

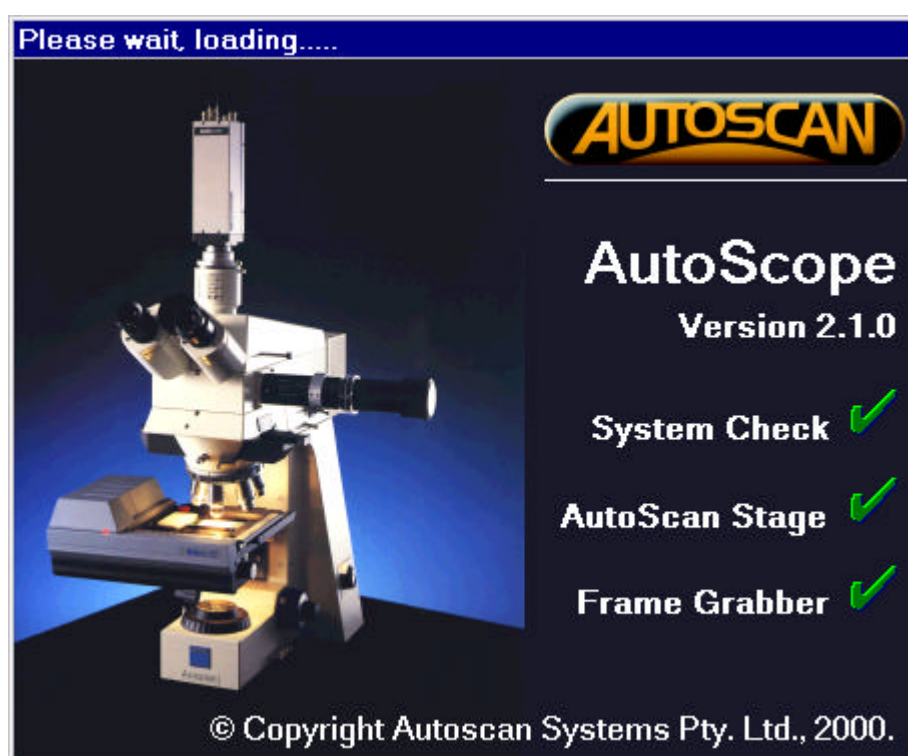
AutoScope

AutoScope is a computerised microscopy system incorporating automatic stage movement control and image processing for object recognition. Autoscope automatically:

- ◆ controls the movement of the AutoScan stage,
- ◆ acquires images of the microscope field-of-view using a camera attached to the microscope,
- ◆ processes the acquired image, identifying pre-defined objects,
- ◆ stores the identified objects in a database for later review, and
- ◆ provides manipulation and export of stored data for post-processing.

The following is a brief overview of AutoScope computer screens and function.

While the application is loading the following splash screen is displayed. Checks for required files and the presence of stage and frame grabber hardware are performed while this screen is displayed. Warnings are issued if required system software and hardware components are not correctly detected.



Following a successful system check, the main system screen is then displayed (as shown below). The main screen is dedicated to the configuration, processing and review of a slide 'batch', that is, a group of related slides. New batches are created, and existing batches opened using the Batch menu.

A slide batch is a hierarchical structure containing one or more slides. Each slide can contain one or more regions of interest (ROI), which, in turn, can contain one or more object definitions. An object definition contains the pre-defined characteristics used for identifying an object in the image acquired from the camera. Once created, an object definition is added to the library of definitions available for use in any subsequent batches.

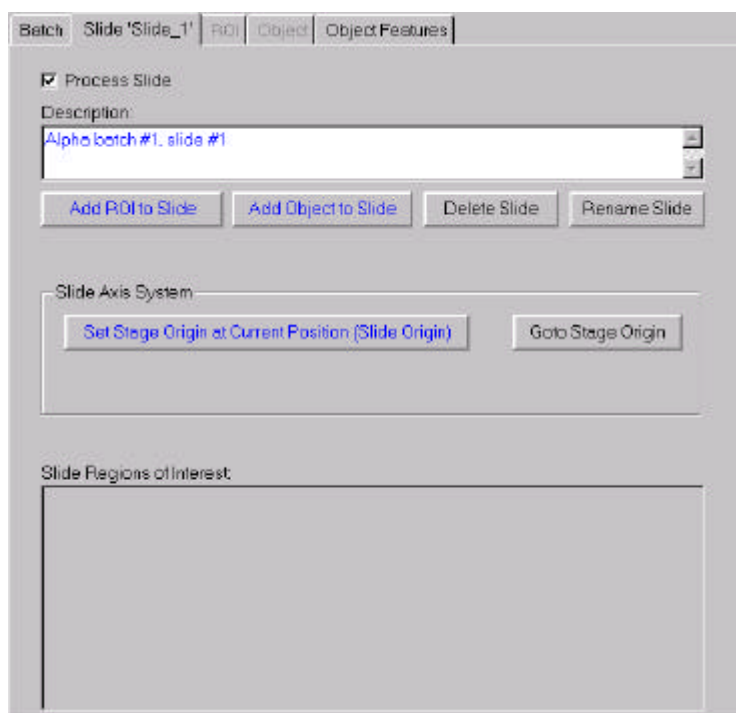
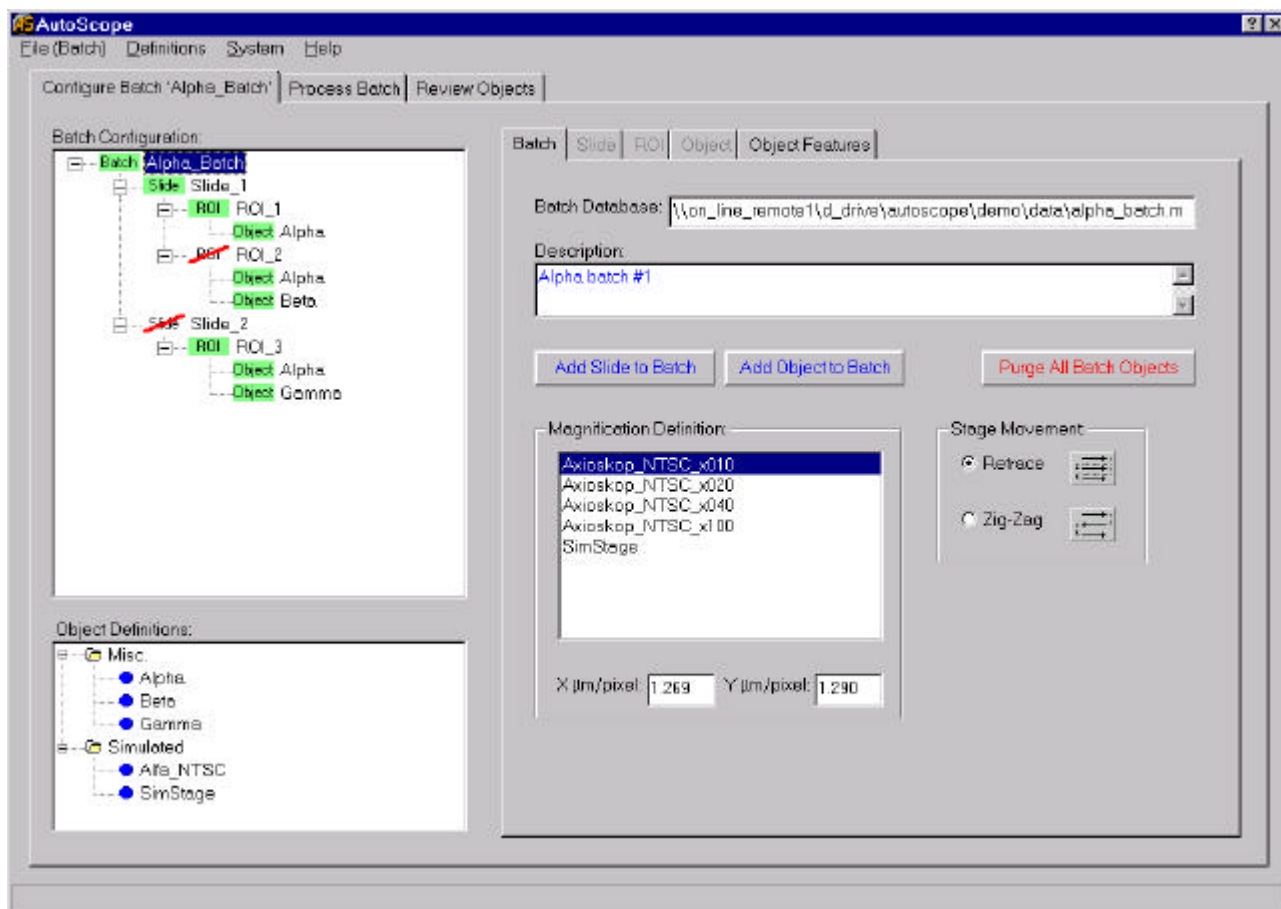
The library of object definitions, grouped by object category, is displayed in the Object Definitions tree control. Object definitions can be added to any batch node. When an object definition is added to a node, it is automatically added to all lower level nodes. For example, when an object definition is added to a slide, it is added to all ROI's under that slide.

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When a batch, slide, ROI or object node is selected, an associated tab is enabled which displays node details and appropriate actions. When the batch node is selected, the batch tab is enabled. The batch tab allows batch name and free-format text description to be entered, batch magnification selected, new slides to be created under the batch and particular object definitions applied to the complete batch.



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When a slide node is selected, the slide tab is enabled (as shown above). The slide tab allows slide name and description to be entered, slide orientation to be defined, new ROI's to be created under the slide, the slide to be deleted and object definitions applied to the complete slide.

When an ROI node is selected, the ROI tab is enabled (as shown below). The ROI tab allows ROI name and description to be entered, the coordinates of the ROI area/volume to be defined, the ROI to be deleted and object definitions applied to the complete ROI. An option allows the entire ROI, or a grid (defined by an X,Y step size) of field of view (FOV) frames within the ROI to be processed.

Batch | Slide 'Slide_1' | ROI 'ROI_1' | Object | Object Features

☒ Process ROI

Description:
Alpha batch #1, slide #1, first ROI
Search 1mmx1mm for for Alpha objects

Add Object to ROI | Delete ROI | Rename ROI

Region of Interest Coordinates:

Start At X: 1000 Y: 1000 Z: 100 | Set from Stage | Goto
End At X: 2000 Y: 2000 Z: 100 | Set from Stage | Goto

☒ Process Entire ROI

Step in X: 0 Step in Y: 0 Step in Z: 0
No. in X: 0 No. in Y: 0 No. in Z: 0

☒ XY Plane Focus Adjustment

X	Y	Focus

Define Minimum Points
Add Point
Delete Point

When an object node is selected, the object tab is enabled. This tab contains an image showing the results of applying the selected object definition to the current microscope field-of-view acquired from the camera.

Batch | Slide 'Slide_1' | ROI 'ROI_1' | Object 'Alpha' | Object Features

☒ Process Object

Acquire | Apply Object Def. | Modify Object Def. | Delete Object From ROI

Camera View (with Applied Object Definition):

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The Object Features tab allows the selection of which object parameters will be added with an object to the batch database when it is identified during processing .

Select object features to be recorded in the batch objects database.

Object General

- ☒ Area
- ☐ Perimeter
- ☐ Circularity
- ☒ Object Thumbnail

User Information

- ☒ Text
- ☒ Flag 1
- ☐ Flag 2
- ☐ Flag 3
- ☐ Flag 4

Ellipsoid Data

- ☒ Major Axis Length
- ☒ Minor Axis Length
- ☒ Major Axis Angle
- ☐ Eccentricity
- ☐ Angle to Max. Quadrant

Radius (Centre to Perimeter)

- ☐ Radius Average
- ☐ Radius Std. Dev.
- ☐ Radius Minimum
- ☐ Radius Maximum

Pixel (Gray-Level) Data

- ☐ Pixel Average
- ☐ Pixel Std. Dev.
- ☐ Pixel Minimum
- ☐ Pixel Maximum

When a batch has been configured, processing is started on the Process Batch tab.

AutoScope

File (Batch) Definitions System Help

Configure Batch 'Alpha_Batch' Process Batch Review Objects

Process Selection:

☒ Process Single Slide ☐ Process Slide Coarse

Index	Slide
#1	Slide_1
#2	
#3	
#4	
#5	
#6	

Run Name:

Make Slide Mosaic ☐ Fill Selected Objects ☒ Show Thumbnail Frame ☒

Time Taken: FOV's: Objects:

Time Remaining:

Processed Field of View:

Processing ROI 'Slide_1_ROI_1'

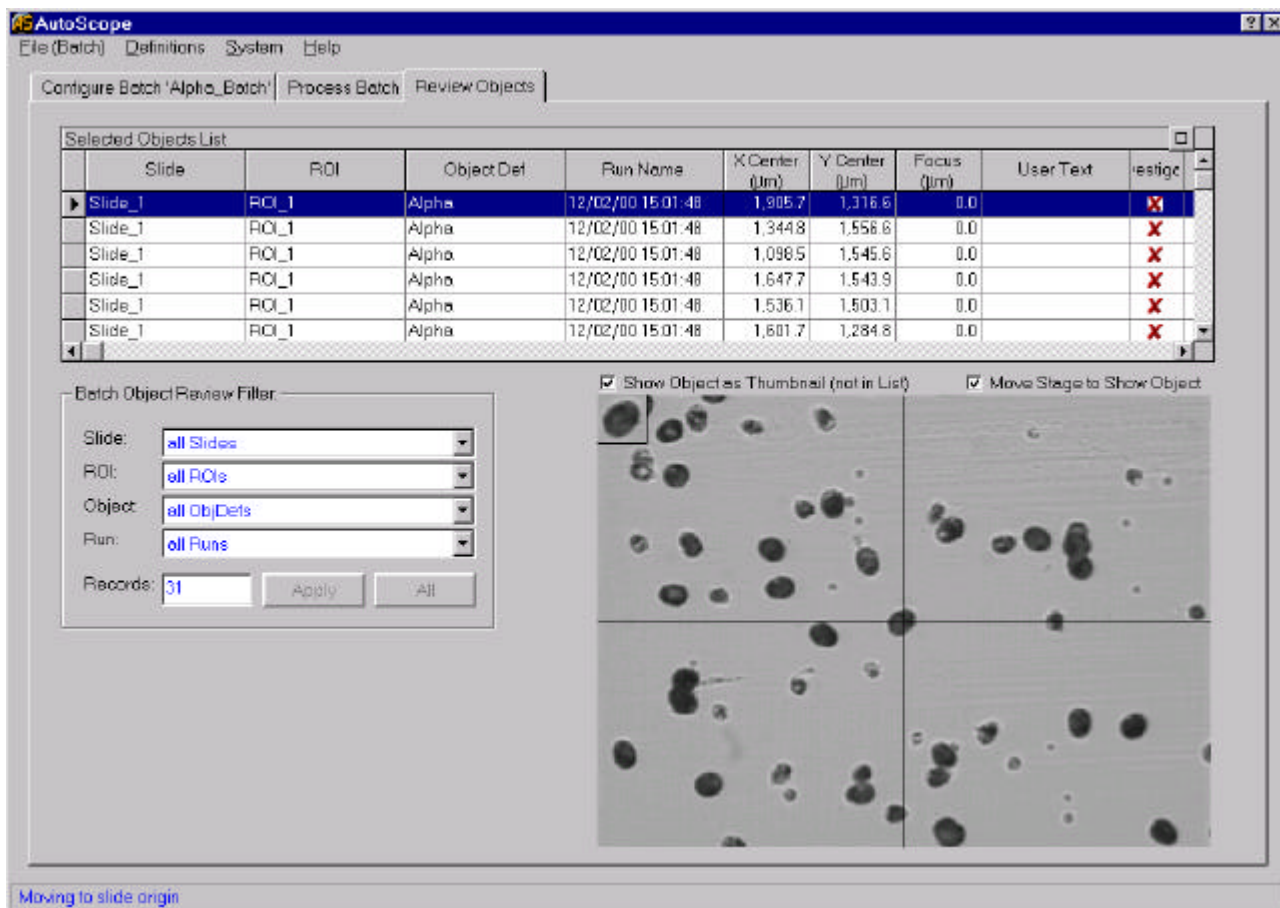
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During processing, a progress bar and the current FOV being analysed is displayed.

When a batch has been processed, the location (slide and ROI), thumbnails and details of the objects found are displayed in a tabular listing on the Review Objects tab. The listing can be filtered to display objects found in the entire batch, or a particular slide or ROI, using the Object Review Filter controls. When an object is selected in the review list, the stage is moved to centre that object in a live FOV from the camera.

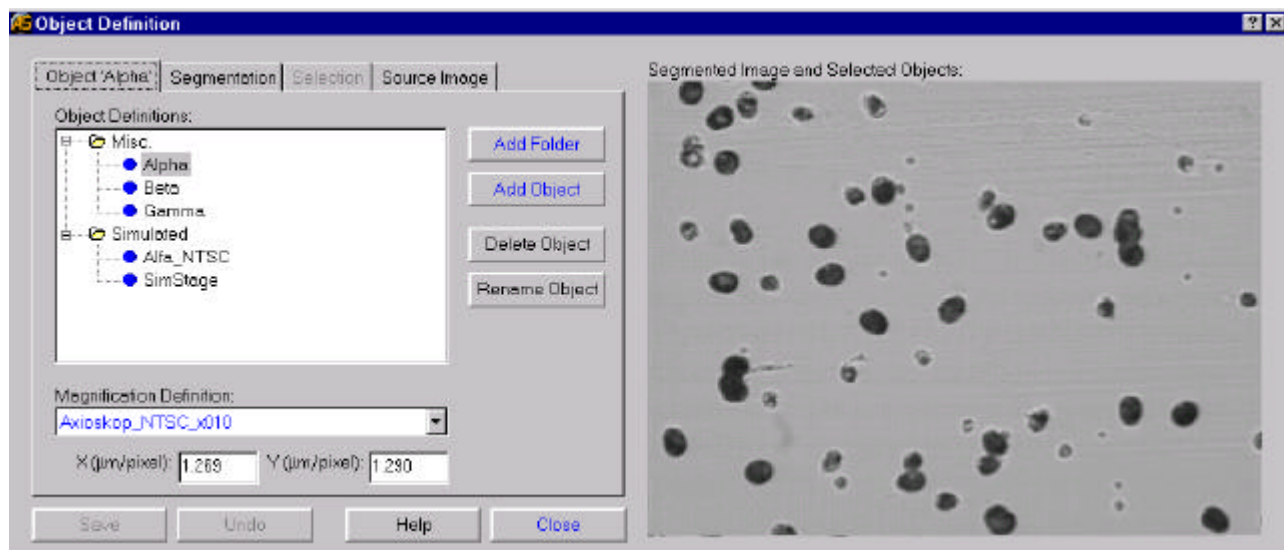


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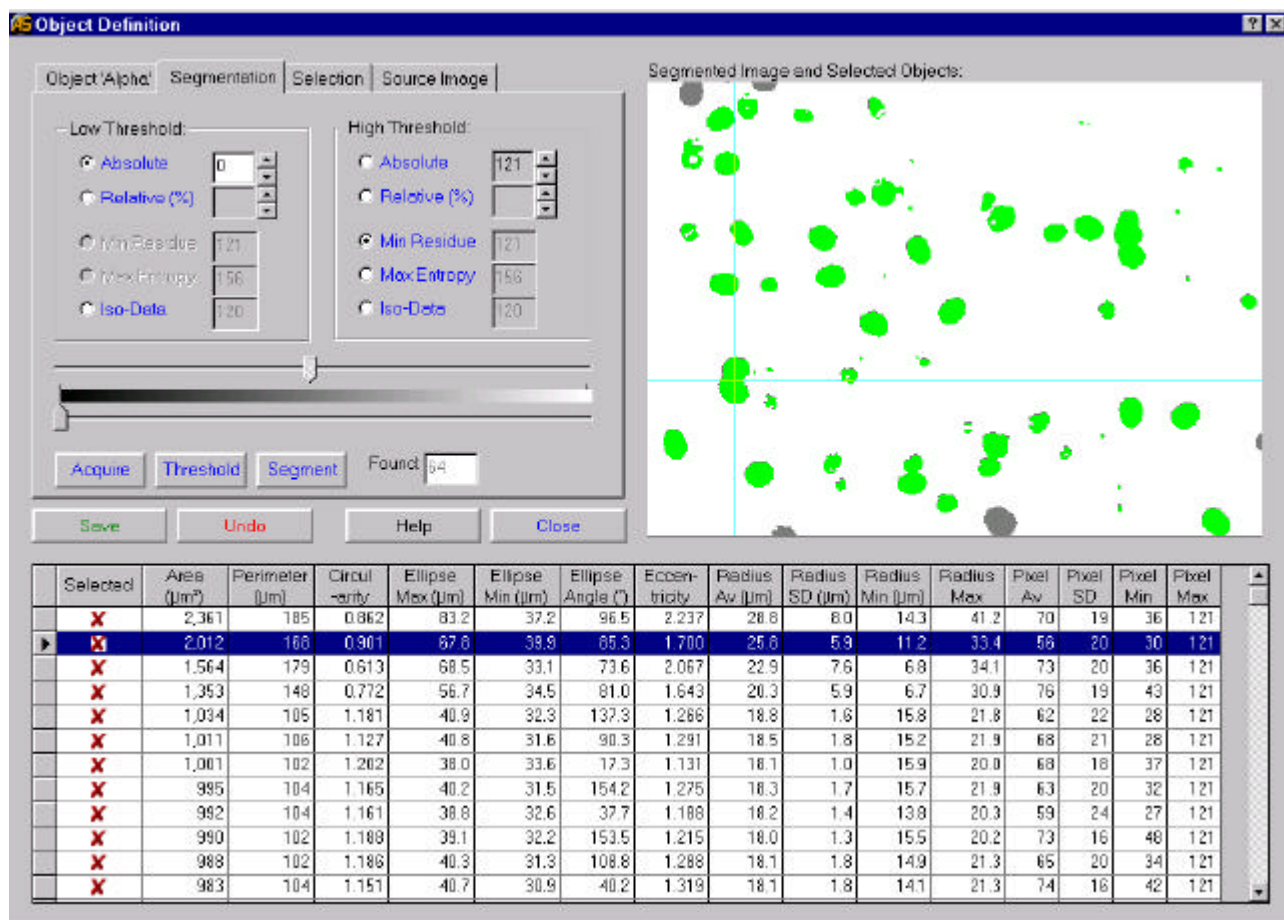
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Object categories and definitions are created and modified using the Object Definition screen. An object definition contains the image thresholding and object geometric features used to characterise an object, and is used during processing to locate all instances of that object in the camera image.



An object definition is created, and applied to an image during processing, in two steps. In the first step the image is segmented using the low and high grey-scale thresholds. Low and high grey-scale thresholds can be a fixed value (*Absolute*), or adaptive (*Relative*, *Minimum Residue*, *Maximum Entropy*, or *Iso-Data*) where the thresholds vary according to the grey-scale distribution within any particular image.



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When the image is segmented a list of all objects, and their geometric characteristics, is displayed. Segmented objects may be of any size and shape. In the second step, desirable objects are selected from the list of all segmented objects on the basis of their geometric features. Multiple selections based on object properties can be applied as part of an object selection script which successively refines the selection of objects from all those segmented.

AS Object Definition

Object 'Alpha' | Segmentation | Selection | Source Image

Select Operation: Remove From Selected If Area > 1300

Feature Selection Criteria: Area > 1300

Add Script Item Update Script Item Delete Script Item

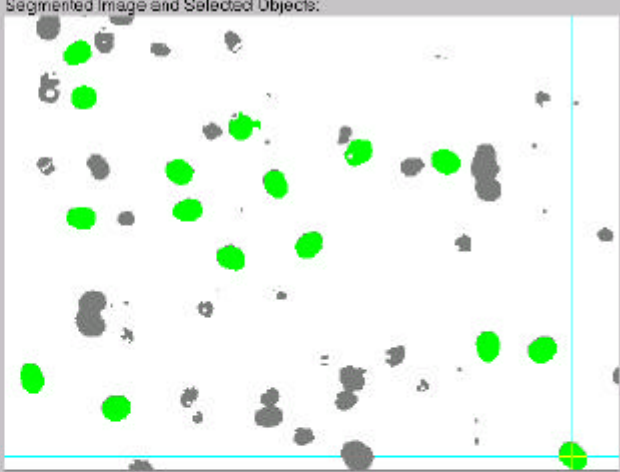
Object Selection Script:

```
Select From Seg. List If Area > 800
Remove From Selected If Area > 1300
```

Apply Item Apply Script Selected: 16 Deselect All

Save Undo Help Close

Segmented Image and Selected Objects:



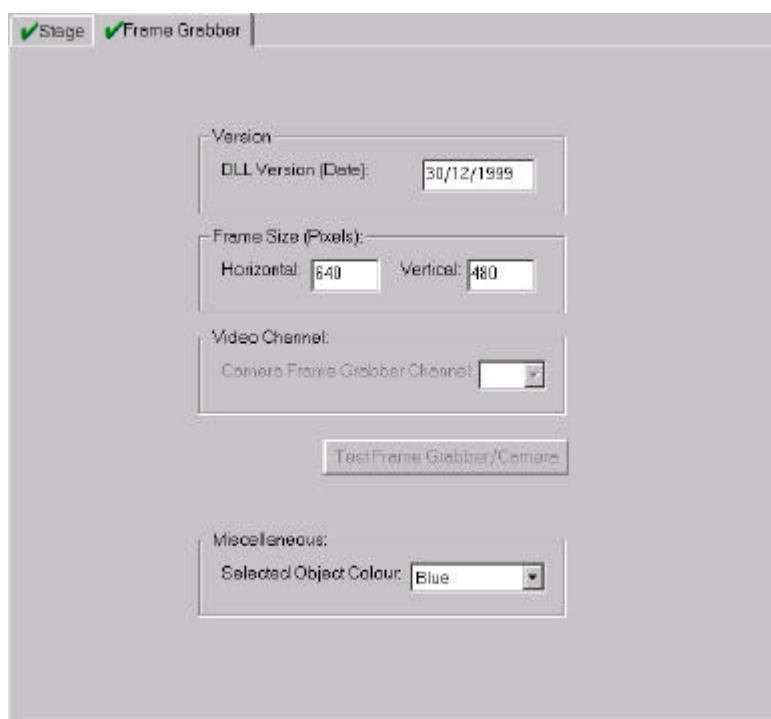
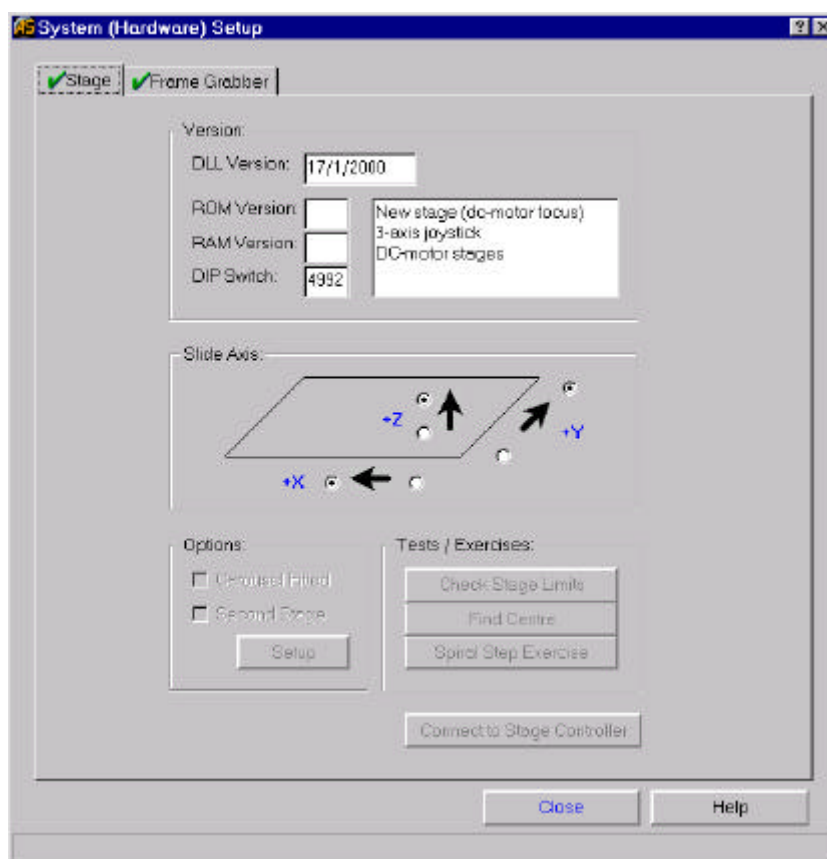
Selected	Area (µm²)	Perimeter (µm)	Circularity	Ellipse Max (µm)	Ellipse Min (µm)	Ellipse Angle (°)	Eccentricity	Radius Av (µm)	Radius SD (µm)	Radius Min (µm)	Radius Max (µm)	Pixel Av	Pixel SD	Pixel Min	Pixel Max
X	2,361	185	0.862	83.2	37.2	96.5	2.237	28.8	8.0	14.3	41.2	70	19	36	121
X	2,012	166	0.901	67.6	38.9	85.3	1.700	25.8	5.9	11.2	33.4	56	20	30	121
X	1,564	179	0.613	68.5	33.1	73.6	2.067	22.9	7.6	6.8	34.1	73	20	36	121
X	1,353	148	0.772	56.7	34.5	81.0	1.643	20.3	5.9	6.7	30.9	76	19	43	121
✓	1,034	105	1.181	40.9	32.3	137.3	1.266	18.8	1.6	15.8	21.8	62	22	28	121
✓	1,011	106	1.127	40.8	31.6	90.3	1.291	18.5	1.8	15.2	21.9	68	21	28	121
✓	1,001	102	1.202	38.0	33.6	17.3	1.131	18.1	1.0	15.9	20.0	68	18	37	121
✓	995	104	1.165	40.2	31.5	154.2	1.275	18.3	1.7	15.7	21.9	63	20	32	121
✓	992	104	1.161	38.8	32.6	37.7	1.188	18.2	1.4	13.8	20.3	59	24	27	121
✓	990	102	1.188	39.1	32.2	153.5	1.215	18.0	1.3	15.5	20.2	73	16	48	121
✓	988	102	1.186	40.3	31.3	108.8	1.288	18.1	1.8	14.9	21.3	65	20	34	121
✓	983	104	1.151	40.7	30.9	40.2	1.319	18.1	1.8	14.1	21.3	74	18	42	121

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System hardware, stage and frame grabber/camera, is configured and tested using the System Setup screen invoked from the menu bar on the main screen. Screens related to hardware configuration and testing are shown below.



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