Version 2.0

Software Development Kit

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User Notes…

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Introduction

cellAnalyst from AssaySoft Inc. analyzes images and builds a database of cell information captured from microscopy images. It has the following capabilities:

- Creates hierarchical albums/image structure. Images from hard disk can be imported into albums.
- Analyzes images (counts cells) and stores the output in a database.
- Enables users to query the database and retrieve images with relevant cell information.

AssaySoft's *Software Development Kit (SDK)* is based on the image analysis unit of cellAnalyst called Pixcavator. The SDK is provided for programmers to build their own applications and contains the following set of dlls.

.NET Environment	Java	C++
PixcavatorFrameworkNET_F.dll	PixcavatorFrameworkJAVA_F.dll	PixcavatorFrameworkCPP_F.dll
PixcavatorWrapper_F.dll	PixcavatorWrapper_F.dll	PixcavatorWrapper_F.dll

Please read the "License.txt" file in the "Documentation" folder before using the SDK.

IF YOU ARE NOT WILLING TO BE BOUND BY THE TERMS OF THIS AGREEMENT, YOU SHOULD REFRAIN FROM ACCESSING OR USING THIS SDK.

SDK Folder Content

The SDK contains the following 4 folders.

- AssaySoft SDK
  - The folder contains the following 2 dlls
    - PixcavatorFramework_L.dll
    - PixcavatorWrapper_L.dll
- Documentation
  - The folder contains the documentation (this document) and the "License" file.
- SampleApplicationCsharp
  - This folder contains a complete Visual Studio/Windows/C# project that uses the SDK
- SampleImage
  - This folder contains a sample image (less than 200x200 pixels) to test the application built using SDK

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Software Development Kit (SDK)

As of August 8, 2008, we have released only the .NET SDK. We plan to release the other SDKs shortly.

Based on this SDK a programmer can build a .NET C# application. The source code (the complete project) of a sample program built using Microsoft Visual Studio 2005 is provided. Using the sample source code the programmer can see how the dll functions are called. The complete procedure of building the sample application is also described in this manual. Once the interaction between the C# code and the dll is understood, the programmer can build his own application.

The SDK comes with two dlls that can be incorporated in your application:

1. PixcavatorFrameworkNET_F.dll
2. PixcavatorWrapper_F.dll

Your application needs to communicate with "PixcavatorFrameworkNET_F.dll" only.

Full capability and Limited capability SDK

AssaySoft Inc. provides SDKs of 2 different kinds.

1. Full capability:
    a. PixcavatorFramework_F.dll
    b. PixcavatorWrapper_F.dll
2. Limited capability:
    a. PixcavatorFramework_L.dll
    b. PixcavatorWrapper_L.dll

The letters "F" and "L indicates "Full capability" and "Limited capability". The difference between these two flavors is that the "Limited capability" dll analyzes images that is restricted to "40,000" pixels (e.g., 200 x 200 pixels). The "Limited capability" SDK is available for a free download from AssaySoft.com.

After you have built an application using the "Limited capability" SDK, you should contact AssaySoft to license the "Full capability" SDK. After the license agreement is signed, you will be able to replace the "Limited capability" dlls with the "Full capability" dlls and your application will become capable of analyzing images of all sizes.

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Analysis of Images

The SDK analyzes images and computes the number of objects of different kinds found in the image. In biological images objects found in an image are referred to as cells.

The user can vary the following parameters of the objects to get the optimal analysis of an image:

- Size
- Contrast

The analysis data about the objects found in the image is displayed in a table and the contours of the objects are mapped on the image. This data includes the following:

Descriptor	Definition
Type	Dark or light object
Location	The X, Y-coordinates of the geometric center of mass of the object
Size	The area of the object measured in pixels
Perimeter	The length of the boundary of the object
Roundness	A perfect circle will have a value of 100
Intensity	The highest or the lowest intensity of the pixels for light or dark objects respectively
Contrast	The difference between the intensity of the object and the intensity of the surrounding area
Saliency	The combination of size and contrast; indicates how important this object is relative to other objects
Center of Mass	If the object intensity values are not homogeneous, this indicates the true center of mass
Average Contrast	The difference between the average intensity of the object and the intensity of the surrounding area

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AnalyzeImage Method

The Pixcavator SDK object has a method called “AnalyzeImage”.

```
PixcavatorFramework pf = new PixcavatorFramework();
List<PixObject> objects = null;
try
{
    objects = pf.AnalyzeImage
      ( fileName, size, contrast, saliency, roundness );
}
catch (MaximumSizeExceededException)
{
    MessageBox.Show(
      "Maximum image size for the demo version is 40000 pixels.",
      "Image size", MessageBoxButtons.OK, MessageBoxIcon.Error);
    return;
}
```

The 5 input parameters to this method are as follows.

1. file name
2. size
3. contrast
4. saliency
5. roundness

- The “file name” parameter indicates the input image file name.
- The “size” parameter indicates the threshold size value below which objects will be ignored.
- The “contrast” parameter indicates the threshold contrast value below which objects will be ignored.
- Saliency is a combination of size and contrast
- Ignore the roundness parameter. It may be used in the next release.

If the input image is greater than 40,000 pixels, the “Limited capability” SDK will raise an exception.

The “AnalyzeImage” method returns a table that contains the object statistics. Declare a DataGridView in your GUI and the objects returned from the SDK will be mapped on the data grid.

```
dataGridView1.DataSource = objects;
```

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The SDK also returns the contour information about all the objects. These contours are mapped on the image to display all the objects.

```
Bitmap bmp = new Bitmap(_fileName);
foreach (PixObject obj in objects)
{
    Color color =
      obj.Type == PixObjectType.Dark ? Color.Red : Color.Green;
    foreach (Point p in obj.Contour)
        if (
            p.X > 0 &&
            p.X < pictureBox1.Image.Width &&
            p.Y > 0 &&
            p.Y < pictureBox1.Image.Height)

            bmp.SetPixel(p.X, p.Y, color);
}
pictureBox1.Image = bmp;
```

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How to Build the Sample Application

The following procedure describes how the sample application was built.

Create a project using Visual Studio

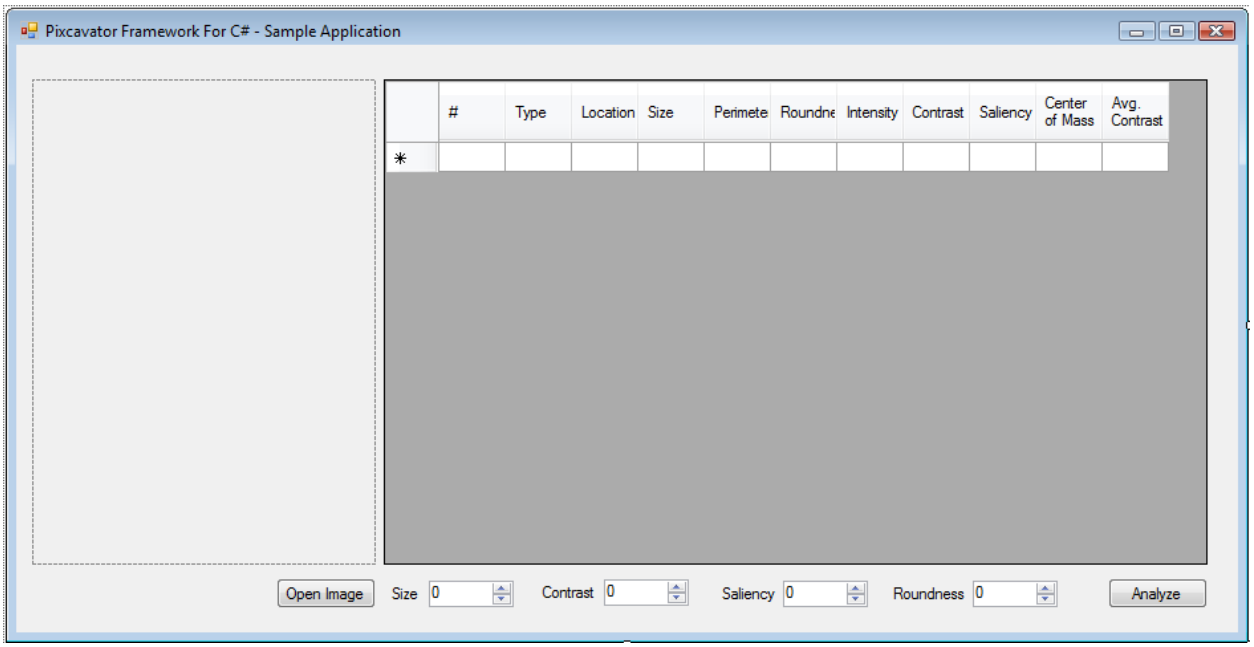
Start Microsoft Visual Studio 2005 or 2008

Menu command: File/New/Project
Visual C#
Windows/Windows Application

Name: SampleApp
Location: D:\t\t1

GUI

Build the following Graphical User Interface (GUI) using Forms Interface



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1. PictureBox
   (Name): pictureBox1
   BorderStyle: FixedSingle
   SizeMode: Zoom

2. Button:
   (Name): button1
   Text: Open Image

3. NumericUpDown Counter
   (Name): numericUpDown1

4. NumericUpDown Counter
   (Name): numericUpDown2

5. NumericUpDown Counter
   (Name): numericUpDown3

6. NumericUpDown Counter
   (Name): numericUpDown4

7. Label
   Text: Size

8. Label
   Text: Contrast

9. Label
   Text: Saliency

10. Label
    Text: Roundness

11. Button:
    (Name): button2
    Text: Analyze
    Enabled: False

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12. DataGridView
    (Name): dataGridView1
    ReadOnly: True

    Columns should be specified as follows.

	(Name)	Header Text	Visible	Data Property Name	Width
1	Column1	#	True	Number	50
2	Column2	Type	True	Type	50
3	Column3	Location	True	Location	50
4	Column4	Size	True	Size	50
5	Column5	Perimeter	True	Perimeter	50
6	Column6	Roundness	True	Roundness	50
7	Column7	Intensity	True	Intensity	50
8	Column8	Contrast	True	Contrast	50
9	Column9	Saliency	True	Saliency	50
10	Column10	Center of Mass	True	AltLocation	50
11	Column11	Avg. Contrast	True	AvgContrast	50
12	Column12	Curvature	False	Curvature	100
13	Column13	Death	False	Death	100
14	Column14	Border	False	Border	100
15	Column15	Id	False	Id	100
16	Column16	Size	False	Size	100
17	Column17	Contour Count	False	ContourCount	100
18	Column18	Length	False	Length	100
19	Column19	Frame	False	Frame	100
20	Column20	Dim	False	Dim	100
21	Column21	Birth	False	Birth	100

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Source Code

Class "Form1" global variable:

```
private string _fileName;
```

Event button1_Click

```
private void button1_Click(object sender, EventArgs e)
        {
            OpenFileDialog fd = new OpenFileDialog();
            fd.Filter = "All Image
                  Files|*.tif;*.tiff;*.bmp;*.png;*.jpg;*.jpeg;*.gif;*.stk";
            DialogResult res = fd.ShowDialog();
            if (res != DialogResult.OK)
                return;
            _fileName = fd.FileName;
            pictureBox1.Load(_fileName);
            numericUpDown1.Maximum =
                  (decimal)Math.Round(0.15 * pictureBox1.Image.Height *
            pictureBox1.Image.Width);
            numericUpDown3.Maximum =
                  (decimal)Math.Round((double)pictureBox1.Image.Height *
                  pictureBox1.Image.Width / 2500 * 40);
            numericUpDown3.Value = numericUpDown3.Maximum;
            button2.Enabled = true;
        }
```

Add the cellAnalyst SDK "dll" to the code.

Copy the cellAnalyst SDK

Copy "PixcavatorFrameworkNET_L.dll" and "PixcavatorWrapper_L.dll" to the following folder.

SampleApp\SampleApp\Lib

Add Reference:

Add reference to the "PixcavatorFrameworkNET_L.dll"

Include "PixcavatorWrapper_L.dll" in the project, and change the property of this file such that it gets copied in the "bin" folder whenever project is compiled.

Add the following name spaces:

```
using PixcavatorFrameworkNET;
using System.IO;
```

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Event button2_Click

```
private void button2_Click(object sender, EventArgs e)
 {
     PixcavatorFramework pf = new PixcavatorFramework();
     List<PixObject> objects = null;
     try
     {
         objects = pf.AnalyzeImage(_fileName,
           (int)numericUpDown1.Value, (int)numericUpDown2.Value,
             (int)numericUpDown3.Value, (int)numericUpDown4.Value);
     }
     catch (MaximumSizeExceededException)
     {
         MessageBox.Show(
           "Maximum image size for the demo version is 40000 pixels.",
           "Image size", MessageBoxButtons.OK, MessageBoxIcon.Error);
         return;
     }

     dataGridView1.DataSource = objects;

     Bitmap bmp = new Bitmap(_fileName);
     foreach (PixObject obj in objects)
     {
         Color color =
           obj.Type == PixObjectType.Dark ? Color.Red : Color.Green;
         foreach (Point p in obj.Contour)
             if (
                 p.X > 0 &&
                 p.X < pictureBox1.Image.Width &&
                 p.Y > 0 &&
                 p.Y < pictureBox1.Image.Height)

                 bmp.SetPixel(p.X, p.Y, color);
     }
     pictureBox1.Image = bmp;
 }
```

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Compile the Application:

Select the compile mode: Debug or Release.

Invoke the following menu command.
Build/Build Solution

To Run the Application inside Visual Studio

Invoke the following menu command.
Debug/Start Without Debugging

To Run the Application

Go to the “Debug” or “Release” folder (depending upon in which mode the application was compiled and double click on the SampleApp.exe file.

SampleApp\SampleApp\bin\Debug\SampleApp.exe

Make sure that the “Debug” or the “Release” folder contains both the dlls.

a. PixcavatorFramework_L.dll
b. PixcavatorWrapper_L.dll

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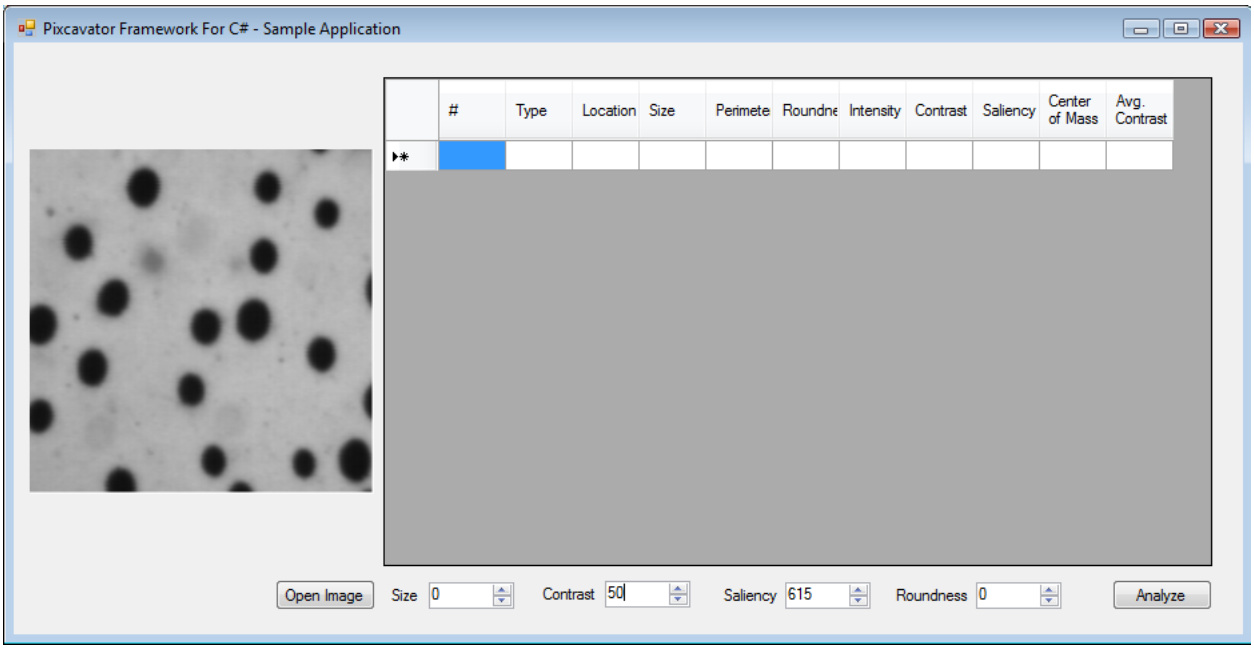
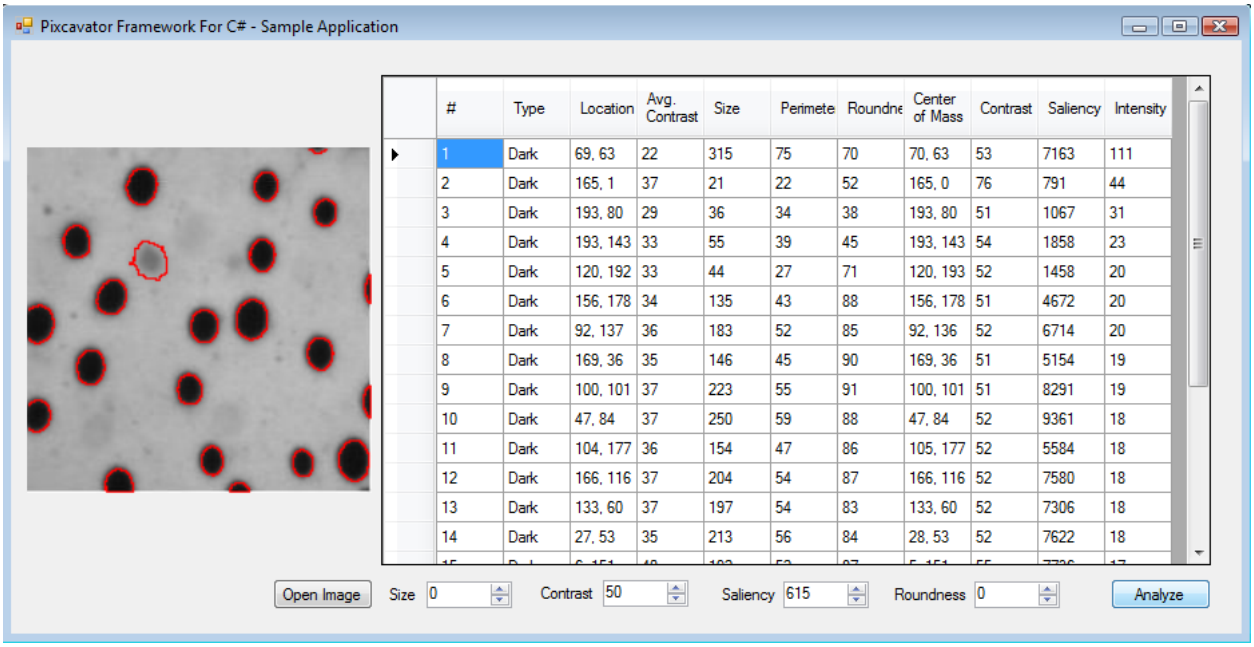
Select the “Open Image” button and then select the sample image provided with the SDK called “Sample200x200.bmp”



Change the contrast counter value to 50 and then press the “Analyze” button. The sample image will be analyzed and the following analysis will be displayed.



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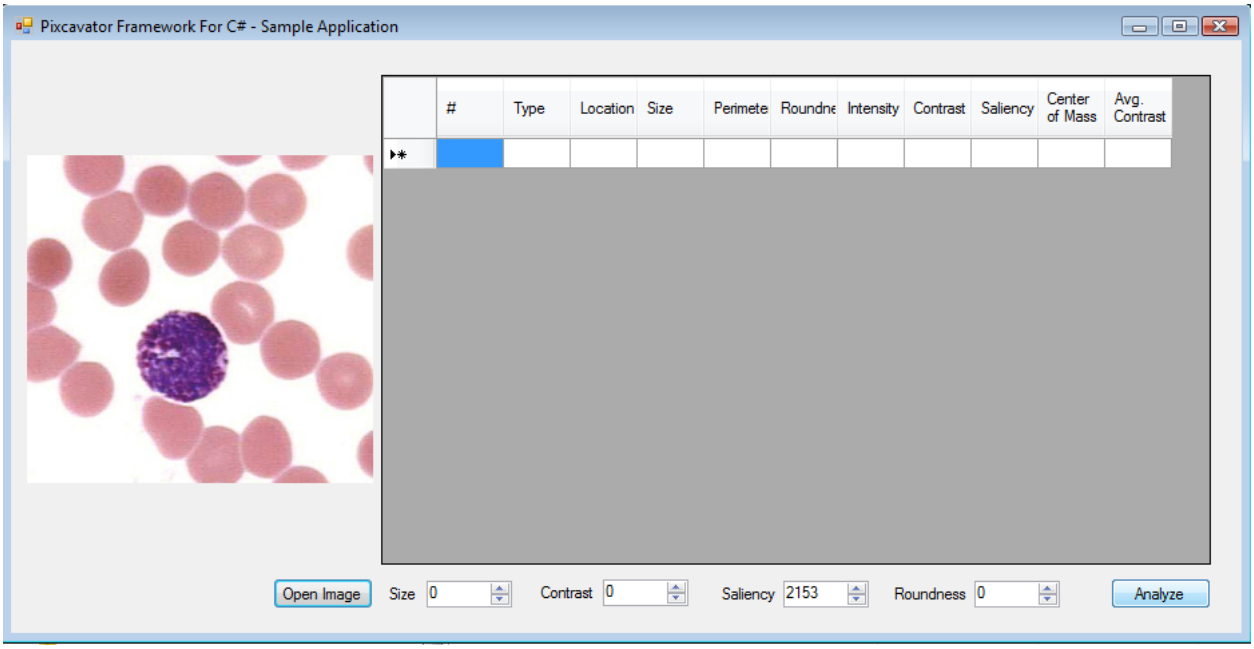
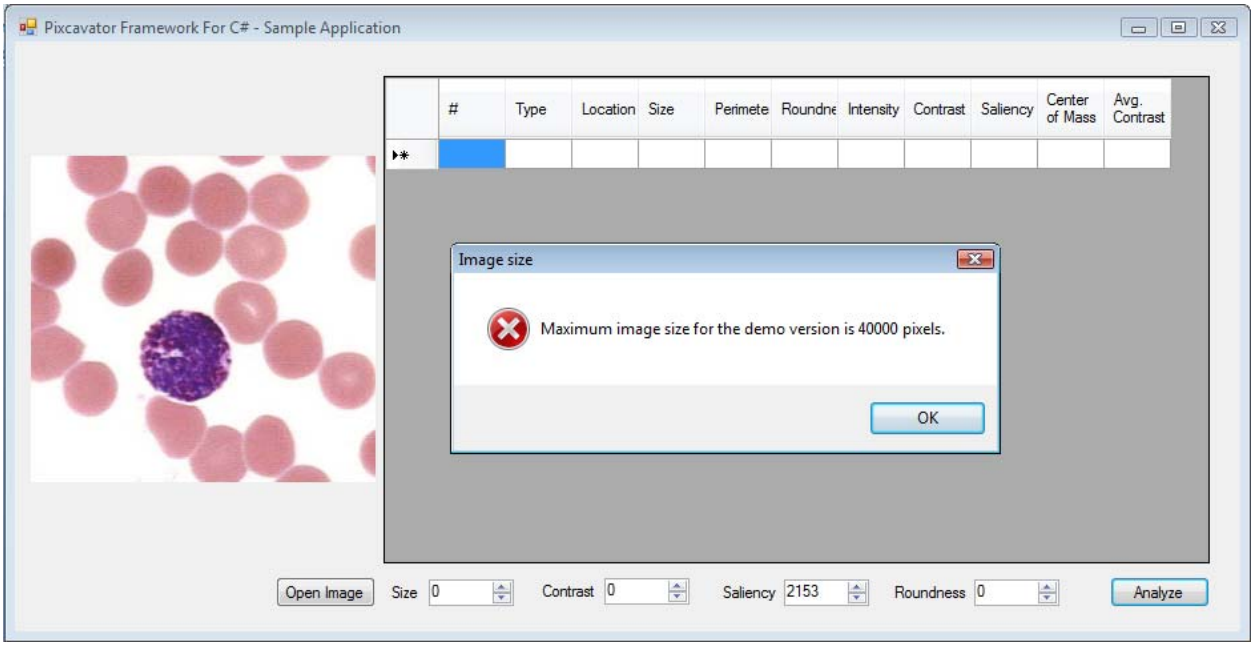
Select the “Open Image” button and then select any other image that has the dimension greater than 40,000 pixels.



Press the “Analyze” button. An error message will be displayed indicating that the image cannot be analyzed because it is larger than 40,000 pixels.



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Product Evolution

AssaySoft will continue to refine its SDK. User feedback provides valuable insight into user outcomes and expectations. We invite your comments and suggestions for individual as well as community use. Feel free to use the “Contact Us” page of our web site for this purpose.

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