Semi-Automated Analysis Software for a Novel Biochemistry Assay

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in Computer Science

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Special Thanks

- Dr. Frederick C. Harris, Jr.
- Dr. Sergiu Dascalu
- Dr. Josh Baker

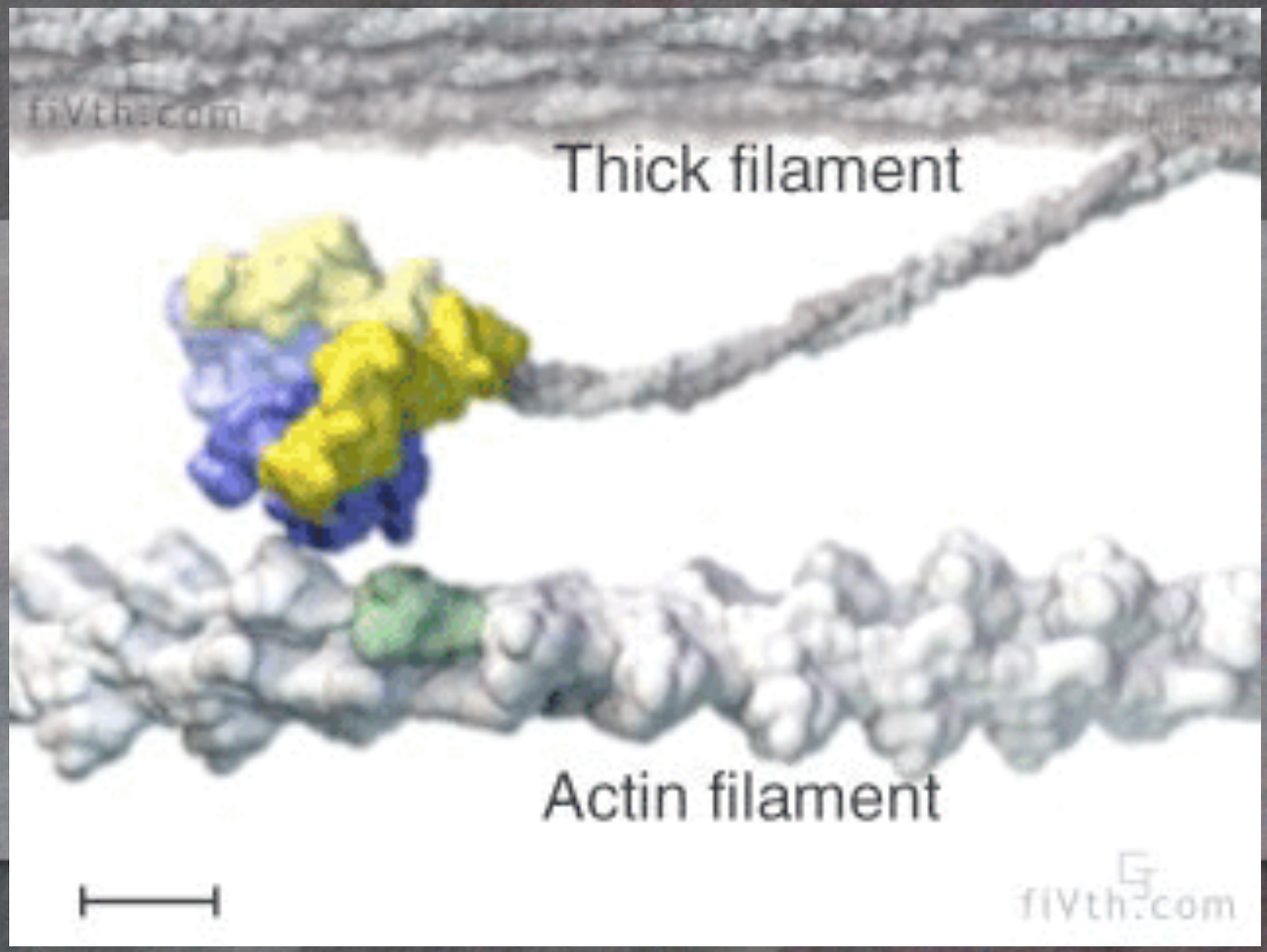
- Del Jackson

Overview

- Background
- SANoBA
  - Software Design And Implementation
  - Walkthrough
- Conclusions
- Future Work

Background

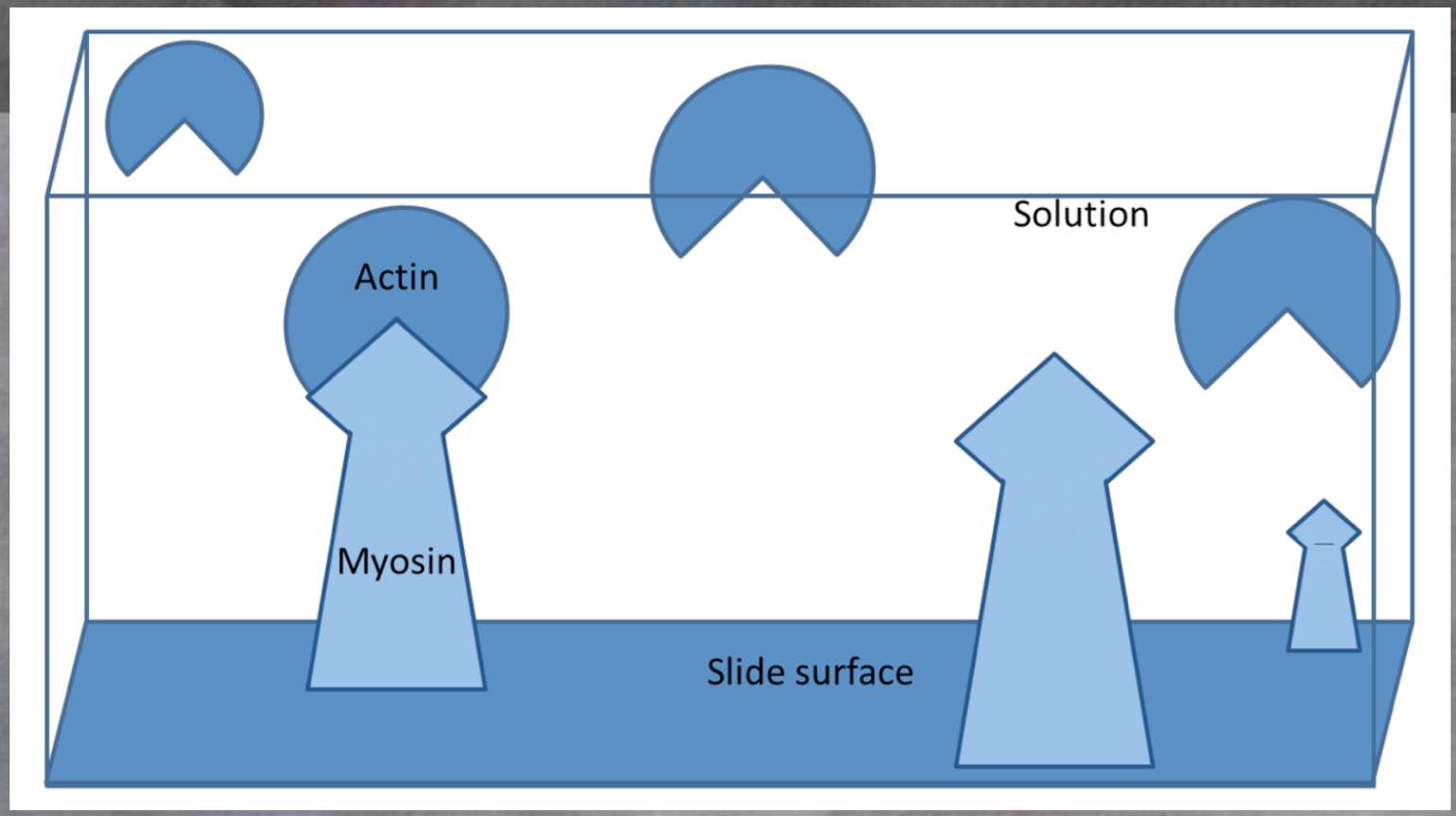
Study of Muscle Tissue



Movie 1 - A general muscle model.

SiMBA

"Single Molecule Binding Assay"



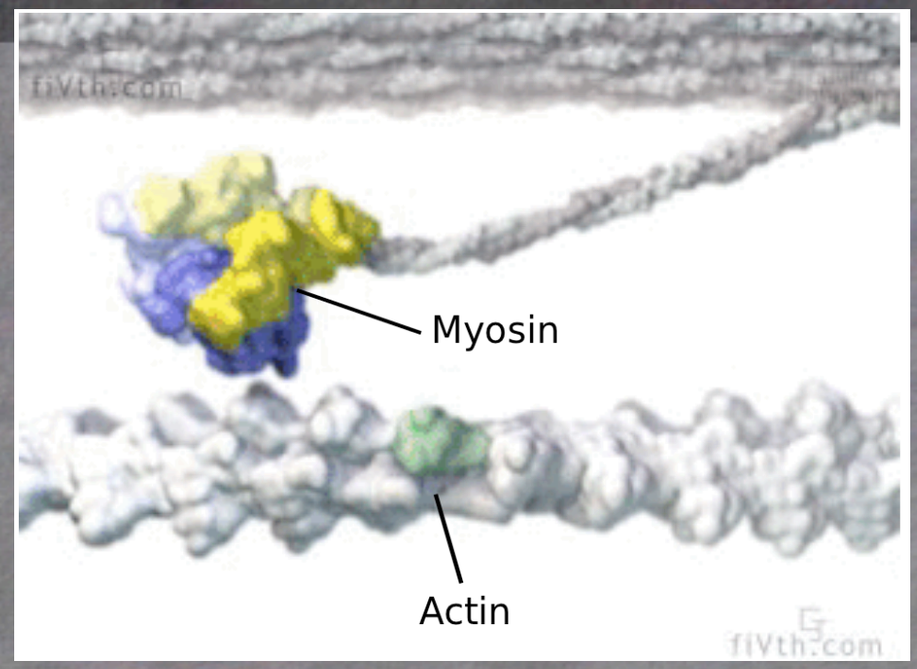


Figure 1 - SiMBA Model And Screen Shot From Movie 1.

Desired Results: T_{on}, T_{off}

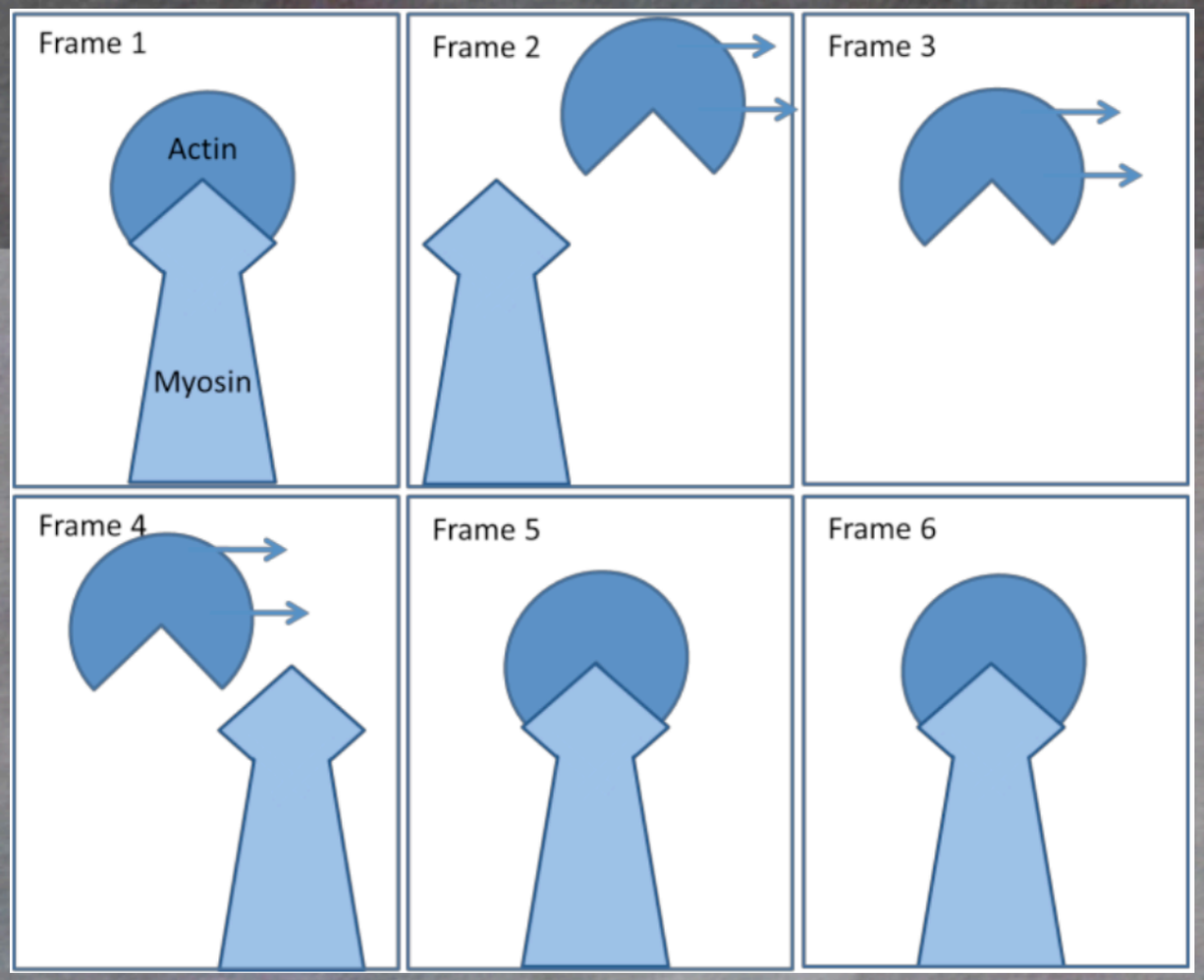


Figure 2 - Basic representation of a t_{Off} Event

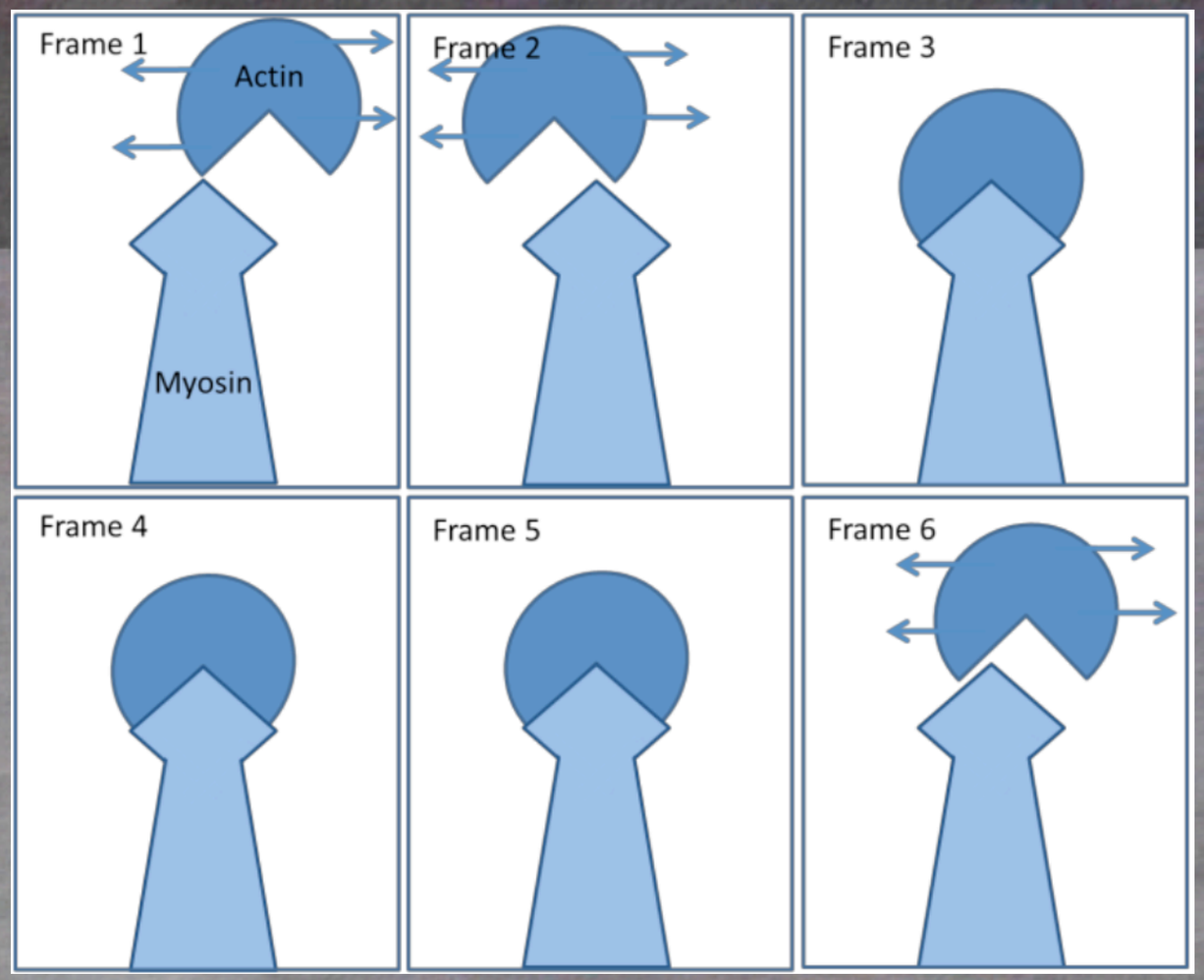


Figure 3 - Basic representation of a t_{On} Event

Lab Process

Figure 4 - Frozen Samples

Figure 5 - Thawing The Samples

Lab Process

Figure 6 - Buffer Preparation

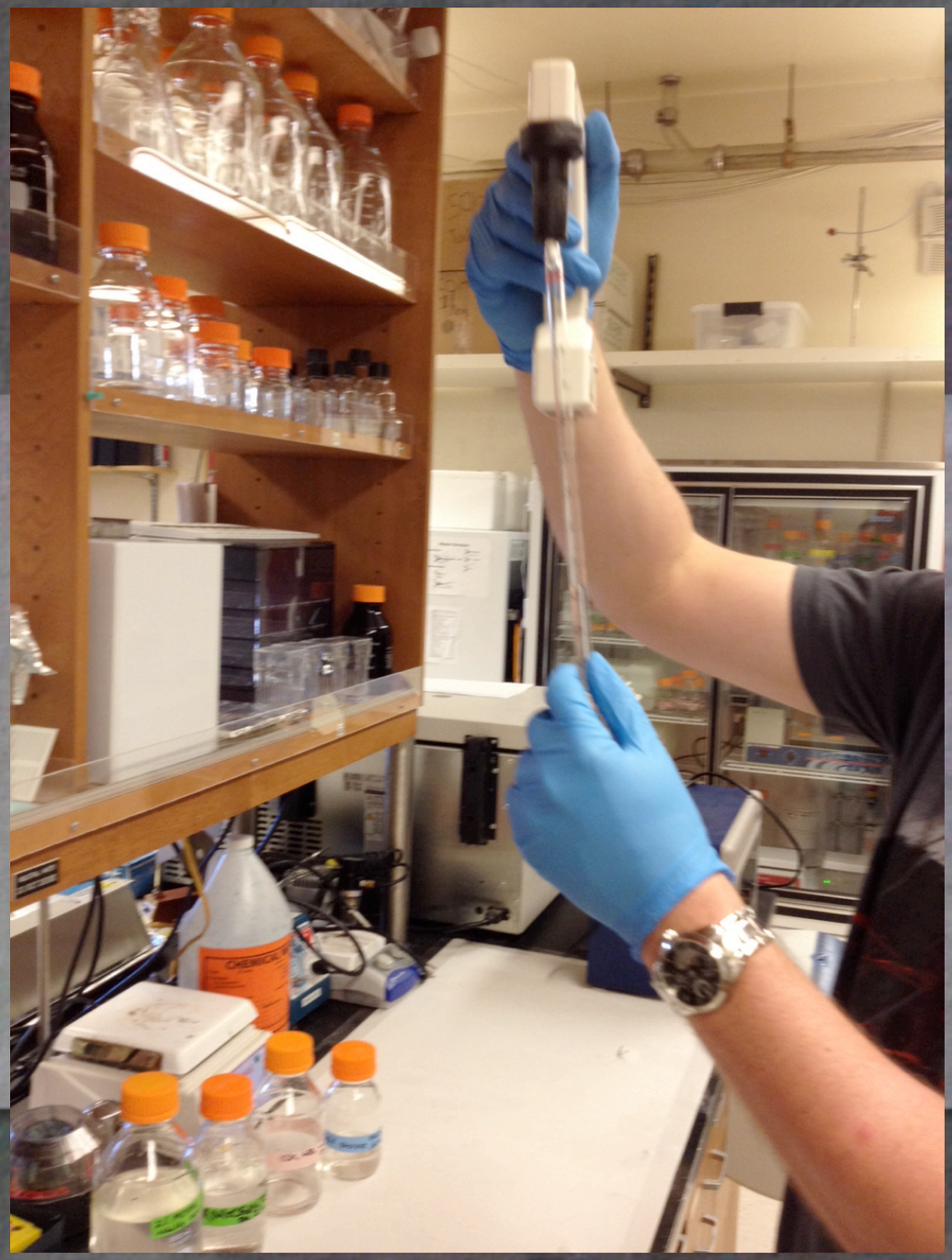


Figure 7 - All Samples Are Identical Except For A Varying Parameter

Lab Process

Figure 8 -Rotational Mixer

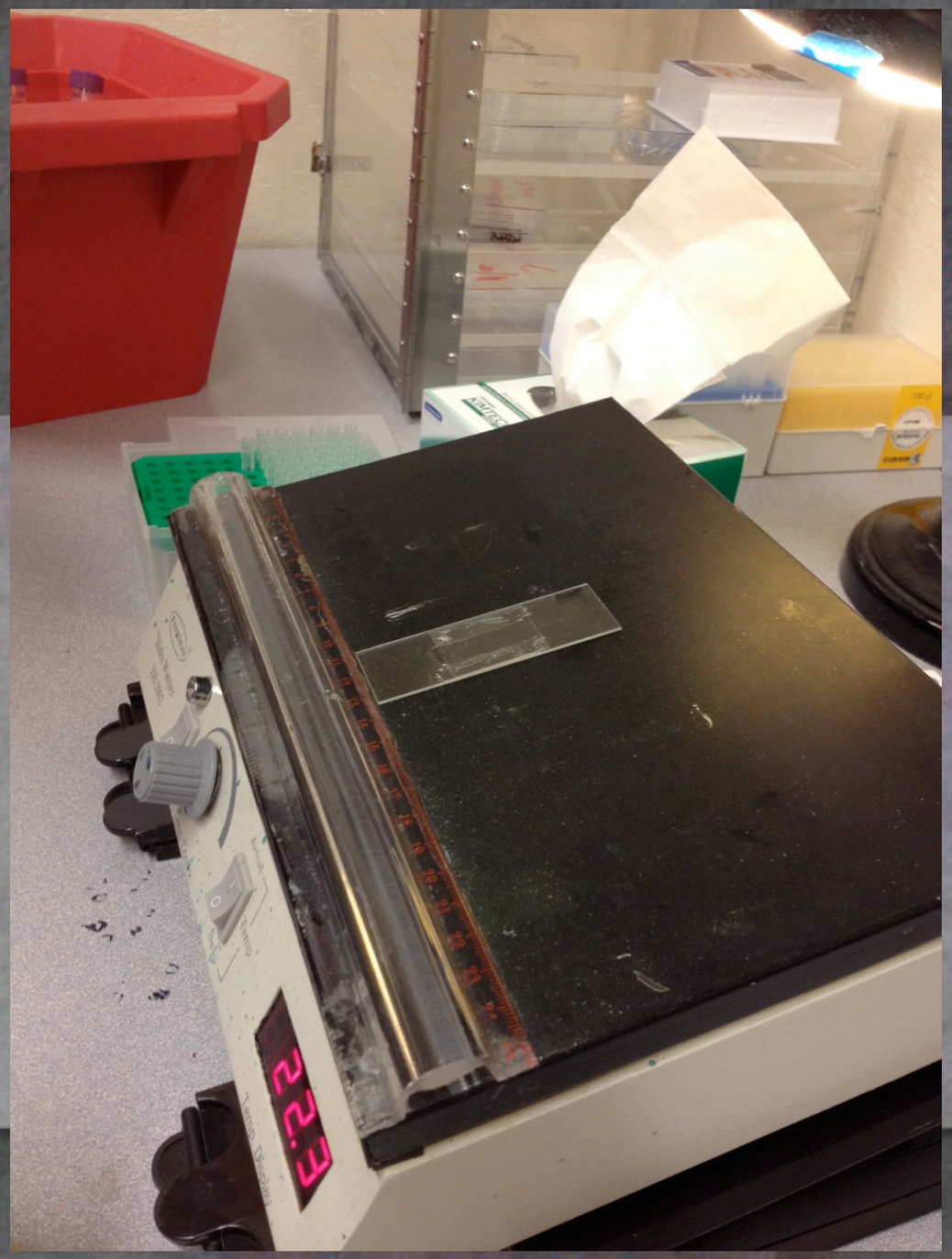


Figure 9 - Samples Brought To Experimental Temperature

Lab Process

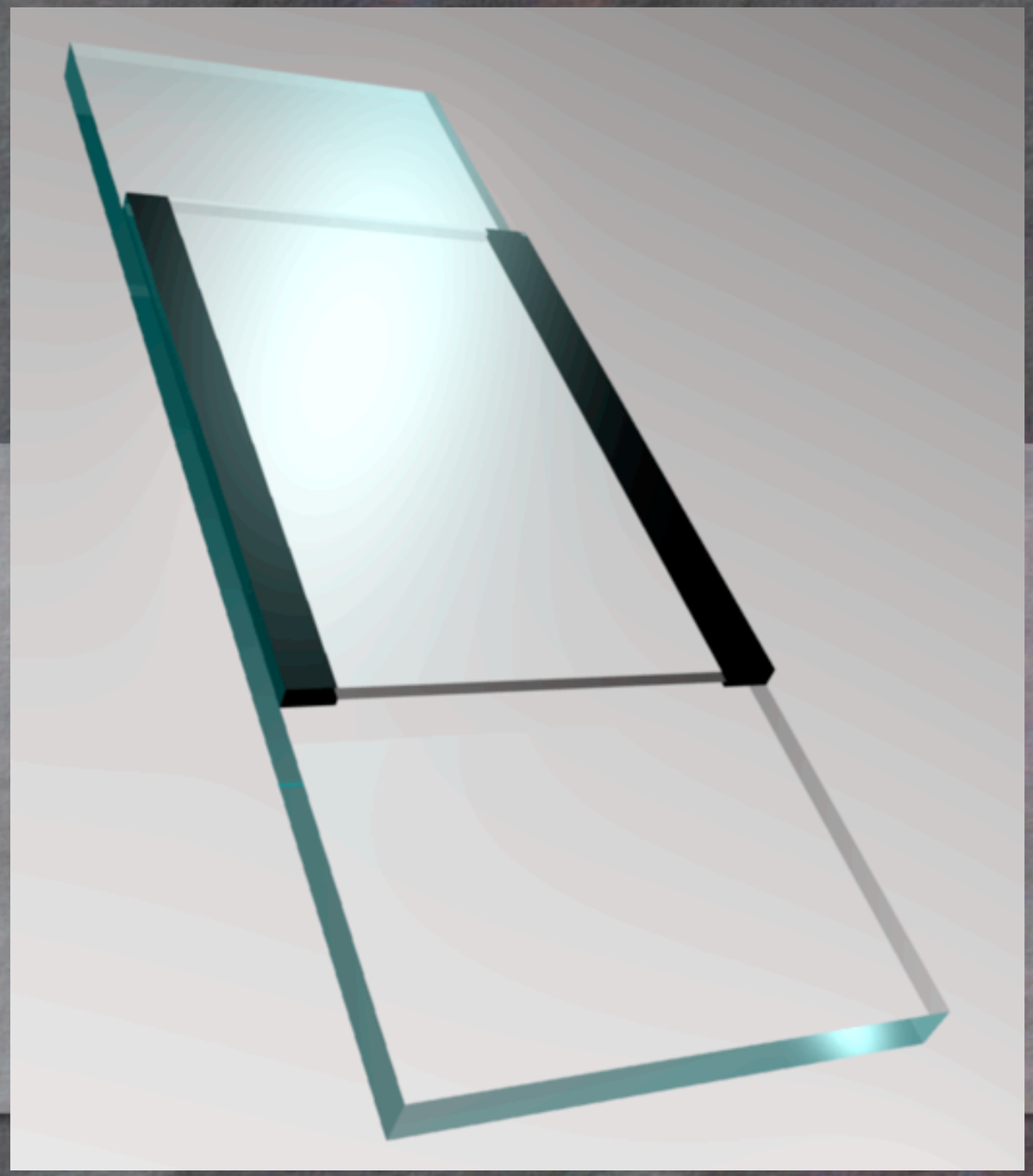


Figure 10 -Flow Slide Mockup

Lab Process

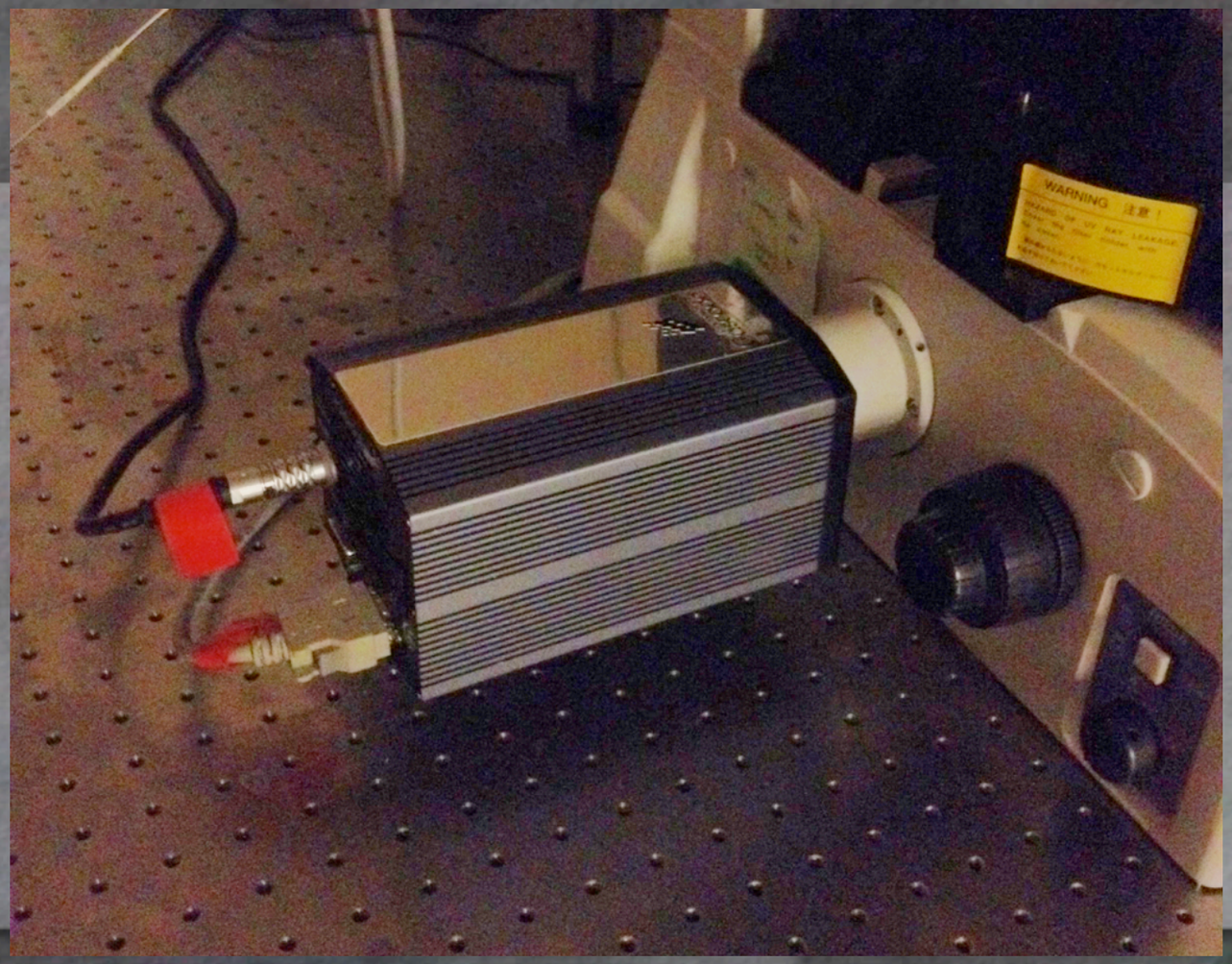
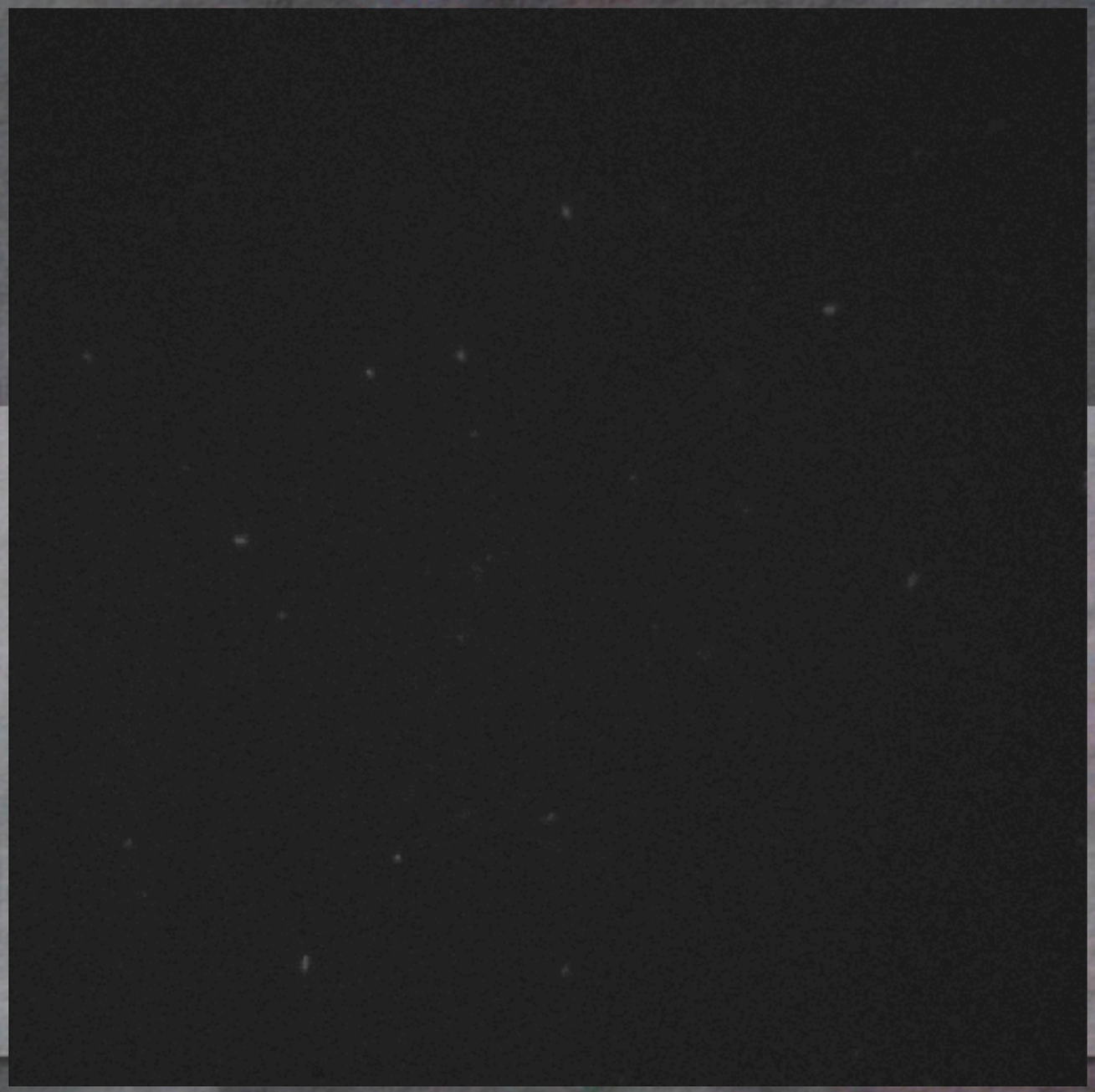


Figure 11 - Roper 512B CCD

Figure 12 - Nikon 2000-TE Inverted Microscope

Lab Process

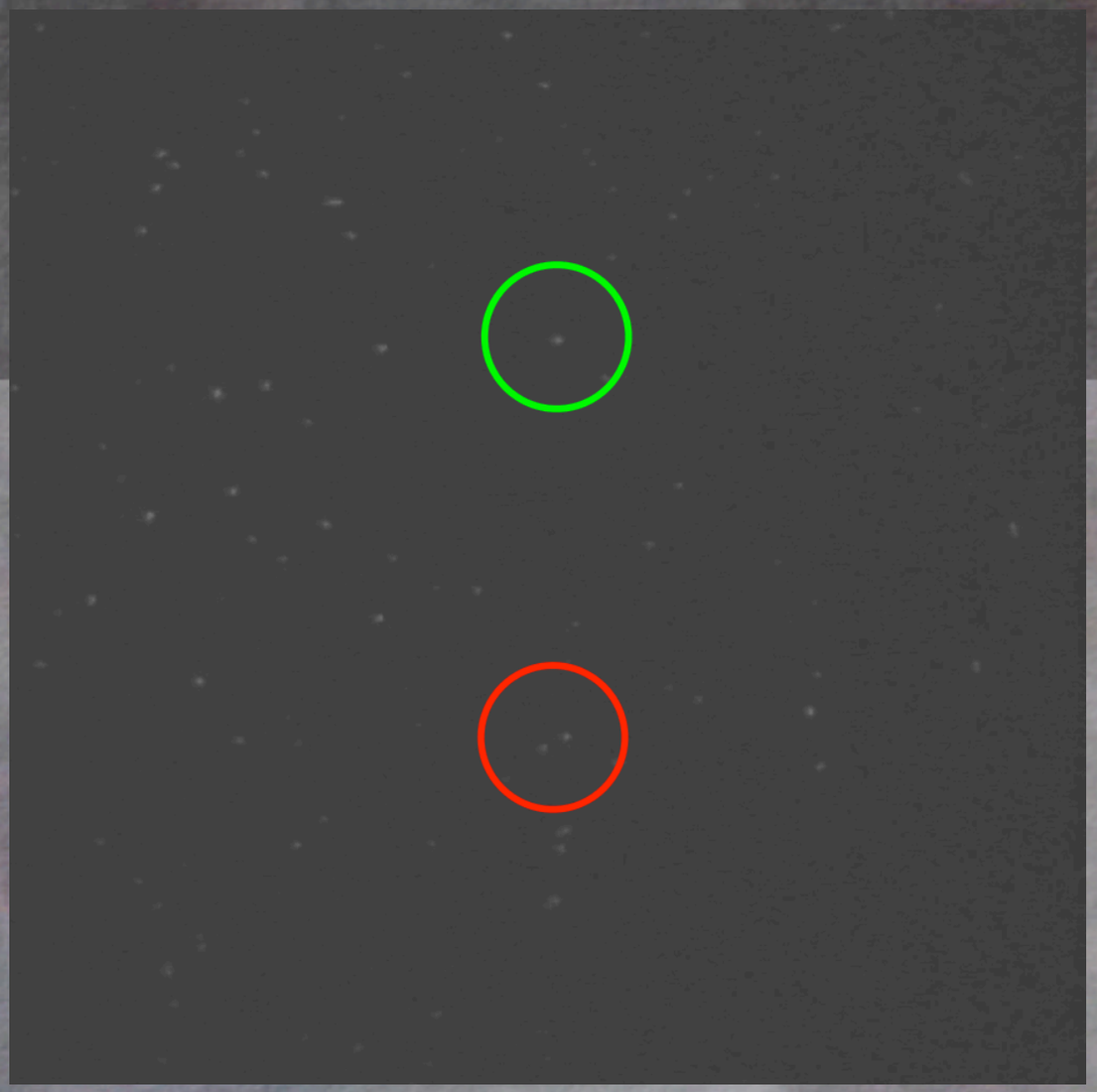


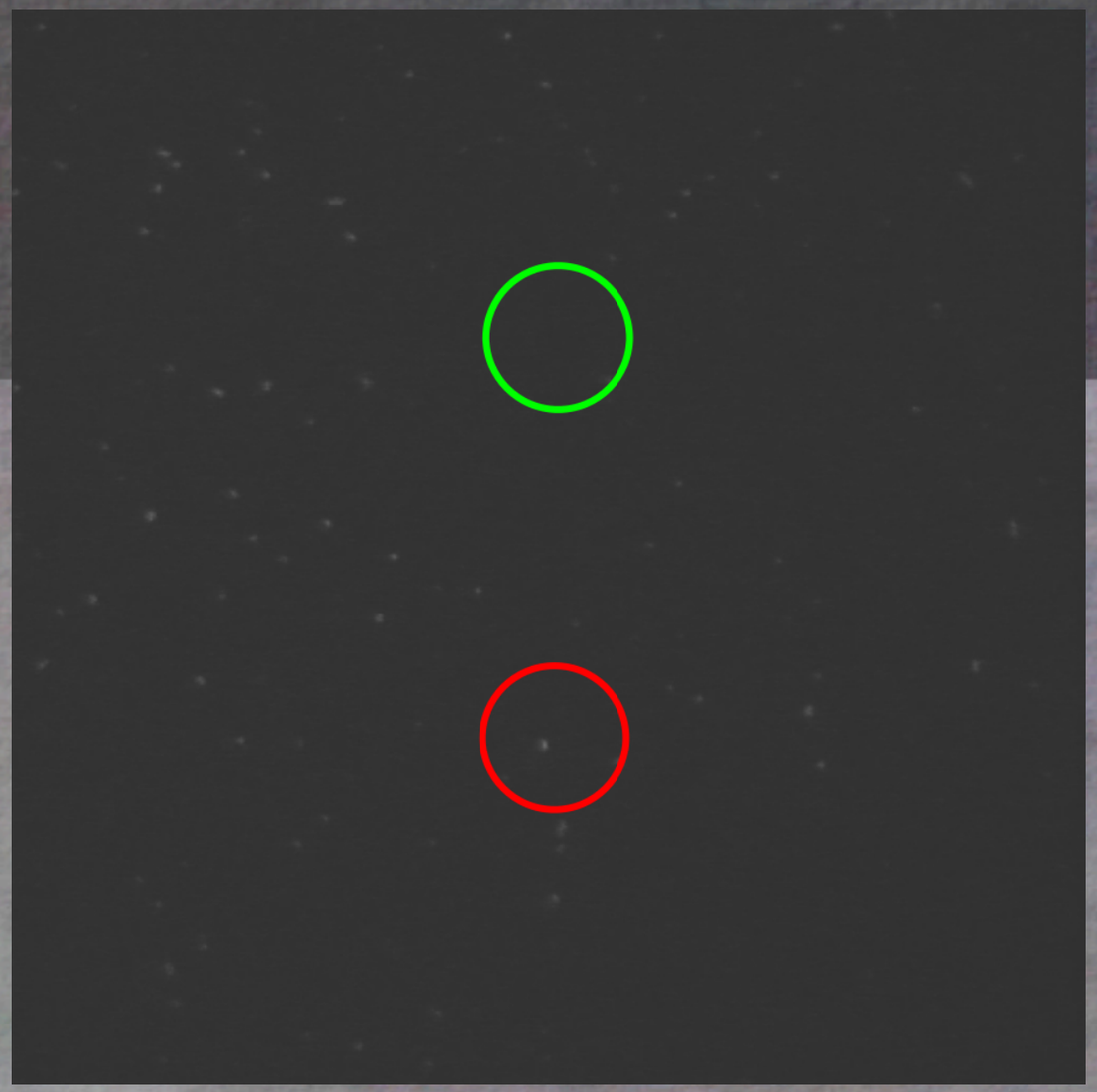
Movie 2 - SiMBA Output

Lab Process

Figures 13/14 - Comparison Of Consecutive Frames
From Movie 0.5ugmLS1-2.5nMAct-0.9 MC1.avi, Frame 22 And 23

Lab Process





Figures 15/16 - Movie 0.5ugmLS1-2.5nMAct-0.9 MC1.avi, Frame 22 And 23
Object Visible Then Not Visible (green)
Two Object Over Lap (red)

Functional Requirements

The System Will:

- Process Raw Images.
- Locate Objects In The Images.
- Track Objects Over Consecutive Images.
- Disregard Objects Outside Of A Set Size Threshold.
- Allow Size Threshold Adjustments At Run Time.
- Allow Adjustment For Amount Of Time Object Can Be Gone.
- Store Unique Sets Of Frames Based On Threshold Settings.
- Allow The User To Remove Objects.
- Allow The User To Add Objects.
- Allow The User To Rename Objects.
- Allow The User To Navigate Movie.
- Allow The User To Animate Movie.
- Output Event Data To A File For Further Analysis.

Threshold

- Removes pixels in certain range
- Convert grey scale image to B&W

Figures 17/18 - Before And After Threshold

Blur

- Reduces image noise
- Reduce pixelation

Figures 19/20 - Before And After Blur

SANoBA
Software Design And Implementation

SANoBA

"Semi-Automated Analysis Software for a Novel Biochemistry Assay"

1. Import data
2. Image processing
3. Image analysis and object coordination
4. Data analysis and event coordination
5. User interaction and control
6. Final output and analysis

1. Import Data

- SimplePCI output to CXD and converts to AVI
- This is then converted to individual frames in a PGM format

2. Image Processing

- Threshold - Based on samples taken of each frame (slide 16).
- Blur - Helps reduce data loss (slide 17).

3. Image Analysis

- Object location of each frame
- BFS algorithm
- Each object given ID and color
- Stored with location and size in a vector

4. Data Analysis

- Determine behavior across frames
- In motion, being still, gone?

5. User Interaction

- Adjust settings.
- Add or remove objects if necessary.
- Save state of program.
- Ensure best possible outcome for unforeseeable situations.

6. Final Output & Analysis

- Desired output is t_{On} and t_{Off} and duration of each occurrence.
- Imported to Origin software.

Software Design

- Functional requirements (previously discussed on slide 15).
- Use cases
- Traceability matrix

Use Cases

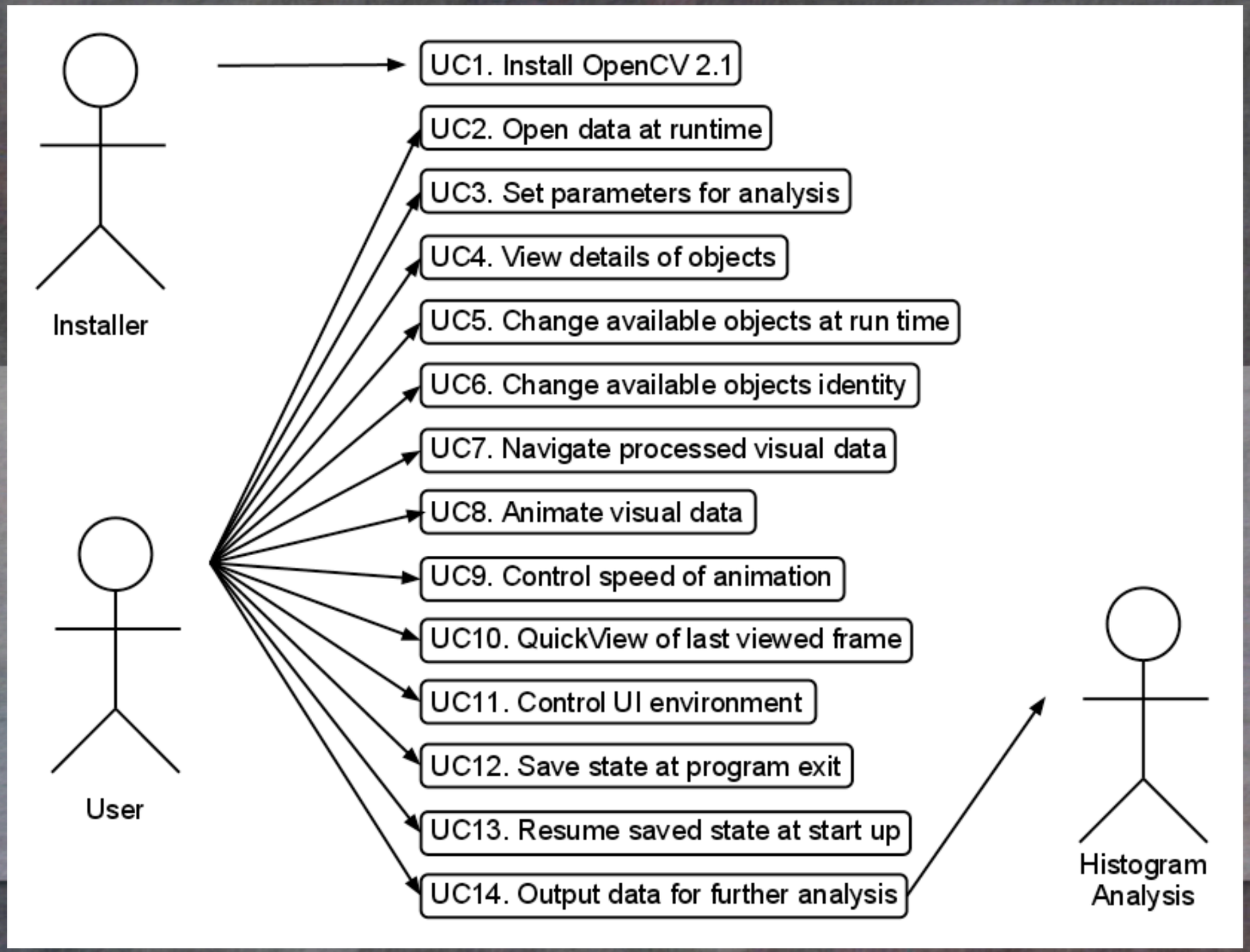
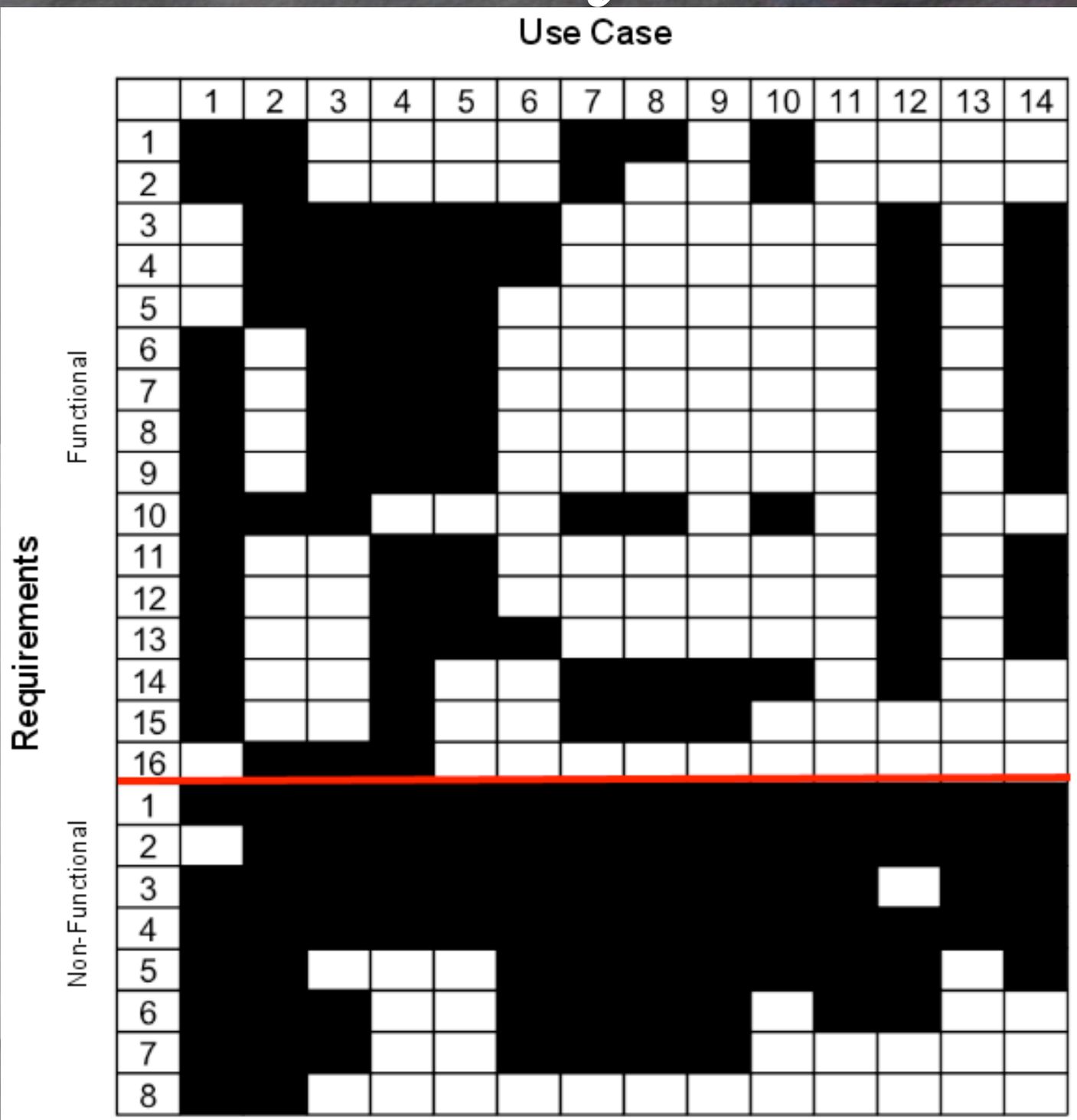
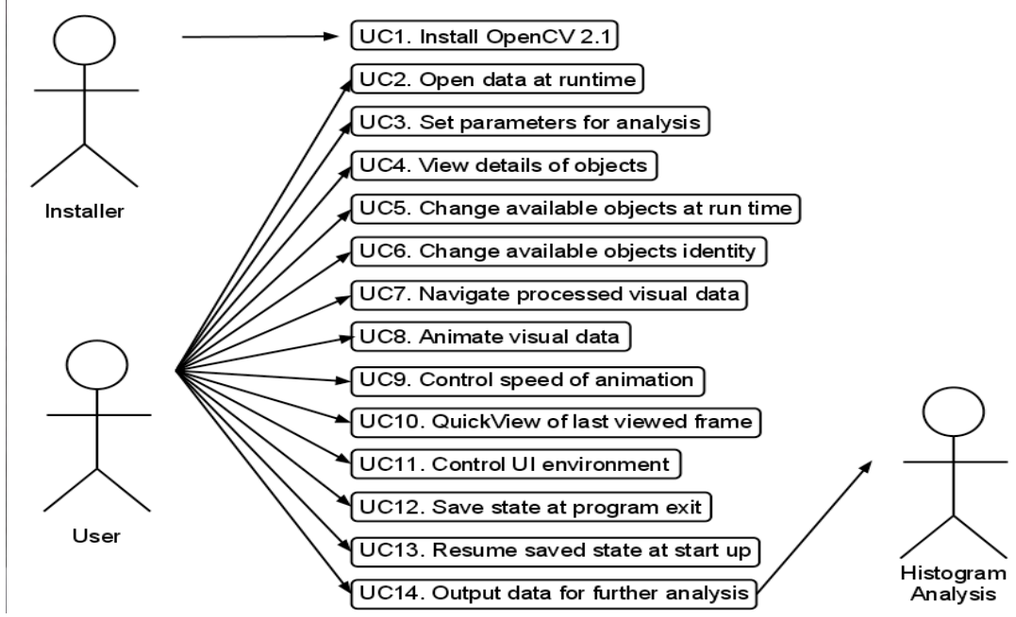


Figure 21 - Use Case Diagram

Traceability Matrix





F1. The system will convert movie files to image files.

F2. The system will process raw images.

F3. The system will locate objects in the images.

F4. The system will track objects over consecutive images.

F5. The system will disregard objects outside of a set size threshold.

F6. The system will allow size threshold adjustments at run time.

F7. The system will allow motion threshold adjustments at run time.

F8. The system will allow adjustment for amount of time object can be gone.

F9. The system will allow blur adjustments at run time.

F10. The system will store unique sets of frames based on threshold settings.

F11. The system will allow the user to add objects.

F12. The system will allow the user to remove objects.

F13. The system will allow the user to rename objects.

F14. The system will allow the user to navigate movie.

F15. The system will allow the user to animate movie.

F16. The system will output event data to a file.

Figure 22 - Traceability Matrix Between Use Cases And Functional And Non-functional Requirements

SANoBA
Walkthrough

Walkthrough

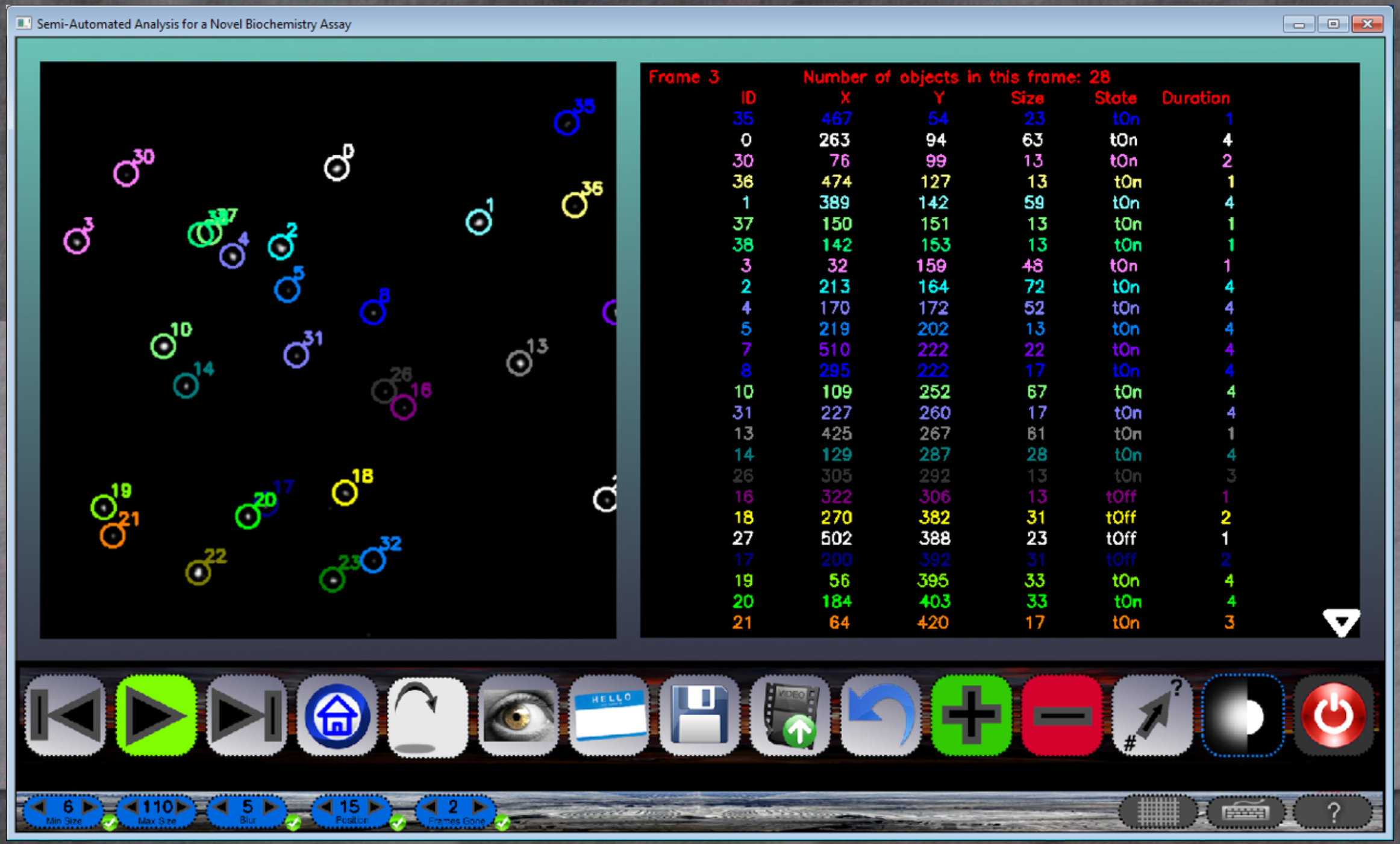
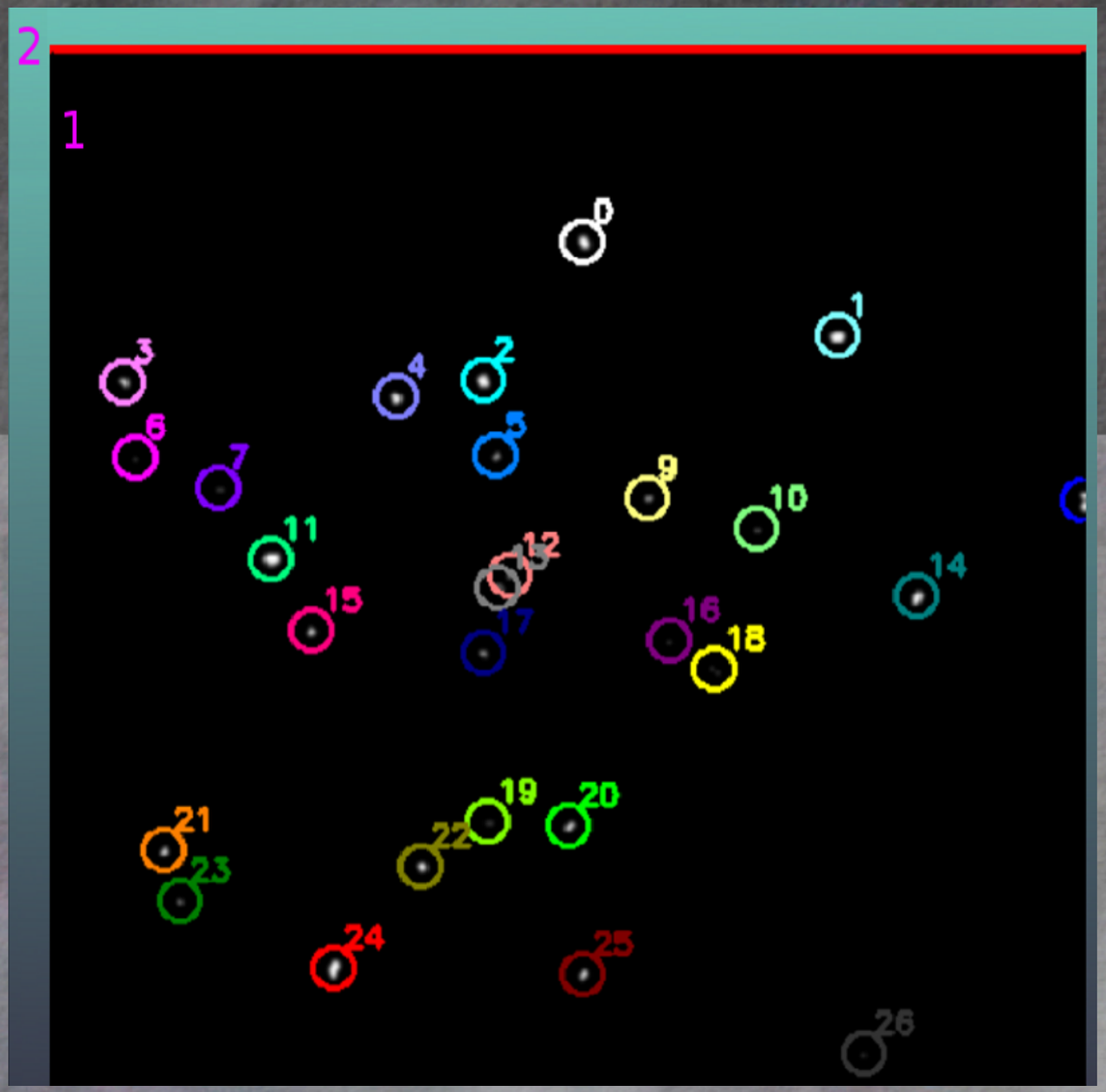


Figure 23 - Main User Interface

Walkthrough

Figure 24 - Label UI

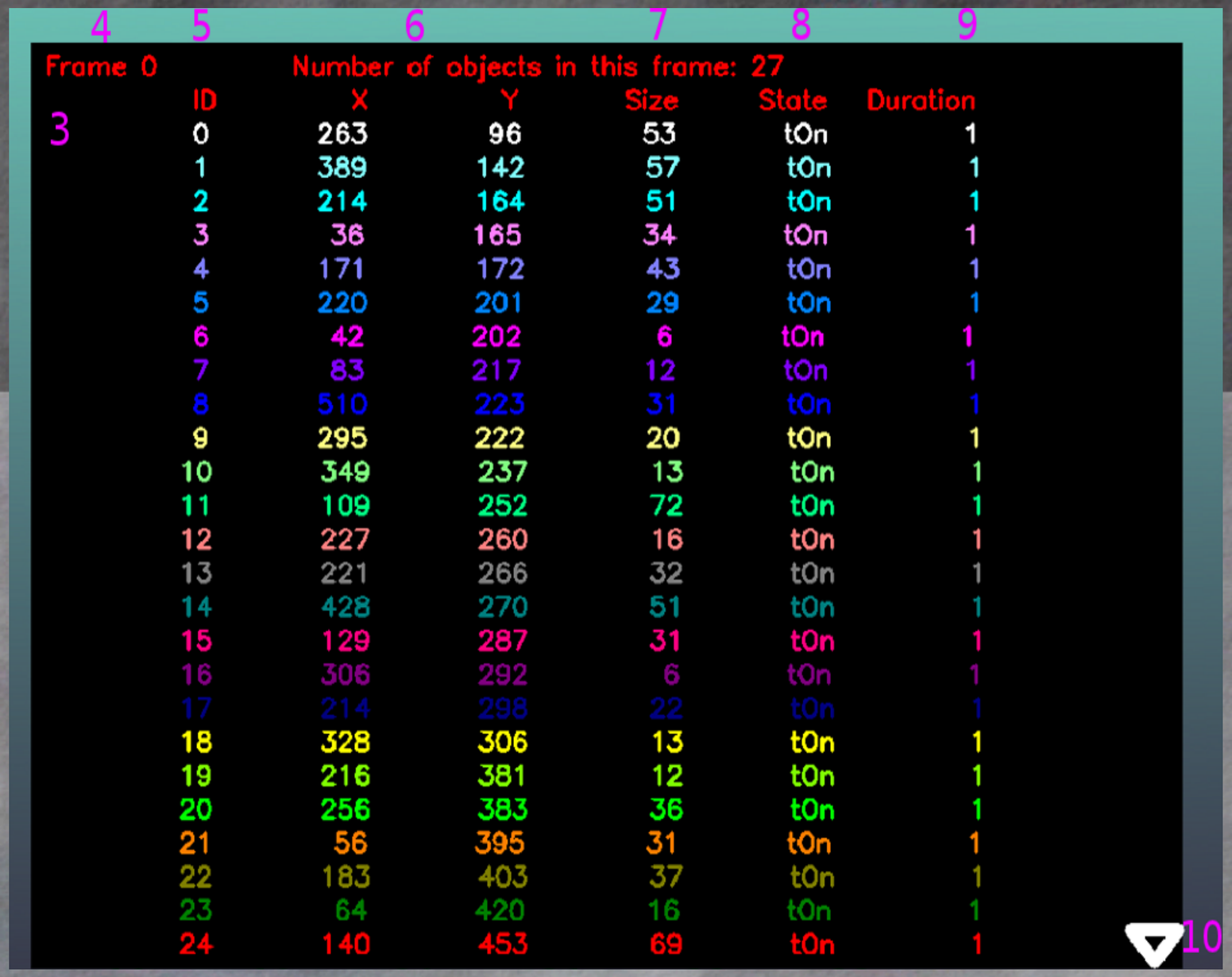
Walkthrough



1 - Main Object Window
2 - Red Bar On First Frame

Figure 25 - Object Window

Walkthrough



3 - Information Window.

4 - The Current Frame Number.

5 - Object IDs In Current Frame.

6 - X / Y Coordinates Of Each Object Present In Current Frame.

7 - The Size Of Each Object In The Current Frame.

8 - The State Of Each Object In The Current Frame.

9 - How Long That Object Has Been In Its Current State.

10 - Arrows Present When More Objects Than List Can Display.

Figure 26 - Info Window

Walkthrough

11 Go Back / Slow Down.
12 Start / Stop Animation.
13 Go Forward / Speed Up.
14 Go To First Frame.
15 Go To Specific Frame.
16 See The Last Frame Viewed.
17 Show / Hide IDs / Circles.
18 Save.
19 Open Movie / Saved File.
20 Undo, Currently Unavailable.
21 Add Object.
22 Remove Object.
23 Rename Object
(Activates Secondary Window).
24 Turn Blur On / Off.
25 Quit Program.

Figure 27 - Functions

Walkthrough

27 - Adjust The Minimum Object Size.

28 - Adjust The Maximum Object Size.

29 - Adjust The Amount Of Blur.

30 - Adjust How Far An Object Moves Before Considered To Be Moving.

31 - Adjust How Many Frames An Object Is Gone Before Considered To Be Gone.

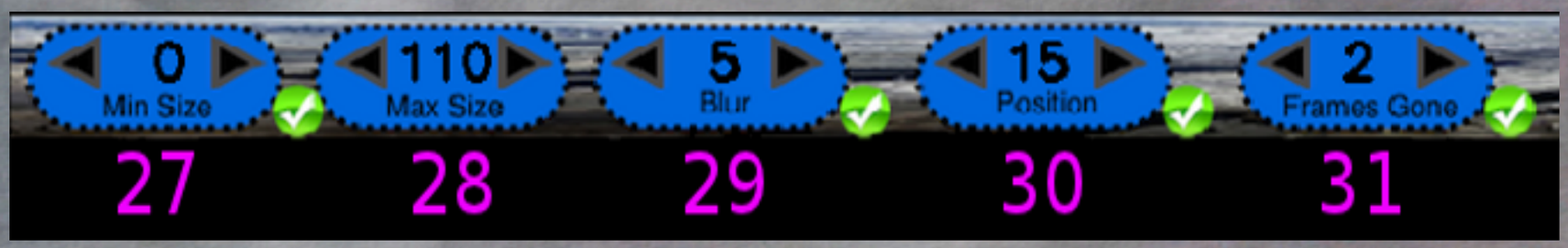


Figure 28 - Threshold Settings

Walkthrough

32 - Show/ Hide Grid

33 - Show / Hide Keyboard Shortcuts

34 - Show Help Screen

Figure 29 - Threshold Settings

Walkthrough

36 - Close Secondary UI Window.

37 - Entered Value Of New ID.

38 - Show / Hide Grid.

39 - Go Back.

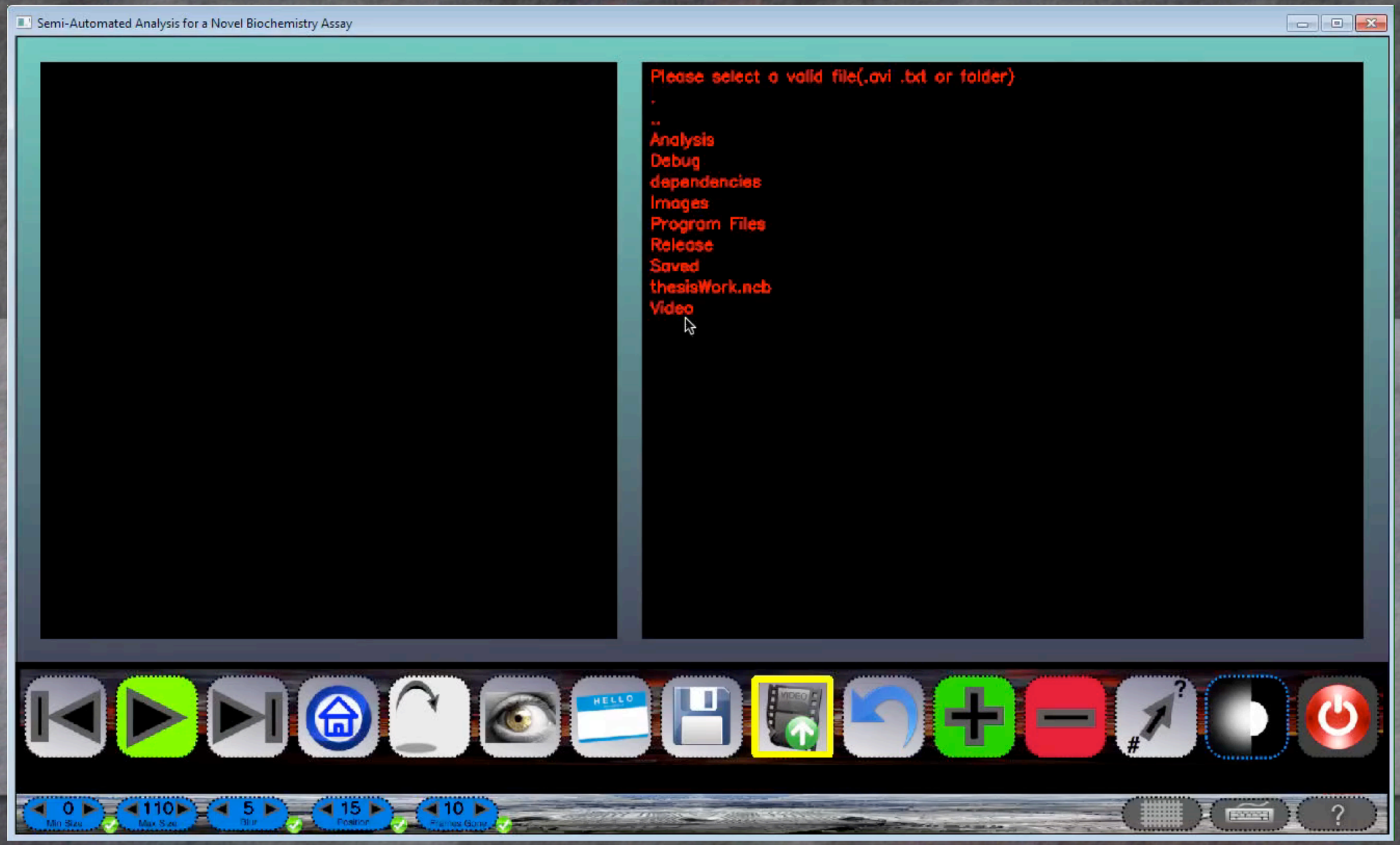
40 - Go Forward.

Figure 29 - Secondary UI

Walkthrough

Movie 2 - Open Movie

Walkthrough

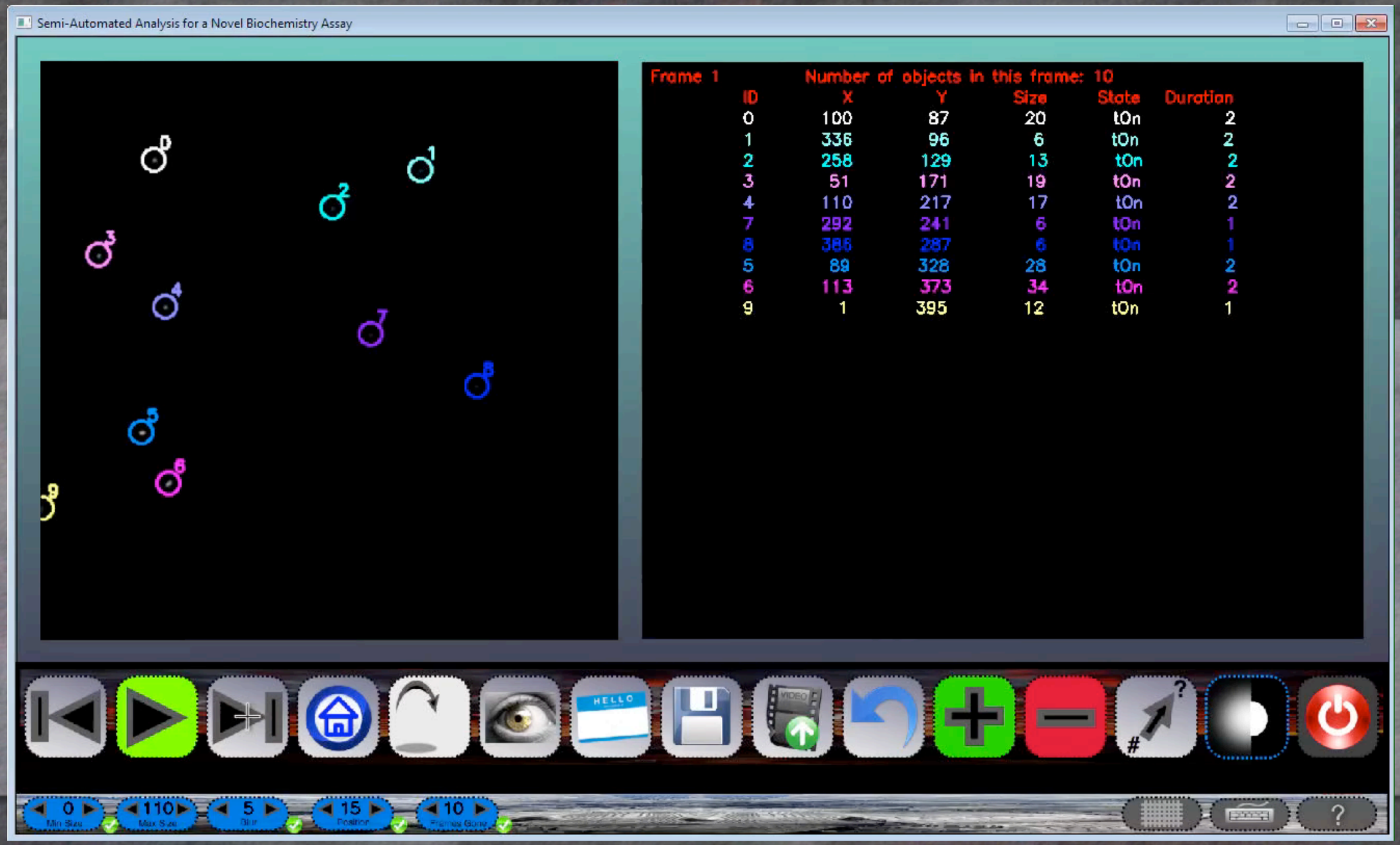


Movie 2 - Open Movie

Walkthrough

Movie 3 - Remove Object

Walkthrough



Movie 3 - Remove Object

Walkthrough

Movie 4 - Add Object

Walkthrough

Movie 4 - Add Object

Walkthrough

Movie 5 - Rename Object

Walkthrough

Movie 5 - Rename Object

Walkthrough

Movie 6 - Save And Open File

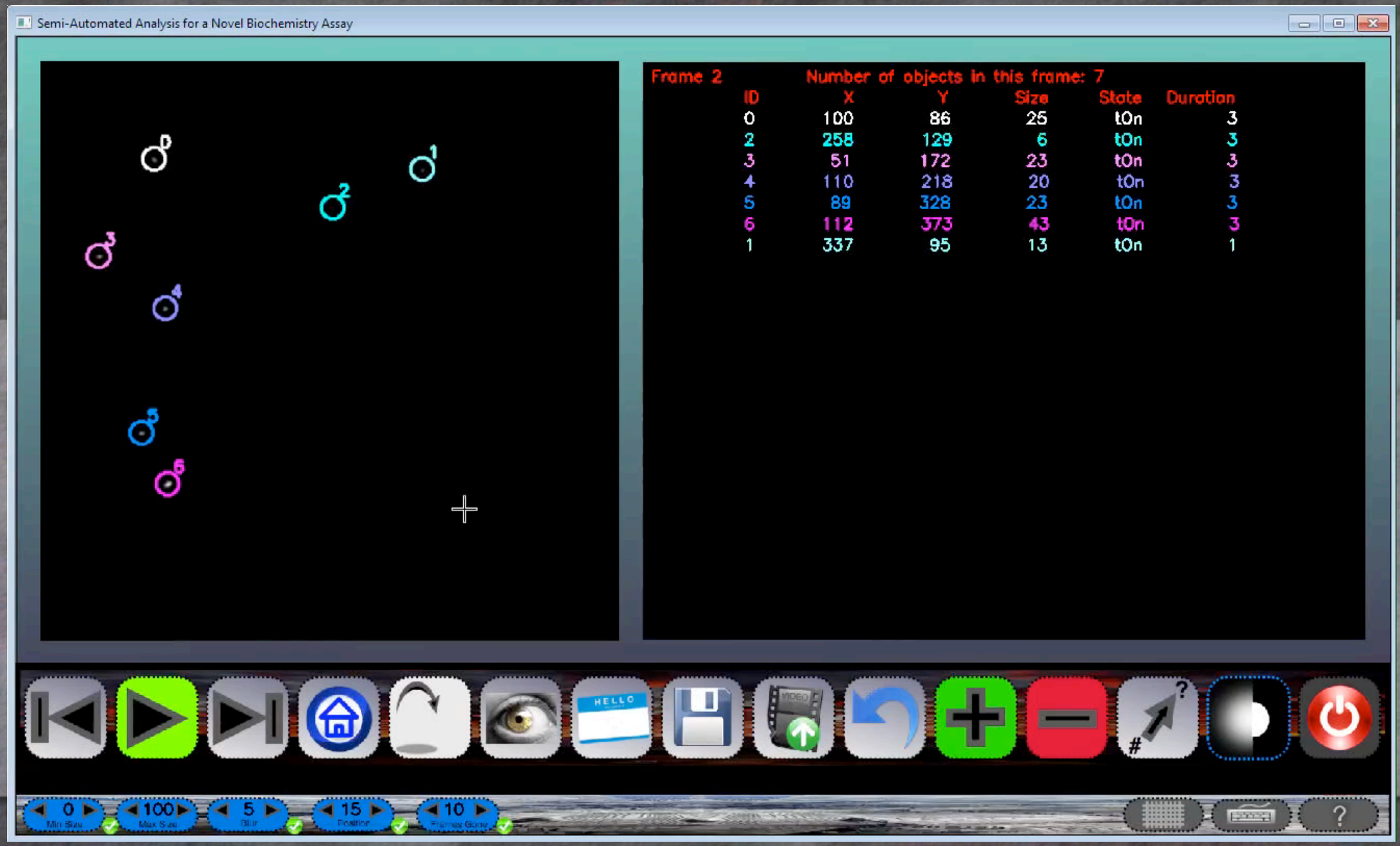
Walkthrough

Movie 6 - Save And Open File

Walkthrough

Movie 6 - Show/ Hide IDs

Walkthrough



Movie 6 - Show/ Hide IDs

Walkthrough

Movie 7 - Home And Goto...

Walkthrough

Movie 7 - Home And Goto...

Walkthrough

Movie 8 - Blur

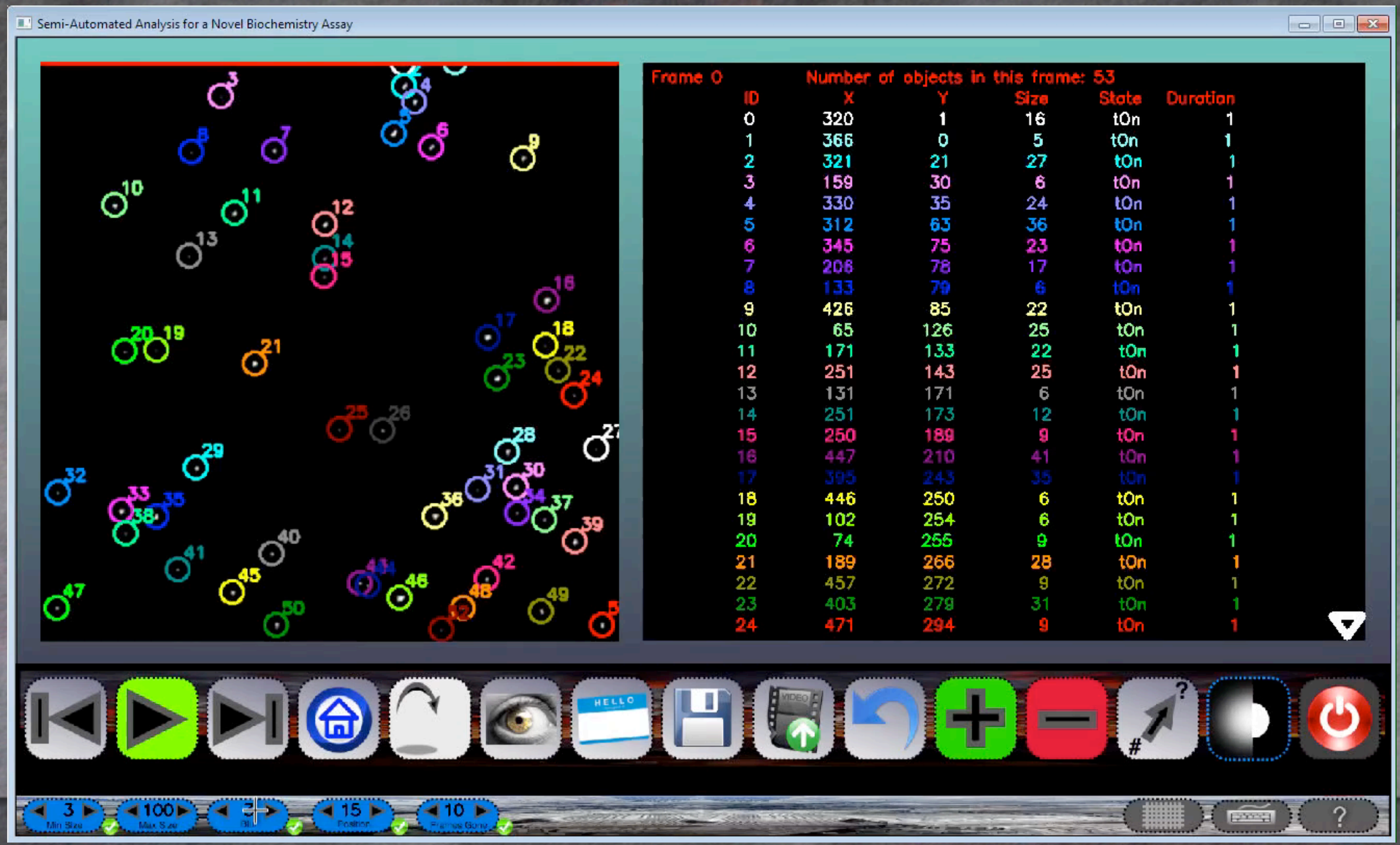
Walkthrough

Movie 8 - Blur

Walkthrough

Movie 9 - Size Threshold

Walkthrough

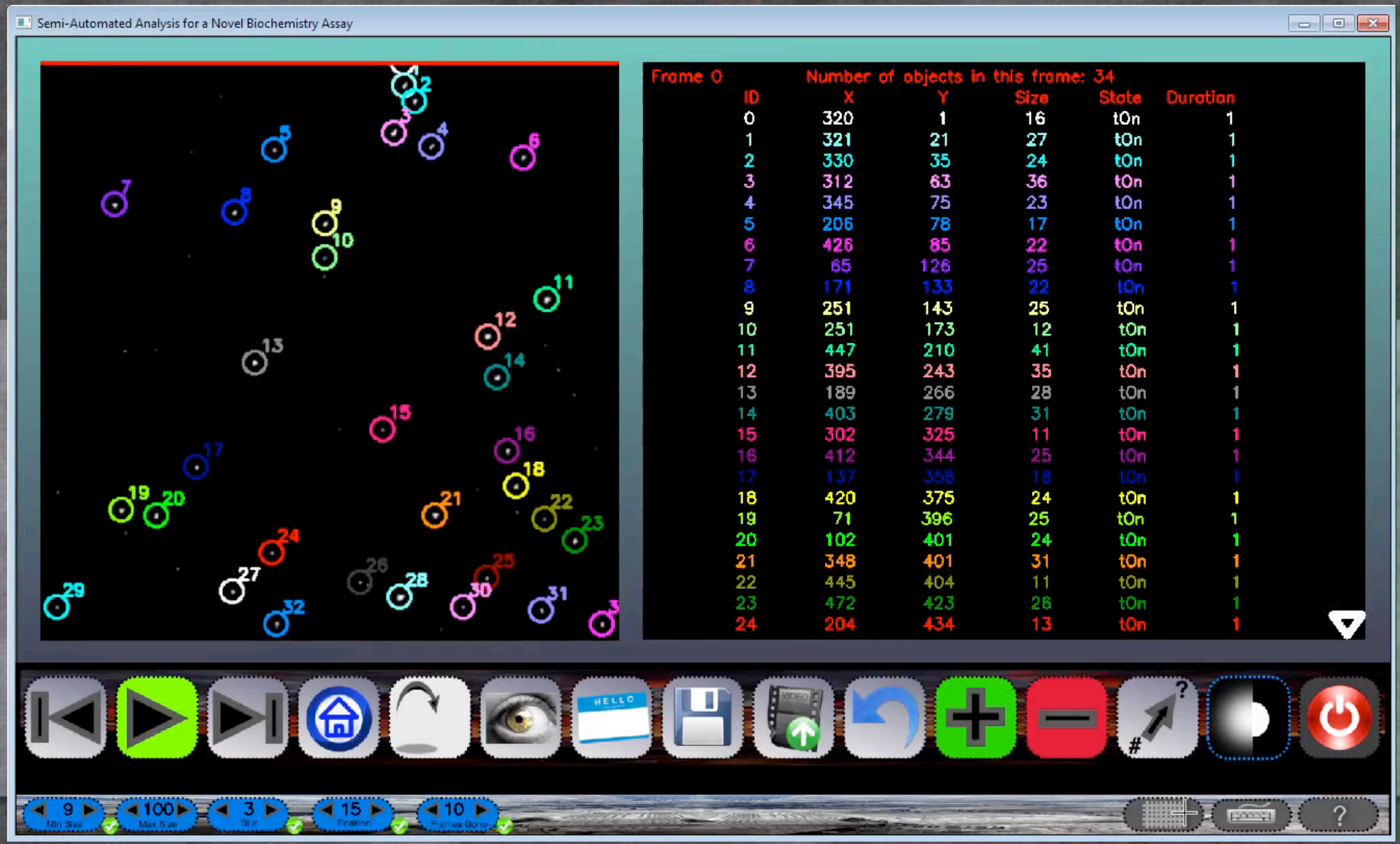


Movie 9 - Size Threshold

Walkthrough

Movie 10 - Grid, Keyboard, Help, And Quit

Walkthrough



Movie 10 - Grid, Keyboard, Help, And Quit

Conclusions

Conclusions

- Dr. Baker's Lab and SiMBA
- SANoBA

Future Work

Future Work

OpenCV GPU
Kymograph Image
Add Function Expansion
Undo Function
Status Bar
Object History
Multiple Operating Systems
Recent Projects Listing
Run Time Format Conversions
Zoom Function
Adaptability To Other Problems
Motion Analysis
Threshold Refinement

Future Work

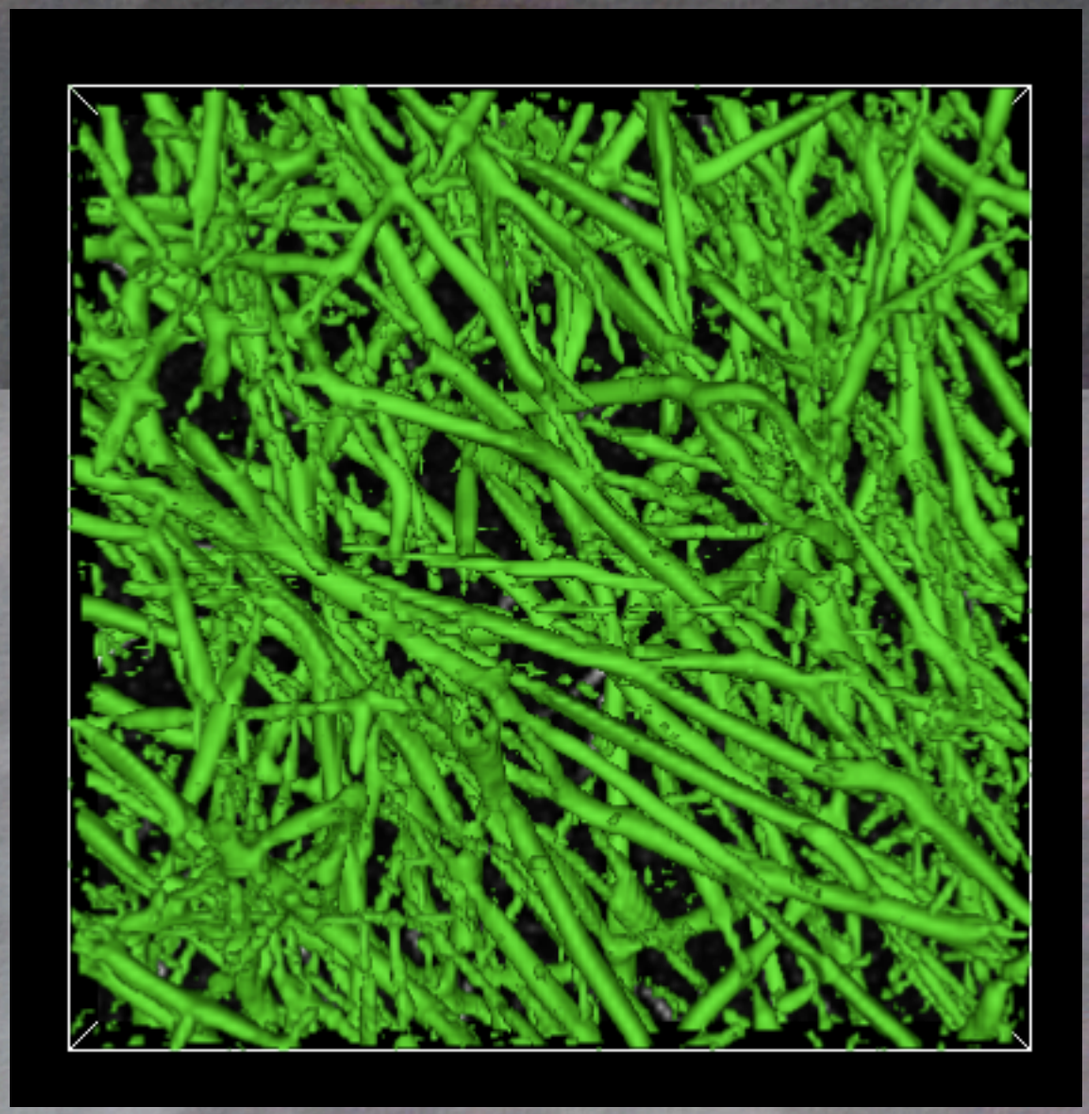


Figure 30 - Example Of A Kymograph