in a zigzag manner until the desired area on the glass slide is explored. Figure 23 shows examples of images acquired using this macro.
Figure 23. Pictures Extracted from Unprocessed Sequences of Boca Grande Beach Sand Particles (Cartagena, Colombia) Obtained Using the Macro Acquisititon. Only NIR Radiation Was Captured Under a 4x Magnification and the 0.65x C-Mount Adapter Attached to the Camera.

*EnhanceEdges.* This macro opens all the raw sequences from a specified folder and enhances the images for better edge detection. The deconvolution process applied to the images varies according to the objective lens used. The 4x magnification has to use the Nearest Neighbor algorithm whereas the 10x and 20x magnifications are set to use the Inverse Filter Algorithm. The enhancement process is based on the routines explained in chapter 3, but the process is simplified by applying the same settings to all the image sequences within a folder. This macro:

1) Deconvolves the image sequences

2) Creates Extended Depth of Field test strips,

3) Then applies the Local Best Fit Contrast Enhancement routine (it has been set for 3 iterations) followed by the variance filter plus the respective averaging (refer to the scheme for macro EdgeDetec on
figure 21). The final outputs are Improved EDF test strips. Figure 24 shows examples of images enhanced using this macro.

![Figure 24. Pictures of Improved Frames from Extended Depth of Field Test Strips (Edfts) for Boca Grande Beach Sand Particles (Cartagena, Colombia) Using the Macro EnhanceEdges. Frame Information: Maximum Local Contrast, Normalized Illumination, Sequence Arranged From Top to Bottom.](image)

**Sort_GSIs.** This macro is designed to detect shapes and sort the particles found on an image. The macro requests the user to open a random Edfts and select which frame to extract (it will extract the same frame number on all Edfts within the folder), then opens the threshold window and the user sets the intensity range that define the shape of Gray Scale Images (GSIs) of the particles. The histogram values range from 0 to 255 on the 8 bit grayscale. Once the user selects an appropriate value, it will be applied to all the extracted frames. The macro will detect several shapes and including background noise, but it will filter those shapes that do not match certain area and/or minimum caliper specified on the macro (refer to figure 29 for more information on measurements that can be obtained with IPP). The best way to minimize defective shapes is to set properly a
suitable type of illumination and keep dust out of the lenses, camera and glass slides used. Figure 25 shows examples of particles sorted using this macro.

![Fig 25](image)

*Figure 25. Pictures of Sorted Sand Particles from Boca Grande Beach (Cartagena, Colombia) Obtained Using the Macro `Sort_GSIs`. Even Though Shapes Were Filtered by Area and Minimum Caliper, Some Background Noise Was Recognized as Shapes.*

**Edit_GSIs.** This macro allows the user to edit sorted GSIs of particles on each image. The operator evaluates each image in a folder. Undesired shapes can be removed by via detecting those forms as AOIs and filling them with white color. Before converting to binary, even if the editing process is not perfect, the filtering process will get rid of those shapes. Figure 26 shows examples of edited images prior binarization using this macro.
Figure 26. Edited Pictures of Sand Particles from Boca Grande Beach (Cartagena, Colombia) Using Macro Edit_GSIs. Undesired Shapes Are Marked as AoIs and Filled White by the Operator. Although Some Defective Shapes Are Still Present, They Will Not Pass the Filter Settings for Area and Minimum Caliper Prior Becoming Binary Images

MakeBINI_C_M. This name stands for Make Binary Images plus Count and Measure. This macro converts to binary all those shapes recognized and edited on previous macros, and then it saves the binary images in a specified folder. Figure 27 shows that only useful shapes are shown on the final images. After converting to binary, several measurements can be obtained from those shapes. IPP has an interesting Count and Measure tool that can give useful measurements for all those shapes recognized in a text format, which can later be opened in a spreadsheet. This macro will also include a file called SORTED_BINDATA.txt in the folder containing the following measurements: perimeter, area, roundness, radius (maximum, minimum and average), diameter (maximum, minimum and average), axis (major and minor), aspect ratio, caliper (maximum, minimum and mean), fractal dimension, perimeter of the equivalent ellipse and perimeter of the convex outline of the object. Figure 28 shows diagrams and a brief
explanation of the measurements that are included in this text file. Appendix F shows a comparison of measurements for a Rhode Island particle shape obtained under different magnifications.

Figure 27. Binary Images of Shapes of Sand Particles from Boca Grande Beach (Cartagena, Colombia) Using Macro MakeBINI_C_M.

<table>
<thead>
<tr>
<th>Perimeter</th>
<th>Area</th>
<th>Roundness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of the object's outline. More accurate than previous version. Old version now called Perimeter2.</td>
<td>Area of object. Does not include holes option is turned off.</td>
<td>(perimeter&quot;2&quot;) / (4 * pi * area). Uses &lt;Perimeter2&gt; and &lt;Area&gt; by default. Select &lt;Perimeter&gt; and &lt;Area (polygon)&gt; for more accurate roundness.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Axis (major)</th>
<th>Axis (minor)</th>
<th>Aspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of major axis of ellipse with same moments of order 1 and 2 as object.</td>
<td>Length of minor axis of ellipse with same moments of order 1 and 2 as object.</td>
<td>Ratio of major axis and minor axis of ellipse equivalent to object.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Feret (linear)</th>
<th>Feret (min)</th>
<th>Feret (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longest caliper (linear) length.</td>
<td>Smallest caliper (linear) length.</td>
<td>Average caliper (Feret) length.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Radius (max)</th>
<th>Radius (min)</th>
<th>Radius Ratio</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Fractal Dimension</th>
<th>Perimeter (ellipse)</th>
<th>Perimeter (convex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fractal dimension of the object's outline.</td>
<td>Perimeter of the equivalent ellipse.</td>
<td>Perimeter of the convex outline of the object.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diameter (max)</th>
<th>Diameter (min)</th>
<th>Diameter (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of longest line joining two points of object's outline and passing through the centroid.</td>
<td>Length of shortest line joining two points of object's outline and passing through the centroid.</td>
<td>Average length of diameters measured at 2 degree intervals and passing through object's centroid.</td>
</tr>
</tbody>
</table>

Figure 28. Description of the Measurements Obtained from Binary Shapes Using Media Cybernetics’ Image-Pro Plus
CHAPTER FIVE: THREE-DIMENSIONAL SHAPE RECONSTRUCTION OF GRANULAR PARTICLES: REVIEW OF SOME ALTERNATIVES

Shape reconstruction in three dimensions for small particles is not an easy task. It is a tedious and very time consuming process. Extensive research has been done in the fields of biology, medicine, paleontology and material sciences. This work has gathered information about three methods available: X-Ray NanoTomography Systems, 3D reconstruction from images acquired at incremental focal planes and Serial Sectioning.

5.1 X-Ray Nano Tomography

This exciting technology provides fast and easy collection of tomographic data sets for 3D reconstructions. Now days, with the use of nano technology, machines have the possibility of obtaining higher resolutions than on previous models. It has become a powerful tool for examining particles in three-dimensions.

Thanks to Micro Photonics, Inc., we were able to obtain several images of a Rhode Island sand sample. Unfortunately, at the time the sample was sent, only X-Ray microtomographs were available on the market. Figures 29 and 30 show images taken with the Skyscan 1072 micro-CT. The resolution is poor (+/- 5 µm at low contrast) and the fact that particles are in touch with each other makes it difficult to obtain an accurate 3D reconstruction. The latest model is the Skyscan Nano2011 High-Resolution Nano-CT.
The smallest detail available on this model has a resolution of 400nm at low contrast.

Both equipments do not require any special specimen preparation.

Figure 29. Sample Images of Rhode Island Sand Particles Obtained with a Skyscan 1072 Micro-CT
Figure 30. Tile Images of Rhode Island Sand Particles Obtained with a SkyScan 1072 Micro-CT. Space Interval between These Sample Images Is 35um
5.2 3-D Reconstruction from Images Acquired at Incremental Focal Planes

This is another nondestructive method in which an image of an object is acquired and stored every time the distance between the object and the objective is constantly changed (at known intervals). Then a “shape from focus” (Nayard, 1989) can be obtained. There are other programs for different operative system platforms and with different reconstruction schemes, algorithms and levels of performance that are used for both the calculation of a sharp composite in focus image of the particle and its three dimensional shape from image sets at different planes of focus. These methods are widely used in with satisfactory levels of success in fluorescent microscopy and objects acquired in bright field environments.

For our study, we had no success in obtaining satisfactory 3D reconstructions by using 3D Constructor on Image-Pro Plus. The excess of glare, haziness and blur from sand particles such as quartz, makes it impossible for the algorithms to detect depth for some regions on the surface of the particle. Also, it is necessary to acquire images from at least three views to get a detailed 3D composition of the particle. 3D Constructor, can not handle a volume reconstructions from three views (top, bottom and one side) but only from two (top and bottom). This can be done by acquiring a set of images from one side, then flipping the particle 180° and then taking another set of images. After the process of acquisition is finished, a macro can arrange both sets of images and use 3D Constructor for visualizing the volume. It is unpractical to reconstruct particles one by one using this method. It not only would require a high level of precision to flip and locate the particle in the right position, but also the process would be extremely slow.
5.3 Serial Sectioning

This is a destructive process, which means that particles will be disintegrated for the purpose of their 3D characterization. In this method, a series of thin slices of known thickness are removed successively from the particles, which are inlaid in a hard medium. The removal process of thin slices is done with a very fine and precise polishing machine. At each interval of the removal process, a 2D image of the cross-section is acquired and stored (See figure 31).

Figure 31. Representation of Cross Sections during Serial Sectioning. Images Are Acquired After Each Pass on the Polishing Machine
By using those 2D images to obtain X and Y coordinates, and by knowing the depth at which each image was obtained, 3D reconstruction can be performed via software (See figure 32).

![3D reconstruction diagram](image)

Figure 32. Representation of A 3-D Reconstruction of a Glass Micro Sphere and a Sand Particle by Means of Serial Sectioning

3D Constructor can handle this volume reconstruction only if the sectioning interval is kept constant. Figure 33 shows a 3D reconstruction of a low resolution artificial sphere created for illustration purposes. Several other software packages compatible with MS Windows XP have been evaluated. **3D-DOCTOR**, from Able Software Corp., is an advanced 3D modeling, image processing and measurement software for Magnetic Resonance Imaging (MRI), Computed Tomography (CT), Positron Emission Tomography (PET), microscopy, scientific, and industrial imaging applications. It uses vector-based technologies to create 3D models from volumetric images. It is capable of
handling uneven slice thicknesses and variable spacing between slices. Then it can create new images with even slice thickness and spacing between slices using an optimal interpolation algorithm. This is of great advantage for accurate 3D rendering and quantitative analysis.

It can export the polygonal mesh models to STereoLithographies (STL), AutoCAD (DXF), Initial 2D/3D Graphics Exchange Specification (IGES), 3D Studio (3DS), Wavefront (OBJ), raw triangles, Virtual Reality Modeling Language (VRML), Graph Coordinates (XYZ) and other formats for simulation and 3D measurements for quantitative analysis.

![Figure 33. 3D Visualization of an Artificial Sphere Using 3D Constructor 5.0](image)

5.3.1 Selection of a Medium for Serial Sectioning: Epoxy Systems

A serial sectioning procedure is an alternative utilized to achieve a three-dimensional digital reconstruction of sand particles. One of the objectives of this research
is to find a material where sand particles can be completely inlaid for future serial sectioning. The success of 3D reconstruction in this method relies in the quality of specimen preparation. A strategy for the procedure is presented to be used as a baseline. Undoubtedly, new tools, equipment, materials and ideas that could improve, benefit or even substitute the method may come along the way and they undoubtedly should be implemented.

The procedure and material used to create the specimens should not disturb the particles. In a series of repetitive procedures, this material must be capable of holding firmly the particles as an abrasive surface removes extremely thin layers successively, until the particles are completely sectioned. The wearing surface of the specimen must be kept homogenous and remain flat and even for every step of the sectioning. This means that gaps, voids and uneven surfaces should be avoided. Also, high contrast between the surrounding material and sand particles is required for accurate and precise edge detection.

Epoxy resins are indeed a versatile and tenacious material that could be used for this application. In recent years, innovation and advances in its formulation and the multiple array of chemical and physical properties, offer a wide variety of applications. Diverse formulations of epoxy resins are customized to meet unique requirements according to the purpose of its specific application. Jang, Frost and Park (1999) developed a comprehensive procedure for preserving sand specimens with epoxy resin. Specimen coupons were latterly prepared for surface analysis using a variety of grinding and polishing procedures.
In this case, some of the general characteristics found in epoxy systems are suitable for this application:

1) They come as an individual component or in a two-component presentation: Part A- Resin and Part B- Hardener

2) They are homogenous materials, non toxic and non-reactive with sands

3) Most systems present good handling characteristics and some can be cured at room temperature and have minimal shrinkage

4) They can be easily poured into a mold and will adopt and sustain a molded shape when cured

5) Some systems have low viscosity, present resistance to air entrainment, offer excellent edge retention and can fill small holes and voids

6) Offer good bonding and adhesion

7) Give the hardest cured mounts and polishing properties

8) If the system is clear, they have the ability to be dyed in different colors. This is useful to enhance contrast and facilitate edge detection

Several brands and types of epoxy have been evaluated. Most of them have the properties mentioned above, but the epoxy system that ranks better in the overall is EPO-TEK 301 from Epoxy Technology, Inc., Billerica, MA. Table 5 shows some typical properties of this epoxy system.

Table 5. Some Properties for the Epotek 301 Epoxy System

<table>
<thead>
<tr>
<th>Brand</th>
<th>Model</th>
<th>Color</th>
<th>Consistency</th>
<th>Viscosity (cP)</th>
<th>Hardness</th>
<th>Cure Schedule</th>
<th>Shrinkage Measurement</th>
<th>Mixing ratio</th>
<th>Parts by weight</th>
<th>Pot Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoxy Technology</td>
<td>Epotek 301</td>
<td>Transparent</td>
<td>Low viscosity liquid</td>
<td>100-200</td>
<td>81</td>
<td>65°C: 1 hr Room temp. Overnight</td>
<td>1% (ASTM D2596-86)</td>
<td>Resin Hardener</td>
<td>20:5</td>
<td>1 yr</td>
</tr>
</tbody>
</table>
There are several ways to dye epoxy. For this particular use, dark and light tones are needed for light and dark particles respectively. The goal is to differentiate the particles from the medium in which they are inlaid. These products are compatible with epoxy: photocopier/laser printer toner (very black and fine), lamp black powder, aniline, microfine cement, cement color additives and special pigments. All these products, when added in small quantities, result in minimal loss in strength or hardness of the epoxy and they do not alter the properties or chemical composition of the epoxy. Also, their average particle size is only a few microns.

The process for dying epoxy for this research has been achieved by using an Epotek #11 Color Concentrates (black and white). These pigments have the smallest particle size from all the options mentioned and are very opaque. They consist of color pigments ground and crushed into a liquid epoxy carrier.

For any type of epoxy system, it is very important to read and follow the instructions and use the proper mixing ratios specified by the manufacturer. Documentation made available by Epoxy Technologies, Inc. provides good information on how to handle epoxy systems.

In case of epoxy components that have been stored at low temperatures (this can considerably extend the shelf life of epoxy), they must be brought to room temperature prior to use. The containers should not be opened until they have reached ambient temperature. If this step is not done, moisture will condense on the surface of the materials, and it will cause deterioration of the epoxy. If the epoxy shows signs of
crystallization, the closed containers with the epoxy components can be heated individually at 40°C-55°C, until crystals are dissolved. The covers are kept on but loosely fitting so air can escape from the container.

5.3.2 Sample Preparation: Cold Casting

Cold cast means that no extreme heat is used to cure the epoxy. It is a simple casting method since no special equipment is needed for sample preparation. Special care must be taken if too little product is used, because any imprecise weigh of the resin and/or hardener will make vary the mixing ratios, thus affecting the characteristics of the cured epoxy. The minimum amount of epoxy mixture used was 2.5 grams. This amount will be enough to prepare more than one sample. The following list summarizes steps proposed by the Epoxy Technologies, Inc. for sample preparation:

1) Make sure sands are dry and clean. It may be necessary to wash sand particles with 100% pure water. Then dry sand in an oven at 100°C for 24 hours. It is recommended to store the sand in closed containers in order to keep them in a dry and clean environment

2) Before mixing the epoxy resin and hardener together, mix the materials on each of the containers

3) Lower viscosities can be obtained in the epoxy resin if it is heated at temperatures between 50°C and 60°C. This step is optional but greatly recommended if the mold can handle the temperature without inconvenience
4) Prior to use, gently stir the resin. Do not mix vigorously. This could entrap air into the resin.

5) Add the Epotek #11 Colored Concentrate to the *epoxy resin* only. Pigments should be mixed 1%-5% by weight of the entire formulation. Then mix both the dyed resin and the hardener materials for 1-2 minutes in a clockwise fashion and 1-2 min in counter-clockwise fashion. All mixings should be done gently in order to avoid bubbles in mixture.

6) Apply a thin layer of release agent to the surfaces of the mold. Release agent is a low viscosity chemical that does not allow adhesion of the epoxy to the mold.

7) Pour a small amount of dyed epoxy in molds.

8) Add few sand particles to the epoxy medium. Make sure most of the particles are not in touch each other. Note that pigment and some sand particles will settle at the bottom and sides of the mold.

9) Place the samples on a flat surface and allow curing in a dry and clean environment. Remember that Epotek 301 can also be cured in one hour at a temperature of 65º C. Generally speaking, epoxy systems can reach higher adhesive strength by curing at high temperatures. Bubbles can also be removed or minimized if some heat is applied.

10) In case bubbles affect the quality of the samples and no heat is applied, vacuum may help this effect. Vacuum Degassing can be accomplished.
by placing the sample in a vacuum chamber with a volume at least 5 times larger than the sample. Use a pump and apply a vacuum of 29 inches of Hg quickly. The key for good degassing is to hold the vacuum for a short period of time and not to apply too much vacuum. Otherwise, the “rolling boil” effect will appear, adding bubbles to the epoxy. Vacuum and vibration will remove bubbles even faster.

The conditions for optimal sample preparation depend on many variables. The process can be optimized only through a trial and error process and experience.

5.3.3 Equipment for Serial Sectioning

Serial sectioning is a complicated procedure that requires a very high level of precision. During this research, information has been gathered about equipment suitable for this task. Polishing machines such as Buehler PowerPro 5000, Logitech PM5 Precision Lapping/Polishing Machine and Allied High Tech Multiprep System are used for serial sectioning in diverse applications.

The most outstanding polishing machine from the ones mentioned above is the Multiprep System from Allied High Tech Products, Inc (See figure 34). This semi-automatic system is ideal for parallel polishing, preparation of thin sections and sample preparation for microscopic evaluation (optical, Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), etc.). This machine comes with a digital micrometer that allows the user to monitor the amount of
material that has been removed from the specimen in real time. This is useful because the operator has more control on the thicknesses and spacing of the layers to be removed. The specimen can be turning on its axis and oscillate across the rotating platen simultaneously, if necessary. These characteristics provide uniform abrasive pattern and equal wearing of surfaces. The spindle is calibrated perpendicular to the abrasive and the specimen is calibrated parallel to the abrasive. If required, samples can be polished at specific angles.

According to the manufacturer, with this fine polishing equipment, material can be removed at 1 µm increments. One advantage is that it does not require any hand held polishing jigs, allowing only the sample to be in contact with the abrasive surface. The control panel manages all functions such as platen speed (5-350 RPM clockwise or counterclockwise), timer, oscillation and rotation settings. Its cam-locking system allows the exact repositioning of several types of fixtures and holders available. An automatic dosing system can be used to apply abrasive suspensions and/or lubricants.

Figure 34. Allied High Tech Multiprep System
CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Results and Conclusions

The light reflected and the glare emitted under illumination for some sand particles, like quartz grains, were found to be the most challenging issues for shape digitization. The use of an infrared pass filter for acquiring images proved to be an effective alternative for better edge detection and faster processing. Figure 35 shows a U.S Silica #1 Dry sand particle seen through different filters. Note how some image artifacts are omitted when seen through an IR pass filter.

![Composite Image Obtained Using the Extended Depth of Field Function on Image-Pro Plus For an U.S. Silica #1 Dry Sand Particle: a) Only Visible Light Captured, and b) Only NIR Light Captured. Scale Not Shown](image)

Figure 35. Composite Image Obtained Using the Extended Depth of Field Function on Image-Pro Plus For an U.S. Silica #1 Dry Sand Particle: a) Only Visible Light Captured, and b) Only NIR Light Captured. Scale Not Shown
For some of the sands collected where there was enough material, a sieve analysis was performed. Results for the percentages by weight retained on sieves are presented on Appendix E. If the density of the particles is assumed equal, these percentage values can be used to obtain the same percentage of size ranges for shape digitization (2D and 3D). By using shapes based on a realistic grain size distribution, the numerical modeling process will be more reliable. In addition to grain size distribution, it would be interesting to examine how shape behavior varies for different size ranges on sands composed of different minerals and from different origins.

The grain size distribution obtainable from images requires the digitization of a large number of randomly selected particles. It has observed that smaller sand particles tend to get adhered to the surface of the glass slide, possibly due to electrostatic forces. Larger particles tend to fall off the surface during manipulation of the specimen and have fewer possibilities to be included in the acquisition process. This problem can be solved by putting a retaining barrier on the edges of the glass slide before placing sand particles on it. This barrier will prevent sand particles from falling off the surface.

The routines implemented on Image-Pro Plus proved to be effective and reliable for image processing in two dimensions. The process of massive acquisition has started with some sands collected from around the world. Appendix G shows grayscale pictures of these sands.

The main application of this work is related to the characterization and digitization of angular particles. The procedures implemented can be used to obtain such shapes in two dimensions. Similar techniques for edge enhancement and detection of cross sections
of sample submitted to serial sectioning can be used for three-dimensional reconstructions.

Shapes obtained from the application of this work can be used for numerical modeling of angular particles using the Discrete Element Method. The creation and publication of a shape database for different types of granular soils will allow several researchers to use those shapes for modeling and testing of soils. This modeling process will be the first steps towards the process of revealing aspects like the influence of particle shape on the liquefaction behavior of soils. Even though particle shape is not enough to predict completely the mechanical response of an assembly of soil particles (note that porosity, roughness and multiple particle densities are other parameters that influence soil behavior), it could become a promising process that will encourage geotechnical engineers to model granular soils particle by particle.

6.2 Recommendations for Future Work

Manual relocation of the X-Y stage is time consuming and limits the accuracy, and reproducibility of the stage movements. The use of an automated stage would make the process of acquisition faster for digitization of shapes of sand particles. In case of 2D, Image-Pro can control the zigzag process and more shapes can be digitized for each type of sand collected in less time. It is recommended to get a compatible automated stage for the microscope that can be controlled with the hardware and software available. An imaginary mesh can divide the glass slide where particles are hold, into zones of certain
area, according to the field of view of the objective lens. A macro could be implemented to maneuver the movement of the stage in such a way that the particle shapes can be acquired automatically for each zone.

In case of 3D reconstruction, the recommended procedure to utilize is serial sectioning. This alternative is more affordable than using a nano tomograph and more compatible with the equipment available. An automatic stage could also be used to acquire images of small zones on the surface of the epoxy specimen at specific and reproducible coordinates. These small images can be later stitched via software to create a larger picture, thus, incrementing the number of particles that can be reconstructed after each layer removal process.

Figure 36 is the cross section of a sample containing Rhode Island sand particles. The medium is acrylic that comes in a light amber color. The samples has a diameter of 4 mm (this size can not be seen entirely with the microscope) and was arranged from individual images acquired with Image-Pro at a 4x magnification and using a 0.65x C-mount adapter for the camera. By using Microsoft PowerPoint, a combined image was created. The stitching process could have been done using Image-Pro but it was not able to handle 6 images at maximum resolution (1.4 Mega pixels per image). It is noted how smooth and even looks the composite image by using the Bright field Incident Illumination System, even though the surface of the specimen is not completely parallel to the objective lens (notice the shadow on the specimen). Definitely
Figure 36. Tiled Images of a Cross-Section of a Polished Sample Containing Rhode Island Sand Particles. The Material Where Particles Were Inlaid is Light Amber Color Acrylic. The Diameter of The Sample is Approximately 4 mm and Was Polished Using the Multiprep System. Sample Was Courtesy of Allied High Tech Products, Inc.

The optimum shape and size of the epoxy specimen will be defined by trial and error. At the lowest magnification (4x Objective Lens + 0.65 C-Mount adapter for the camera), the maximum diameter of the sample that can be stitched using 4 images on Image-Pro is about 3 mm. A suitable mold for this size could be a ½ cc or 1cc disposable syringe. This mold would give a cylindrical sample. The polishing machine can also be used to make four flat cuts in the walls of the cylinder, which can be used for image alignment.
Optimum results for a fine polishing process can be achieved using a 0.5 \( \mu \text{m} \) Cerium Oxide Lapping film and to keep the wearing surface of the specimen parallel to the abrasive surface. The key factor to obtain accurate binary shapes in less time is to achieve good contrast between particles and their background. Black and white pigments might work better if they are seen under visible light. NIR opaque dyes or pigments may give better contrast. It is recommended to try dyes such as Epolight 9151 (See figure 37) from Epolin, Inc. This dye is compatible with epoxy and suitable for injection molding.

![Normalized Absorption Spectrum of Epolight 9151 In Chloroform](Source: Epolin Technical Data Sheet for Epolight 9151 Anthraquinone Dye)

At the same time, image alignment has to be done precisely before any attempt for 3D reconstruction. Solid glass micro spheres can be used as reference objects that can also be inlaid in epoxy. The center of each circular cross section in a micro sphere remains the same during the serial sectioning process. Five hundred grams of glass micro spheres with a mean diameter of 203 \( \mu \text{m} \), obtained from of Potters Industries, Inc., will be
used for testing. Another alternative is to mark a shape on the material with an indenter. Chawla and Wunsch (2004) used a Vickers micro-hardness indenter to create fiducial marks. If the indenter can mark a well defined pyramidal volume on the sample, then the measurements of cross sections at different stages of the serial sectioning process can be related to thicknesses of the material removed.

The creation of an exploratory routine for acquisition, tiling and alignment, enhancement, conversion to binary and 3D reconstruction is recommended. After a methodology and procedure is achieved and refined, that basic routine can be adapted for a massive quantification of 3D structures of sand particles. The edge detection and image enhancement routines implemented for two-dimensional digitization of shapes can also be applied to the cross section images used for 3D reconstruction from serial sectioning.
REFERENCES


Allied High Tech Products, Inc., 2003, “Catalogue of Products for Precision Surface and Sample Preparation”.


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Prior Scientific Inc., 2001, “ProScan II Operating Instructions”.


APPENDICES
Appendix A. Source Code for the Script File “GET2DSAHPE.SCR”

Option Explicit
' THIS SCRIPT WORKS ONLY WITH NEAR INFRARED (NIR) WAVELENGHTS ONLY. MAKE SURE YOUR CAMERA HAS THE PROPER WRATTEN FILTER IN PLACE.
' First Macro: Generate an image sequence of sand particles
Sub Acquisition()
    ret = IpMacroStop("Place the sample on the microscope stage. Locate a sand particle using the Preview screen.",0)
    ret = IpTemplateMode(0)
    ret = IpAcqShow(ACQ_SNAP, 1)
    ret = IpAcqShow(ACQ_LIVE, 1)
    IpArray(0) = 0 'left
    IpArray(1) = 0 'top
    IpArray(2) = 1391 'right
    IpArray(3) = 1039 'bottom
    ret = IpAcqControl(88, 1, IpArray(0)) ' ACQCMD_AUTOEXPOSURE
    ret = IpTemplateMode(0)
    ret = IpScopeShow(1)
    ret = IpMacroStop("Select a setting according to current objective magnification. Set Top and Bottom limits and click on Acquire. Save all files as SEQUENCES.",1)
End Sub

' Second Macro: Open Image Sequence, deconvolves, obtains EDF TestStrips, applies Local Best Fit Contrast Enhancement routine, filters with Variance Filter
Sub EdgeDetec()
    ret = IpTemplateMode(1)
    Dim ori%, decon%, edfd%, cedfd%
    ' Open file and select Rectangular AOI
    ret = IpMacroStop("Select a sequence of images.",0)
    ret = IpWsLoad("C:\Documents and Settings\All Users\Documents","seq")
    ret = IpMacroStop("Adjust the following rectangular AOI(Area of Interest) to cover the entire particle.",0)

    ipRect.Left = 490
    ipRect.top = 236
    ipRect.Right = 1154
    ipRect.bottom = 894
    ret = IpAoiCreateBox(ipRect)
Appendix A (continued)

ret = IpWsDuplicate()
    ret = IpDocCloseEx(0)
    ori = IpAppSelectDoc(1)

'Deconvolution using SharpStack

ret = IpmacroStop("Select the Spatial Calibration.",0)
ret = IpSCalShowEx(SCAL_DLG_SELECT, SCAL_SHOW)

'Dim docInfo As IPDOCINFO, scaleVal As Single
Dim gmagnum As Integer
Begin Dialog UserDialog 380,112,"Select Magnification(4x,10x or 20x)"
    Text 40,21,90,14,"Magnification",.Text1
    TextBox 140,14,90,21,.magnum
    OKButton 80,84,90,21
End Dialog
Dim dlg1 As UserDialog
' Apply to dialog
dlg1.magnum = Str(gmagnum)

' Get user values
Dialog dlg1

' Get back from dialog
gmagnum = Val(dlg1.magnum)

Select Case gmagnum
    Case 4
        ret = IpDcnvShow(1)
        ret = IpLensApply("4x_065", APPLYTO_IMAGE)
        ret = IpSCalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
        ret = IpDCnvSet(DCNV_TYPE, DCTYPE_NEAREST)
        ret = IpDCnvSetSng(DCNV_NA, 0.100000)
        ret = IpDCnvSetSng(DCNV_RI, 1.000000)
        ret = IpDCnvSetSng(DCNV_WL, 775.000000)
        ret = IpDCnvSetSng(DCNV_XSPACING, 1.739130)
        ret = IpDCnvSetSng(DCNV_YSPACING, 1.739130)
        ret = IpDCnvSet(DCNV_BRIGHTFIELD, 0)
        ret = IpDCnvSet(DCNV_PHASEOBJECTS, 0)
        ret = IpDCnvSet(DCNV_NEIGHBORSSpacing, 1)
        ret = IpDCnvSet(DCNV_HAZEREMOVAL, 10)
        ret = IpDCnvSet(DCNV_USEACTIVEPORTION, 0)
Appendix A (continued)

```c
ret = IpDcnvSet(DCNV_CONVERTTOFLOAT, 1)
ret = IpDcnvSet(DCNV_SPHERICALABERRATION, -9)
ret = IpMacroStop("Input appropriate parameters to SharpStack, Deconvolve image, then Continue.",0)
ret = IpDcnvShow(0)

Case 10
ret = IpDcnvShow(1)
ret = IpLensApply("10x_065", APPLYTO_IMAGE)
ret = IpScalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
ret = IpDcnvSet(DCNV_TYPE, DCTYPE_INVERSE)
ret = IpDcnvSetSng(DCNV_NA, 0.250000)
ret = IpDcnvSetSng(DCNV_RI, 1.000000)
ret = IpDcnvSetSng(DCNV_WL, 775.000000)
ret = IpDcnvSetSng(DCNV_XSPACING, 0.714286)
ret = IpDcnvSetSng(DCNV_YSPACING, 0.714286)
ret = IpDcnvSet(DCNV_BRIGHTFIELD, 0)
ret = IpDcnvSet(DCNV_PHASEOBJECTS, 0)
ret = IpDcnvSet(DCNV_NEIGHBORSspacing, 1)
ret = IpDcnvSet(DCNV_HAZEREMOVAL, 10)
ret = IpDcnvSet(DCNV_USEACTIVEPORTION, 0)
ret = IpDcnvSet(DCNV_CONVERTTOFLOAT, 1)
ret = IpDcnvShow(0)

Case 20
ret = IpDcnvShow(1)
ret = IpLensApply("20x_065", APPLYTO_IMAGE)
ret = IpScalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
ret = IpDcnvSet(DCNV_TYPE, DCTYPE_INVERSE)
ret = IpDcnvSetSng(DCNV_NA, 0.400000)
ret = IpDcnvSetSng(DCNV_RI, 1.000000)
ret = IpDcnvSetSng(DCNV_WL, 775.000000)
ret = IpDcnvSetSng(DCNV_XSPACING, 0.350877)
ret = IpDcnvSetSng(DCNV_YSPACING, 0.350877)
ret = IpDcnvSet(DCNV_BRIGHTFIELD, 0)
ret = IpDcnvSet(DCNV_PHASEOBJECTS, 0)
ret = IpDcnvSet(DCNV_NEIGHBORSspacing, 1)
ret = IpDcnvSet(DCNV_HAZEREMOVAL, 10)
ret = IpDcnvSet(DCNV_USEACTIVEPORTION, 0)
ret = IpDcnvSet(DCNV_CONVERTTOFLOAT, 1)
```

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Appendix A (continued)

    ret = IpDCnvSet(DCNV_SPHERICALABERRATION, -14.6862)
    ret = IpMacroStop("Input appropriate parameters to SharpStack, Deconvolve
image, then Continue.", 0)
    ret = IpDCnvShow(0)
    Case Else

End Select

'Filtering
'EDF(Extended Depth of Field TestStrips
    ret = IpTemplateMode(0)
    Dim nNorm%, nCriteria%, nOrder%
    Dim sNorm(0 To 1) As String, valNorm(0 To 1) As Integer
    Dim sCriteria(0 To 3) As String, valCriteria(0 To 3) As Integer
    Dim sOrder(0 To 1) As String, valOrder(0 To 1) As Integer
    Dim nSeries%, nEDF%, nFrames%
    Dim textStr As String

'Set source to the current document?
    ret = IpEDFRemove(DOCSEL_ALL)
    ret = IpEDFNew(1)

'Set values and strings for the various parameters
    valNorm(0) = 0
    sNorm(0) = "Not Normalized"
    valNorm(1) = 1
    sNorm(1) = "Normalized"

    valCriteria(0) = EDF_MAX_LOCALCONTRAST
    sCriteria(0) = "Local Contrast"
    valCriteria(1) = EDF_MAX_DEPTHCONTRAST
    sCriteria(1) = "Depth Contrast"
    valCriteria(2) = EDF_MAX_INTENSITY
    sCriteria(2) = "Maxmimum"
    valCriteria(3) = EDF_MIN_INTENSITY
    sCriteria(3) = "Minimum"

    valOrder(0) = EDF_TOPDOWN
    sOrder(0) = "Top Down"
    valOrder(1) = EDF_BOTTOMUP
    sOrder(1) = "Bottom Up"
Appendix A (continued)

'Initialize value for series
nSeries = -1

' Loop through the various criteria
' Various criteria
For nCriteria = 0 To 3
' Various orders
For nOrder = 0 To 1
' Normalize or not
For nNorm = 0 To 1

' Set parameters and create the EDF
ret = IpEDFSet(EDF_NORMALIZE, valNorm(nNorm), 0)
ret = IpEDFSet(EDF_CRITERIA, valCriteria(nCriteria), 0)
ret = IpEDFSet(EDF_ORDER, valOrder(nOrder), 0)
ret = IpEDFCreate(EDF_COMPOSITE)
ret = IpDocGet(GETACTDOC, 0, nEDF)

' Write description onto images
textStr = sCriteria(nCriteria) + Chr$(10)
textStr = textStr + sNorm(nNorm) + Chr$(10)
textStr = textStr + sOrder(nOrder)
ret = IpAnCreateObj(GO_OBJ_TEXT)
ret = IpAnMove(0, 20, 20)
ret = IpAnText(textStr)
ret = IpAnSet(GO_ATTR_ZOOM, 0)
ret = IpAnSet(GO_ATTR_RECTSTYLE, GO_RECTSTYLE_NOBORDER_FILL)
ret = IpAnSet(GO_ATTR_TEXTAUTOSIZE, 1)
ret = IpAnSet(GO_ATTR_FONTBOLD, 700)
ret = IpAnSet(GO_ATTR_BRUSHCOLOR, 12632256)
ret = IpAnSet(GO_ATTR_TEXTCOLOR, 0)
ret = IpAnBurn()

' Add to the series
If nSeries < 0 Then
' Set series to this image
nSeries = nEDF
Else
' Or append EDF to the accumulated series
Appendix A (continued)

ret = IpAppSelectDoc(nEDF)
ret = IpWsCopyFrames(0,1)
ret = IpAppSelectDoc(nSeries)
ret = IpWsPasteFrames(-1)

' Set to the last frame of the series
ret = IpSeqGet(SEQ_NUMFRAMES, nFrames)
ret = IpSeqSet(SEQ_ACTIVEFRAME, nFrames-1)

' Clean up the EDF
ret = IpAppSelectDoc(nEDF)
ret = IpDocClose()

End If

Next nNorm
Next nOrder
Next nCriteria

'Delete original sequence
ret = IpDocCloseEx(ori)

'Image enhancement
'User set variables are kept as globals so that they can be preserved between calls to this
routine
Dim gnumIter As Integer

'Local Best Fit Contrast Enhancement
'Sub MinMaxscale()
Dim source%, dest%, minima%, maxima%, diff%, floor%, scaled%
Dim docInfo As IPDOCINFO, scaleVal As Single

Begin Dialog UserDialog 320,112,"Local contrast enhancement"
  Text 40,21,90,14,"Iterations",.Text1
  TextBox 140,14,90,21,.numIter
  OKButton 80,84,90,21
End Dialog
Dim dlg2 As UserDialog

If gnumIter=0 Then
  ' Set up initial values
  gnumIter = 5
Appendix A (continued)

End If

' Apply to dialog
dlg2.numIter = Str(gnumIter)

' Get user values
Dialog dlg2

' Get back from dialog
gnumIter = Val(dlg2.numIter)

' Check image class and scaling
ret = IpDocGet(GETDOCINFO, DOCSEL_ACTIVE, docInfo)
Select Case docInfo.Class
    Case IMC_GRAY, IMC_RGB
        scaleVal = 255.0
    Case IMC_GRAY12, IMC_RGB36
        scaleVal = 4095.0
    Case IMC_GRAY16, IMC_RGB48
        scaleVal = 65535.0
    Case Else
        ' Not supported!
        Beep
        ret = IpMacroStop("Image type not supported!", vbOK)
        Exit Sub
End Select

'Create working images
ret = IpDocGet(GETACTDOC, DOCSEL_ACTIVE, source)
dest = IpWsDuplicate()
minima = IpWsDuplicate()
maxima = IpWsDuplicate()

'Smoothed minima
ret = IpAppSelectDoc(minima)
ret = IpFltErode(MORPHO_7x7OCTAGON, gnumIter)
ret = IpFltGauss(7, 10, gnumIter*2)

'Smoothed maxima
ret = IpAppSelectDoc(maxima)
ret = IpFltDilate(MORPHO_7x7OCTAGON, gnumIter)
ret = IpFltGauss(7, 10, gnumIter*2)
Appendix A (continued)

'Difference (local contrast scale)
   diff = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 1)
   ret = IpAppSelectDoc(dest)
   ret = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 0)
   ret = IpOpImageArithmetics(diff, scaleVal, OPA_DIV, 0)

'Clean up
   ret = IpAppSelectDoc(minima)
   ret = IpDocClose()
   ret = IpAppSelectDoc(maxima)
   ret = IpDocClose()
   ret = IpAppSelectDoc(diff)
   ret = IpDocClose()

'Average with image after enhancement
   ret = IpOpImageArithmetics(source, 0.0, OPA_AVG, 0)

'Application of Variance Filter
   ret = IpTemplateMode(0)
   ret = IpWsDuplicate()
   ret = IpFltVariance(7, 7)
   ret = IpDocCloseEx(1)

'Average with EDF TestStrip sequence
   ret = IpTemplateMode(0)
   ret = IpAppSelectDoc(18)
   ret = IpDocClose()
   ret = IpAppSelectDoc(19)
   ret = IpOpImageArithmetics(2, 0.0, OPA_AVG, 0)
   ret = IpOpShow(0)

'Convert image to grayscale 8
   ret = IpTemplateMode(0)
   ret = IpAoiShow(FRAME_NONE)
   ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
   ret = IpHstEqualize(EQ_BESTFIT)
   'ret = IpAppSelectDoc(19)
   'ret = IpDocClose()

'Choose grayscale value where edge is defined
   ret = IpMacroStop("Select best frame from any of the EDF Test Strips. Select MakeBIN macro when the AOI is defined.",0)
Appendix A (continued)

    ret = IpTemplateMode(1)
    ret = IpAoiCreateIrregular(Pts(0), 7)

    End Sub

'Third Macro: Extracts best frame from EDF TestStrips, defines particle contour, makes a binary image.
Sub MakeBIN()

    ret = IpMacroStop("A Grayscale 8 sequence will be created from selected Image sequence. Try an Irregular AOI around the particle.",0)

    'Convert image to grayscale 8
    ret = IpTemplateMode(0)
    ret = IpAoiShow(FRAME_NONE)
    ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
    ret = IpHstEqualize(EQ_BESTFIT)

    'Extract frame of interest
    ret = IpTemplateMode(1)
    Dim gnFrame As Long
    ' Set up initial values
    gnFrame = 16
    Begin Dialog UserDialog 320,112,"Select frame to extract."
        Text 40,21,90,14,"Frame number",.Frame
        TextBox 140,14,90,21,.nFrame
        OKButton 80,84,90,21
    End Dialog
    Dim dlg3 As UserDialog
    ' Apply to dialog
    dlg3.nFrame = Str(gnFrame)

    ' Get user values
    Dialog dlg3

    ' Get back from dialog
    gnFrame = Val(dlg3.nFrame) - 1
    ret = IpSeqExtractFrames(gnFrame, 1)
    ret = IpAoiCreateIrregular(Pts(0), 7)
    ret = IpMacroStop("A binary image will be created.",0)
Appendix A (continued)

'Fill particle shape with white color
  ret = IpWsFill(0, 2, 0)
  ret = IpTemplateMode(0)

'Make binary image
  ret = IpAoiShow(FRAME_RESET)
  ret = IpAoiShow(FRAME_NONE)
  ret = IpSegShow(1)
  ret = IpSegSetAttr(SETCURSEL, 0)
  ret = IpSegSetAttr(CHANNEL, 0)
  ret = IpSegPreview(ALL_C_B)
  ret = IpSegSetRange(0, 0, 255)
  ret = IpSegPreview(ALL_C_B)
  ret = IpSegSetRange(0, 255, 255)
  ret = IpSegPreview(ALL_C_B)
  ret = IpSegCreateMask(5, 0, 1)
  ret = IpSegShow(0)
  ret = IpWsSaveAs("", "BMP")

End Sub
Appendix B. Source Code for the Script File Mult2DShape4x

Option Explicit

' Global directory starting string, for saving where we last processed
Dim gDirStart As String
' This Macro: Helps the operator to generate sets of images at different focal planes

Sub Acquisition()
    ret = IpMacroStop ("Place the sample on the microscope stage. Locate a group of
sand particles using the Preview screen and start acquiring image sets.",0)
    ret = IpTemplateMode(0)
    ret = IpAcqShow(ACQ_SNAP, 1)
    ret = IpAcqShow(ACQ_LIVE, 1)
    IpArray(0) = 0 'left
    IpArray(1) = 0 'top
    IpArray(2) = 1391 'right
    IpArray(3) = 1039 'bottom
    ret = IpAcqControl(88, 1, IpArray(0)) ' ACQCMD_AUTOEXPOSURE
    ret = IpTemplateMode(0)
    ret = IpScopeShow(1)
    ret = IpMacroStop ("Select a setting according to current objective magnification. Set
Top and Bottom limits and click on Acquire. Save all files as SEQUENCES. Click on
Continue now.",0)
End Sub

' This Macro: Automatically opens image sequences one by one. Then it deconvolves
each sequence, creates an EDF Test Strip, applies a Local Best Fit Contrast
Enhancement
' routine, Applies a Variance Filter and then saves edge enhanced images

Sub EnhanceEdges()

' Scan through and process all files in a directory
    Dim IName As String*255
    Dim fName As String
    Dim workStr As String
    Dim savefol As String
    Dim docID As Integer
    Dim X As Integer
    X = 0
Appendix B (continued)

'Operator selects a folder where to store enhanced images
savefol = GetFilePath("EDFs", "", "C:\", "Select a file where to store enhanced images", 2)

' Creating starting point
    If gDirStart = "" Then
        gDirStart = "C:"
    End If

' Get a file name in the desired directory
    workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find image sequences", 0)

' Check if user did not cancel
    If workStr = "" Then
        Exit Sub
    End If

' Close all open images prior to processing
    ret = IpAppCloseAll()

' Extract the directory name from the full file name
    gDirStart = Left(workStr, InStrRev(workStr, ")

'debugclear-clear the Output For work purposes
    ret = IpOutputClear()

' Select Calibration for 4X Objective Lens
    ret = IpSCalSelect("4x_065")

    ret = IpTemplateMode(0)
    fName = Dir(gDirStart + "*.SEQ", 32)

While fName <> ""
    X = X + 1

    ' Print out the file name and its attributes
    Debug.Print GetAttr(gDirStart + fName); " "; fName

    ' Load the image sequence
    docID = IpSeqOpen(gDirStart + fName, "SEQ", 0,15)
    ret = IpSMShowNav(SM_HIDE)
Appendix B (continued)

ret = IpTemplateMode(0)
ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
ret = IpDocCloseEx(0)

' Start routine
If docID >= 0 Then

ret = IpTemplateMode(0)

' Deconvolution using SharpStack for 4X Objective Lens parameters
ret = IpDcnvShow(0)
ret = IpLensApply("4x_065", APPLYTO_IMAGE)
ret = IpSCalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
ret = IpDcnvSet(DCNV_TYPE, DCTYPE_NEAREST)
ret = IpDcnvSetSng(DCNV_NA, 0.100000)
ret = IpDcnvSetSng(DCNV_RI, 1.000000)
ret = IpDcnvSetSng(DCNV_WL, 775.000000)
ret = IpDcnvSetSng(DCNV_XSPACING, 1.739130)
ret = IpDcnvSetSng(DCNV_YSPPACING, 1.739130)
ret = IpDcnvSet(DCNV_BRIGHTFIELD, 0)
ret = IpDcnvSet(DCNV_PHASEOBJECTS, 0)
ret = IpDcnvSet(DCNV_NEIGHBORSPPACING, 1)
ret = IpDcnvSet(DCNV_HAZEREMOVAL, 10)
ret = IpDcnvSet(DCNV_USEACTIVEPORTION, 0)
ret = IpDcnvSet(DCNV_CONVERTTOFLOAT, 0)
ret = IpDcnvDeconvolve
ret = IpDcnvShow(0)

' Filtering
' EDF(Extended Depth of Field Test Strip)
ret = IpTemplateMode(0)
Dim nNorm%, nCriteria%, nOrder%
Dim sNorm(0 To 1) As String, valNorm(0 To 1) As Integer
Dim sCriteria(0 To 3) As String, valCriteria(0 To 3) As Integer
Dim sOrder(0 To 1) As String, valOrder(0 To 1) As Integer
Dim nSeries%, nEDF%, nFrames%
Dim textStr As String

' Set source to the current document?
ret = IpEDFRemove(DOCSEL_ALL)
ret = IpEDFNew(1)
Appendix B (continued)

' Set values and strings for the various parameters
valNorm(0) = 0
sNorm(0) = "Not Normalized"
valNorm(1) = 1
sNorm(1) = "Normalized"
valCriteria(0) = EDF_MAX_LOCALCONTRAST
sCriteria(0) = "Local Contrast"
valCriteria(1) = EDF_MAX_DEPTHCONTRAST
sCriteria(1) = "Depth Contrast"
valCriteria(2) = EDF_MAX_INTENSITY
sCriteria(2) = "Maxmimum"
valCriteria(3) = EDF_MIN_INTENSITY
sCriteria(3) = "Minimum"
valOrder(0) = EDF_TOPDOWN
sOrder(0) = "Top Down"
valOrder(1) = EDF_BOTTOMUP
sOrder(1) = "Bottom Up"

' Initialize value for series
nSeries = -1

' Loop through the various criteria
' Various criteria
For nCriteria = 0 To 3
' Various orders
  For nOrder = 0 To 1
' Normalize or not
  For nNorm = 0 To 1
' Set parameters and create the EDF
    ret = IpEDFSet(EDF_NORMALIZE, valNorm(nNorm), 0)
    ret = IpEDFSet(EDF_CRITERIA, valCriteria(nCriteria), 0)
    ret = IpEDFSet(EDF_ORDER, valOrder(nOrder), 0)
    ret = IpEDFCreate(EDF_COMPOSITE)
    ret = IpDocGet(GETACTDOC, 0, nEDF)
' Write description onto images
    textStr = sCriteria(nCriteria) + Chr$(10)
    textStr = textStr + sNorm(nNorm) + Chr$(10)
    textStr = textStr + sOrder(nOrder)
  Next
Next
Next
Appendix B (continued)

ret = IpAnCreateObj(GO_OBJ_TEXT)
ret = IpAnMove(0, 20, 20)
ret = IpAnText(textStr)

ret = IpAnSet(GO_ATTR_ZOOM, 0)
ret = IpAnSet(GO_ATTR_RECTSTYLE, GO_RECTSTYLE_NOBORDER_FILL)
ret = IpAnSet(GO_ATTR_TEXTAUTOSIZE, 1)
ret = IpAnSet(GO_ATTR_FONTBOLD, 700)
ret = IpAnSet(GO_ATTR_BRUSHCOLOR, 12632256)
ret = IpAnSet(GO_ATTR_TEXTCOLOR, 0)
ret = IpAnBurn()

' Add to the series
If nSeries < 0 Then
  ' Set series to this image
  nSeries = nEDF
Else
  ' Or append EDF to the accumulated series
  ret = IpAppSelectDoc(nEDF)
  ret = IpWsCopyFrames(0,1)
  ret = IpAppSelectDoc(nSeries)
  ret = IpWsPasteFrames(-1)
  
  ' Set to the last frame of the series
  ret = IpSeqGet(SEQ_NUMFRAMES, nFrames)
  ret = IpSeqSet(SEQ_ACTIVEFRAME, nFrames-1)
  
  ' Clean up the EDF
  ret = IpAppSelectDoc(nEDF)
  ret = IpDocClose()
End If

Next nNorm
Next nOrder
Next nCriteria

' Delete previous sequences
ret = IpDocCloseEx(1)
ret = IpDocCloseEx(2)

' Further Image enhancement
Appendix B (continued)

' Local Best Fit Contrast Enhancement

Dim source%, dest%, minima%, maxima%, diff%, floor%, scaled%
Dim docInfo As IPDOCINFO, scaleVal As Single

' Check image class and scaling
ret = IpDocGet(GETDOCINFO, DOCSEL_ACTIVE, docInfo)
Select Case docInfo.Class
    Case IMC_GRAY, IMC_RGB
        scaleVal = 255.0
    Case IMC_GRAY12, IMC_RGB36
        scaleVal = 4095.0
    Case IMC_GRAY16, IMC_RGB48
        scaleVal = 65535.0
    Case Else
        ' Not supported!
        Beep
        ret = IpMacroStop("Image type not supported!", vbOK)
        Exit Sub
End Select

' Create working images
ret = IpDocGet(GETACTDOC, DOCSEL_ACTIVE, source)
dest = IpWsDuplicate()
minima = IpWsDuplicate()
maxima = IpWsDuplicate()

' Smoothed minima
ret = IpAppSelectDoc(minima)
ret = IpFltErode(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Smoothed maxima
ret = IpAppSelectDoc(maxima)
ret = IpFltDilate(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Difference (local contrast scale)
diff = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 1)
ret = IpAppSelectDoc(dest)
ret = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 0)
ret = IpOpImageArithmetics(diff, scaleVal, OPA_DIV, 0)
Appendix B (continued)

' Clean up
  ret = IpAppSelectDoc(minima)
  ret = IpDocClose()
  ret = IpAppSelectDoc(maxima)
  ret = IpDocClose()
  ret = IpAppSelectDoc(diff)
  ret = IpDocClose()

' Average with image after enhancement
  ret = IpOpImageArithmetics(source, 0.0, OPA_AVG, 0)

' Application of Variance Filter
  ret = IpTemplateMode(0)
  ret = IpWsDuplicate()
  ret = IpFltVariance(7, 7)
  ret = IpDocCloseEx(1)

' Average with EDF TestStrip sequence
  ret = IpTemplateMode(0)
  ret = IpAppSelectDoc(18)
  ret = IpDocClose()
  ret = IpAppSelectDoc(19)
  ret = IpOpImageArithmetics(2, 0.0, OPA_AVG, 0)
  ret = IpOpShow(0)

' Convert image to grayscale 8
  ret = IpTemplateMode(0)
  ret = IpAoiShow(FRAME_NONE)
  ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
  ret = IpHstEqualize(EQ_BESTFIT)

' User selects folder where to save image
  ' DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
  ret = IpStAutoName(savefol + "###.SEQ", X, IName)
  ret = IpWsSaveAs(IName, "SEQ")
  ret = IpAppCloseAll()

Else
  Debug.Print "Error loading ": gDirStart + fName
End If
Appendix B (continued)

' Get the next file name
fName = Dir()
Wend
ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)
End Sub

' This Macro: Opens EDF Test Strips from a folder in successive order, Uses the
 Count/Measure tool to filter by area and min caliper.

Sub Sort_GSIs()
ret = IpMacroStop("This macro starts a new session.", 0)
Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim savefol2 As String
Dim docID As Integer
Dim X As Integer

' Initialize X
X = 0

' Select a folder where to store the outputs
savefol2 = GetFilePath("SortedGSIs", ",", "C:\\", "Select a folder where to store sorted Gray Scale Images", 2)

' Creating starting point
If gDirStart = "" Then
    gDirStart = "C:\\"
End If

' Get a file name from a folder
workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find EDF Test Strips", 0)

' Check if the user did not cancel
If workStr = "" Then
    Exit Sub
Appendix B (continued)

End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")

' Clear the output for work purposes
debugclear
ret = IpOutputClear()

' Select Calibration for 4x Objective Lens
ret = IpSCalSelect("4x_065")
ret = IpTemplateMode(1)

fName = Dir(gDirStart + "*.SEQ", 32)
ret = IpMacroStop ("Explore any Test Strip from the group. Select the best frame where particles outlines are well defined.",0)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")

' Determine Frame number to extract for all the Test Strips within the folder

Dim gnFrame As Long

' Set up initial values
gnFrame = 2
Begin Dialog UserDialog 320,112,"Select frame number to extract."
Text 40,21,90,14,"Frame number",.Frame
TextBox 140,14,90,21,.nFrame
OKButton 80,84,90,21
End Dialog
Dim dlg3 As UserDialog

' Apply to dialog
dlg3.nFrame = Str(gnFrame)

' Get user values
Dialog dlg3
Appendix B (continued)

' Get back from dialog
    gnFrame = Val(dlg3.nFrame) - 1
    ret = IpSeqExtractFrames(gnFrame, 1)

' Determine Intensity range manually

    Dim gnInte As Long

    'ret = IpBlbShow(1)
    ret = IpSegShow(1)
    ret = IpSegSetAttr(SETCURSEL, 0)
    ret = IpSegSetAttr(CHannel, 0)
    ret = IpSegPreview(CURRENT_C_T)

    ' Set up initial values
    gnInte = 123
    Text 40,21,90,14,"Select a value for intensity",.Inte
    TextBox 140,14,90,21,.nInte
    OKButton 80,84,90,21
    End Dialog
    Dim dlg4 As UserDialog

    ' Apply to dialog
    dlg4.nInte = Str(gnInte)

    ' Get user values
    Dialog dlg4

    ' Get back from dialog
    gnInte = Val(dlg4.nInte)

    ret = IpTemplateMode(0)
    ret = IpAppCloseAll()
    While fName <> ""
        X = X + 1

        ' Print out the file name and its attributes
        Debug.Print GetAttr(gDirStart + fName); " "; fName

        ' Load the image sequence
Appendix B (continued)

docID = IpSeqOpen(gDirStart + fName, "SEQ", 0, 16)

' Start routine
If docID >= 0 Then

' Extract frame of interest
ret = IpSeqExtractFrames(gnFrame, 1)

' Measuring and sorting
ret = IpTemplateMode(0)
' Select Calibration for 4x Objective Lens
ret = IpSCalSelect("4x_065")
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
' Apply Filter Ranges
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
' Do accumulate count
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
' Reset for no measurements
ret = IpBlbEnableMeas(BLBM_ALL, 0)
' Measure Min Feret
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Measure Area
ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Count range for particles (obtained from the manual intensity selection)
ret = IpBlbSetAttr(BLOB_AUTORANGE, 0)
ret = IpSegSetRange(-1, 0, gnInte)
' Apply smoothing
ret = IpBlbSetAttr(BLOB_SMOOTHING, 2)
' Apply 8-connected objects
ret = IpBlbSetAttr(BLOB_8CONNECT, 1)
' Fill holes
ret = IpBlbSetAttr(BLOB_FILLHOLES, 0)
ret = IpBlbSetAttr(BLOB_MINAREA, 1)
ret = IpBlbSetAttr(BLOB_CLEANBORDER, 1)
ret = IpSortAttr(SORT_LABELS, 0)
' Measure Min Feret
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Measure Area
ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Filter that excludes particles outside the range 75<Min Caliper<2000 um and
1500<Area<50000 um^2
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
Appendix B (continued)

    ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
    ' Count
    ret = IpBlbCount()
    ' Sort by Area
    ret = IpSortAttr(SORT_AUTO, 1)
    ret = IpSortAttr(SORT_LABELS, 0)
    ret = IpSortAttr(SORT_COLOR, 0)
    ret = IpSortAttr(SORT_INDEX, 255)
    ret = IpSortAttr(SORT_MEAS, 10)
    ret = IpSortAttr(SORT_ROTATE, 1)
    ret = IpSortObjects()

    'User selected folder where to save image
    'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret= IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret= IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()

Else
    Debug.Print "Error loading "; gDirStart + fName
End If

' Get the next file name
fName = Dir()
Wend

    ret = IpMacroStop("All images in directory processed.", MS_MODAL)
End Sub

' This Macro: Opens sorted GSI's and lets the operator get rid of spots and defected shapes
Sub EditGSIs()

    ret = IpMacroStop("This macro will help you edit GSIs before starting the process of measuring and counting.", 0)

    Dim IName As String*255
    Dim fName As String
    Dim workStr As String
Appendix B (continued)

Dim savefol2 As String
Dim docID As Integer
Dim F As Integer
Dim B As Integer

' Initialize X
Dim X As Integer

Dim gnFrame1 As Long

' Select a folder where to store the outputs
savefol2 = GetFilePath("EdGSIs", ",", ",", "Select a folder where to store edited images", 2)
' Set up initial values
  gnFrame1 = 000
Begin Dialog UserDialog 520,112,"What is the number of the last image saved?"
  Text 40,21,90,14,"Image number",.Frame1
  TextBox 140,14,90,21,.nFrame1
  OKButton 80,84,90,21
End Dialog

Dim dlg31 As UserDialog
' Apply to dialog
dlgl.nFrame1 = Str(gnFrame1)

' Get user values
Dialog dlg31

' Get back from dialog
gnFrame1 = Val(dlg31.nFrame1)

X = gnFrame1

' Creating starting point
If gDirStart = "" Then
  gDirStart = "C:\"
End If

' Get a file name from a folder
workStr = GetFilePath("", "BMP", gDirStart, "Specify a folder where to find the sorted GSIs", 0)
Appendix B (continued)

' Check if the user did not cancel
If workStr = "" Then
    Exit Sub
End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, "\"))

' Clear the output for work purposes
debugclear
ret = IpOutputClear()

' Select Calibration for 4x Objective Lens
ret = IpSCalSelect("4x_065")
ret = IpTemplateMode(0)

fName = Dir(gDirStart + "*.BMP", 32)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")
While fName <> ""
    ret = IpTemplateMode (1)
    ret = IpWsLoad(gDirStart + fName, "BMP")
    ret = IpMacroStop ("If this image presents undesirable areas that can be filled, select them as AOIs.", 0)
    'ret = IpAoiCreateIrregular(Pts(0), 7)
    ret = IpMacroStop ("Click continue to go to the next step", 0)
    B = IpMacroStop ("Do you want to fill AOIs with white?", MS_MODAL + MS_YESNO + MS_QUEST)
    If B = 1 Then
        ret = IpWsFill(0,2,0)
        ret = IpAoiShow(FRAME_RESET)
        ret = IpAoiShow(FRAME_NONE)
        ret = IpAoiMultAppend(0)
Appendix B (continued)

    ret = IpAoiMultShow(0)

    Else
    End If

    F = IpMacroStop("Click on YES to save and open next image.", MS_MODAL + MS_YESNO)

    ret = IpTemplateMode(0)

    If F = 1 Then
        X = X + 1
        'User selected folder where to save image
        'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
        ret = IpTemplateMode (1)
        ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
        ret = IpWsSaveAs(IName, "BMP")
        ret = IpAppCloseAll()
    Else
        Exit Sub
    End If
    ret = IpTemplateMode (0)
    ' Get the next file name
    fName = Dir()

    Wend

    ret = IpMacroStop("All images in directory processed.", MS_MODAL)

End Sub

' This Macro: Opens one by one all edited GSI's in a folder, counts and measures all particle shapes found in each image, saves all data calculated as a text file (DATA.TXT)
' within folder

Sub MakeBINI_C_M()

    ' Scan through and process all files in a folder

    ret = IpAoiMultShow(0)
Appendix B (continued)

ret = IpMacroStop ("This macro will open all binary images within a folder and will count, measure and sort all particles found. Important: Make sure all BINIs have been audited before applying this macro.", 0)

Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim docID As Integer
Dim savefol2 As String
Dim X As Integer

X = 0

' Creating starting point
If gDirStart = "" Then
gDirStart = "C:\"
End If

' Select a folder where to store the outputs
savefol2 = GetFilePath("SORTED_BIN", ",", "C:\", "Select a folder where to store binary images with edited particles", 2)

' Get a file name from a folder
workStr = GetFilePath("," BMP, gDirStart, "Specify a folder where to find the edited GSIs", 0)

' Check that user did not cancel
If workStr = "" Then
Exit Sub
End If

' Close all open images prior to processing
ret = IpAppCloseAll()

' Extract the directory name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")")

' Clear the output for work purposes
ddebugclear
ret = IpOutputClear()

'Select Calibration For 4x Objective Lens
ret = IpSCalSelect("4x_065")
Appendix B (continued)

ret = IpTemplateMode(0)

fName = Dir(gDirStart + "*.BMP", 32)

While fName <> ""
X = X + 1

' Print out the file name and its attributes
Debug.Print GetAttr(gDirStart + fName); " "; fName

' Load the image sequence
docID = IpWsLoad(gDirStart + fName, "BMP")

' Start routine
If docID >= 0 Then

ret = IpTemplateMode(0)

' Selecting Spatial Calibration
ret = IpSCalSelect("4x_065")
ret = IpSCalShowEx(SCAL_DLG_SELECT, SCAL_HIDE)

' Start Count/Size Process
ret = IpBlbShow(1)
ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 0)
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
ret = IpBlbSetAttr(BLOB_8CONNECT,1)
ret = IpBlbSetAttr(BLOB_MINAREA,0)
ret = IpBlbSetAttr(BLOB_CLEANBORDER,1)
ret = IpBlbSetAttr(BLOB_FILLHOLES, 1)
ret = IpBlbSetAttr(BLOB_LABELMODE, 1)

' Reset for no measurements
ret = IpBlbEnableMeas(BLBM_ALL, 0)

' Feret(min): Smallest caliper(feret) length
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)

' Area of particle
ret = IpBlbEnableMeas(BLBM_AREA, 1)

' Filter that excludes particles outside the range 75<Min Caliper<2000 um and Area<2435 um^2
ret = IpBlbSetFilterRange(BLBM_MINFERRET, 75, 50000)
Appendix B (continued)

```c
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
ret = IpBlbUpdate(0)

ret = IpBlbCount()
'Create a Mask
ret = IpBlbCreateMask()
ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 1)
' ***MEASUREMENTS**
' Reset for no measurements
ret = IpBlbEnableMeas(BLM_ALL, 0)
' Perimeter: Length of particle's outline.
ret = IpBlbEnableMeas(BLM_PERIMETER, 1)
' Area of particle
ret = IpBlbEnableMeas(BLM_AREA, 1)
' Roundness: Perimeter^2 / (4*pi*Area).
ret = IpBlbEnableMeas(BLM_ROUNDNESS, 1)

' Perimeter(ellipse): Perimeter of the equivalent ellipse
ret = IpBlbEnableMeas(BLM_PELLIPSE, 1)
' Perimeter(convex): Perimeter of the convex outline of the particle
ret = IpBlbEnableMeas(BLM_PCONVEX, 1)

' Fractal dimension of the particle's outline
ret = IpBlbEnableMeas(BLM_FRACTDIM, 1)

' Axis(major): Length of major axis of ellipse with same moments of order 1 and
2 as particle
ret = IpBlbEnableMeas(BLM_MAJORAX, 1)
' Axis(minor): Length of major axis of ellipse with same moments of order 1 and
2 as particle
ret = IpBlbEnableMeas(BLM_MINORAX, 1)
' Aspect: Ratio between major axis and minor axis of ellipse equivalent to particle
ret = IpBlbEnableMeas(BLM_ASPECT, 1)

' Diameter(max): Length of longest line joining two points of object's outline and
passing through the centroid
ret = IpBlbEnableMeas(BLM_MAXFERRET, 1)
' Diameter(min): Length of shortest line joining two points of object's outline and
passing through the centroid
ret = IpBlbEnableMeas(BLM_MINFERRET, 1)
```
Appendix B (continued)

' Diameter (mean): Average length of diameters measured at 2 degrees intervals and passing through particle's centroid
ret = IpBlbEnableMeas(BLM_MEANFERRET, 1)

' Feret(max): Longest caliper(feret) length
ret = IpBlbEnableMeas(BLM_MAXCALIP, 1)
' Feret(min): Smallest caliper(feret) length
ret = IpBlbEnableMeas(BLM_MINCALIP, 1)
' Feret(mean): Average caliper(feret) length
ret = IpBlbEnableMeas(BLM_MEANCALIP, 1)

' Radius(max): Maximum distance between particle's centroid and outline
ret = IpBlbEnableMeas(BLM_MAXRADIUS, 1)
' Radius (min): Minimum distance between particle's centroid and outline
ret = IpBlbEnableMeas(BLM_MINRADIUS, 1)
' Radius Ratio: Ratio between Max. Radius and Min. Radius
ret = IpBlbEnableMeas(BLM_RADIUSRATIO, 1)

' Filter that excludes particles outside the range 50<Min Caliper<3000 um and 1500<Area<1000000 um^2
ret = IpBlbSetFilterRange(BLM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLM_AREA, 1500, 1000000)

' Sort by Area
ret = IpSortAttr(SORT_AUTO, 1)
' ret = IpSortAttr(SORT_INDEX, 255)
ret = IpSortAttr(SORT_MEAS, 10)

' count BINIs
ret = IpBlbUpdate(0)
ret = IpBlbCount()

If X = 1 Then
ret = IpBlbSaveData(savefol2 + "DATA.TXT", S_APPEND+S_HEADER+S_Y_AXIS)
ret = IpBlbShow(0)
Else
IpBlbSaveData(savefol2 + "DATA.TXT", S_APPEND+S_Y_AXIS)
ret = IpBlbShow(0)
End If
Appendix B (continued)

'User selected folder where to save image
'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret = IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()
    Else
        Debug.Print "Error loading "; gDirStart + fName
    End If

' Get the next file name
fName = Dir()

Wend
    ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)

End Sub
Appendix C. Source Code for the Script File Mult2DShape10x

Option Explicit

' Global directory starting string, for saving where we last processed
Dim gDirStart As String
' This Macro: Helps the operator to generate sets of images at different focal planes

Sub Acquisition()
    ret = IpMacroStop("Place the sample on the microscope stage. Locate a group of
sand particles using the Preview screen and start acquiring image sets.",0)
    ret = IpTemplateMode(0)
    ret = IpAcqShow(ACQ_SNAP, 1)
    ret = IpAcqShow(ACQ_LIVE, 1)
    IpArray(0) = 0 'left
    IpArray(1) = 0 'top
    IpArray(2) = 1391 'right
    IpArray(3) = 1039 'bottom
    ret = IpAcqControl(88, 1, IpArray(0)) ' ACQCMD_AUTOEXPOSURE
    ret = IpTemplateMode(0)
    ret = IpScopeShow(1)
    ret = IpMacroStop("Select a setting according to current objective magnification. Set
Top and Bottom limits and click on Acquire. Save all files as SEQUENCES.",0)

End Sub

' This Macro: Automatically opens image sequences one by one. Then it deconvolves
each sequence, creates an EDF Test Strip, applies a Local Best Fit Contrast
Enhancement
' routine, Applies a Variance Filter and then saves edge enhanced images

Sub EnhanceEdges()

' Scan through and process all files in a directory
Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim savefol As String
Dim docID As Integer
Dim X As Integer
X = 0
Appendix C (continued)

'Operator selects a folder where to store enhanced images
savefol = GetFilePath("EDFts", "", "C:\", "Select a file where to store enhanced images", 2)

' Creating starting point
  If gDirStart = "" Then
    gDirStart = "C:\"
  End If

' Get a file name in the desired directory
  workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find image sequences", 0)

' Check if user did not cancel
  If workStr = "" Then
    Exit Sub
  End If

' Close all open images prior to processing
  ret = IpAppCloseAll()

' Extract the directory name from the full file name
  gDirStart = Left(workStr, InStrRev(workStr, ")")

' Clear the output for work purposes
  debugclear
  ret = IpOutputClear()

' Select Calibration for 10x Objective Lens
  ret = IpSCalSelect("10x_065")

      ret = IpTemplateMode(0)
      fName = Dir(gDirStart + "*.SEQ", 32)

    While fName <> ""
      X = X + 1

      ' Print out the file name and its attributes
        Debug.Print GetAttr(gDirStart + fName); " "; fName

      ' Load the image sequence
        docID = IpSeqOpen(gDirStart + fName, "SEQ",0,15)
Appendix C (continued)

    ret = IpSMShowNav(SM_HIDE)
    ret = IpTemplateMode(0)
    ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
    ret = IpDocCloseEx(0)

' Start routine
If docID >= 0 Then
    ret = IpTemplateMode(0)

' Deconvolution using SharpStack for 10X Objective Lens parameters
    ret = IpDcnvShow(0)
    ret = IpLensApply("10x_065", APPLYTO_IMAGE)
    ret = IpSCalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
    ret = IpDCnvSet(DCNV_TYPE, DCTYPE_INVERSE)
    ret = IpDCnvSetSng(DCNV_NA, 0.250000)
    ret = IpDCnvSetSng(DCNV_RI, 1.000000)
    ret = IpDCnvSetSng(DCNV_WL, 775.000000)
    ret = IpDCnvSetSng(DCNV_XSPACING, 0.714286)
    ret = IpDCnvSetSng(DCNV_YSPACING, 0.714286)
    ret = IpDCnvSetSng(DCNV_ZSPACING, 1.5000000)
    ret = IpDCnvSet(DCNV_BRIGHTFIELD, 0)
    ret = IpDCnvSet(DCNV_PHASEOBJECTS, 0)
    ret = IpDCnvSet(DCNV_NEIGHBORSPACING, 1)
    ret = IpDCnvSet(DCNV_HAZEREMOVAL, 10)
    ret = IpDCnvSet(DCNV_USEACTIVEPORTION, 0)
    ret = IpDCnvSet(DCNV_CONVERTTOFLOAT, 0)
    ret = IpDCnvSet(DCNV_SPHERICALABERRATION, -9)
    ret = IpDCnvDeconvolve
    ret = IpDcnvShow(0)

' Filtering
' EDF(Extended Depth of Field Test Strip)
    ret = IpTemplateMode(0)
    Dim nNorm%, nCriteria%, nOrder%
    Dim sNorm(0 To 1) As String, valNorm(0 To 1) As Integer
    Dim sCriteria(0 To 3) As String, valCriteria(0 To 3) As Integer
    Dim sOrder(0 To 1) As String, valOrder(0 To 1) As Integer
    Dim nSeries%, nEDF%, nFrames%
    Dim textStr As String

' Set source to the current document?
    ret = IpEDFRmove(DOCSEL_ALL)
Appendix C (continued)

    ret = IpEDFNew(1)

    ' Set values and strings for the various parameters
    valNorm(0) = 0
    sNorm(0) = "Not Normalized"
    valNorm(1) = 1
    sNorm(1) = "Normalized"

    valCriteria(0) = EDF_MAX_LOCALCONTRAST
    sCriteria(0) = "Local Contrast"
    valCriteria(1) = EDF_MAXDEPTHCONTRAST
    sCriteria(1) = "Depth Contrast"
    valCriteria(2) = EDF_MAX_INTENSITY
    sCriteria(2) = "Maximum"
    valCriteria(3) = EDF_MIN_INTENSITY
    sCriteria(3) = "Minimum"

    valOrder(0) = EDF_TOPDOWN
    sOrder(0) = "Top Down"
    valOrder(1) = EDF_BOTTOMUP
    sOrder(1) = "Bottom Up"

    ' Initialize value for series
    nSeries = -1

    ' Loop through the various criteria
    ' Various criteria
    For nCriteria = 0 To 3
    '   Various orders
    '   For nOrder = 0 To 1
    '      Normalize or not
    '      For nNorm = 0 To 1

    '      Set parameters and create the EDF
    ret = IpEDFSet(EDF_NORMALIZE, valNorm(nNorm), 0)
    ret = IpEDFSet(EDF_CRITERIA, valCriteria(nCriteria), 0)
    ret = IpEDFSet(EDF_ORDER, valOrder(nOrder), 0)
    ret = IpEDFCreate(EDF_COMPOSITE)
    ret = IpDocGet(GETACTDOC, 0, nEDF)

    '      Write description onto images
    textStr = sCriteria(nCriteria) + Chr$(10)
Appendix C (continued)

textStr = textStr + sNorm(nNorm) + Chr$(10)
textStr = textStr + sOrder(nOrder)

ret = IpAnCreateObj(GO_OBJ_TEXT)
ret = IpAnMove(0, 20, 20)
ret = IpAnText(textStr)

ret = IpAnSet(GO_ATTR_ZOOM, 0)
ret = IpAnSet(GO_ATTR_RECTSTYLE, GO_RECTSTYLE_NOBORDER_FILL)
ret = IpAnSet(GO_ATTR_TEXTAUTOSIZE, 1)
ret = IpAnSet(GO_ATTR_FONTBOLD, 700)
ret = IpAnSet(GO_ATTR_BRUSHCOLOR, 12632256)
ret = IpAnSet(GO_ATTR_TEXTCOLOR, 0)
ret = IpAnBurn()

' Add to the series
If nSeries < 0 Then
  ' Set series to this image
  nSeries = nEDF
Else
  ' Or append EDF to the accumulated series
  ret = IpAppSelectDoc(nEDF)
  ret = IpWsCopyFrames(0,1)
  ret = IpAppSelectDoc(nSeries)
  ret = IpWsPasteFrames(-1)

  ' Set to the last frame of the series
  ret = IpSeqGet(SEQ_NUMFRAMES, nFrames)
  ret = IpSeqSet(SEQ_ACTIVEFRAME, nFrames-1)

  ' Clean up the EDF
  ret = IpAppSelectDoc(nEDF)
  ret = IpDocClose()
End If

Next nNorm
Next nOrder
Next nCriteria

' Delete previous sequences
ret = IpDocCloseEx(1)
Appendix C (continued)

ret = IpDocCloseEx(2)

' Further Image enhancement
' Local Best Fit Contrast Enhancement

Dim source%, dest%, minima%, maxima%, diff%, floor%, scaled%
Dim docInfo As IPDOCINFO, scaleVal As Single

' Check image class and scaling
ret = IpDocGet(GETDOCINFO, DOCSEL_ACTIVE, docInfo)
Select Case docInfo.Class
Case IMC_GRAY, IMC_RGB
    scaleVal = 255.0
Case IMC_GRAY12, IMC_RGB36
    scaleVal = 4095.0
Case IMC_GRAY16, IMC_RGB48
    scaleVal = 65535.0
Case Else
    ' Not supported!
    Beep
    ret = IpMacroStop("Image type not supported!", vbOK)
    Exit Sub
End Select

' Create working images
ret = IpDocGet(GETACTDOC, DOCSEL_ACTIVE, source)
dest = IpWsDuplicate()
minima = IpWsDuplicate()
maxima = IpWsDuplicate()

' Smoothed minima
ret = IpAppSelectDoc(minima)
ret = IpFltErode(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Smoothed maxima
ret = IpAppSelectDoc(maxima)
ret = IpFltDilate(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Difference (local contrast scale)
diff = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 1)
Appendix C (continued)

```plaintext
ret = IpAppSelectDoc(dest)
    ret = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 0)
    ret = IpOpImageArithmetics(diff, scaleVal, OPA_DIV, 0)

' Clean up
    ret = IpAppSelectDoc(minima)
    ret = IpDocClose()
    ret = IpAppSelectDoc(maxima)
    ret = IpDocClose()
    ret = IpAppSelectDoc(diff)
    ret = IpDocClose()

' Average with image after enhancement
    ret = IpOpImageArithmetics(source, 0.0, OPA_AVG, 0)

' Application of Variance Filter
    ret = IpTemplateMode(0)
    ret = IpWsDuplicate()
    ret = IpFltVariance(7, 7)
    ret = IpDocCloseEx(1)

' Average with EDF TestStrip sequence
    ret = IpTemplateMode(0)
    ret = IpAppSelectDoc(18)
    ret = IpDocClose()
    ret = IpAppSelectDoc(19)
    ret = IpOpImageArithmetics(2, 0.0, OPA_AVG, 0)
    ret = IpOpShow(0)

' Convert image to grayscale 8
    ret = IpTemplateMode(0)
    ret = IpAoiShow(FRAME_NONE)
    ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
    ret = IpHstEqualize(EQ_BESTFIT)

' User selects folder where to save image
    ' DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret = IpStAutoName(savefol + "###.SEQ", X, IName)
    ret = IpWsSaveAs(IName, "SEQ")
    ret = IpAppCloseAll()
```
Appendix C (continued)

Else
    Debug.Print "Error loading "; gDirStart + fName
End If

' Get the next file name
fName = Dir()

Wend

ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)

End Sub

' This Macro: Opens EDF Test Strips from a folder in successive order, Uses the Count/Measure tool to filter by area and min caliper.

Sub Sort_GSIs()

    ret = IpMacroStop("This macro starts a new session.", 0)

    Dim IName As String*255
    Dim fName As String
    Dim workStr As String
    Dim savefol2 As String
    Dim docID As Integer
    Dim X As Integer

    ' Initialize X
    X = 0

    ' Select a folder where to store the outputs
    savefol2 = GetFilePath("SortedGSIs", ",", "C:\", "Select a folder where to store sorted Gray Scale Images", 2)

    ' Creating starting point
    If gDirStart = "/" Then
        gDirStart = "C:\"
    End If

    ' Get a file name from a folder
    workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find EDF Test Strips", 0)
Appendix C (continued)

' Check if the user did not cancel
If workStr = "" Then
    Exit Sub
End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ""))

' Clear the output for work purposes
dbgeclear
ret = IpOutputClear()

' Select Calibration for 10x Objective Lens
ret = IpSCalSelect("10x_065")
ret = IpTemplateMode(1)

fName = Dir(gDirStart + "*.SEQ", 32)
ret = IpMacroStop("Explore any Test Strip from the group. Select the best frame where particles outlines are well defined.",0)
' ret = IpTemplateMode(1)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")

' Determine Frame number to extract for all the Test Strips within the folder

Dim gnFrame As Long

' Set up initial values
 gnFrame = 2
Begin Dialog UserDialog 320,112,"Select frame number to extract."
 Text 40,21,90,14,"Frame number",.Frame
 TextBox 140,14,90,21,.nFrame
 OKButton 80,84,90,21
End Dialog
Dim dlg3 As UserDialog

' Apply to dialog
dlg3.nFrame = Str(gnFrame)
Appendix C (continued)

' Get user values
Dialog dlg3

' Get back from dialog
gnFrame = Val(dlg3.nFrame) - 1
ret = IpSeqExtractFrames(gnFrame, 1)

' Determine Intensity range manually
Dim gnInte As Long

'ret = IpBlbShow(1)
ret = IpSegShow(1)
ret = IpSegSetAttr(SETCURSEL, 0)
ret = IpSegSetAttr(CHannel, 0)
ret = IpSegPreview(CURRENT_C_T)

' Set up initial values
gnInte = 123
Text 40,21,90,14,"Select a value for intensity",.Inte
TextBox 140,14,90,21,.nInte
OKButton 80,84,90,21
End Dialog
Dim dlg4 As UserDialog

' Apply to dialog
dlg4.nInte = Str(gnInte)

' Get user values
Dialog dlg4

' Get back from dialog
gnInte = Val(dlg4.nInte)

ret = IpTemplateMode(0)
ret = IpAppCloseAll()
While fName <> ""
X = X + 1

' Print out the file name and its attributes
Debug.Print GetAttr(gDirStart + fName); " "; fName

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Appendix C (continued)

' Load the image sequence
docID = IpSeqOpen(gDirStart + fName, "SEQ",0,16)

' Start routine
If docID >= 0 Then

' Extract frame of interest
ret = IpSeqExtractFrames(gnFrame, 1)

' Converting to binary
ret = IpTemplateMode(0)
' Select Calibration for 10x Objective Lens
ret = IpSCalSelect("10x_065")
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
' Apply Filter Ranges
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
' Do accumulate count
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
' Reset for no measurements
ret = IpBlbEnableMeas(BLM_ALL, 0)
' Measure Min Feret
ret = IpBlbEnableMeas(BLM_MINCALIP, 1)
' Measure Area
ret = IpBlbEnableMeas(BLM_AREA, 1)
' Count range for particles (obtained from the manual intensity selection)
ret = IpBlbSetAttr(BLOB_AUTORANGE, 0)
ret = IpSegSetRange(-1, 0, gnInte)
' Apply smoothing
ret = IpBlbSetAttr(BLOB_SMOOTHING,1)
' Apply 8-connected objects
ret = IpBlbSetAttr(BLOB_8CONNECT,1)
' Fill holes
ret = IpBlbSetAttr(BLOB_FILLHOLES,0)
ret = IpBlbSetAttr(BLOB_MINAREA,1)
ret = IpBlbSetAttr(BLOB_CLEANBORDER,1)
ret = IpSortAttr(SORT_LABELS, 0)
' Count
ret = IpBlbCount()
' Measure Min Feret
ret = IpBlbEnableMeas(BLM_MINCALIP, 1)
' Measure Area
ret = IpBlbEnableMeas(BLM_AREA, 1)
Appendix C (continued)

' Filter that excludes particles outside the range 50<Min Caliper<3000 um and 1500<Area<1000000 um^2
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
' Count
ret = IpBlbCount()
' New counting properties
ret = IpBlbSetAttr(BLOB_AUTORANGE, 0)
ret = IpSegSetRange(0, 0, gnInte)
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
ret = IpBlbSetAttr(BLOB_8CONNECT, 1)
ret = IpBlbSetAttr(BLOB_MINAREA, 1)
ret = IpBlbSetAttr(BLOB_CLEANBORDER, 1)
ret = IpBlbSetAttr(BLOB_SMOOTHING, 1)
ret = IpSortAttr(SORT_ROTATE, 1)
' Filter that excludes particles outside the range 75<Min Caliper<2000 um and 1500<Area<100000 um^2
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
' Count again before sorting
ret = IpBlbCount()
ret = IpBlbUpdate(0)
' Set automatic background
ret = IpSortAttr(SORT_AUTO, 0)
ret = IpSortAttr(SORT_INDEX, 255)
' Sort by Min feret
ret = IpSortAttr(SORT_MEAS, 10)
'ret = IpBlbUpdate(0)
ret = IpSortObjects()

' User selected folder where to save image
'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
ret= IpStAutoName(savefol2 + "###.BMP", X, IName)
ret= IpWsSaveAs(IName, "BMP")
ret = IpAppCloseAll()

Else
    Debug.Print "Error loading "; gDirStart + fName
End If
Appendix C (continued)

' Get the next file name
fName = Dir()
Wend

ret = IpMacroStop("All images in directory processed.", MS_MODAL)

End Sub

' This Macro: Opens sorted GSI's and lets the operator get rid of spots and defected shapes

Sub EditGSIs()

ret = IpMacroStop("This macro will help you edit GSIs before starting the process of measuring and counting.", 0)

Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim savefol2 As String
Dim docID As Integer
Dim F As Integer
Dim B As Integer
Dim X As Integer

' Initialize X
Dim gnFrame1 As Long

' Select a folder where to store the outputs
savefol2 = GetFilePath("EdGSIs", ",", "C:\", "Select a folder where to store edited images", 2)
' Set up initial values
gnFrame1 = 000
Begin Dialog UserDialog 520,112,"What is the number of the last image saved?"
Text 40,21,90,14,"Image number",.Frame1
TextBox 140,14,90,21,.nFrame1
OKButton 80,84,90,21
End Dialog

Dim dlg31 As UserDialog
' Apply to dialog
Appendix C (continued)

dl31.nFrame1 = Str(gnFrame1)

' Get user values
Dialog dlg31

' Get back from dialog
gnFrame1 = Val(dlg31.nFrame1)

X = gnFrame1

' Creating starting point
If gDirStart = "" Then
    gDirStart = "C:\"
End If

' Get a file name from a folder
workStr = GetFilePath("", "BMP", gDirStart, "Specify a folder where to find the sorted GSIs", 0)

' Check if the user did not cancel
If workStr = "" Then
    Exit Sub
End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")

' Clear the output for work purposes
ddebugclear
ret = IpOutputClear()

' Select Calibration for 10x Objective Lens
ret = IpSCalSelect("10x_065")
ret = IpTemplateMode(0)

fName = Dir(gDirStart + "*.BMP", 32)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")
Appendix C (continued)

While fName <> ""

ret = IpTemplateMode(1)
ret = IpWsLoad(gDirStart + fName, "BMP")

ret = IpMacroStop("If this image presents undesirable areas that can be filled, select them as AOIs.", 0)
ret = IpMacroStop("Click continue to go to the next step", 0)
B = IpMacroStop("Do you want to fill AOIs with white?", MS_MODAL + MS_YESNO + MS_QUEST)

If B = 1 Then
    ret = IpWsFill(0,2,0)
    ret = IpAoiShow(FRAME_RESET)
    ret = IpAoiShow(FRAME_NONE)
    ret = IpAoiMultAppend(0)
    ret = IpAoiMultShow(0)
Else
    End If

F = IpMacroStop("Click on YES to save and open next image.", MS_MODAL + MS_YESNO)
ret = IpTemplateMode(0)

If F = 1 Then
    X = X + 1
    'User selected folder where to save image
    'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret = IpTemplateMode(1)
    ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret = IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()
Else
    Exit Sub
    End If
ret = IpTemplateMode(0)

' Get the next file name
Appendix C (continued)

fName = Dir()
Wend

ret = IpMacroStop("All images in directory processed.", MS_MODAL)
End Sub

' This Macro: Opens one by one all binary images in a folder, counts and measures all
particle shapes found in each image, saves all data calculated as a text file
(DATA.TXT)
' within folder

Sub MakeBINI_C_M()

' Scan through and process all files in a folder

ret = IpMacroStop ("This macro will open all binary images within a folder and will
count, measure and sort all particles found. Important: Make sure all BINIs have been
audited before applying this macro.", 0)

Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim docID As Integer
Dim savefol2 As String
Dim X As Integer

X = 0

' Creating starting point
If gDirStart = "" Then
    gDirStart = "C:\"
End If
' Select a folder where to store the outputs
savefol2 = GetFilePath("SORTED_BIN", ",", ",C:\", "Select a folder where to store binary images with edited particles", 2)

' Get a file name from a folder
workStr = GetFilePath("", "BMP", gDirStart, "Specify a folder where to find the edited GSIs", 0)
Appendix C (continued)

' Check that user did not cancel
If workStr = "" Then
  Exit Sub
End If

' Close all open images prior to processing
ret = IpAppCloseAll()

' Extract the directory name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")")

' Clear the output for work purposes
debugclear
ret = IpOutputClear()

'Select Calibration For 10x Objective Lens
ret = IpSCalSelect("10x_065")

ret = IpTemplateName(0)

fName = Dir(gDirStart + "*.BMP", 32)

While fName <> ""
  X = X + 1
  ' Print out the file name and its attributes
  Debug.Print GetAttr(gDirStart + fName); " " ; fName
  ' Load the image sequence
  docID = IpWsLoad(gDirStart + fName, "BMP")

  ' Start routine
  If docID >= 0 Then
    ret = IpTemplateName(0)

    ' Selecting Spatial Calibration
    ret = IpSCalSelect("10x_065")
    ret = IpSCalShowEx(SCAL_DLG_SELECT, SCAL_HIDE)
    ' Start Count/Size Process
    'ret = IpBlbShow(1)
    ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
  End If

End While
Appendix C (continued)

ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 0)
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
ret = IpBlbSetAttr(BLOB_8CONNECT, 1)
ret = IpBlbSetAttr(BLOB_MINAREA, 0)
ret = IpBlbSetAttr(BLOB_CLEANBORDER, 1)
ret = IpBlbSetAttr(BLOB_FILLHOLES, 1)
ret = IpBlbSetAttr(BLOB_LABELMODE, 1)
ret = IpSortAttr(SORT_ROTATE, 1)
' Reset for no measurements
ret = IpBlbEnableMeas(BLM_ALL, 0)
' Feret(min): Smallest caliper(feret) length
ret = IpBlbEnableMeas(BLM_MINCALIP, 1)
' Area of particle
ret = IpBlbEnableMeas(BLM_AREA, 1)
' Filter that excludes particles outside the range 50<Min Caliper<0000 um<1500<Area<1000000 um^2
ret = IpBlbSetFilterRange(BLM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLM_AREA, 1500, 1000000)
ret = IpBlbUpdate(0)
ret = IpBlbCount()
'Create a Mask
ret = IpBlbCreateMask()
ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 1)
' ***MEASUREMENTS**
' Reset for no measurements
ret = IpBlbEnableMeas(BLM_ALL, 0)
' Perimeter: Length of particle's outline.
ret = IpBlbEnableMeas(BLM_PERIMETER, 1)
' Area of particle
ret = IpBlbEnableMeas(BLM_AREA, 1)
' Roundness: Perimeter^2/ (4*pi*Area).
ret = IpBlbEnableMeas(BLM_ROUNDESS, 1)
' Perimeter(ellipse): Perimeter of the equivalent ellipse
ret = IpBlbEnableMeas(BLM_PELLIPSE, 1)
' Perimeter(convex): Perimeter of the convex outline of the particle
ret = IpBlbEnableMeas(BLM_PCONVEX, 1)
' Fractal dimension of the particle's outline
ret = IpBlbEnableMeas(BLM_FRACTDIM, 1)
Appendix C (continued)

' Axis(major): Length of major axis of ellipse with same moments of order 1 and 2 as particle
ret = IpBblEnableMeas(BLBM_MAJORAX, 1)
' Axis(minor): Length of major axis of ellipse with same moments of order 1 and 2 as particle
ret = IpBblEnableMeas(BLBM_MINORAX, 1)
' Aspect: Ratio between major axis and minor axis of ellipse equivalent to particle
ret = IpBblEnableMeas(BLBM_ASPECT, 1)
' Diameter(max): Length of longest line joining two points of object's outline and passing through the centroid
ret = IpBblEnableMeas(BLBM_MAXFERRET, 1)
' Diameter(min): Length of shortest line joining two points of object's outline and passing through the centroid
ret = IpBblEnableMeas(BLBM_MINFERRET, 1)
' Diameter (mean): Average length of diameters measured at 2 degrees intervals and passing through particle's centroid
ret = IpBblEnableMeas(BLBM_MEANFERRET, 1)
' Feret(max): Longest caliper(feret) length
ret = IpBblEnableMeas(BLBM_MAXCALIP, 1)
' Feret(min): Smallest caliper(feret) length
ret = IpBblEnableMeas(BLBM_MINCALIP, 1)
' Feret(mean): Average caliper(fert) length
ret = IpBblEnableMeas(BLBM_MEANCALIP, 1)
' Radius(max): Maximum distance between particle's centroid and outline
ret = IpBblEnableMeas(BLBM_MAXRADIUS, 1)
' Radius (min): Minimum distance between particle's centroid and outline
ret = IpBblEnableMeas(BLBM_MINRADIUS, 1)
' Radius Ratio: Ratio between Max. Radius and Min. Radius
ret = IpBblEnableMeas(BLBM_RADIUSRATIO, 1)

' Filter that excludes particles outside the range 50<Min Caliper<3000 um and 1500<Area<1000000 um^2
ret = IpBblSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBblSetFilterRange (BLBM_AREA, 1500, 1000000)

' count BINIs
ret = IpBblUpdate(0)
ret = IpBblCount()

If X = 1 Then
ret = IpBblSaveData(savefol2 + "DATA.TXT", S_APPEND+S_HEADER+S_Y_AXIS)
Appendix C (continued)

    ret = IpBlbShow(0)
    Else
        IpBlbSaveData(savefol2 + "DATA.TXT", S_APPEND+S_Y_AXIS)
        ret = IpBlbShow(0)
    End If

    'User selected folder where to save image
    'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret= IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret= IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()
    Else
        Debug.Print "Error loading "; gDirStart + fName
    End If

    ' Get the next file name
    fName = Dir()

    Wend
    ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)

End Sub
Appendix D. Source Code for the Script File Mult2DShape20x

Option Explicit

' Global directory starting string, for saving where we last processed
Dim gDirStart As String
' This Macro: Helps the operator to generate sets of images at different focal planes

Sub Acquisition()
    ret = IpMacroStop("Place the sample on the microscope stage. Locate a group of
sand particles using the Preview screen and start acquiring image sets.",0)
    ret = IpTemplateMode(0)
    ret = IpAcqShow(ACQ_SNAP, 1)
    ret = IpAcqShow(ACQ_LIVE, 1)
    IpArray(0) = 0 'left
    IpArray(1) = 0 'top
    IpArray(2) = 1391 'right
    IpArray(3) = 1039 'bottom
    ret = IpAcqControl(88, 1, IpArray(0)) ' ACQCMD_AUTOEXPOSURE
    ret = IpTemplateMode(0)
    ret = IpScopeShow(1)
    ret = IpMacroStop("Select a setting according to current objective magnification. Set
Top and Bottom limits and click on Acquire. Save all files as SEQUENCES.",0)
End Sub

' This Macro: Automatically opens image sequences one by one. Then it deconvolves
each sequence, creates an EDF Test Strip, applies a Local Best Fit Contrast
Enhancement
' routine, Applies a Variance Filter and then saves edge enhanced images

Sub EnhanceEdges()

' Scan through and process all files in a directory
Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim savefol As String
Dim docID As Integer
Dim X As Integer
'Initialize X
X = 0
Appendix D (continued)

'Operator selects a folder where to store enhanced images
savefol = GetFilePath("EDFts", "", "C:\", "Select a file where to store enhanced images", 2)

' Creating starting point
If gDirStart = "" Then
    gDirStart = "C:\"
End If

' Get a file name in the desired directory
workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find image sequences", 0)

' Check if user did not cancel
If workStr = "" Then
    Exit Sub
End If

' Close all open images prior to processing
ret = IpAppCloseAll()

' Extract the directory name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")

' Clear the output for work purposes
declear
ret = IpOutputClear()

' Select Calibration for 20x Objective Lens
ret = IpSCalSelect("20x_065")

ret = IpTemplateMode(0)
fName = Dir(gDirStart + "*.SEQ", 32)

While fName <> ""
    X = X + 1
    Debug.Print GetAttr(gDirStart + fName); " "; fName

    ' Load the image sequence
    docID = IpSeqOpen(gDirStart + fName, "SEQ",0,15)
Appendix D (continued)

    ret = IpSMShowNav(SM_HIDE)
    ret = IpTemplateMode(0)
    ret = IpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
    ret = IpDocCloseEx(0)

' Start routine
If docID >= 0 Then
    ret = IpTemplateMode(0)

' Deconvolution using SharpStack for 10X Objective Lens parameters
    ret = IpDcnvShow(0)
    ret = IpLensApply("20x_065", APPLYTO_IMAGE)
    ret = IpSCalSetLong(SCAL_CURRENT_CAL, SCAL_APPLY, 0)
    ret = IpDCnvSet(DCNV_TYPE, DCTYPE_INVERSE)
    ret = IpDCnvSetSng(DCNV_NA, 0.400000)
    ret = IpDCnvSetSng(DCNV_RI, 1.000000)
    ret = IpDCnvSetSng(DCNV_WL, 775.000000)
    ret = IpDCnvSetSng(DCNV_XSPACING, 0.350877)
    ret = IpDCnvSetSng(DCNV_YSPACING, 0.350877)
    ret = IpDCnvSetSng(DCNV_ZSPACING, 1.5000000)
    ret = IpDCnvSet(DCNV_BRIGHTFIELD, 0)
    ret = IpDCnvSet(DCNV_PHASEOBJECTS, 0)
    ret = IpDCnvSet(DCNV_NEIGHBORSPACING, 1)
    ret = IpDCnvSet(DCNV_HAZEREMOVAL, 10)
    ret = IpDCnvSet(DCNV_USEACTIVEPORTION, 0)
    ret = IpDCnvSet(DCNV_CONVERTTOFLOAT, 0)
    ret = IpDCnvSet(DCNV_SPHERICALABERRATION, -14.6862)
    ret = IpDCnvDeconvolve
    ret = IpDCnvShow(0)

' Filtering
' EDF(Extended Depth of Field Test Strip)
    ret = IpTemplateMode(0)
    Dim nNorm%, nCriteria%, nOrder%
    Dim sNorm(0 To 1) As String, valNorm(0 To 1) As Integer
    Dim sCriteria(0 To 3) As String, valCriteria(0 To 3) As Integer
    Dim sOrder(0 To 1) As String, valOrder(0 To 1) As Integer
    Dim nSeries%, nEDF%, nFrames%
    Dim textStr As String

' Set source to the current document?
    ret = IpEDFRemove(DOCSEL_ALL)
Appendix D (continued)

ret = IpEDFNew(1)

' Set values and strings for the various parameters
valNorm(0) = 0
sNorm(0) = "Not Normalized"
valNorm(1) = 1
sNorm(1) = "Normalized"

valCriteria(0) = EDF_MAX_LOCALCONTRAST
sCriteria(0) = "Local Contrast"
valCriteria(1) = EDF_MAXDEPTHCONTRAST
sCriteria(1) = "Depth Contrast"
valCriteria(2) = EDF_MAX_INTENSITY
sCriteria(2) = "Maxmimum"
valCriteria(3) = EDF_MIN_INTENSITY
sCriteria(3) = "Minimum"

valOrder(0) = EDF_TOPDOWN
sOrder(0) = "Top Down"
valOrder(1) = EDF_BOTTOMUP
sOrder(1) = "Bottom Up"

' Initialize value for series
nSeries = -1

' Loop through the various criteria
' Various criteria
For nCriteria = 0 To 3
'   Various orders
   For nOrder = 0 To 1
      ' Normalize or not
      For nNorm = 0 To 1
         ' Set parameters and create the EDF
         ret = IpEDFSet(EDF_NORMALIZE, valNorm(nNorm), 0)
         ret = IpEDFSet(EDF_CRITERIA, valCriteria(nCriteria), 0)
         ret = IpEDFSet(EDF_ORDER, valOrder(nOrder), 0)
         ret = IpEDFCreate(EDF_COMPOSITE)
         ret = IpDocGet(GETACTDOC, 0, nEDF)
         ' Write description onto images
         textStr = sCriteria(nCriteria) + Chr$(10)
      Next nNorm
   Next nOrder
Next nCriteria
Appendix D (continued)

    textStr = textStr + sNorm(nNorm) + Chr$(10)
    textStr = textStr + sOrder(nOrder)

    ret = IpAnCreateObj(GO_OBJ_TEXT)
    ret = IpAnMove(0, 20, 20)
    ret = IpAnText(textStr)

    ret = IpAnSet(GO_ATTR_ZOOM, 0)
    ret = IpAnSet(GO_ATTR_RECTSTYLE, GO_RECTSTYLE_NOBORDER_FILL)
    ret = IpAnSet(GO_ATTR_TEXTAUTOSIZE, 1)
    ret = IpAnSet(GO_ATTR_FONTBOLD, 700)
    ret = IpAnSet(GO_ATTR_BRUSHCOLOR, 12632256)
    ret = IpAnSet(GO_ATTR_TEXTCOLOR, 0)
    ret = IpAnBurn()

    ' Add to the series
    If nSeries < 0 Then
        ' Set series to this image
        nSeries = nEDF
    Else
        ' Or append EDF to the accumulated series
        ret = IpAppSelectDoc(nEDF)
        ret = IpWsCopyFrames(0,1)
        ret = IpAppSelectDoc(nSeries)
        ret = IpWsPasteFrames(-1)

        ' Set to the last frame of the series
        ret = IpSeqGet(SEQ_NUMFRAMES, nFrames)
        ret = IpSeqSet(SEQ_ACTIVEFRAME, nFrames-1)

        ' Clean up the EDF
        ret = IpAppSelectDoc(nEDF)
        ret = IpDocClose()
    End If

    Next nNorm
    Next nOrder
    Next nCriteria

    ' Delete previous sequences
    ret = IpDocCloseEx(1)
Appendix D (continued)

ret = IpDocCloseEx(2)

' Further Image enhancement
' Local Best Fit Contrast Enhancement

Dim source%, dest%, minima%, maxima%, diff%, floor%, scaled%
Dim docInfo As IPDOCINFO, scaleVal As Single

' Check image class and scaling
ret = IpDocGet(GETDOCINFO, DOCSEL_ACTIVE, docInfo)
Select Case docInfo.Class
  Case IMC_GRAY, IMC_RGB
    scaleVal = 255.0
  Case IMC_GRAY12, IMC_RGB36
    scaleVal = 4095.0
  Case IMC_GRAY16, IMC_RGB48
    scaleVal = 65535.0
  Case Else
    ' Not supported!
    Beep
    ret = IpMacroStop("Image type not supported!", vbOK)
    Exit Sub
End Select

' Create working images
ret = IpDocGet(GETACTDOC, DOCSEL_ACTIVE, source)
dest = IpWsDuplicate()
minima = IpWsDuplicate()
maxima = IpWsDuplicate()

' Smoothed minima
ret = IpAppSelectDoc(minima)
ret = IpFltErode(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Smoothed maxima
ret = IpAppSelectDoc(maxima)
ret = IpFltDilate(MORPHO_7x7OCTAGON, 3)
ret = IpFltGauss(7, 10, 3*2)

' Difference (local contrast scale)
diff = IpOpImageArithmetics(minima, 0.0, OPA_SUB, 1)
Appendix D (continued)

    ret = lpAppSelectDoc(dest)
    ret = lpOpImageArithmetics(minima, 0.0, OPA_SUB, 0)
    ret = lpOpImageArithmetics(diff, scaleVal, OPA_DIV, 0)

' Clean up
    ret = lpAppSelectDoc(minima)
    ret = lpDocClose()
    ret = lpAppSelectDoc(maxima)
    ret = lpDocClose()
    ret = lpAppSelectDoc(diff)
    ret = lpDocClose()

' Average with image after enhancement
    ret = lpOpImageArithmetics(source, 0.0, OPA_AVG, 0)

' Application of Variance Filter
    ret = lpTemplateMode(0)
    ret = lpWsDuplicate()
    ret = lpFltVariance(7, 7)
    ret = lpDocCloseEx(1)

' Average with EDF TestStrip sequence
    ret = lpTemplateMode(0)
    ret = lpAppSelectDoc(18)
    ret = lpDocClose()
    ret = lpAppSelectDoc(19)
    ret = lpOpImageArithmetics(2, 0.0, OPA_AVG, 0)
    ret = lpOpShow(0)

' Convert image to grayscale 8
    ret = lpTemplateMode(0)
    ret = lpAoiShow(FRAME_NONE)
    ret = lpWsConvertImage(IMC_GRAY, CONV_SCALE, 0, 0, 0, 0)
    ret = lpHstEqualize(EQ_BESTFIT)

' User selects folder where to save image
    ' DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret= lpStAutoName(savefol + "###.SEQ", X, IName)
    ret= lpWsSaveAs(IName, "SEQ")
    ret = lpAppCloseAll()
Appendix D (continued)

Else

Debug.Print "Error loading "; gDirStart + fName

End If

' Get the next file name
fName = Dir()

Wend

ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)

End Sub

' This Macro: Opens EDF Test Strips from a folder in succesive order, Uses the Count/Measure tool to filter by area and min caliper.

Sub Sort_GSIs()

ret = IpMacroStop("This macro starts a new session.", 0)

Dim IName As String*255
Dim fName As String
Dim workStr As String
Dim savefol2 As String
Dim docID As Integer
Dim X As Integer

' Initialize X
X = 0

' Select a folder where to store the outputs
savefol2 = GetFilePath("SortedGSIs", ",", "C:\", "Select a folder where to store sorted Gray Scale Images", 2)

' Creating starting point
If gDirStart = "" Then
gDirStart = "C:\"
End If

' Get a file name from a folder
workStr = GetFilePath("", "SEQ", gDirStart, "Specify a folder where to find EDF Test Strips", 0)
Appendix D (continued)

' Check if the user did not cancel
If workStr = "" Then
   Exit Sub
End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, "))

' Clear the output for work purposes
debugclear
ret = IpOutputClear()

' Select Calibration for 20x Objective Lens
ret = IpSCalSelect("20x_065")
ret = IpTemplateMode(1)

fName = Dir(gDirStart + "*.SEQ", 32)
ret = IpMacroStop ("Explore any Test Strip from the group. Select the best frame where particles outlines are well defined.",0)
'ret = IpTemplateMode(1)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")

' Determine Frame number to extract for all the Test Strips within the folder

Dim gnFrame As Long

' Set up initial values
gnFrame = 2
Begin Dialog UserDialog 320,112,"Select frame number to extract."
Text 40,21,90,14,"Frame number",.Frame
TextBox 140,14,90,21,.nFrame
OKButton 80,84,90,21
End Dialog
Dim dlg3 As UserDialog

' Apply to dialog
dlg3.nFrame = Str(gnFrame)
Appendix D (continued)

' Get user values
Dialog dlg3

' Get back from dialog
  gnFrame = Val(dlg3.nFrame) - 1
  ret = IpSeqExtractFrames(gnFrame, 1)

' Determine Intensity range manually

Dim gnInte As Long

'ret = IpBlbShow(1)
ret = IpSegShow(1)
ret = IpSegSetAttr(SETCURSEL, 0)
ret = IpSegSetAttr(CHANNEL, 0)
ret = IpSegPreview(CURRENT_C_T)

' Set up initial values
  gnInte = 123
  Text 40,21,90,14,"Select a value for intensity",.Inte
  TextBox 140,14,90,21,.nInte
  OKButton 80,84,90,21
End Dialog
Dim dlg4 As UserDialog

' Apply to dialog
  dlg4.nInte = Str(gnInte)

' Get user values
Dialog dlg4

' Get back from dialog
  gnInte = Val(dlg4.nInte)

ret = IpTemplateMode(0)
ret = IpAppCloseAll()
While fName <> ""
  X = X + 1
  ' Print out the file name and its attributes
  Debug.Print GetAttr(gDirStart + fName); " "; fName
  X = X + 1
Appendix D (continued)

' Load the image sequence
docID = IpSeqOpen(gDirStart + fName, "SEQ",0,16)

' Start routine
If docID >= 0 Then

' Extract frame of interest
ret = IpSeqExtractFrames(gnFrame, 1)

' Converting to binary
ret = IpTemplateMode(0)
' Select Calibration for 20x Objective Lens
ret = IpSCalSelect("20x_065")
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
' Apply Filter Ranges
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
' Do accumulate count
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
' Reset for no measurements
ret = IpBlbEnableMeas(BLBM_ALL, 0)
' Measure Min Feret
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Measure Area
ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Count range for particles (obtained from the manual intensity selection)
ret = IpBlbSetAttr(BLOB_AUTORANGE, 0)
ret = IpSegSetRange(-1, 0, gnInte)
' Apply smoothing
ret = IpBlbSetAttr(BLOB_SMOOTHING,3)
' Apply 8-connected objects
ret = IpBlbSetAttr(BLOB_8CONNECT,1)
' Fill holes
ret = IpBlbSetAttr(BLOB_FILLHOLEs,0)
ret = IpBlbSetAttr(BLOB_MINAREA,1)
ret = IpBlbSetAttr(BLOB_CLEANBORDER,1)
ret = IpSortAttr(SORT_LABELS, 0)
' Count
ret = IpBlbCount()
' Measure Min Feret
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Measure Area
Appendix D (continued)

ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Filter that excludes particles outside the range 75<Min Caliper<2000 um and
1500<Area<50000 um^2
ret = IpBlbSetFilterRange(BLBM_MINFERRET, 75, 2000)
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
' Count again after filtering min feret
ret = IpBlbCount()
' Create a Mask
'ret = IpBlbCreateMask()
'ret = IpBlbUpdate(0)

' Create BINIs with particles sorted
' Open Sort window
' ret = IpSortShow(1)

' Sort
' Select Calibration for 20x Objective Lens
'ret = IpSCalSelect("20x_065")
' New counting properties
ret = IpBlbSetAttr(BLOB_AUTORANGE, 0)
ret = IpSegSetRange(0, 0, gnInte)
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
ret = IpBlbSetAttr(BLOB_8CONNECT,1)
ret = IpBlbSetAttr(BLOB_MINAREA,1)
ret = IpBlbSetAttr(BLOB_CLEANBORDER,1)
ret = IpBlbSetAttr(BLOB_SMOOTHING,1)
ret = IpSortAttr(SORT_ROTATE, 1)
' Count again before sorting
ret = IpBlbCount()
ret = IpBlbUpdate(0)
' Set automatic background
ret = IpSortAttr(SORT_AUTO, 0)
ret = IpSortAttr(SORT_INDEX, 255)
' Sort by Min feret
ret = IpSortAttr(SORT_MEAS, 10)
'ret = IpBlbUpdate(0)
ret = IpSortObjects()

'User selected folder where to save image
Appendix D (continued)

'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!
ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
ret = IpWsSaveAs(IName, "BMP")
ret = IpAppCloseAll()

Else
    Debug.Print "Error loading "; gDirStart + fName
End If

' Get the next file name
fName = Dir()
Wend
ret = IpMacroStop("All images in directory processed.", MS_MODAL)
End Sub

' This Macro: Opens sorted GSI's and lets the operator get rid of spots and defected shapes
Sub EditGSIs()

    ret = IpMacroStop("This macro will help you edit GSIs before starting the process of measuring and counting.", 0)

    Dim IName As String*255
    Dim fName As String
    Dim workStr As String
    Dim savefol2 As String
    Dim docID As Integer
    Dim F As Integer
    Dim B As Integer
    Dim ori%, decon%, edfd%, cedfd%
    Dim X As Integer
    ' Initialize X
    Dim gnFrame1 As Long

    ' Select a folder where to store the outputs
    savefol2 = GetFilePath("EdGSIs", ",", "C:\", "Select a folder where to store edited images", 2)
Appendix D (continued)

' Set up initial values
gnFrame1 = 000

Begin Dialog UserDialog 520,112,"What is the number of the last image saved?"
   Text 40,21,90,14,"Image number",.Frame1
   TextBox 140,14,90,21,.nFrame1
   OKButton 80,84,90,21
End Dialog

Dim dlg31 As UserDialog
' Apply to dialog
dlg31.nFrame1 = Str(gnFrame1)

' Get user values
Dialog dlg31

' Get back from dialog
gnFrame1 = Val(dlg31.nFrame1)

X = gnFrame1

' Creating starting point
If gDirStart = "" Then
   gDirStart = "C:\"
End If

' Get a file name from a folder
workStr = GetFilePath("", "BMP", gDirStart, "Specify a folder where to find the sorted GSIs", 0)

' Check if the user did not cancel
If workStr = "" Then
   Exit Sub
End If

' Close all open images prior processing
ret = IpAppCloseAll()

' Extract the folder name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")

' Clear the output for work purposes
debugclear

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Appendix D (continued)

ret = IpOutputClear()

' Select Calibration for 20x Objective Lens
ret = IpSCalSelect("20x_065")
ret = IpTemplateMode(0)

fName = Dir(gDirStart + "*.BMP", 32)

' Opening an EDF Test Strip for observation
ret = IpWsLoad(gDirStart, "SEQ")

While fName <> ""
ret = IpTemplateMode (1)
ret = IpWsLoad(gDirStart + fName, "BMP")

ret = IpMacroStop ("If this image presents undesirable areas that can be filled, select them as AOIs.", 0)
ret = IpAoiCreateIrregular(Pts(0), 7)
ret = IpMacroStop ("Click continue to go to the next step", 0)
B = IpMacroStop ("Do you want to fill AOIs with white?", MS_MODAL + MS_YESNO + MS_QUEST)

If B = 1 Then
ret = IpWsFill(0,2,0)
ret = IpAoiShow(FRAME_RESET)
ret = IpAoiShow(FRAME_NONE)
ret = IpAoiMultAppend(0)
ret = IpAoiMultShow(0)

Else
End If

F = IpMacroStop ("Click on YES to save and open next image.", MS_MODAL + MS_YESNO)

ret = IpTemplateMode(0)

If F = 1 Then
X = X + 1
Appendix D (continued)

'User selected folder where to save image
'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE STORED!!!!!
    ret = IpTemplateMode (1)
    ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret = IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()

    Else
        Exit Sub
    End If
    ret = IpTemplateMode (0)
    ' Get the next file name
    fName = Dir()

Wend

    ret = IpMacroStop("All images in directory processed.", MS_MODAL)

End Sub

' This Macro: Opens one by one all binary images in a folder, counts and measures all particle shapes found in each image, saves all data calculated as a text file (DATA.TXT)
' within folder

Sub MakeBINI_C_M()

    ' Scan through and process all files in a folder

    ret = IpMacroStop ("This macro will open all binary images within a folder and will count, measure and sort all particles found. Important: Make sure all BINIs have been audited before applying this macro.", 0)

    Dim IName As String*255
    Dim fName As String
    Dim workStr As String
    Dim docID As Integer
    Dim savefol2 As String
    Dim X As Integer

    X = 0
Appendix D (continued)

' Creating starting point
If gDirStart = "" Then
    gDirStart = "C:\"
End If

' Select a folder where to store the outputs
savefol2 = GetFilePath("SORTED_BIN", "", "C:\", "Select a folder where to store binary images with edited particles", 2)

' Get a file name from a folder
workStr = GetFilePath("", "BMP", gDirStart, "Specify a folder where to find the edited GSIs", 0)

' Check that user did not cancel
If workStr = "" Then
    Exit Sub
End If

' Close all open images prior to processing
ret = IpAppCloseAll()

' Extract the directory name from the full file name
gDirStart = Left(workStr, InStrRev(workStr, ")

' Clear the output for work purposes
ddebugclear
ret = IpOutputClear()

' Select Calibration For 20x Objective Lens
ret = IpSCalSelect("20x_065")
ret = IpTemplateMode(0)

fName = Dir(gDirStart + "*.BMP", 32)
While fName <> ""
    X = X + 1
    ' Print out the file name and its attributes
    Debug.Print GetAttr(gDirStart + fName); " "; fName

    ' Load the image sequence
    docID = IpWsLoad(gDirStart + fName, "BMP")
Appendix D (continued)

' Start routine
If docID >= 0 Then
    ret = IpTemplateMode(0)

' Selecting Spatial Calibration
ret = IpSCalSelect("20x_065")
ret = IpSCalShowEx(SCAL_DLG_SELECT, SCAL_HIDE)
' Start Count/Size Process
'ret = IpBlbShow(1)
ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 0)
ret = IpBlbSetAttr(BLOB_ADDCOUNT, 0)
ret = IpBlbSetAttr(BLOB_MEASUREOBJECTS, 1)
ret = IpBlbSetAttr(BLOB_FILTEROBJECTS, 1)
ret = IpBlbSetAttr(BLOB_8CONNECT, 1)
ret = IpBlbSetAttr(BLOB_MINAREA, 0)
ret = IpBlbSetAttr(BLOB_CLEANBORDER, 1)
ret = IpBlbSetAttr(BLOB_FILLHOLES, 1)
ret = IpBlbSetAttr(BLOB_LABELMODE, 1)
ret = IpSortAttr(SORT_ROTATE, 1)
' Reset for no measurements
ret = IpBlbEnableMeas(BLBM_ALL, 0)
' Feret(min): Smallest caliper(feret) length
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Area of particle
ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Filter that excludes particles outside the range 50<Min Caliper<3000 um and 1500<Area<1000000 um^2
ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)
ret = IpBlbUpdate(0)
ret = IpBlbCount()
'Create a Mask
ret = IpBlbCreateMask()
ret = IpBlbSetAttr(BLOB_AUTORANGE, 1)
ret = IpBlbSetAttr(BLOB_BRIGHTOBJ, 1)
' ***MEASUREMENTS***
' Reset for no measurements
ret = IpBlbEnableMeas(BLBM_ALL, 0)
' Perimeter: Length of particle's outline.
ret = IpBlbEnableMeas(BLBM_PERIMETER, 1)
Appendix D (continued)

' Area of particle
ret = IpBlbEnableMeas(BLBM_AREA, 1)
' Roundness: Perimeter^2/(4*pi*Area).
ret = IpBlbEnableMeas(BLBM_ROUNDNESS, 1)
' Perimeter(ellipse): Perimeter of the equivalent ellipse
ret = IpBlbEnableMeas(BLBM_PELLIPSE, 1)
' Perimeter(convex): Perimeter of the convex outline of the particle
ret = IpBlbEnableMeas(BLBM_PCONVEX, 1)
' Fractal dimension of the particle's outline
ret = IpBlbEnableMeas(BLBM_FRACTDIM, 1)
' Axis(major): Length of major axis of ellipse with same moments of order 1 and 2 as particle
ret = IpBlbEnableMeas(BLBM_MAJORAX, 1)
' Axis(minor): Length of major axis of ellipse with same moments of order 1 and 2 as particle
ret = IpBlbEnableMeas(BLBM_MINORAX, 1)
' Aspect: Ratio between major axis and minor axis of ellipse equivalent to particle
ret = IpBlbEnableMeas(BLBM_ASPECT, 1)
' Diameter(max): Length of longest line joining two points of object's outline and passing through the centroid
ret = IpBlbEnableMeas(BLBM_MAXFERRET, 1)
' Diameter(min): Length of shortest line joining two points of object's outline and passing through the centroid
ret = IpBlbEnableMeas(BLBM_MINFERRET, 1)
' Diameter (mean): Average length of diameters measured at 2 degrees intervals and passing through particle's centroid
ret = IpBlbEnableMeas(BLBM_MEANFERRET, 1)
' Feret(max): Longest caliper(feret) length
ret = IpBlbEnableMeas(BLBM_MAXCALIP, 1)
' Feret(min): Smallest caliper(feret) length
ret = IpBlbEnableMeas(BLBM_MINCALIP, 1)
' Feret(mean): Average caliper(feret) length
ret = IpBlbEnableMeas(BLBM_MEANCALIP, 1)
' Radius(max): Maximum distance between particle's centroid and outline
ret = IpBlbEnableMeas(BLBM_MAXRADIUS, 1)
' Radius (min): Minimum distance between particle's centroid and outline
ret = IpBlbEnableMeas(BLBM_MINRADIUS, 1)
' Radius Ratio: Ratio between Max. Radius and Min. Radius
ret = IpBlbEnableMeas(BLBM_RADIUSRATIO, 1)

' Filter that excludes particles outside the range 50<Min Caliper<3000 um and 1500<Area<1000000 um^2

Appendix D (continued)

    ret = IpBlbSetFilterRange(BLBM_MINCALIP, 50, 3000)
    ret = IpBlbSetFilterRange (BLBM_AREA, 1500, 1000000)

    ' count BINIs
    ret = IpBlbUpdate(0)
    ret = IpBlbCount()

    If X = 1 Then
        ret = IpBlbSaveData(savefol2 + "DATA.TXT",
S_APPEND+S_HEADER+S_Y_AXIS)
        ret = IpBlbShow(0)
    Else
        IpBlbSaveData(savefol2 + "DATA.TXT", S_APPEND+S_Y_AXIS)
        ret = IpBlbShow(0)
    End If

    'User selected folder where to save image
    'DO NOT SAVE IN THE SAME FOLDER WHERE ORIGINAL FILES ARE
    STORED!!!!!
    ret = IpStAutoName(savefol2 + "###.BMP", X, IName)
    ret = IpWsSaveAs(IName, "BMP")
    ret = IpAppCloseAll()

    Else
        Debug.Print "Error loading "; gDirStart + fName
    End If

    ' Get the next file name
    fName = Dir()

    Wend
    ret = IpMacroStop("All images in folder have been processed.", MS_MODAL)

End Sub
Appendix E. Results of Sieve Analysis for Some Sand Samples Collected

Table 6. Percentages Retained by Weight for Different Size Ranges of Some Sand Samples Collected

<table>
<thead>
<tr>
<th>Size Range (mm)</th>
<th>Bell Air Beach Florida, USA</th>
<th>Clearwater Beach Florida, USA</th>
<th>Gulf Beach Florida, USA</th>
<th>Indian Rocks Beach Florida, USA</th>
<th>Madeira Beach Florida, USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.750-2.000</td>
<td>0.00</td>
<td>0.00</td>
<td>0.16</td>
<td>0.73</td>
<td>0.55</td>
</tr>
<tr>
<td>0.600-2.000</td>
<td>0.82</td>
<td>0.00</td>
<td>0.00</td>
<td>1.73</td>
<td>1.00</td>
</tr>
<tr>
<td>0.425-0.600</td>
<td>33.91</td>
<td>0.00</td>
<td>1.54</td>
<td>13.45</td>
<td>2.27</td>
</tr>
<tr>
<td>0.250-0.425</td>
<td>13.91</td>
<td>0.18</td>
<td>4.45</td>
<td>7.09</td>
<td>1.64</td>
</tr>
<tr>
<td>0.180-0.250</td>
<td>18.36</td>
<td>2.91</td>
<td>26.18</td>
<td>14.73</td>
<td>5.64</td>
</tr>
<tr>
<td>0.160-0.180</td>
<td>23.91</td>
<td>37.18</td>
<td>47.00</td>
<td>38.64</td>
<td>62.64</td>
</tr>
<tr>
<td>0.075-0.150</td>
<td>5.55</td>
<td>30.64</td>
<td>13.91</td>
<td>15.00</td>
<td>7.55</td>
</tr>
<tr>
<td>0.075-0.075</td>
<td>2.82</td>
<td>21.00</td>
<td>8.27</td>
<td>18.09</td>
<td></td>
</tr>
<tr>
<td>Pan</td>
<td>-</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Total</td>
<td>99.36</td>
<td>99.91</td>
<td>99.64</td>
<td>99.45</td>
<td>98.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size Range (mm)</th>
<th>Redington Shores Florida, USA</th>
<th>Daytona Beach Florida, USA</th>
<th>US Silica #1 Dry USA</th>
<th>US Silica Std. Melt USA</th>
<th>Rhode Island USA</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.750-2.000</td>
<td>0.73</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.600-2.000</td>
<td>2.09</td>
<td>0.00</td>
<td>0.00</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>0.425-0.600</td>
<td>10.55</td>
<td>0.10</td>
<td>7.26</td>
<td>7.75</td>
<td>9.44</td>
</tr>
<tr>
<td>0.250-0.425</td>
<td>6.73</td>
<td>0.02</td>
<td>36.38</td>
<td>36.30</td>
<td>22.54</td>
</tr>
<tr>
<td>0.180-0.250</td>
<td>14.62</td>
<td>0.20</td>
<td>18.24</td>
<td>15.30</td>
<td>15.36</td>
</tr>
<tr>
<td>0.150-0.180</td>
<td>41.18</td>
<td>14.70</td>
<td>22.41</td>
<td>22.70</td>
<td>42.60</td>
</tr>
<tr>
<td>0.075-0.150</td>
<td>14.46</td>
<td>26.04</td>
<td>4.99</td>
<td>6.72</td>
<td>5.98</td>
</tr>
<tr>
<td>Pan</td>
<td>0.00</td>
<td>1.99</td>
<td>0.75</td>
<td>0.06</td>
<td>0.26</td>
</tr>
<tr>
<td>Total</td>
<td>99.00</td>
<td>99.80</td>
<td>99.97</td>
<td>99.62</td>
<td>99.53</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Size Range (mm)</th>
<th>Toyoura Japan</th>
<th>Loire France</th>
<th>Nice France</th>
<th>Fontainbleau France</th>
<th>Hostun France</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.750-2.000</td>
<td>0.00</td>
<td>1.16</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.600-2.000</td>
<td>0.00</td>
<td>79.53</td>
<td>0.11</td>
<td>0.00</td>
<td>47.33</td>
</tr>
<tr>
<td>0.425-0.600</td>
<td>0.00</td>
<td>18.17</td>
<td>1.34</td>
<td>0.00</td>
<td>40.96</td>
</tr>
<tr>
<td>0.250-0.425</td>
<td>0.00</td>
<td>0.68</td>
<td>11.11</td>
<td>1.06</td>
<td>8.16</td>
</tr>
<tr>
<td>0.180-0.250</td>
<td>7.09</td>
<td>0.06</td>
<td>13.37</td>
<td>5.29</td>
<td>1.65</td>
</tr>
<tr>
<td>0.150-0.180</td>
<td>80.56</td>
<td>0.03</td>
<td>33.73</td>
<td>66.16</td>
<td>1.36</td>
</tr>
<tr>
<td>0.075-0.150</td>
<td>6.82</td>
<td>0.01</td>
<td>6.95</td>
<td>10.38</td>
<td>0.17</td>
</tr>
<tr>
<td>Pan</td>
<td>0.00</td>
<td>0.03</td>
<td>10.26</td>
<td>0.18</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>99.73</td>
<td>100.00</td>
<td>99.69</td>
<td>99.99</td>
<td>95.91</td>
</tr>
</tbody>
</table>
Appendix F. Comparison of Results of a Rhode Island Sand Particle Shape Obtained Using the Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x

Figure 38. Enhanced Image of a Rhode Island Sand Particle Obtained Using the Script Mult2DShape4x

Figure 39. Enhanced Image of a Rhode Island Sand Particle Obtained Using the Script Mult2DShape10x
Figure 40. Enhanced Image of a Rhode Island Sand Particle Obtained Using the Script Mult2DShape20x

Figure 41. Measurement of Areas of a Rhode Island Sand Particle Using Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x
Appendix F (continued)

![Bar chart showing measurements: Axis (major), Axis (minor), Radius (max) and Radius (min) for a Rhode Island Sand Particle using scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x.](image1)

**Figure 42.** Comparison of Measurements Axis (major), Axis (minor), Radius (max) and Radius (min) for a Rhode Island Sand Particle Using Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x

![Bar chart showing measurements: Perimeter, Perimeter (convex) and Perimeter (ellipse) for a Rhode Island Sand Particle using scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x.](image2)

**Figure 43.** Comparison of Measurements Perimeter, Perimeter (convex) and Perimeter (ellipse) for a Rhode Island Sand Particle Using Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x
Appendix F (continued)

Figure 44. Comparison of Measurements Ferret (max), Ferret (min) and Ferret (mean) for a Rhode Island Sand Particle Using Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x

Figure 45. Comparison of Measurements Diameter (max), Diameter (min) and Diameter (mean) for a Rhode Island Sand Particle Using Scripts Mult2DShape4x, Mult2DShape10x and Mult2DShape20x
Appendix G. Grayscale Pictures of Sands Collected

Figure 46. Pictures of Sands from: a) US Silica Standard Melt and b) US Silica #1 Dry

Figure 47. Pictures of Sands from: a) Oxnard and b) Belmont Pier, Long Beach, CA

Figure 48. Pictures of Sands from: a) Rincon Beach, Puerto Rico and b) Rhode Island
Appendix G (continued)

Figure 49. Pictures of Sands from: a) Redington Shores, FL and b) Belleair Beach, FL

Figure 50. Pictures of Sands from: a) Daytona Beach, FL and b) Indian Rocks Beach, FL

Figure 51. Pictures of Sands from: a) Madeira Beach, FL and b) Clearwater Beach, FL
Figure 52. Pictures of Sands from: a) Loire River, France and b) Fontainbleau, France

Figure 53. Pictures of Sands from: a) Hostun, France and b) Nice, France

Figure 54. Pictures of Sands from: a) Arroyo Alamar, Mexico and b) Tecate River, Mexico
Figure 55. Pictures of Sands from: a) Toyoura, Japan and b) Malibu Beach, Panama

Figure 56. Pictures of Sands from Boca Grande Beach, Cartagena, Colombia