

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

Joseph Vesco



Special Thanks

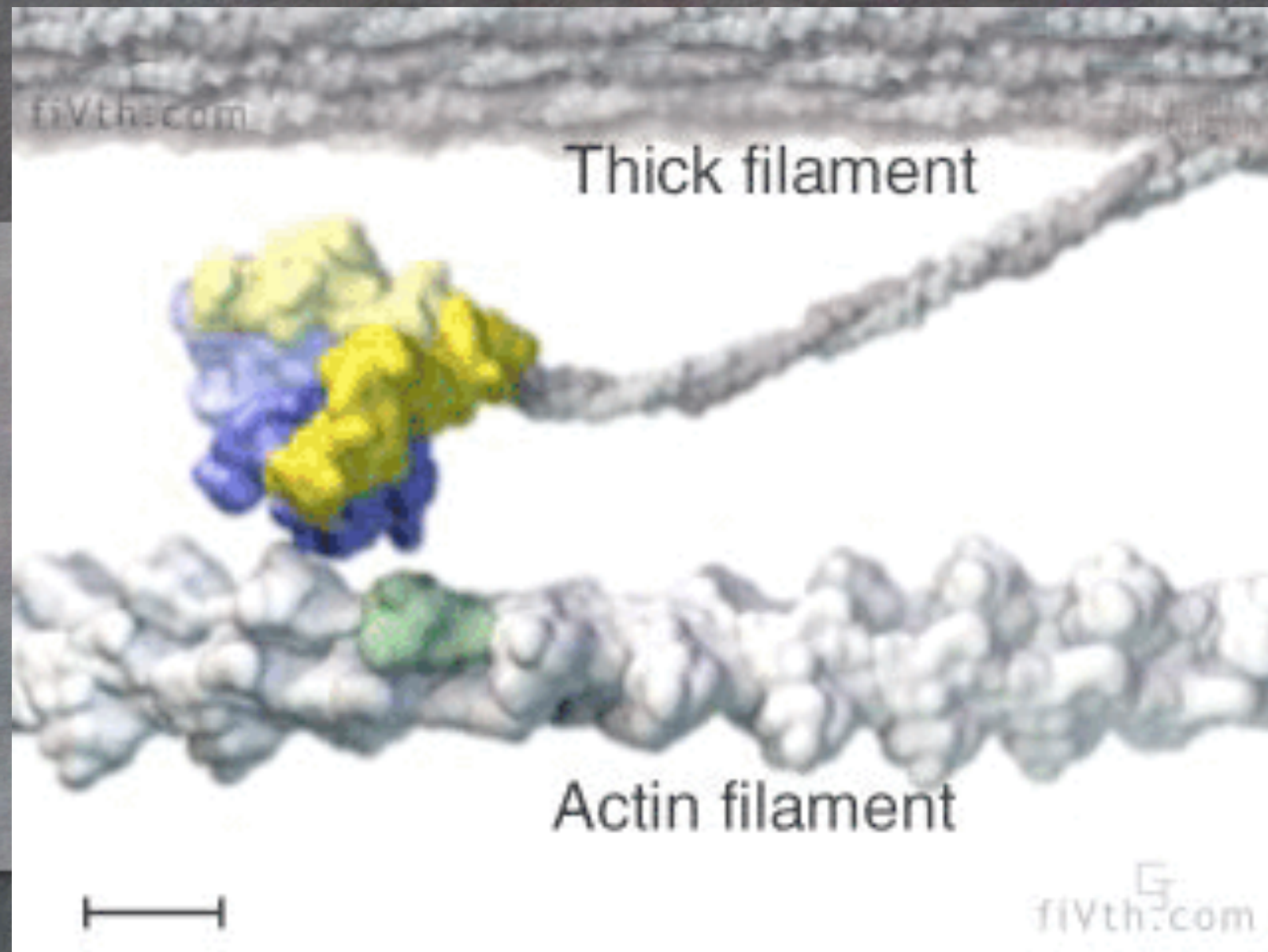
- Dr. Frederick C. Harris, Jr.
 - Dr. Sergiu Dascalu
 - Dr. Josh Baker
-
- Del Jackson

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”

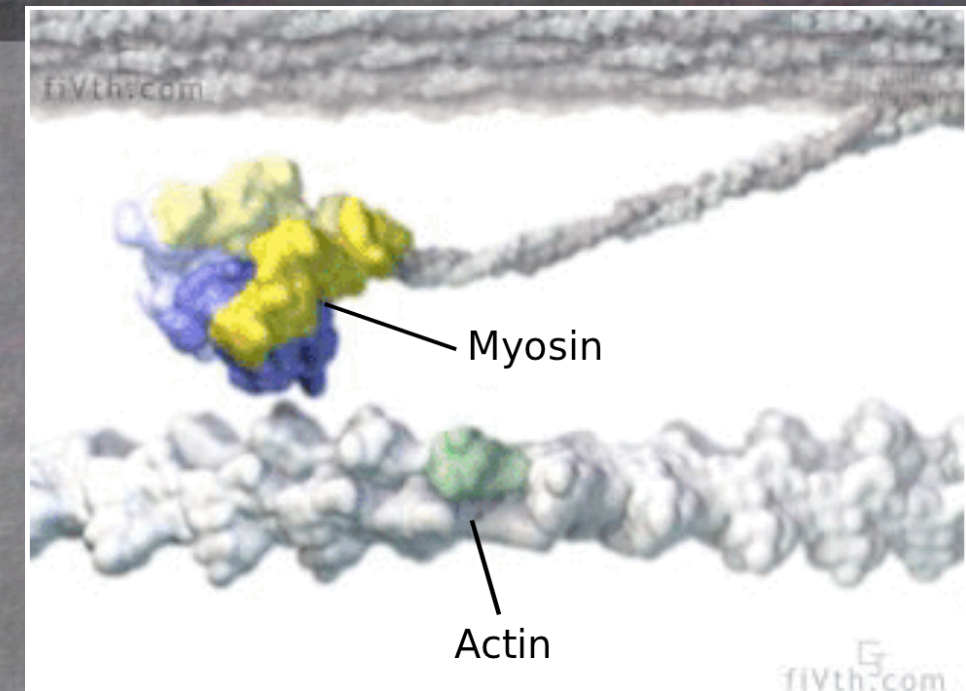
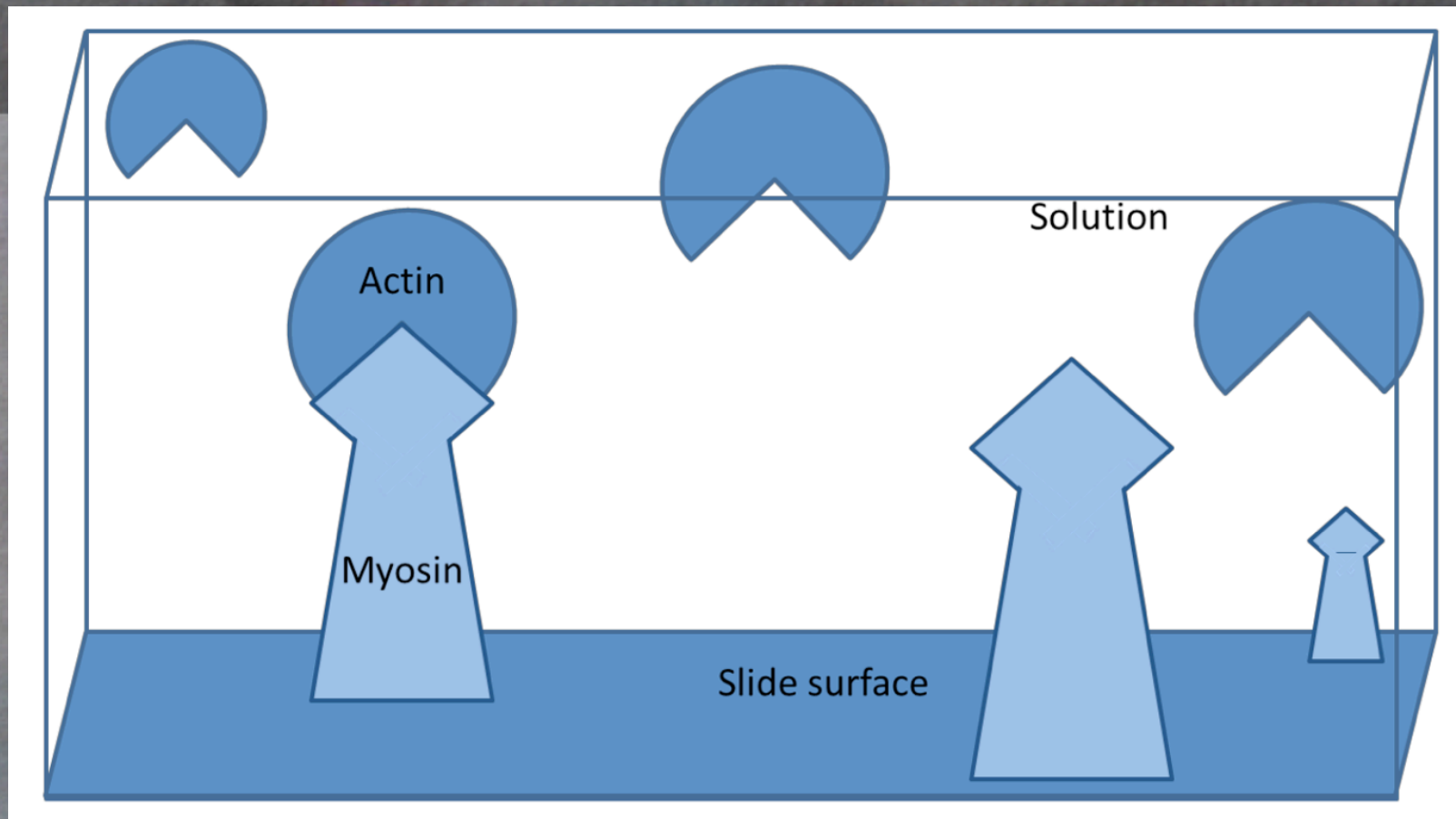


Figure 1 - SiMBA Model And Screen Shot From Movie 1.

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

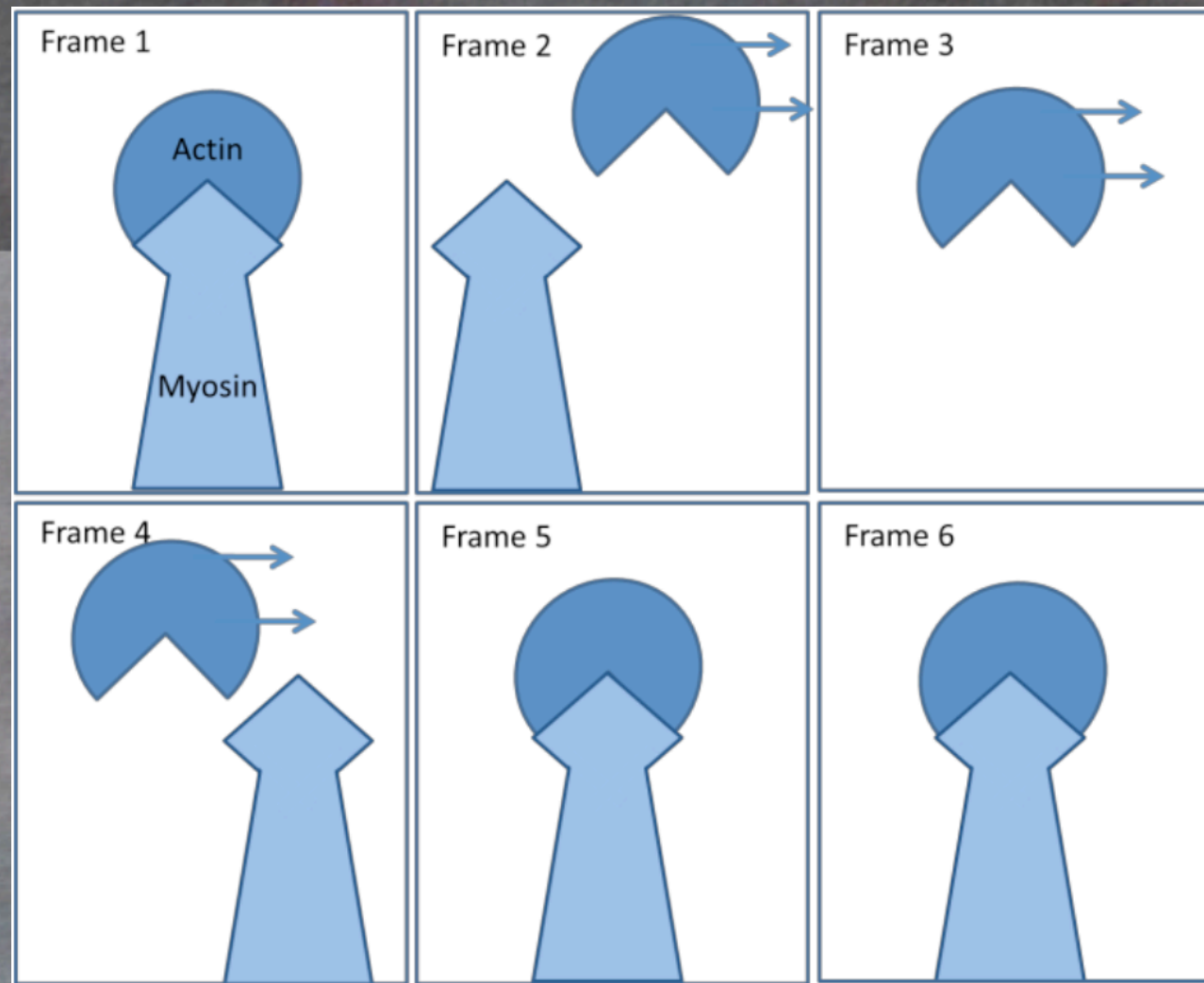


Figure 2 - Basic representation of a t_{off} Event

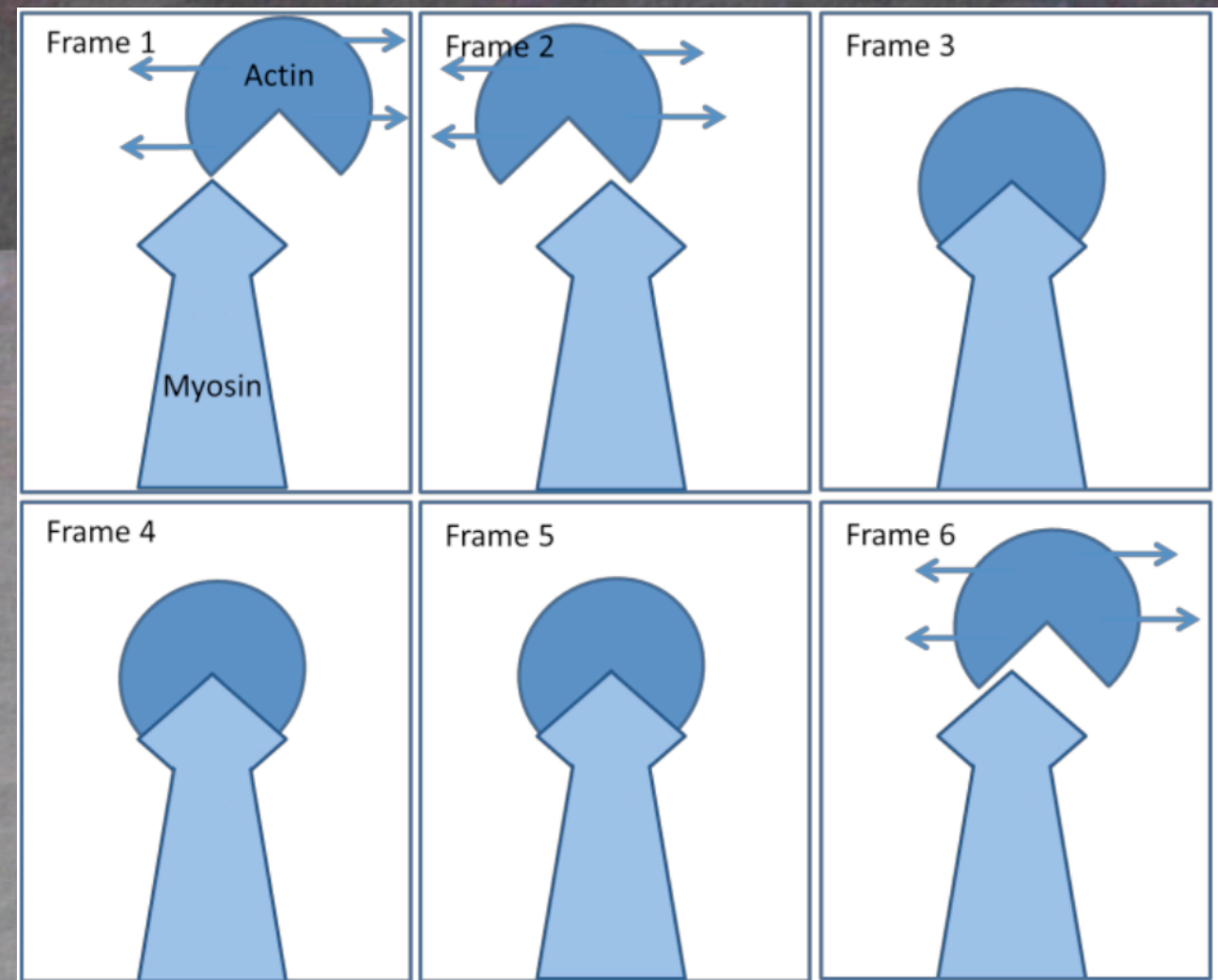


Figure 3 - Basic representation of a t_{on} Event

Lab Process



Figure 4 - Frozen Samples



Figure 5 - Thawing The Samples

Lab Process



Figure 6 - Buffer Preparation

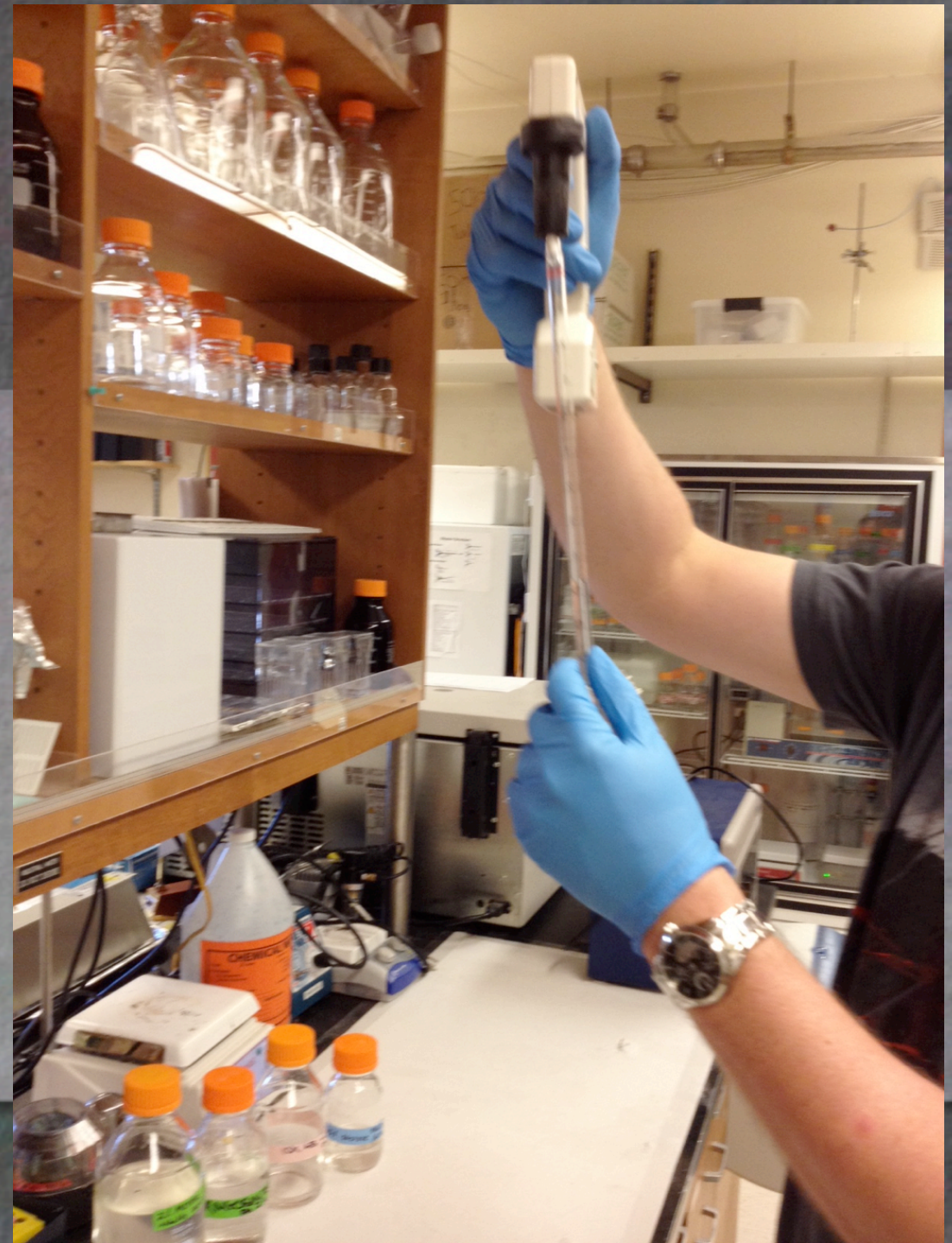


Figure 7 - All Samples Are Identical Except For A Varying Parameter

Lab Process



Figure 8 -Rotational Mixer

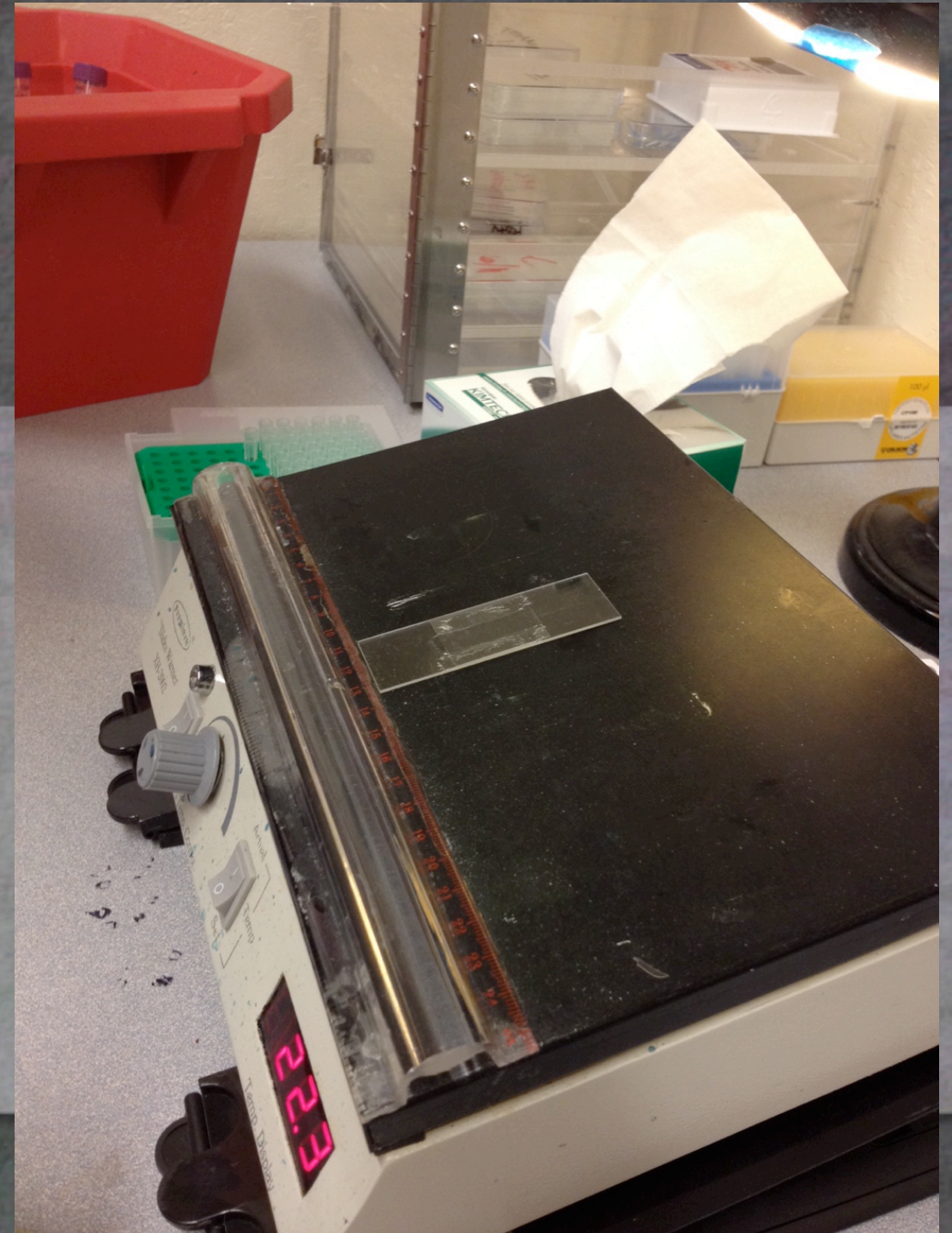


Figure 9 - Samples Brought To
Experimental Temperature

Lab Process

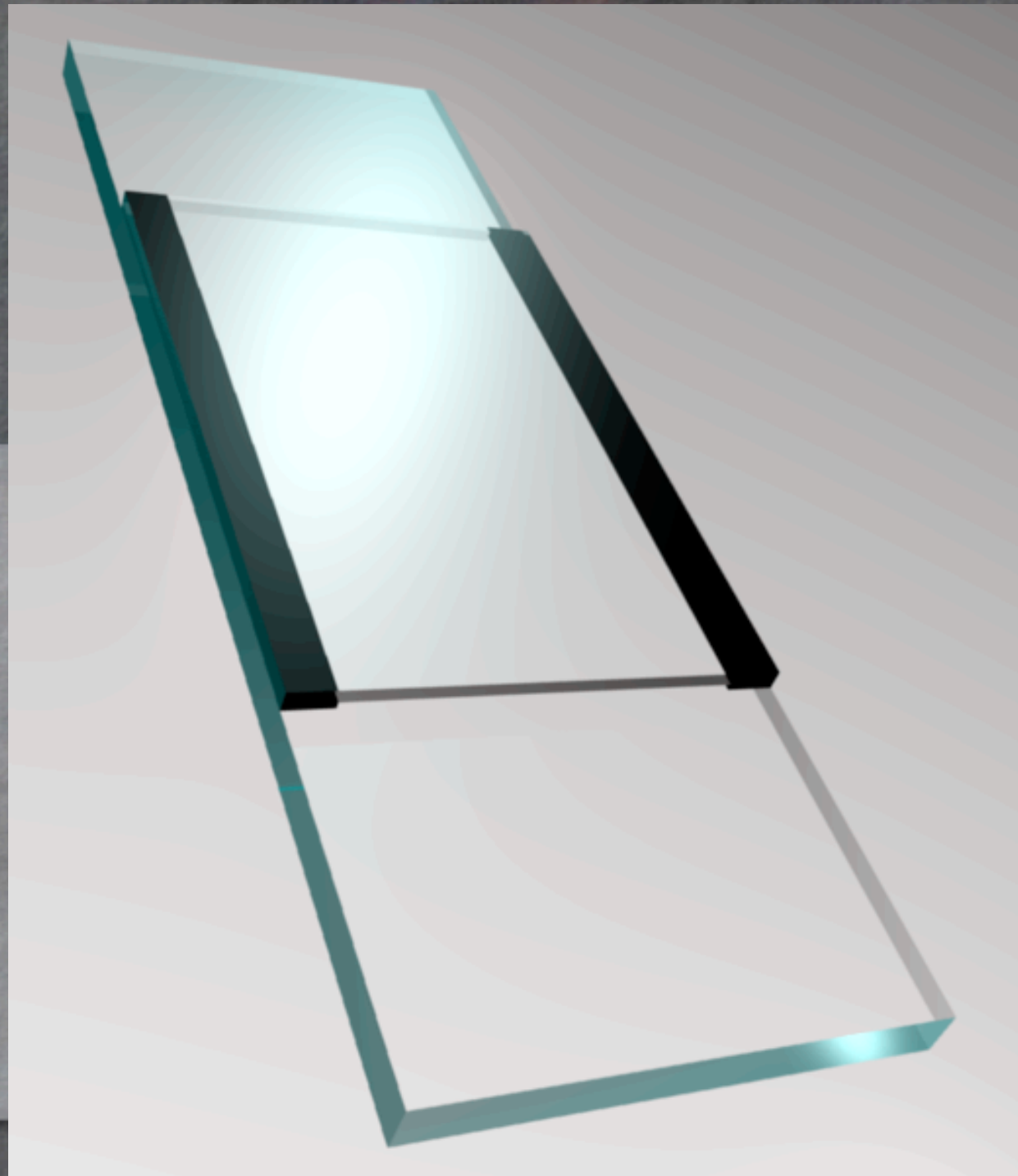


Figure 10 -Flow Slide Mockup

Lab Process

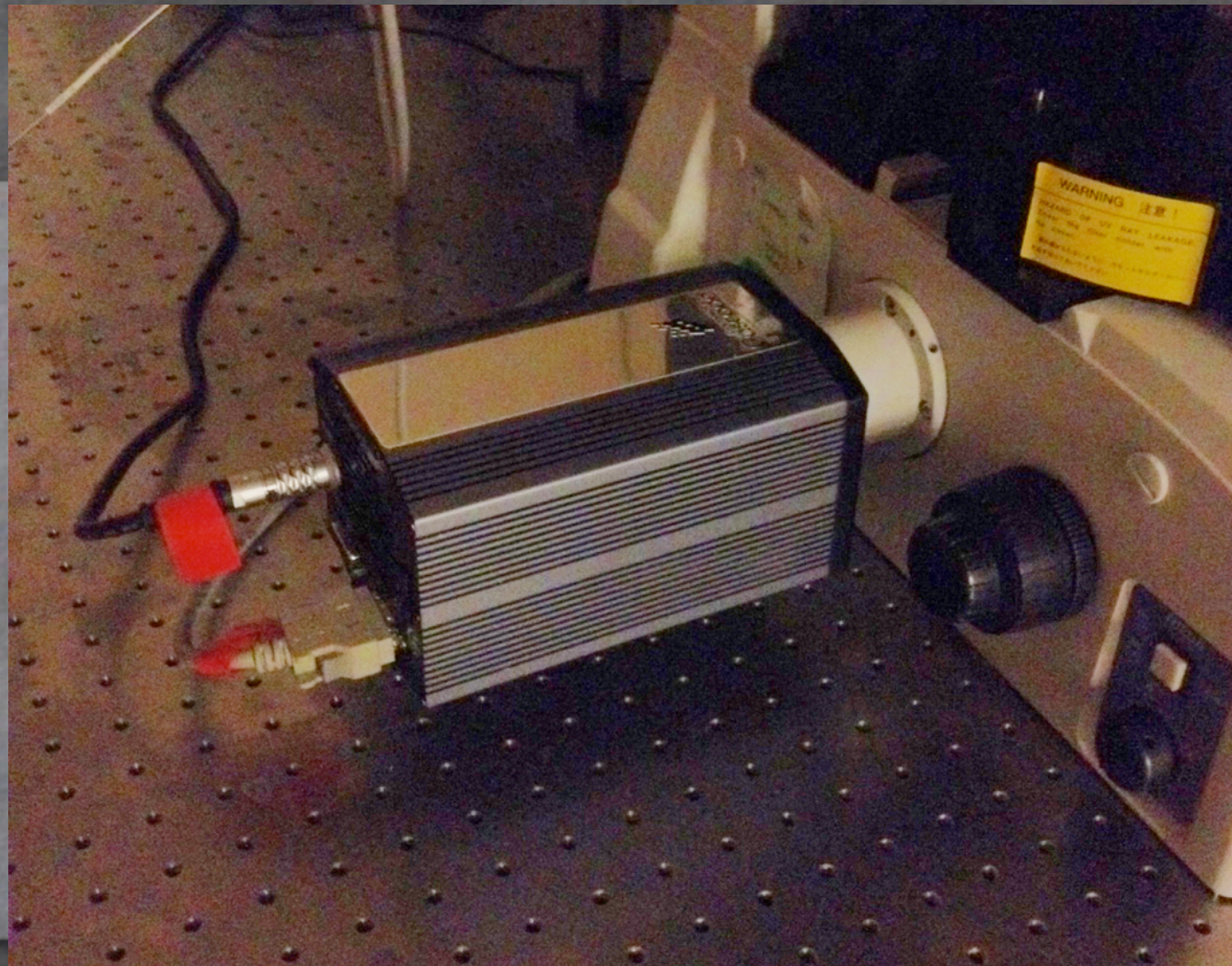
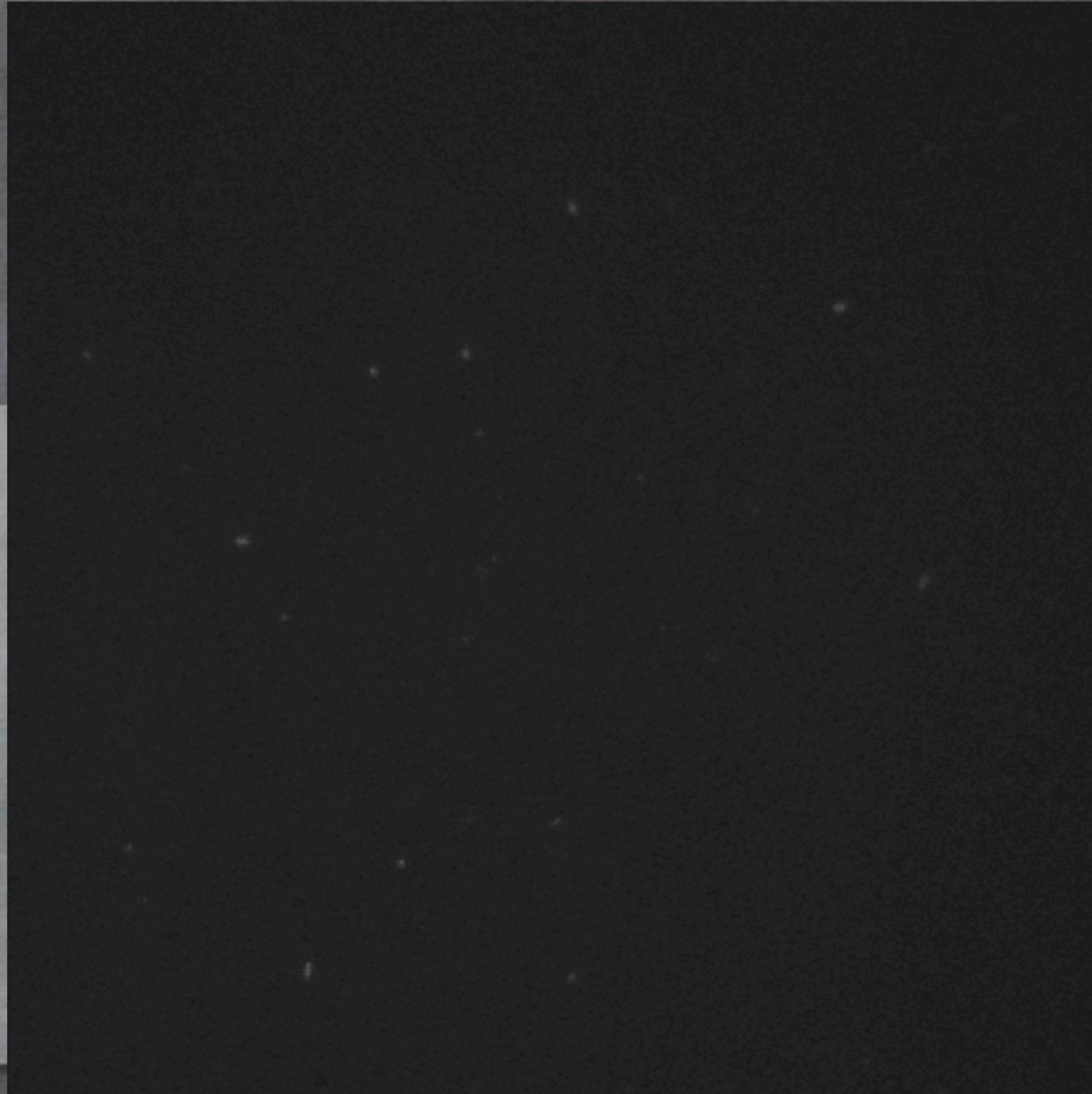


Figure 11 - Roper 512B CCD



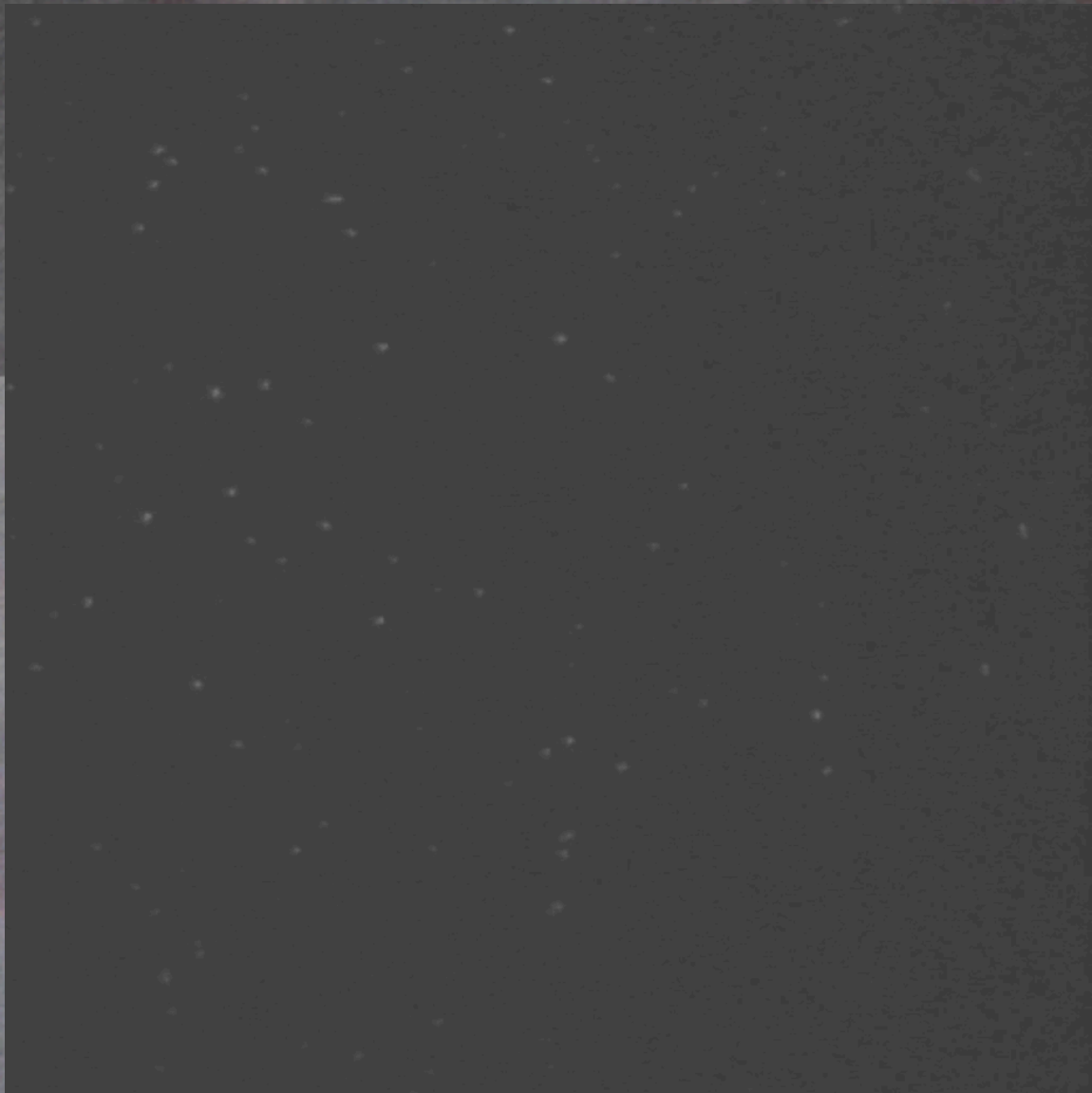
Figure 12 - Nikon 2000-TE
Inverted Microscope

Lab Process



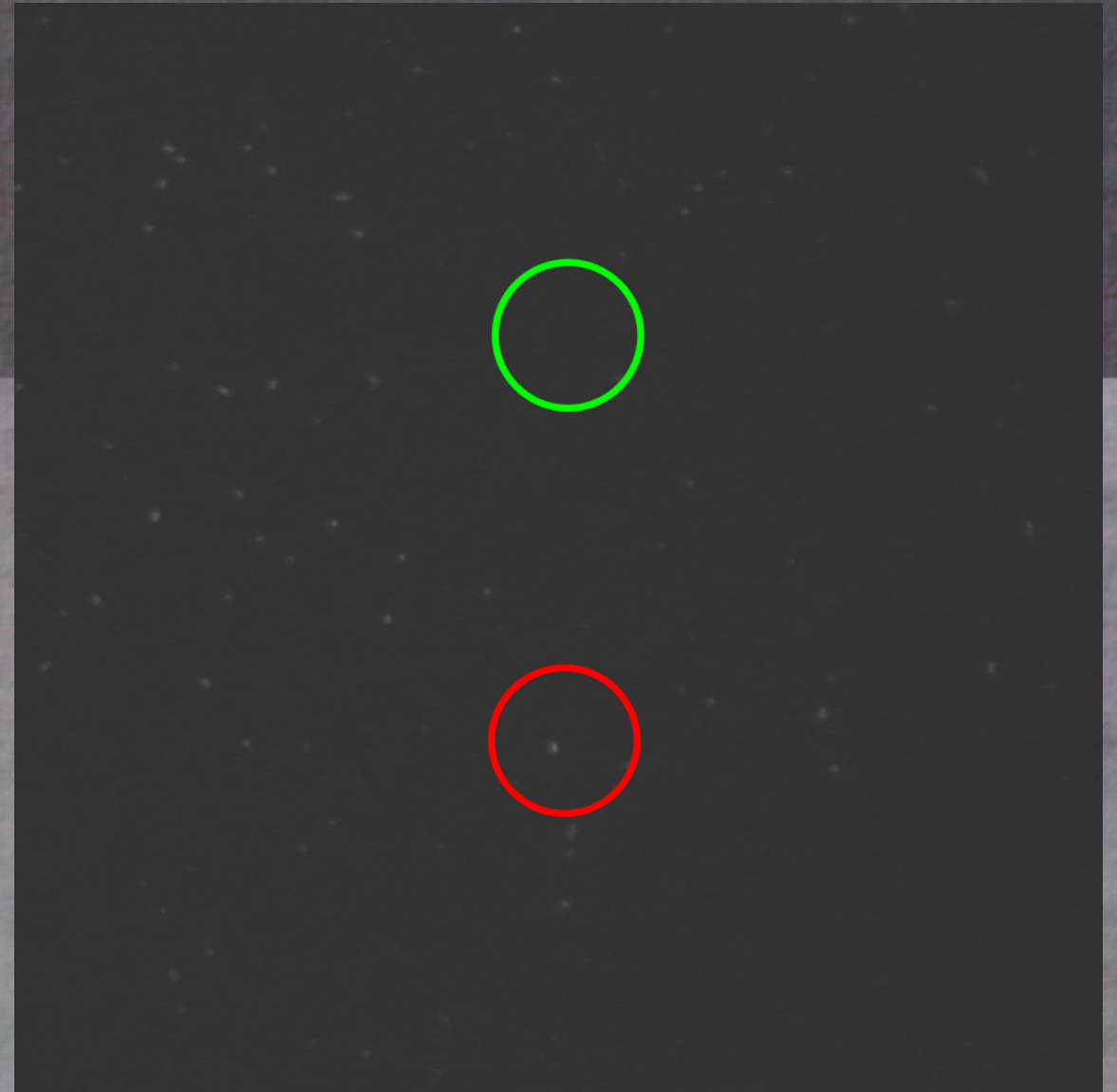
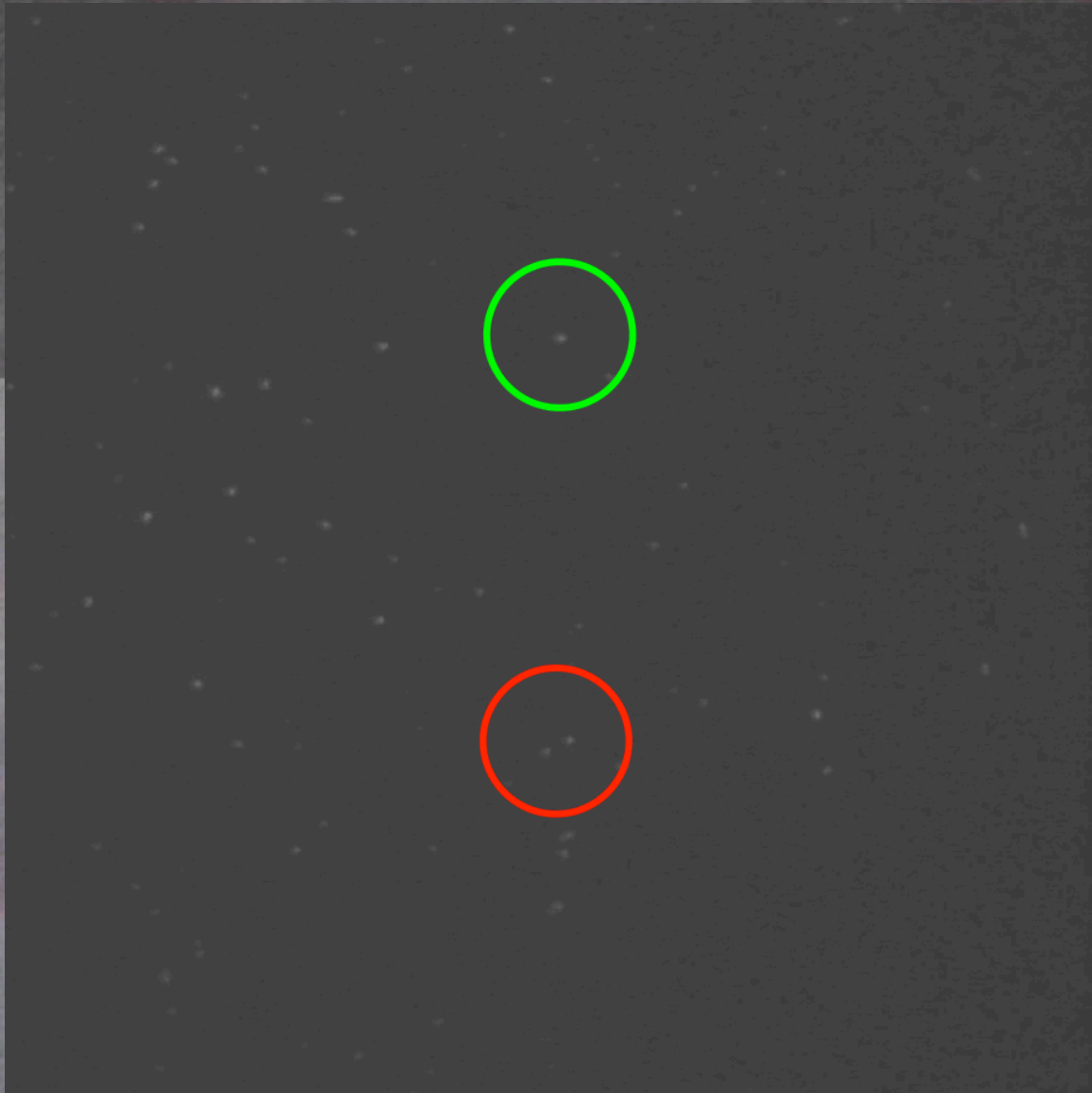
Movie 2 - SiMBA Output

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

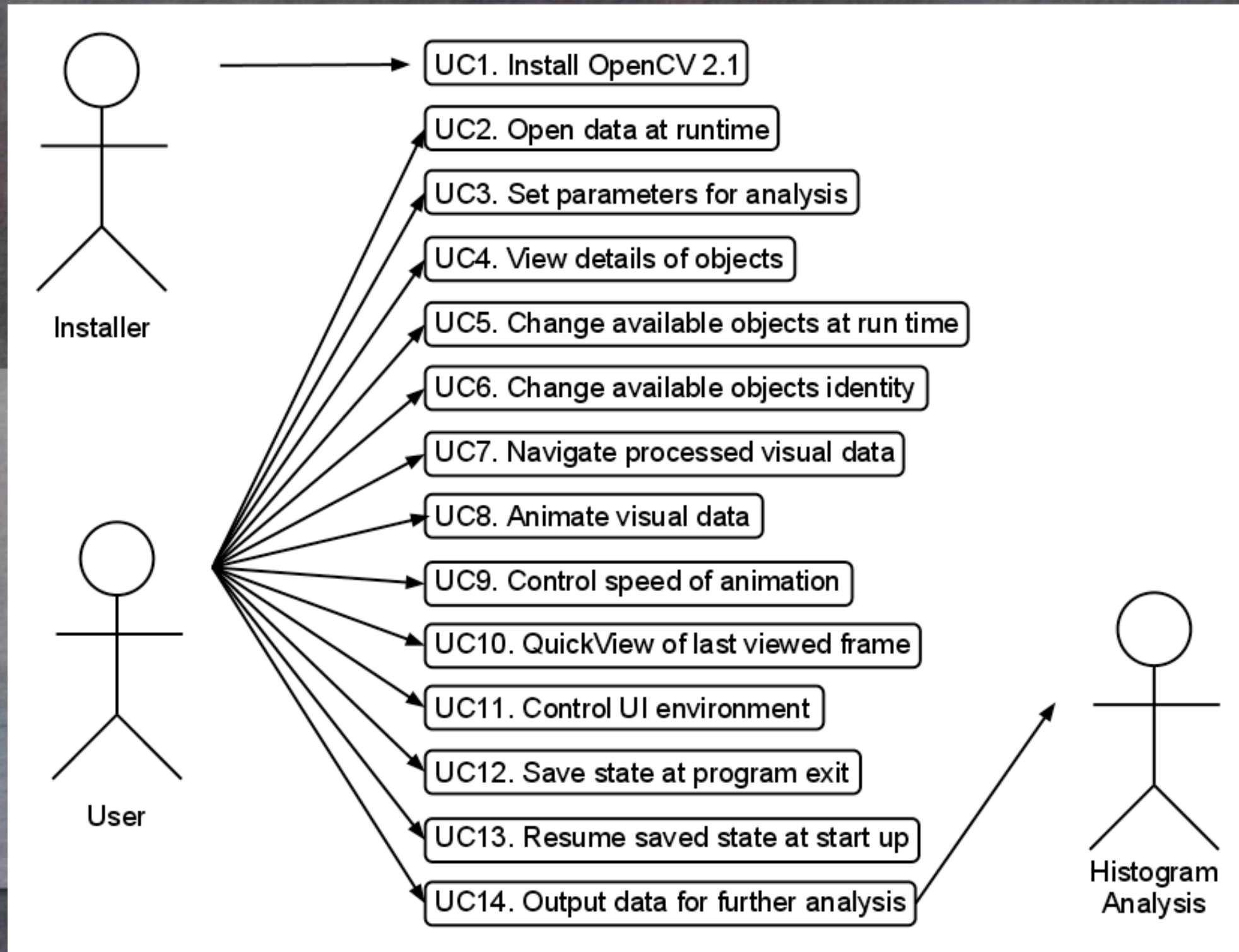


Figure 21 - Use Case Diagram

Traceability Matrix

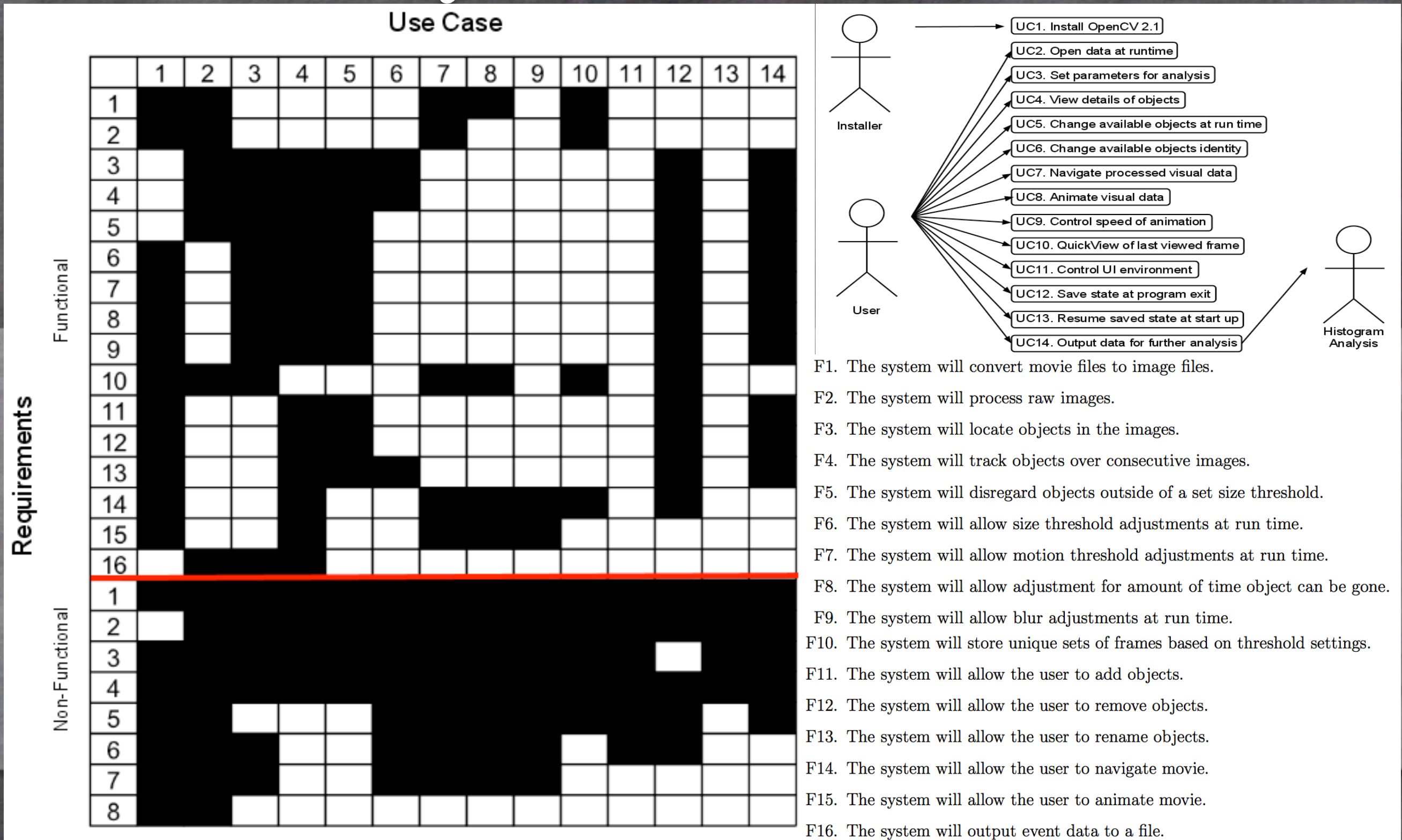


Figure 22 - Traceability Matrix Between Use Cases And Functional And Non-functional Requirements

SANoBA Walkthrough

Walkthrough

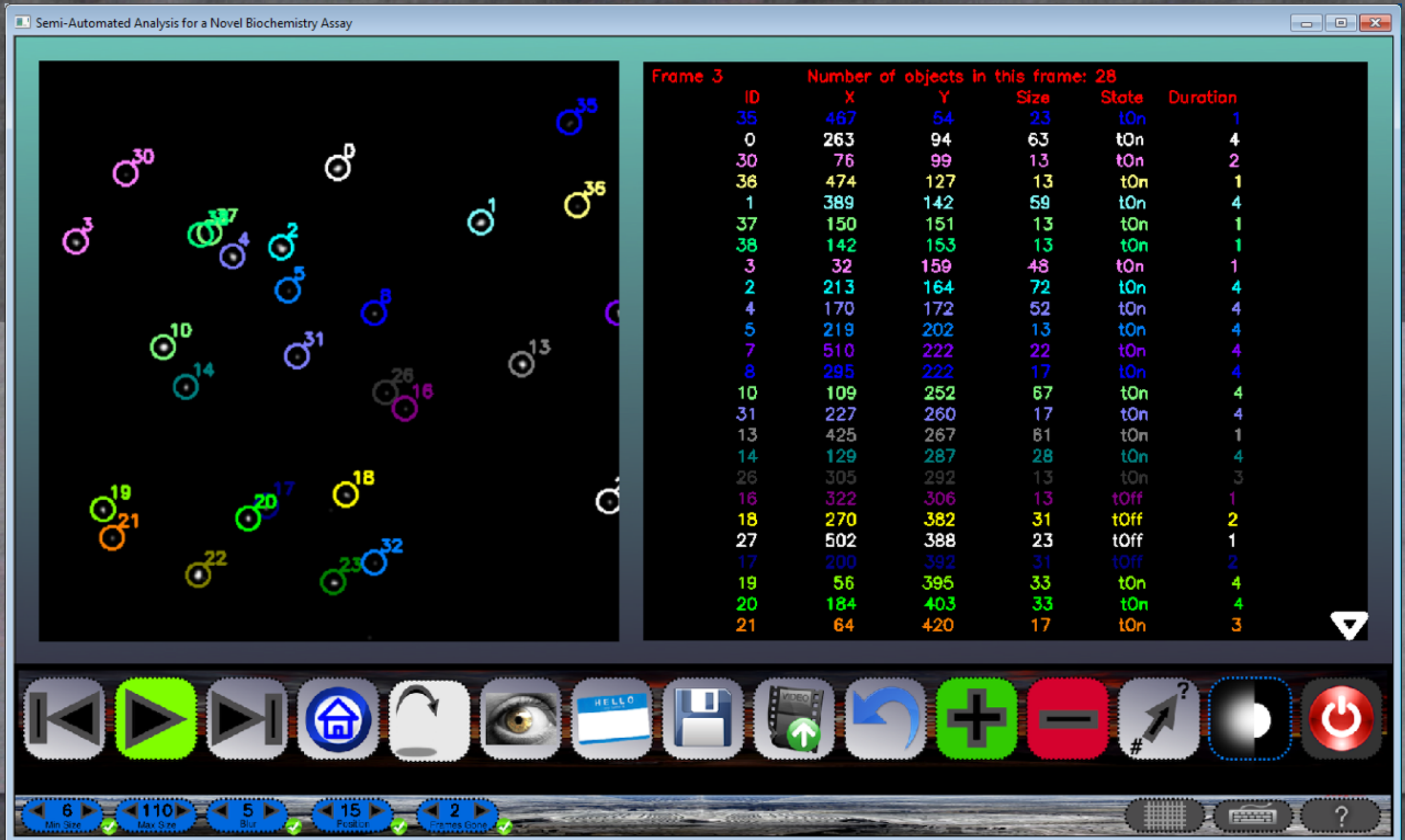


Figure 23 - Main User Interface

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay

1 2

3

4 5 6 7 8 9

Frame 0 Number of objects in this frame: 27

ID	X	Y	Size	State	Duration
0	263	96	53	tOn	1
1	389	142	57	tOn	1
2	214	164	51	tOn	1
3	36	165	34	tOn	1
4	171	172	43	tOn	1
5	220	201	29	tOn	1
6	42	202	6	tOn	1
7	83	217	12	tOn	1
8	510	223	31	tOn	1
9	295	222	20	tOn	1
10	349	237	13	tOn	1
11	109	252	72	tOn	1
12	227	260	16	tOn	1
13	221	266	32	tOn	1
14	428	270	51	tOn	1
15	129	287	31	tOn	1
16	306	292	6	tOn	1
17	214	298	22	tOn	1
18	328	306	13	tOn	1
19	216	381	12	tOn	1
20	256	383	36	tOn	1
21	56	395	31	tOn	1
22	183	403	37	tOn	1
23	64	420	16	tOn	1
24	140	453	69	tOn	1

11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

26 Back(<-) Play(Space) Forward(->) Home(h) Go to...(g) QuickView(q) Show IDs(i) Save(ctrl-s) Open(o) Undo(ctrl-z) Add(a) Remove(s) Rename(r) Blur(b) Quit(Esc)

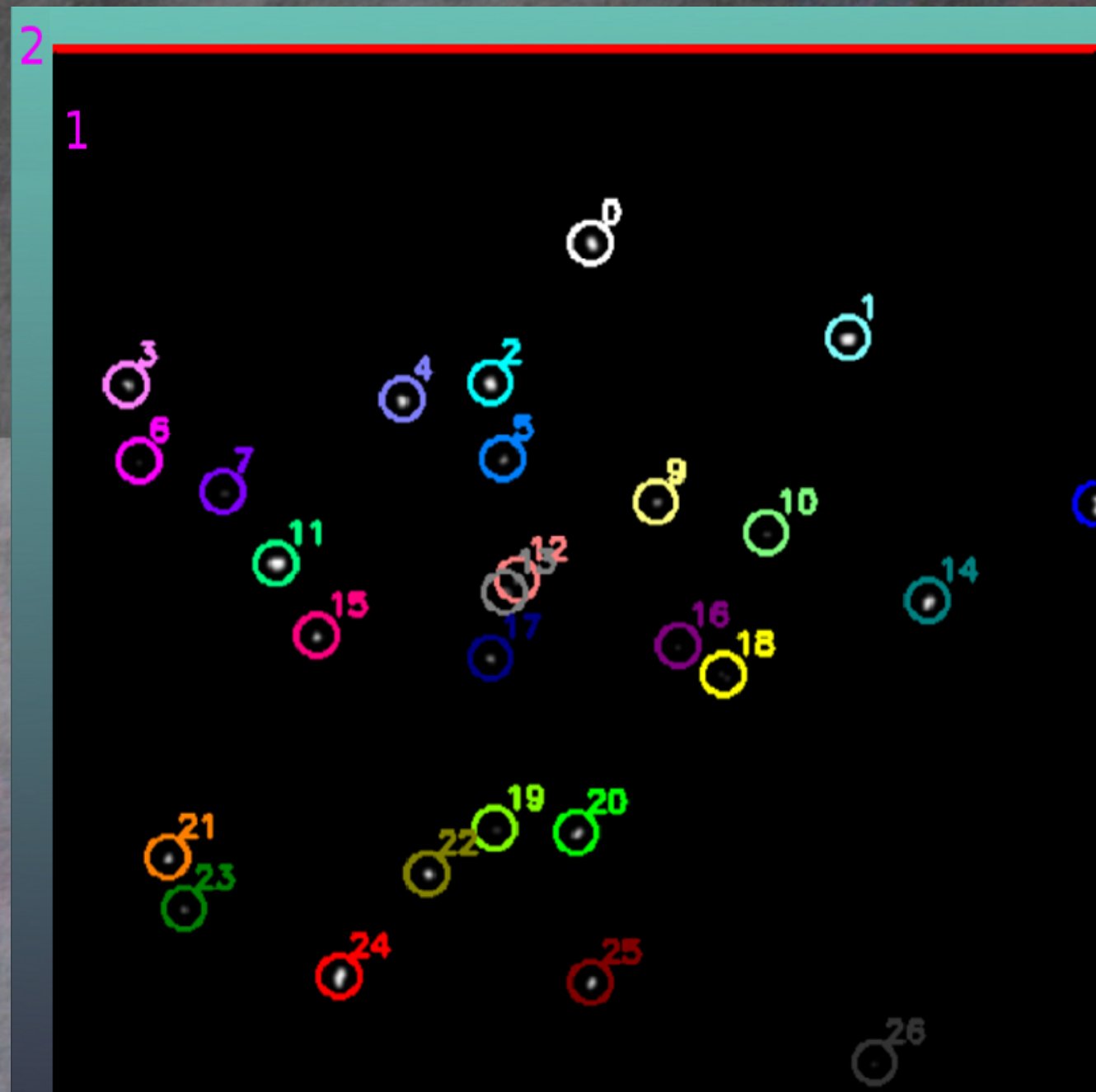
27 28 29 30 31 32 33 34

0 110 5 15 2

Min Size Max Size Blur Position Frames Gone

Figure 24 - Label UI

Walkthrough



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

Figure 25 - Object Window

Walkthrough

4 5 6 7 8 9						
Frame 0	Number of objects in this frame: 27					
3	ID	X	Y	Size	State	Duration
	0	263	96	53	tOn	1
	1	389	142	57	tOn	1
	2	214	164	51	tOn	1
	3	36	165	34	tOn	1
	4	171	172	43	tOn	1
	5	220	201	29	tOn	1
	6	42	202	6	tOn	1
	7	83	217	12	tOn	1
	8	510	223	31	tOn	1
	9	295	222	20	tOn	1
	10	349	237	13	tOn	1
	11	109	252	72	tOn	1
	12	227	260	16	tOn	1
	13	221	266	32	tOn	1
	14	428	270	51	tOn	1
	15	129	287	31	tOn	1
	16	306	292	6	tOn	1
	17	214	298	22	tOn	1
	18	328	306	13	tOn	1
	19	216	381	12	tOn	1
	20	256	383	36	tOn	1
	21	56	395	31	tOn	1
	22	183	403	37	tOn	1
	23	64	420	16	tOn	1
	24	140	453	69	tOn	1

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



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.

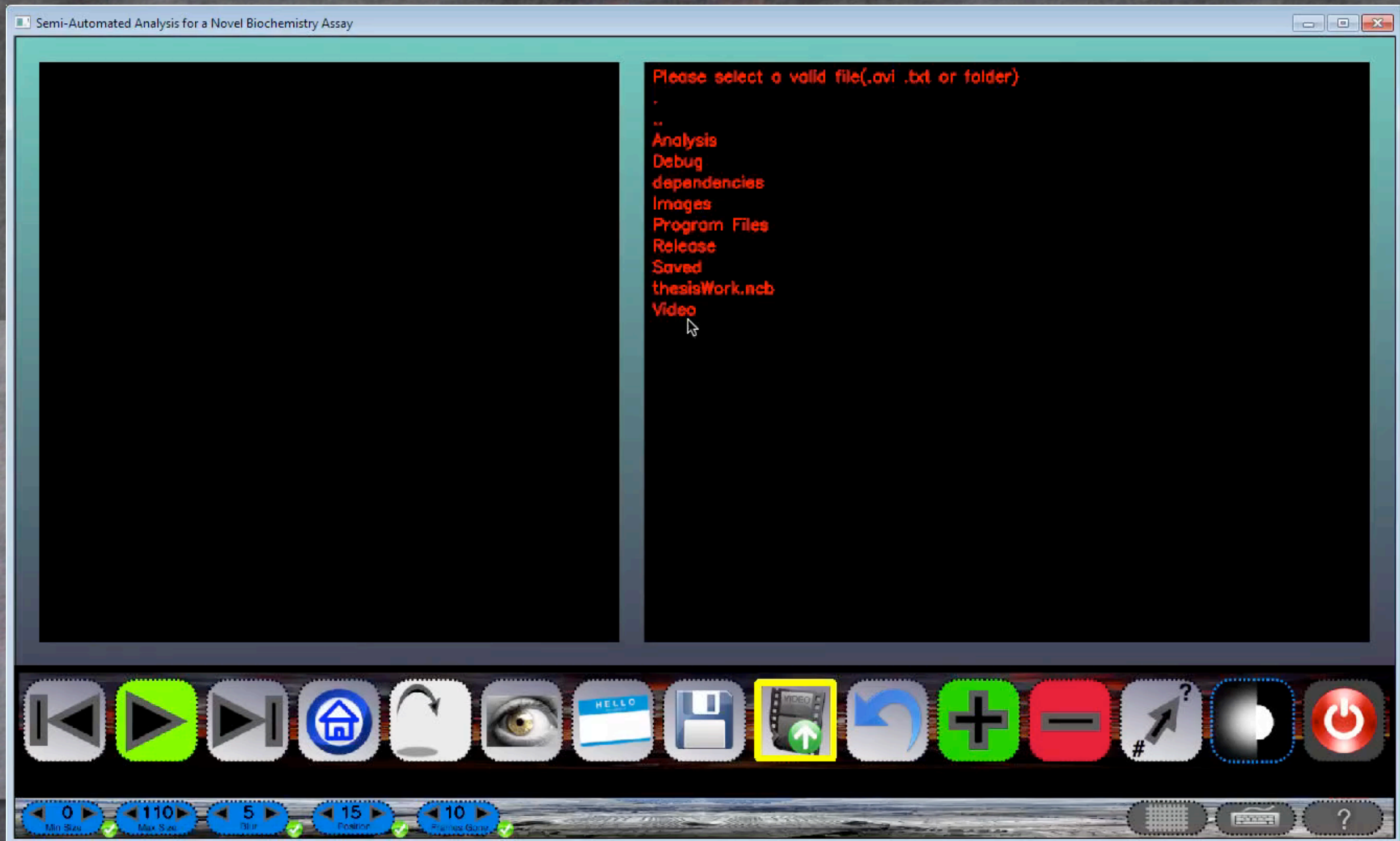
40 - Go Forward.

Figure 29 - Secondary UI

Walkthrough

Movie 2 - Open Movie

Walkthrough



Walkthrough

Movie 3 - Remove Object

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



ID	X	Y	Size	State	Duration
0	100	87	20	tOn	2
1	336	96	6	tOn	2
2	258	129	13	tOn	2
3	51	171	19	tOn	2
4	110	217	17	tOn	2
7	292	241	6	tOn	1
8	388	287	6	tOn	1
5	89	328	28	tOn	2
6	113	373	34	tOn	2
9	1	395	12	tOn	1

Frame 1 Number of objects in this frame: 10

Toolbar icons: Previous, Play, Next, Home, Undo, Eye, Hello, Save, Video, Rotate, Zoom In, Zoom Out, Select, Toggle, Power.

Status bar: 0 Min Size, 110 Max Size, 5 Blur, 15 Position, 10 Rotate, 100%, 100%, ?

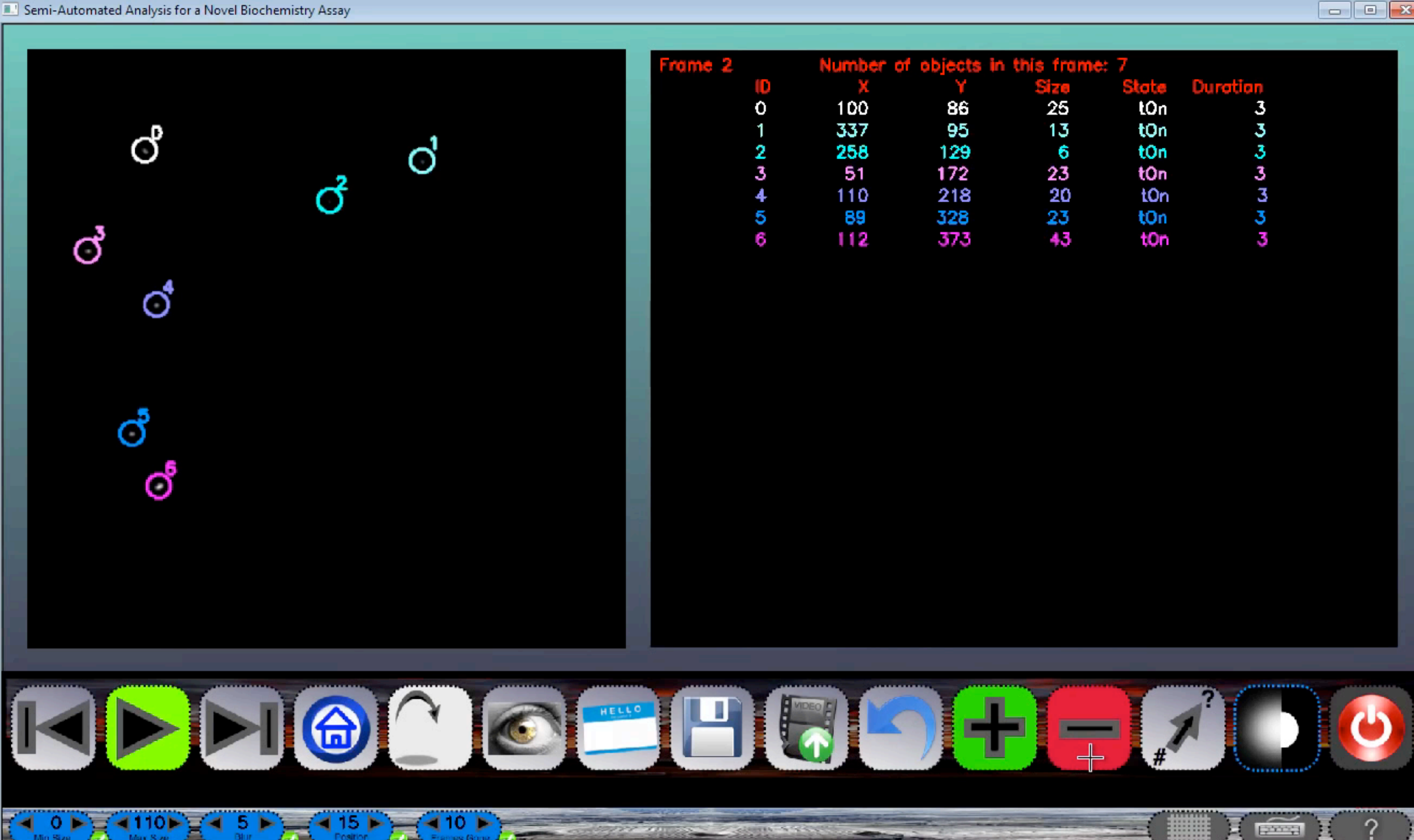
Movie 3 - Remove Object

Walkthrough

Movie 4 - Add Object

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 2						
Number of objects in this frame: 7						
ID	X	Y	Size	State	Duration	
0	100	86	25	tOn	3	
1	337	95	13	tOn	3	
2	258	129	6	tOn	3	
3	51	172	23	tOn	3	
4	110	218	20	tOn	3	
5	89	328	23	tOn	3	
6	112	373	43	tOn	3	

Toolbar icons: Previous, Play, Next, Home, Undo, Eye, Hello, Save, Video, Rotate, Zoom In, Zoom Out, Select, Contrast, Power.

Status bar: Min Size: 0, Max Size: 110, Blur: 5, Position: 15, Frames Gone: 10.

Movie 4 - Add Object

Walkthrough

Movie 5 - Rename Object

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 2

Number of objects in this frame: 7

ID	X	Y	Size	State	Duration
0	100	86	25	tOn	3
2	258	129	6	tOn	3
3	51	172	23	tOn	3
4	110	218	20	tOn	3
5	89	328	23	tOn	3
6	112	373	43	tOn	3
42	337	95	13	tOn	1

Movie 5 - Rename Object

Walkthrough

Movie 6 - Save And Open File

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 2						
Number of objects in this frame: 7						
ID	X	Y	Size	State	Duration	
0	100	86	25	tOn	3	
2	258	129	6	tOn	3	
3	51	172	23	tOn	3	
4	110	218	20	tOn	3	
5	89	328	23	tOn	3	
6	112	373	43	tOn	3	
1	337	95	13	tOn	1	

Toolbar icons: Previous, Play, Next, Home, Undo, Eye, Hello, Save, Video, Rotate, Zoom In, Zoom Out, Select, Contrast, Power.

Status bar: Min Size: 0, Max Size: 110, Blur: 5, Position: 15, Maximal Gain: 10.

Movie 6 - Save And Open File

Walkthrough

Movie 6 - Show/ Hide IDs

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 2						
Number of objects in this frame: 7						
ID	X	Y	Size	State	Duration	
0	100	86	25	tOn	3	
2	258	129	6	tOn	3	
3	51	172	23	tOn	3	
4	110	218	20	tOn	3	
5	89	328	23	tOn	3	
6	112	373	43	tOn	3	
1	337	95	13	tOn	1	

Movie 6 - Show/ Hide IDs

Walkthrough

Movie 7 - Home And Goto...

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 2						
Number of objects in this frame: 7						
ID	X	Y	Size	State	Duration	
0	100	86	25	tOn	3	
2	258	129	6	tOn	3	
3	51	172	23	tOn	3	
4	110	218	20	tOn	3	
5	89	328	23	tOn	3	
6	112	373	43	tOn	3	
1	337	95	13	tOn	1	

Movie 7 - Home And Goto...

Walkthrough

Movie 8 - Blur

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 0 Number of objects in this frame: 53

ID	X	Y	Size	State	Duration
0	320	1	7	tOn	1
1	367	0	2	tOn	1
2	321	21	13	tOn	1
3	159	31	2	tOn	1
4	330	35	11	tOn	1
5	312	62	19	tOn	1
6	345	75	10	tOn	1
7	208	78	7	tOn	1
8	133	80	2	tOn	1
9	426	84	9	tOn	1
10	65	126	12	tOn	1
11	171	133	10	tOn	1
12	251	143	12	tOn	1
13	131	172	2	tOn	1
14	251	173	4	tOn	1
15	250	190	3	tOn	1
16	447	210	20	tOn	1
17	395	243	18	tOn	1
18	446	251	2	tOn	1
19	102	255	2	tOn	1
20	74	256	3	tOn	1
21	189	266	14	tOn	1
22	457	272	3	tOn	1
23	403	278	16	tOn	1
24	471	294	3	tOn	1



0 100 5 15 10
Min Size Max Size Blur Position Pinch Zoom



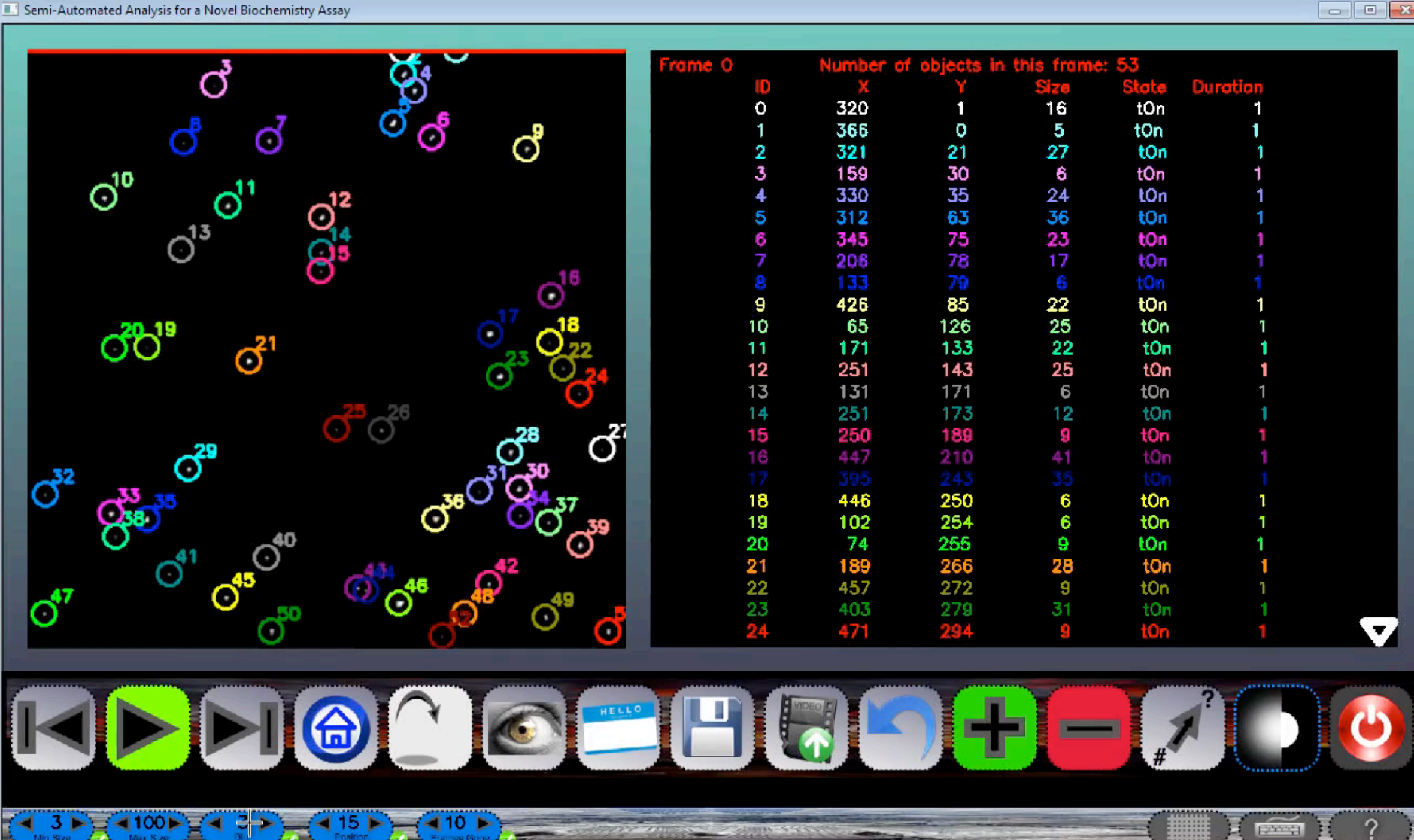
Movie 8 - Blur

Walkthrough

Movie 9 - Size Threshold

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 0	ID	X	Y	Size	State	Duration
	0	320	1	16	tOn	1
	1	366	0	5	tOn	1
	2	321	21	27	tOn	1
	3	159	30	6	tOn	1
	4	330	35	24	tOn	1
	5	312	63	36	tOn	1
	6	345	75	23	tOn	1
	7	208	78	17	tOn	1
	8	133	79	6	tOn	1
	9	426	85	22	tOn	1
	10	65	126	25	tOn	1
	11	171	133	22	tOn	1
	12	251	143	25	tOn	1
	13	131	171	6	tOn	1
	14	251	173	12	tOn	1
	15	250	189	9	tOn	1
	16	447	210	41	tOn	1
	17	395	243	35	tOn	1
	18	446	250	6	tOn	1
	19	102	254	6	tOn	1
	20	74	255	9	tOn	1
	21	189	266	28	tOn	1
	22	457	272	9	tOn	1
	23	403	279	31	tOn	1
	24	471	294	9	tOn	1

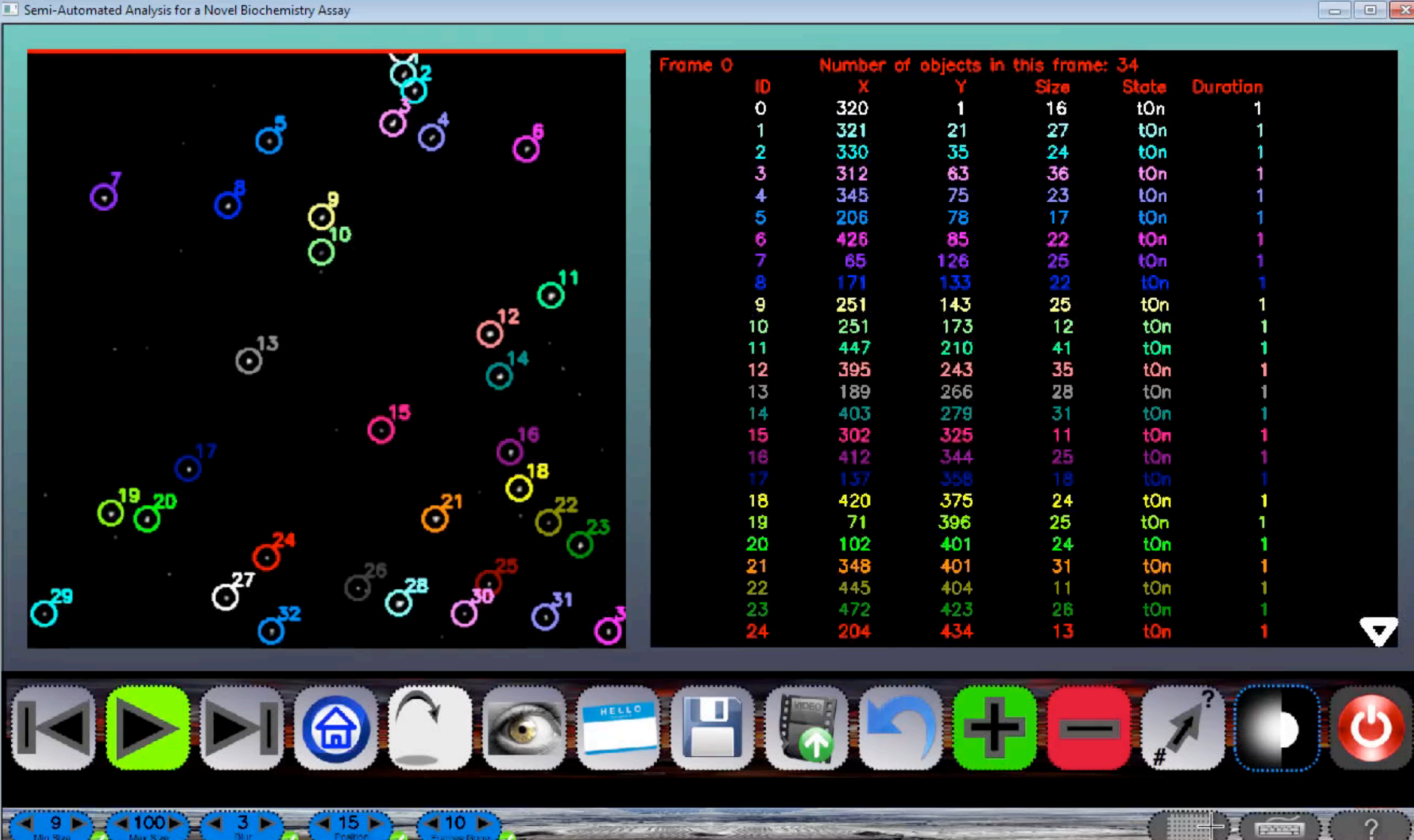
Movie 9 - Size Threshold

Walkthrough

Movie 10 - Grid, Keyboard, Help, And Quit

Walkthrough

Semi-Automated Analysis for a Novel Biochemistry Assay



Frame 0 Number of objects in this frame: 34

ID	X	Y	Size	State	Duration
0	320	1	16	tOn	1
1	321	21	27	tOn	1
2	330	35	24	tOn	1
3	312	63	36	tOn	1
4	345	75	23	tOn	1
5	206	78	17	tOn	1
6	426	85	22	tOn	1
7	65	126	25	tOn	1
8	171	133	22	tOn	1
9	251	143	25	tOn	1
10	251	173	12	tOn	1
11	447	210	41	tOn	1
12	395	243	35	tOn	1
13	189	266	28	tOn	1
14	403	279	31	tOn	1
15	302	325	11	tOn	1
16	412	344	25	tOn	1
17	137	358	18	tOn	1
18	420	375	24	tOn	1
19	71	396	25	tOn	1
20	102	401	24	tOn	1
21	348	401	31	tOn	1
22	445	404	11	tOn	1
23	472	423	26	tOn	1
24	204	434	13	tOn	1

Toolbar icons: Previous, Play, Stop, Home, Undo, Eye, Hello, Save, Video, Rotate, Zoom In, Zoom Out, Select, Contrast, Brightness, Power.

Status bar: Min Size: 9, Max Size: 100, Blur: 3, Position: 15, Rotate: 10, Zoom In, Zoom Out, Help.

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

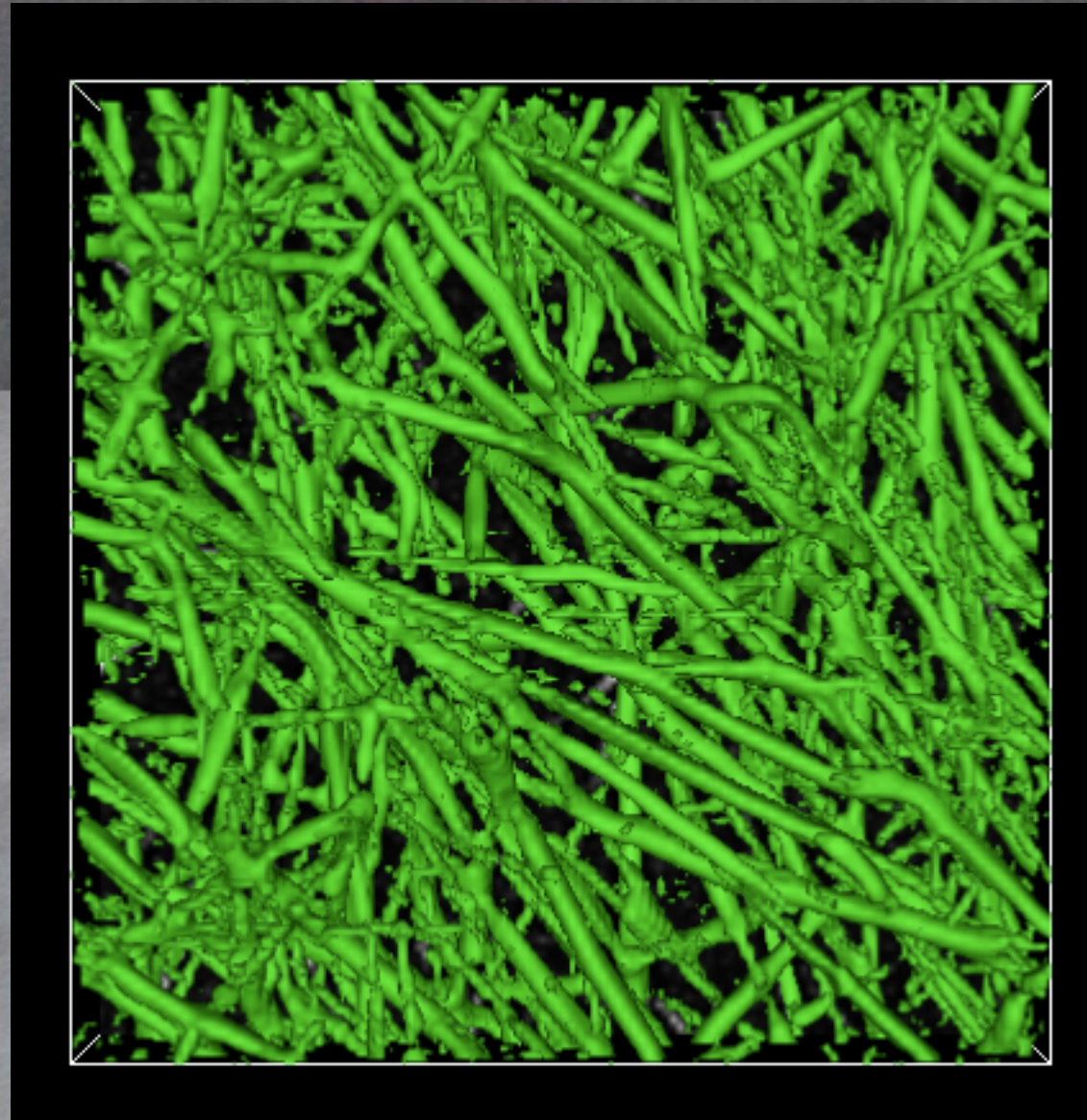


Figure 30 - Example Of A Kymograph

