Automated Biological Image Analysis using Computer Vision and Machine Learning

Shu Kong[1], Edmund Florendo[2], Zhongxuan Mou[1], Evan Lister[3], Olivier Cinquin[3], Charless Fowlkes[1]

[1]Donald Bren School of Information and Computer Science
[2]Henry Samueli School of Engineering
[3]Department of Developmental and Cell Biology

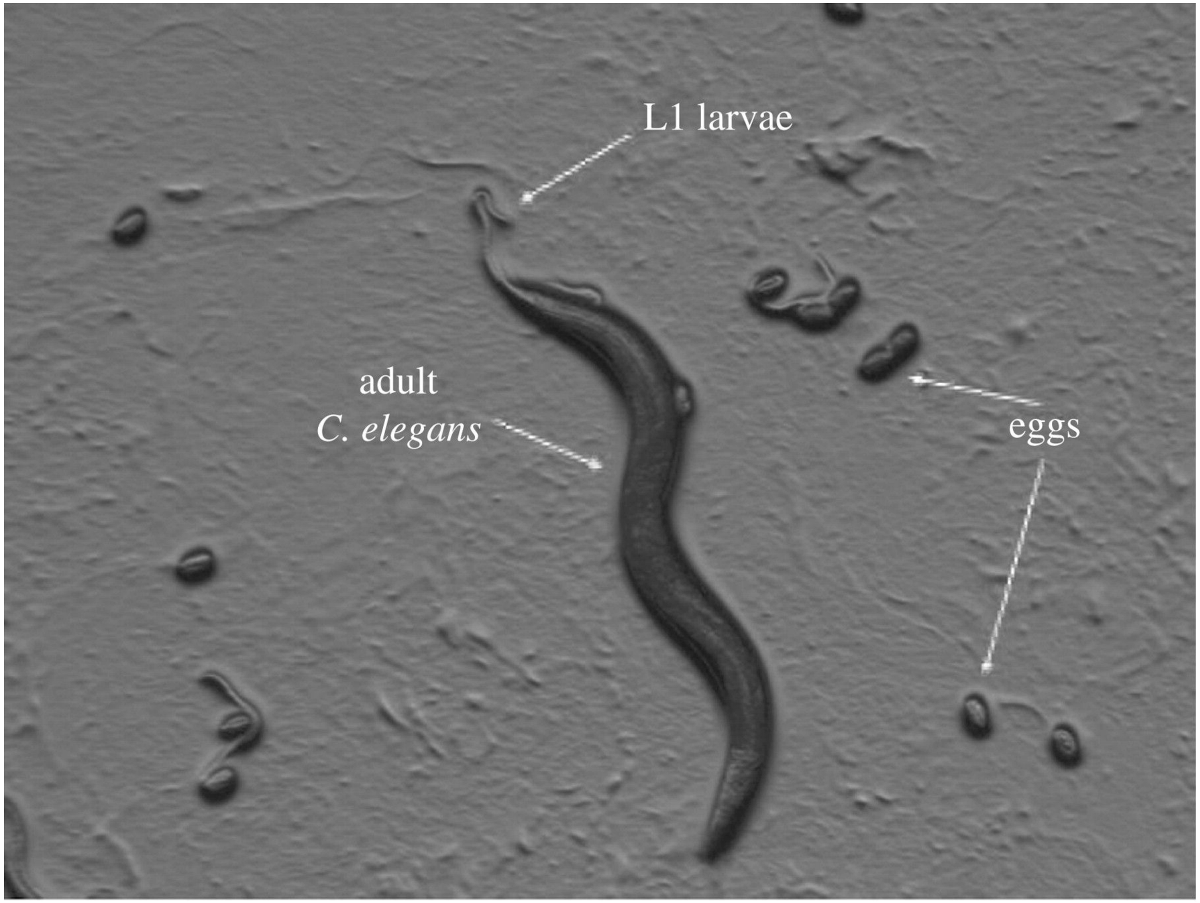
UCIrvine
UNIVERSITY OF CALIFORNIA, IRVINE

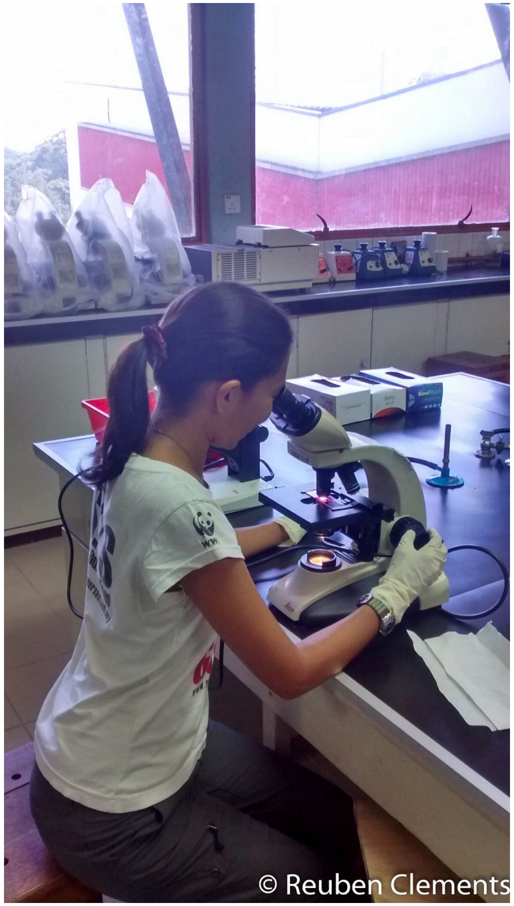
What is our project about?

This project aims to develop a robust system for automatically analyzing images in order to detect, classify and catalog the biological specimens visible in the image.

Why does our project matter?

In order to study how the evironment affects the lifespan of *C. elegans*, people have to detect and count the number of adult worms, larvae and eggs.



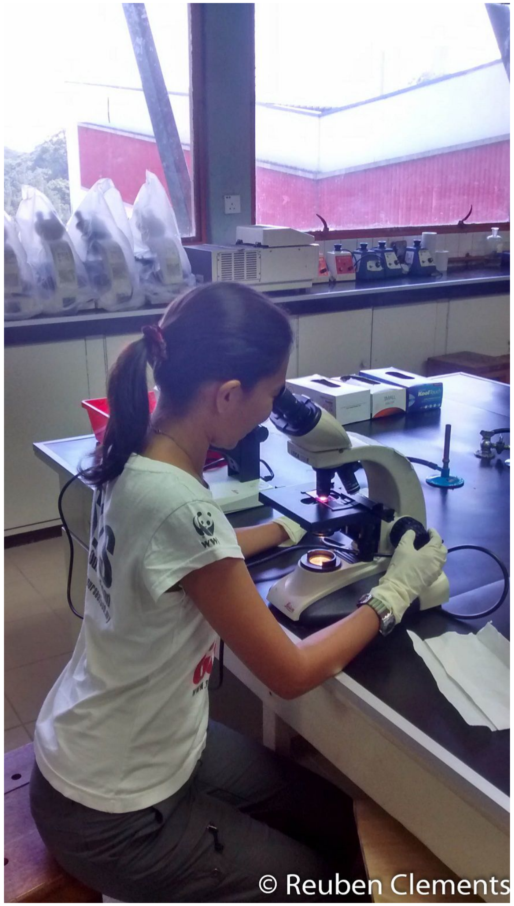

© Reuben Clements

UCIrvine
University of California, Irvine

Why does our project matter?

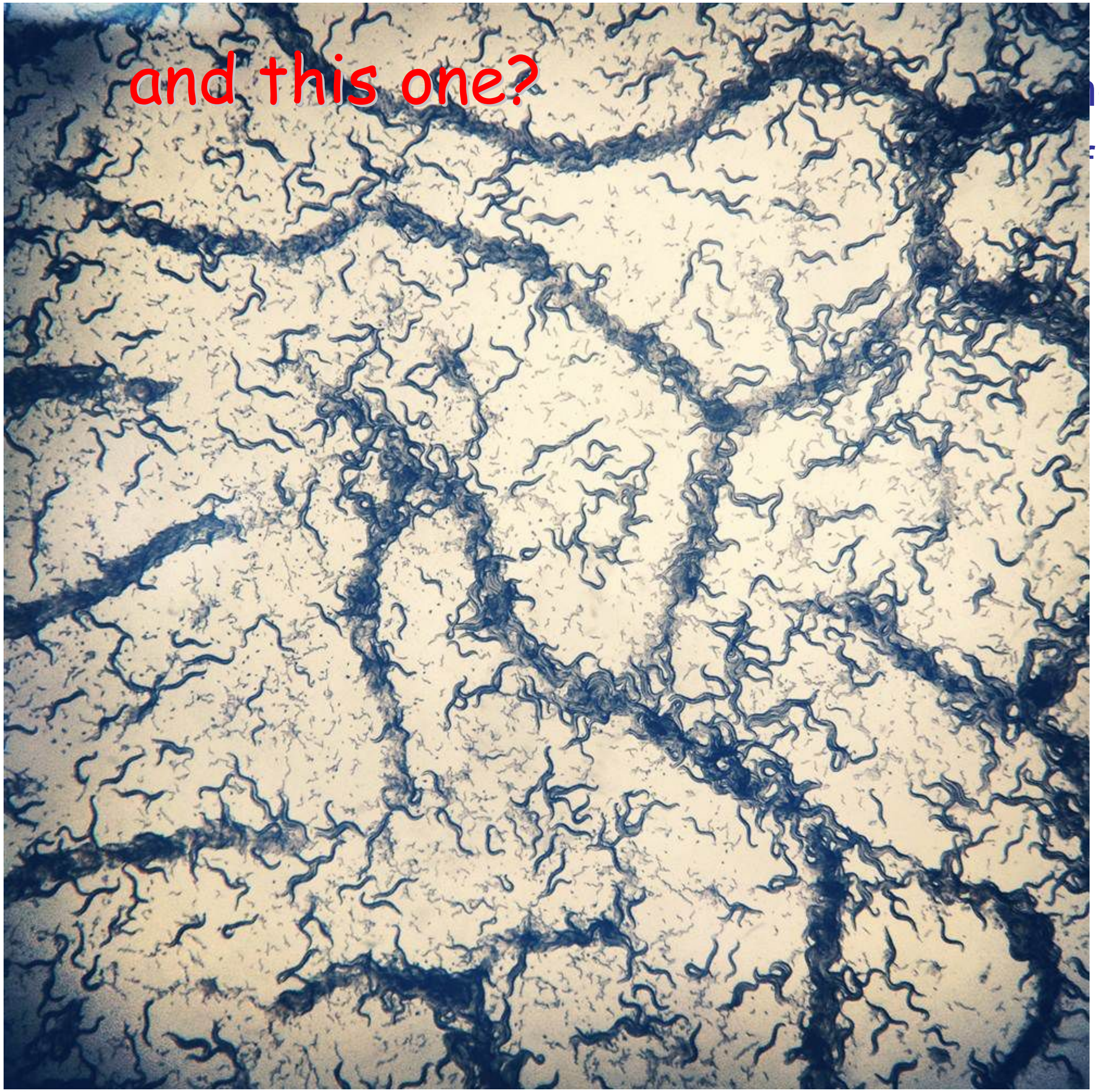
I lifespan of *C. elegans*,
p adult worms, larvae and
e

How about this one?



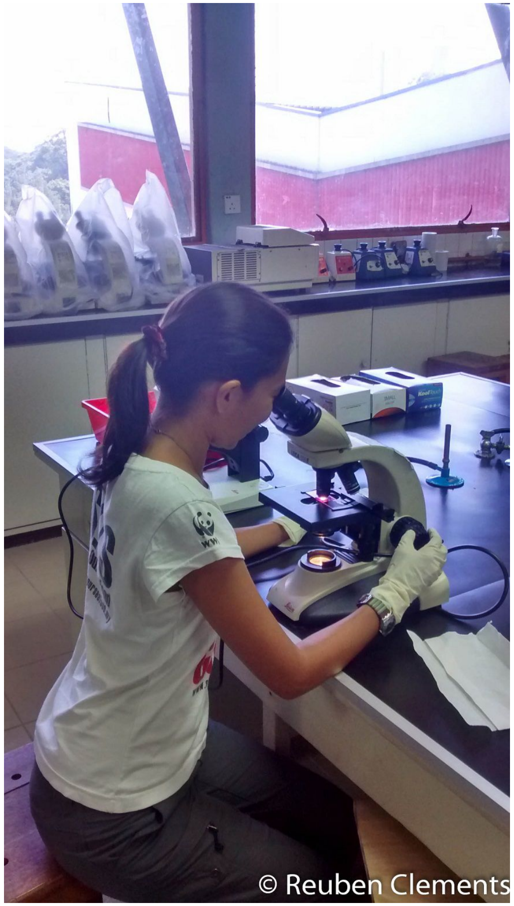
© Reuben Clements

Why does our project matter?



and this one?

e lifespan of *C. elegans*, adult worms, larvae and



© Reuben Clements

Why does our project matter?

In one word, the above examples show that manual observation is very tedious and time-consuming.

This is our motivation to develop some automation.

What do we contribute?

C. elegans detection and counting

- modify low-cost hardware for automatically scanning and acquiring images;
- develop an annotating interface to collect data;
- build a machine learning architecture to learn the worm detector;
- design a *C. elegans* detection and counting system.

C. elegans Detection and Counting

Why is it important to study *C. elegans*?

- *C. elegans* has emerged as a key model system for studying the biological processes that effect aging and animal lifespan.

- High-throughput tracking of worm health and lifespan in large populations is difficult, especially in natural conditions where worms continue to reproduce.

courtesy Kavya Leo Vakkayil

Hardware Modifications

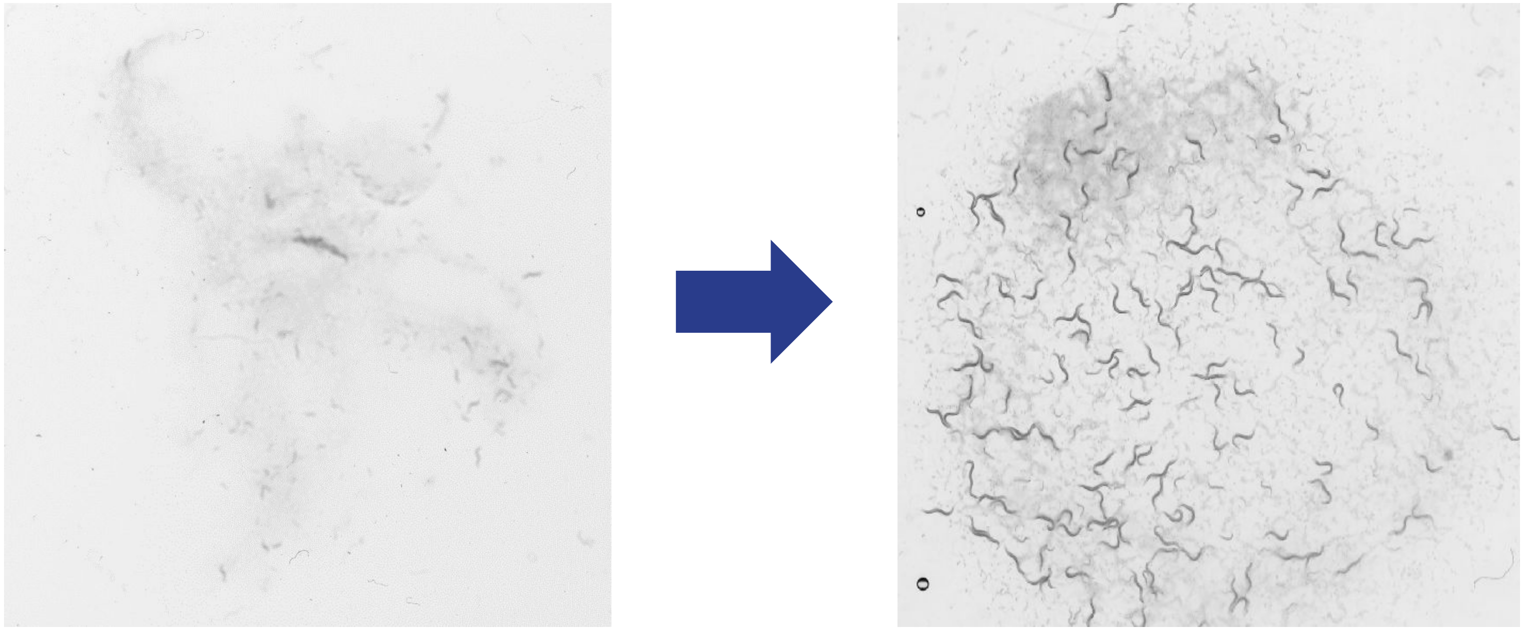
An Epson V700 Perfection Photo Scanner was modified in accordance to the procedures by Stroustrup *et al.*

- Low-cost solution
- High-throughput method
- Very modular and expandable
- 6400 dpi Maximum
- Maximum Scan Area: 8.5" x 11.7"

Stroustrup, Nicholas, Bryne E. Ulmschneider, Zachary M. Nash, Isaac F. L ó pez-Moyado, Javier Apfeld, and Walter Fontana. "The Caenorhabditis Elegans Lifespan Machine." *Nature Methods* 10.7 (2013): 665-70.

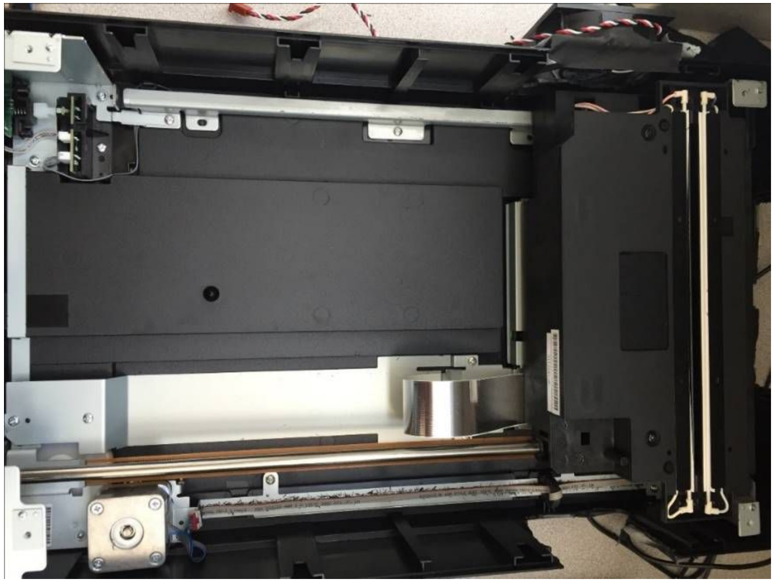
Hardware Modifications

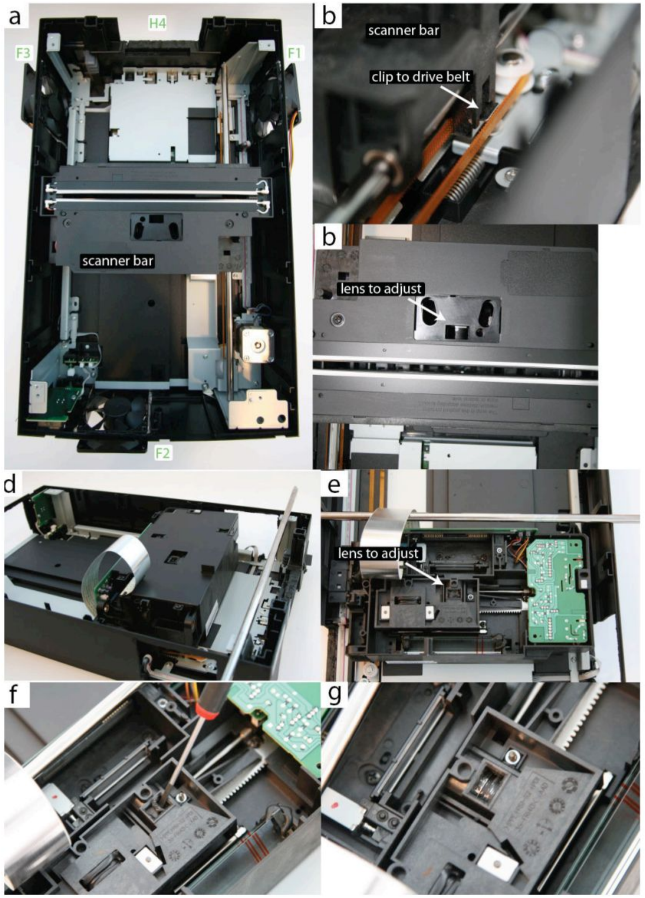
- The position of the scanner lens was modified to optimize the quality of the images



Hardware Modifications

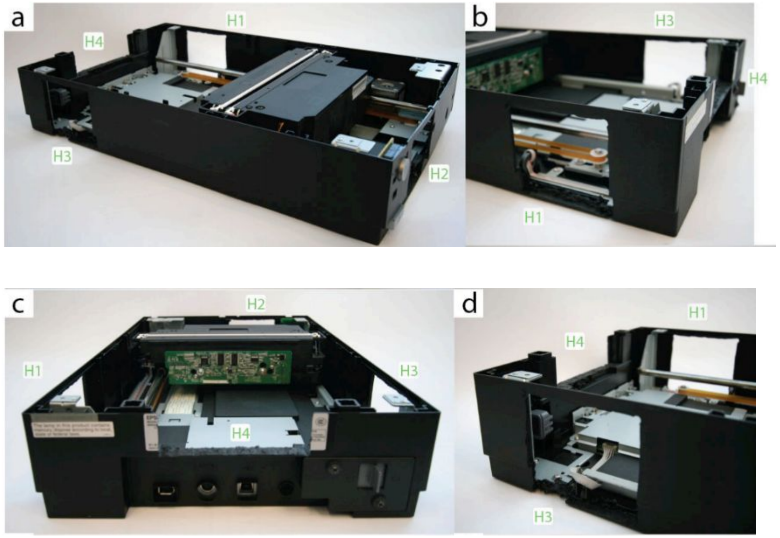
- Adjusting the focus involved opening the scanner and manually moving the lens in the head unit the desired focus was achieved.

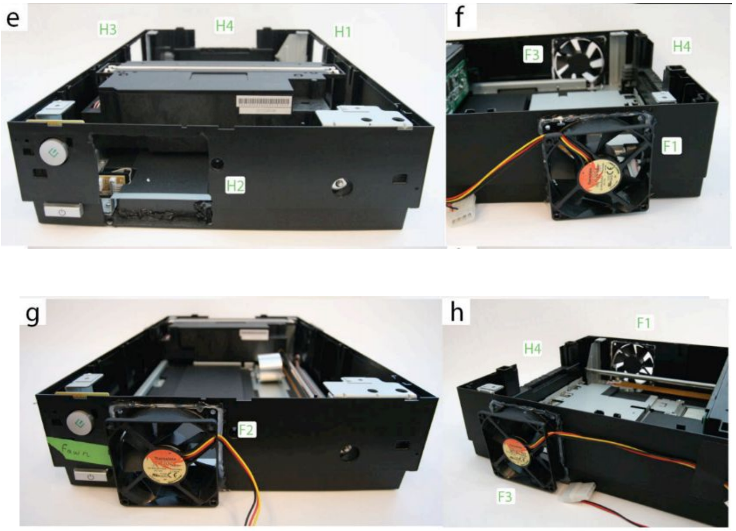


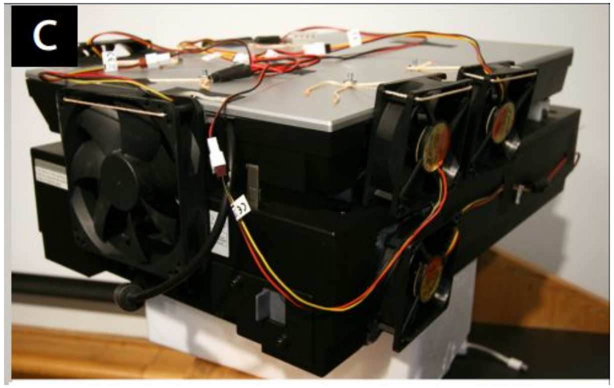


Hardware Modifications

- The scanner was modified to accommodate fans for temperature regulation.







Software Environment

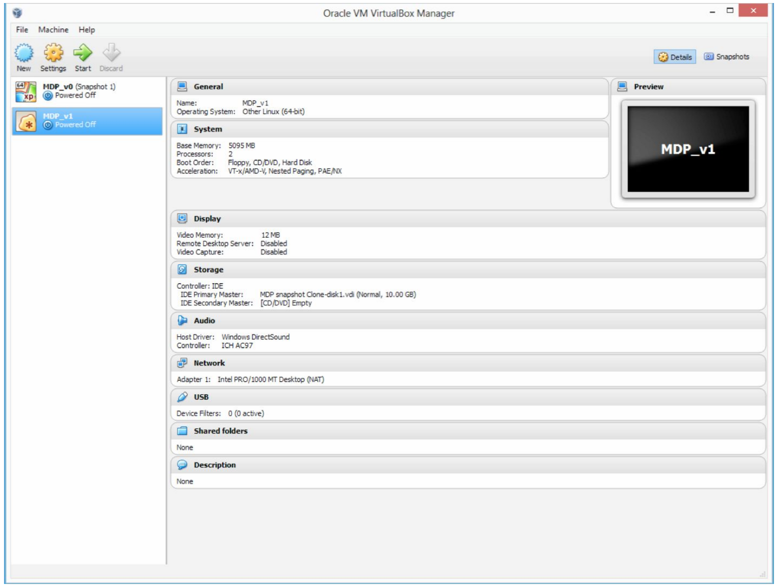
Goal of Automation

- What: Each of the 9 plates
- When: Twice every hour
- Where: Web server

Software Environment

Environment

- Scientific Linux 7 Operating System



General
Name: MDP_v1
Operating System: Other Linux (64-bit)

System
Base Memory: 5095 MB
Processors: 2
Boot Order: Floppy, CD/DVD, Hard Disk
Acceleration: VT-x/AMD-V, Nested Paging, PAE/NX

Display
Video Memory: 12 MB
Remote Desktop Server: Disabled
Video Capture: Disabled

Storage
Controller: IDE
IDE Primary Master: MDP snapshot Clone-disk1.vdi (Normal, 10.00 GB)
IDE Secondary Master: [CD/DVD] Empty

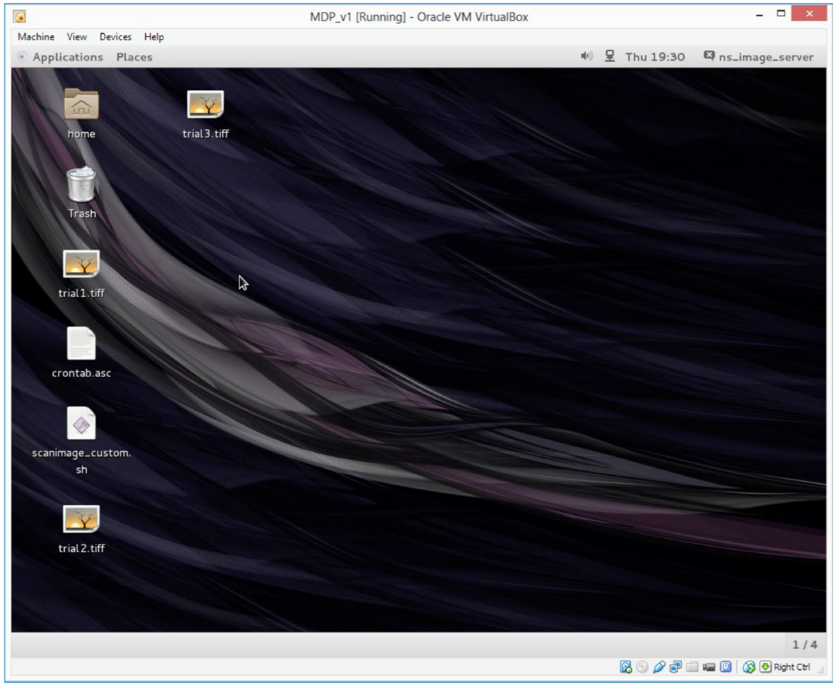
Audio
Host Driver: Windows DirectSound
Controller: ICH AC97

Network
Adapter 1: Intel PRO/1000 MT Desktop (NAT)

USB
Device Filters: 0 (0 active)

Shared folders
None

Description
None

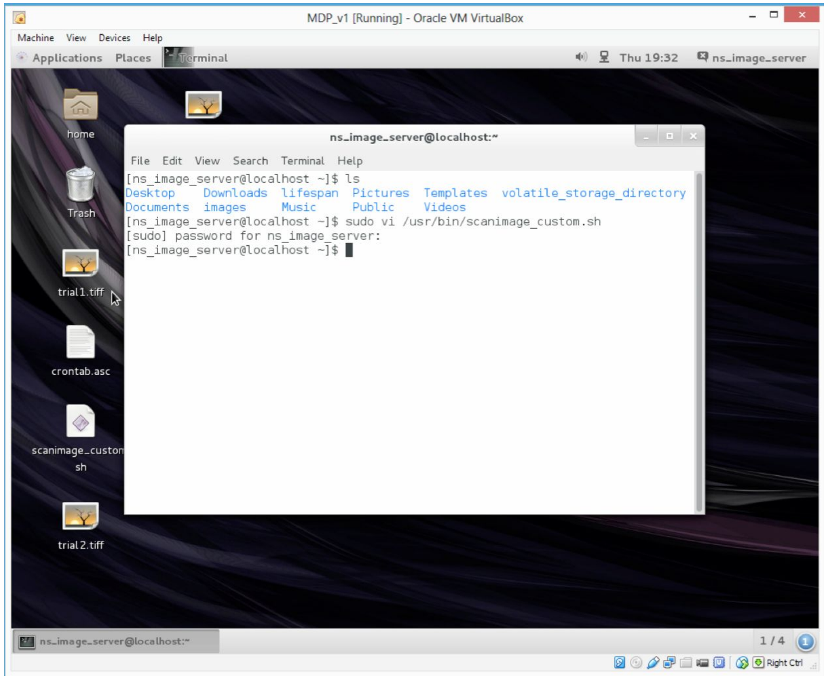


UCIrvine
University of California, Irvine

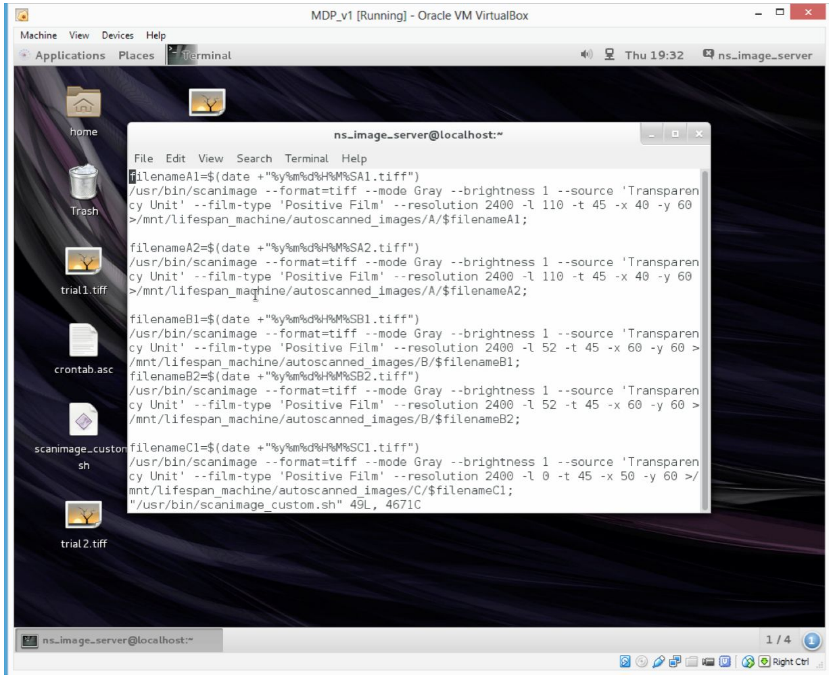
Software Environment

What

- Script: scanimage_custom.sh


MDP_v1 [Running] - Oracle VM VirtualBox
Machine View Devices Help
Applications Places Terminal
Thu 19:32
ns_image_server
home
Trash
trial1.tiff
crontab.asc
scanimage_custom.sh
trial2.tiff
ns_image_server@localhost:~
File Edit View Search Terminal Help
1 / 4
Right Ctrl

```
[ns_image_server@localhost ~]$ ls
Desktop    Downloads  lifespan  Pictures  Templates  volatile_storage_directory
Documents  images     Music     Public    Videos
[ns_image_server@localhost ~]$ sudo vi /usr/bin/scanimage_custom.sh
[sudo] password for ns_image_server:
[ns_image_server@localhost ~]$ 
```

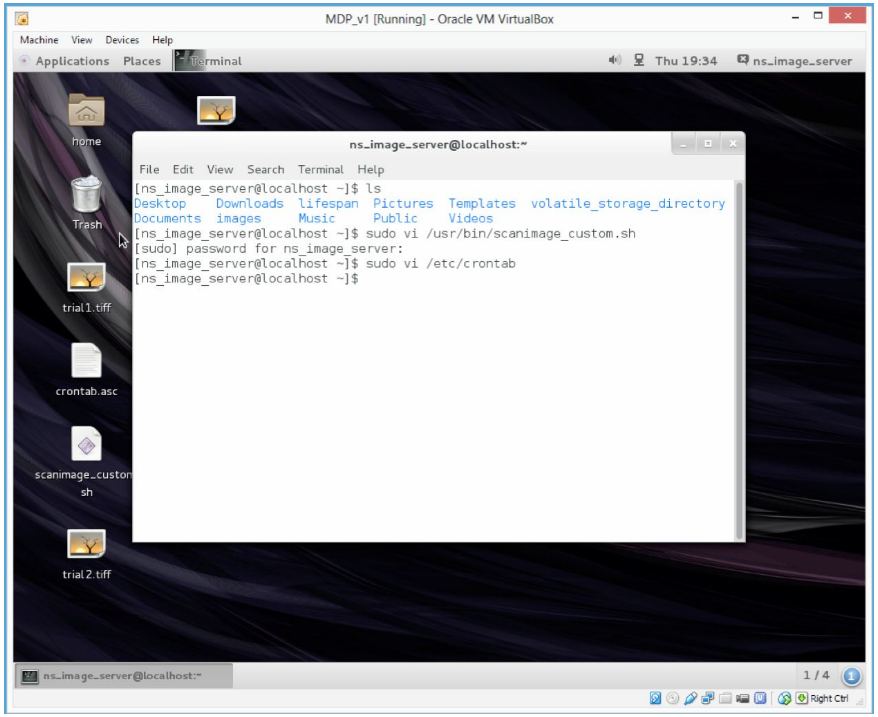

MDP_v1 [Running] - Oracle VM VirtualBox
Machine View Devices Help
Applications Places Terminal
Thu 19:32
ns_image_server
home
Trash
trial1.tiff
crontab.asc
scanimage_custom.sh
trial2.tiff
ns_image_server@localhost:~
File Edit View Search Terminal Help
1 / 4
Right Ctrl

```
filenameA1=$(date +"%y%m%d%H%M%SA1.tiff")
/usr/bin/scanimage --format=tiff --mode Gray --brightness 1 --source 'Transparen
cy Unit' --film-type 'Positive Film' --resolution 2400 -l 110 -t 45 -x 40 -y 60
>/mnt/lifespan_machine/autoscanned_images/A/$filenameA1;

filenameA2=$(date +"%y%m%d%H%M%SA2.tiff")
/usr/bin/scanimage --format=tiff --mode Gray --brightness 1 --source 'Transparen
cy Unit' --film-type 'Positive Film' --resolution 2400 -l 110 -t 45 -x 40 -y 60
>/mnt/lifespan_machine/autoscanned_images/A/$filenameA2;

filenameB1=$(date +"%y%m%d%H%M%SB1.tiff")
/usr/bin/scanimage --format=tiff --mode Gray --brightness 1 --source 'Transparen
cy Unit' --film-type 'Positive Film' --resolution 2400 -l 52 -t 45 -x 60 -y 60 >
/mnt/lifespan_machine/autoscanned_images/B/$filenameB1;
filenameB2=$(date +"%y%m%d%H%M%SB2.tiff")
/usr/bin/scanimage --format=tiff --mode Gray --brightness 1 --source 'Transparen
cy Unit' --film-type 'Positive Film' --resolution 2400 -l 52 -t 45 -x 60 -y 60 >
/mnt/lifespan_machine/autoscanned_images/B/$filenameB2;

filenameC1=$(date +"%y%m%d%H%M%SC1.tiff")
/usr/bin/scanimage --format=tiff --mode Gray --brightness 1 --source 'Transparen
cy Unit' --film-type 'Positive Film' --resolution 2400 -l 0 -t 45 -x 50 -y 60 >/
mnt/lifespan_machine/autoscanned_images/C/$filenameC1;
"/usr/bin/scanimage_custom.sh" 49L, 4671C
```

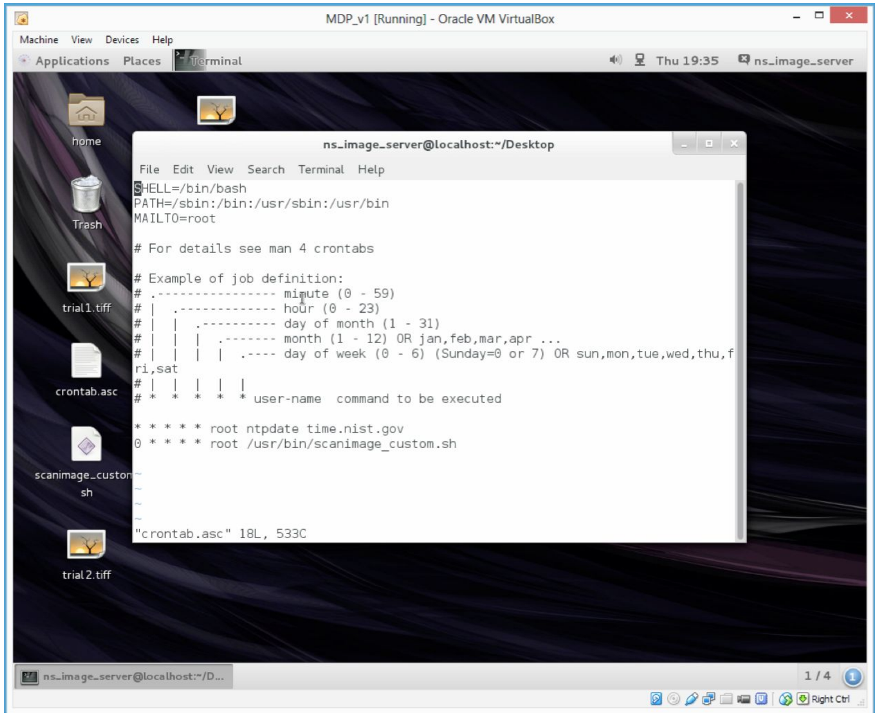
Software Environment

When

- Scheduler: crontab.asc


MDP_v1 [Running] - Oracle VM VirtualBox
Machine View Devices Help
Applications Places Terminal
Thu 19:34
ns_image_server
home
Trash
trial1.tiff
crontab.asc
scanimage_custom.sh
trial2.tiff
ns_image_server@localhost:~
File Edit View Search Terminal Help
1 / 4
Right Ctrl

```
[ns_image_server@localhost ~]$ ls
Desktop    Downloads  lifespan  Pictures  Templates  volatile_storage_directory
Documents  images     Music     Public    Videos
[ns_image_server@localhost ~]$ sudo vi /usr/bin/scanimage_custom.sh
[sudo] password for ns_image_server:
[ns_image_server@localhost ~]$ sudo vi /etc/crontab
[ns_image_server@localhost ~]$
```


MDP_v1 [Running] - Oracle VM VirtualBox
Machine View Devices Help
Applications Places Terminal
Thu 19:35
ns_image_server
home
Trash
trial1.tiff
crontab.asc
scanimage_custom.sh
trial2.tiff
ns_image_server@localhost:~/Desktop
File Edit View Search Terminal Help
ns_image_server@localhost:~/D...
1 / 4
Right Ctrl

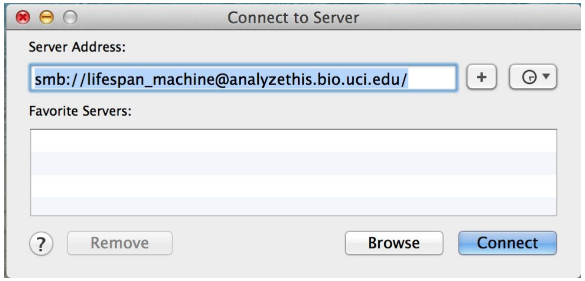
```
SHELL=/bin/bash
PATH=/sbin:/bin:/usr/sbin:/usr/bin
MAILTO=root

# For details see man 4 crontabs

# Example of job definition:
# .---------------- minute (0 - 59)
# |  .------------- hour (0 - 23)
# |  |  .---------- day of month (1 - 31)
# |  |  |  .------- month (1 - 12) OR jan,feb,mar,apr ...
# |  |  |  |  .---- day of week (0 - 6) (Sunday=0 or 7) OR sun,mon,tue,wed,thu,f
ri,sat
# |  |  |  |  |
# *  *  *  *  * user-name  command to be executed

* * * * * root ntpdate time.nist.gov
0 * * * * root /usr/bin/scanimage_custom.sh
~
~
~
~
"crontab.asc" 18L, 533C
```

UCIrvine
University of California, Irvine

Software Environment

Where

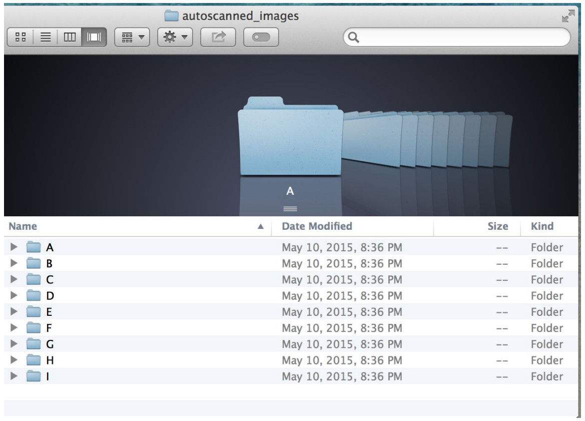
- Symbolic Link + Mount



Connect to Server

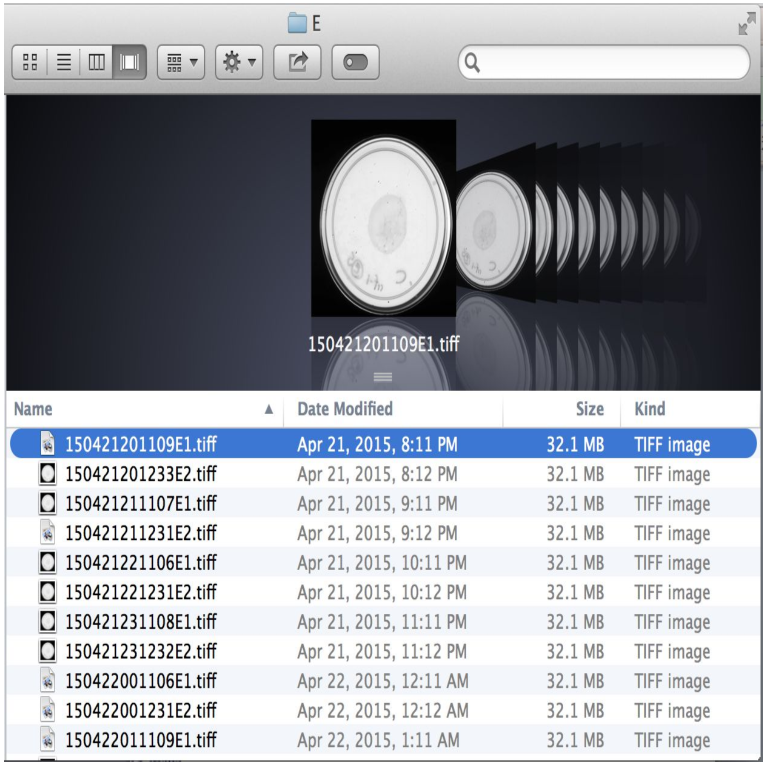
Server Address: smb://lifespan_machine@analyzethis.bio.uci.edu/

Favorite Servers:



autoscanned_images

Name	Date Modified	Size	Kind
A	May 10, 2015, 8:36 PM	--	Folder
B	May 10, 2015, 8:36 PM	--	Folder
C	May 10, 2015, 8:36 PM	--	Folder
D	May 10, 2015, 8:36 PM	--	Folder
E	May 10, 2015, 8:36 PM	--	Folder
F	May 10, 2015, 8:36 PM	--	Folder
G	May 10, 2015, 8:36 PM	--	Folder
H	May 10, 2015, 8:36 PM	--	Folder
I	May 10, 2015, 8:36 PM	--	Folder

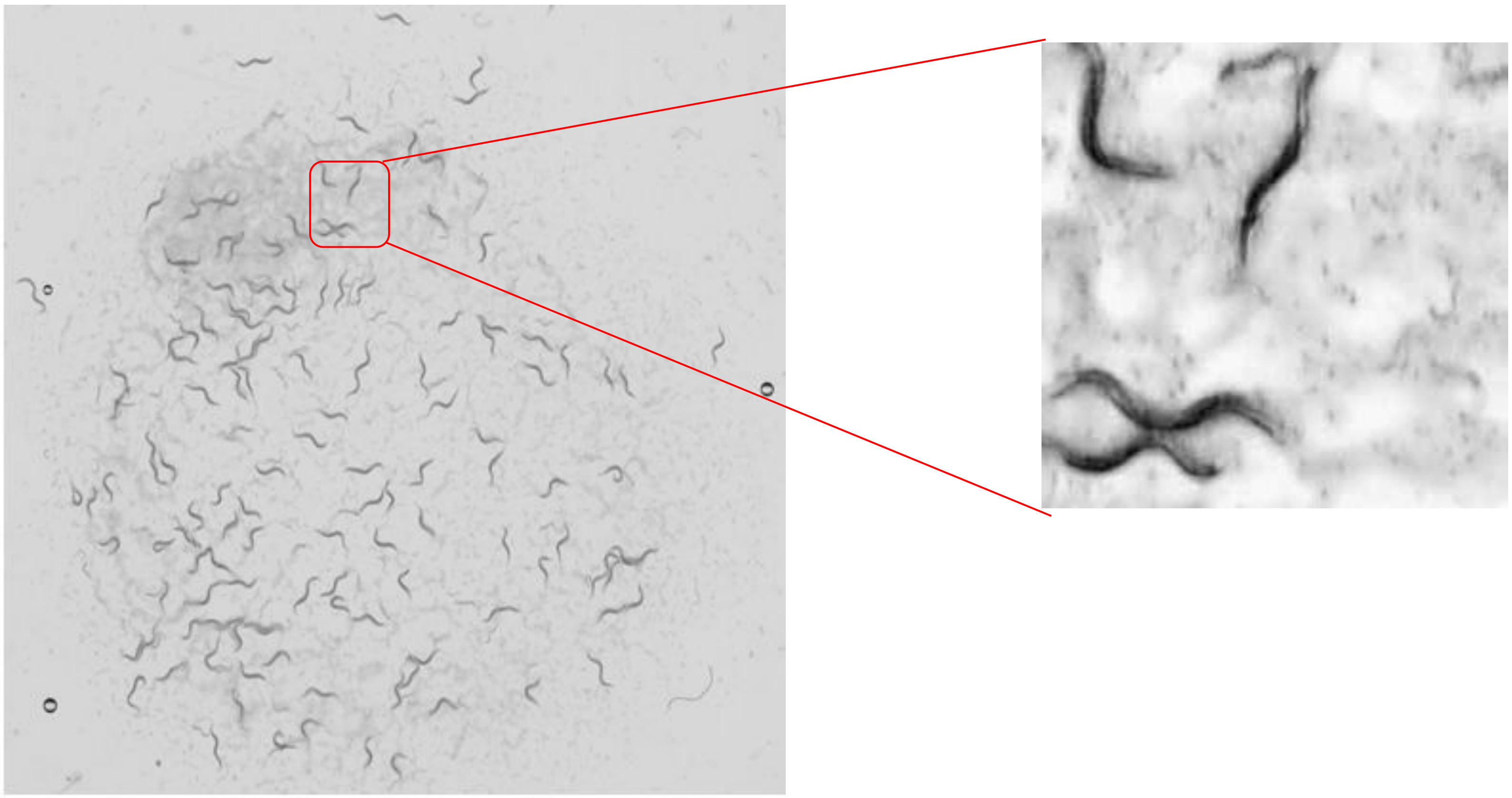


E

Name	Date Modified	Size	Kind
150421201109E1.tiff	Apr 21, 2015, 8:11 PM	32.1 MB	TIFF image
150421201233E2.tiff	Apr 21, 2015, 8:12 PM	32.1 MB	TIFF image
150421211107E1.tiff	Apr 21, 2015, 9:11 PM	32.1 MB	TIFF image
150421211231E2.tiff	Apr 21, 2015, 9:12 PM	32.1 MB	TIFF image
150421221106E1.tiff	Apr 21, 2015, 10:11 PM	32.1 MB	TIFF image
150421221231E2.tiff	Apr 21, 2015, 10:12 PM	32.1 MB	TIFF image
150421231108E1.tiff	Apr 21, 2015, 11:11 PM	32.1 MB	TIFF image
150421231232E2.tiff	Apr 21, 2015, 11:12 PM	32.1 MB	TIFF image
150422001106E1.tiff	Apr 22, 2015, 12:11 AM	32.1 MB	TIFF image
150422001231E2.tiff	Apr 22, 2015, 12:12 AM	32.1 MB	TIFF image
150422011109E1.tiff	Apr 22, 2015, 1:11 AM	32.1 MB	TIFF image

Algorithm for Detection and Counting

Can we detect and segment the worms?



Algorithm for Detection and Counting

Our tool is deep learning --

Deep learning was ranked the first in ten breakthrough technologies 2013 by *MIT Technology Review*.

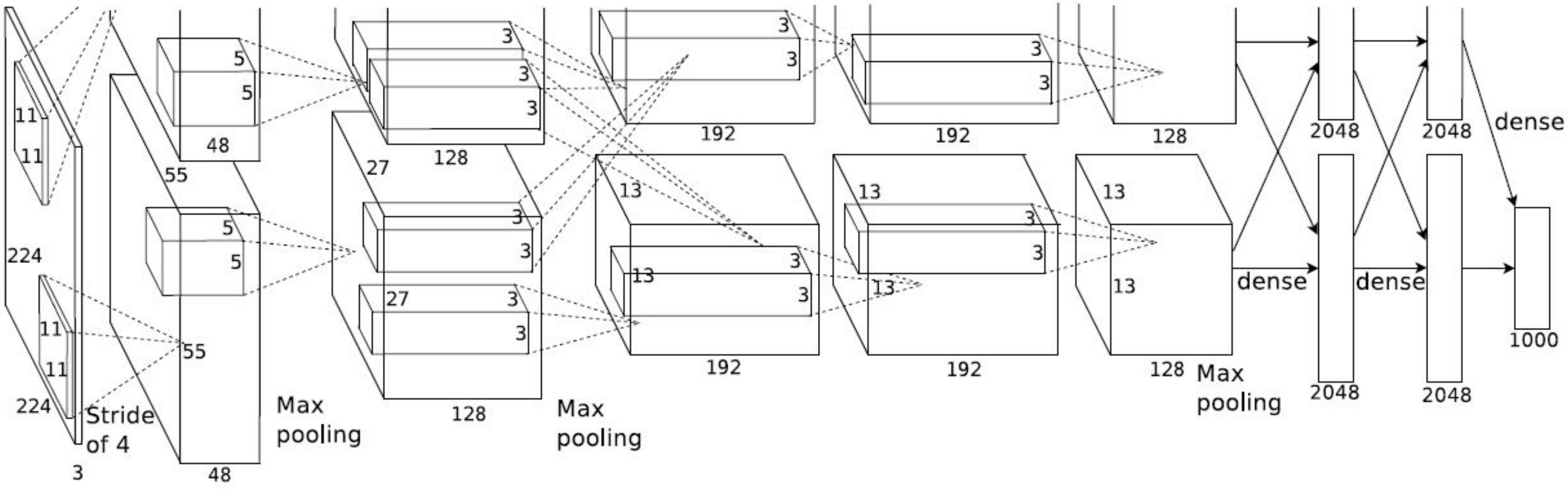
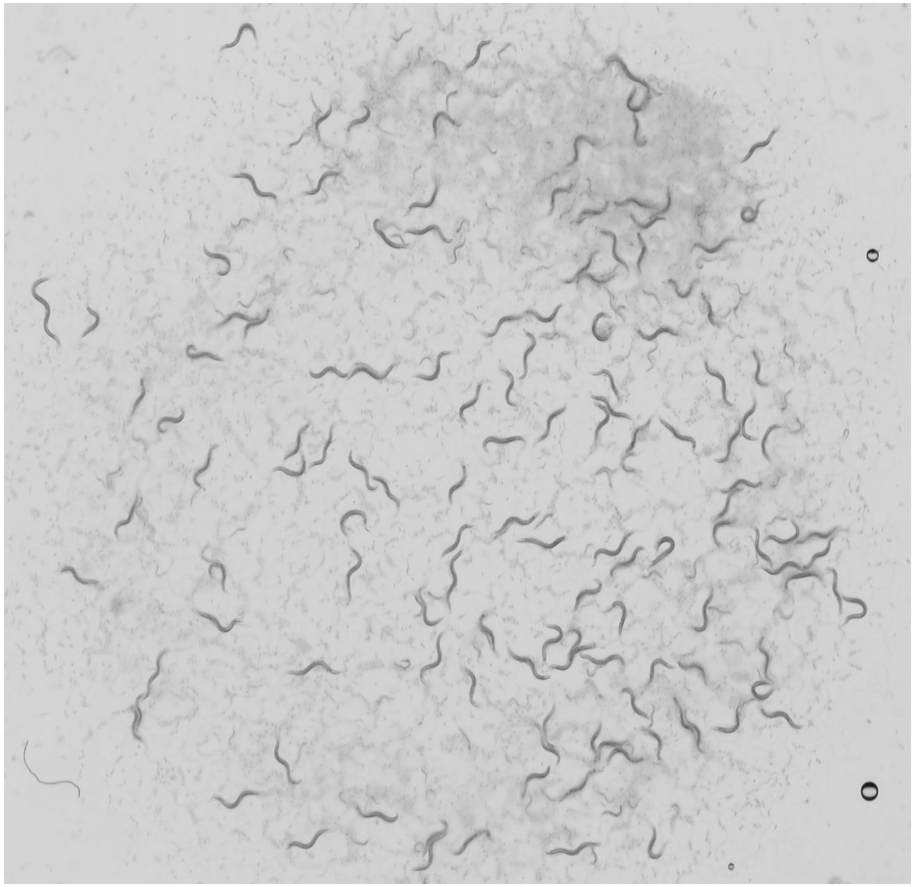


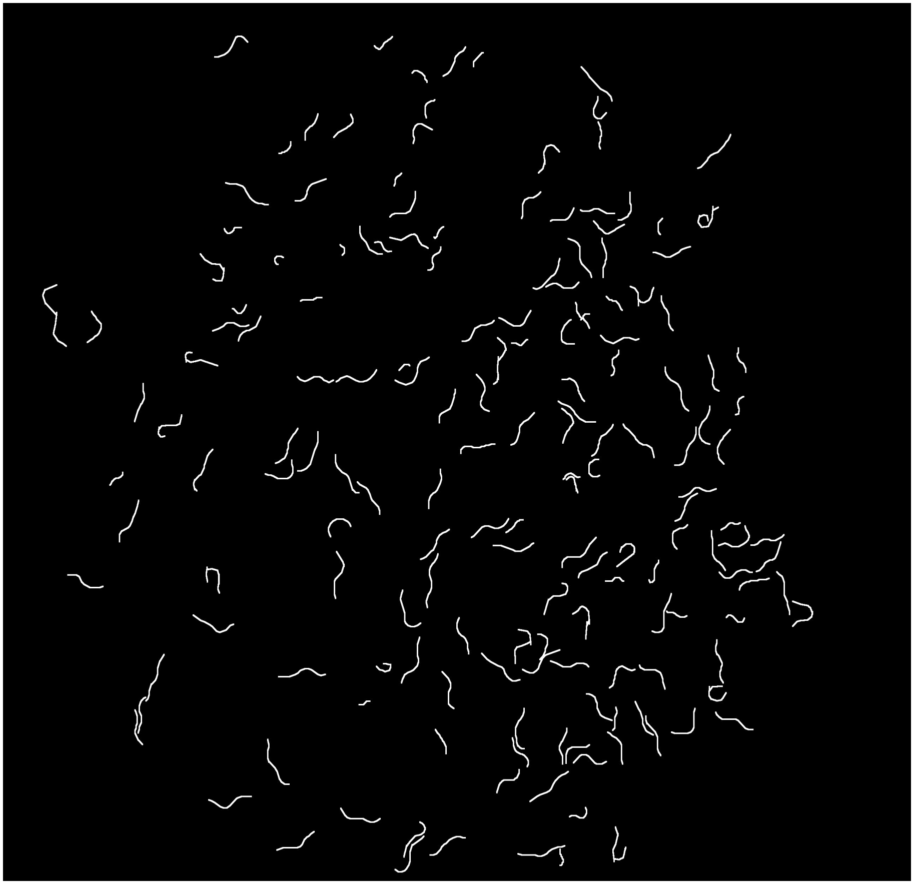
figure from "ImageNet Classification with Deep Convolutional Neural Networks", NIPS, 2012.

Algorithm for Detection and Counting

We develop a semi-automatic interface to collect worm data.

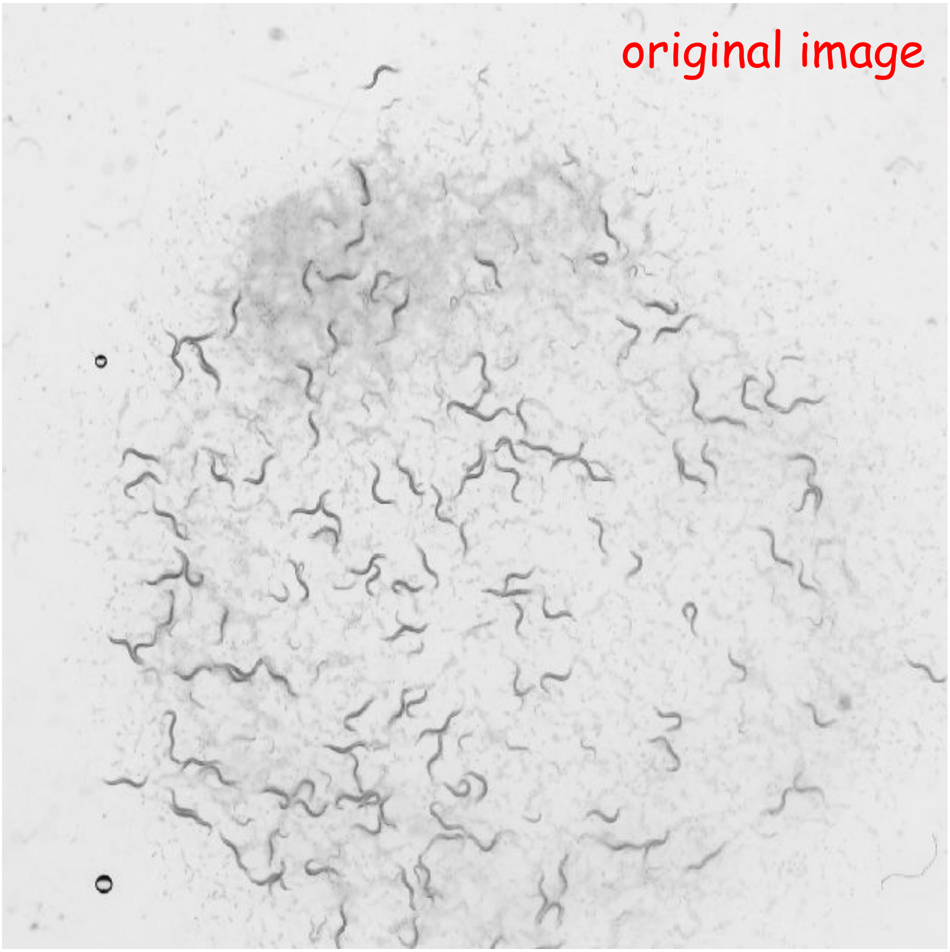
After getting the training set, we can train our model.

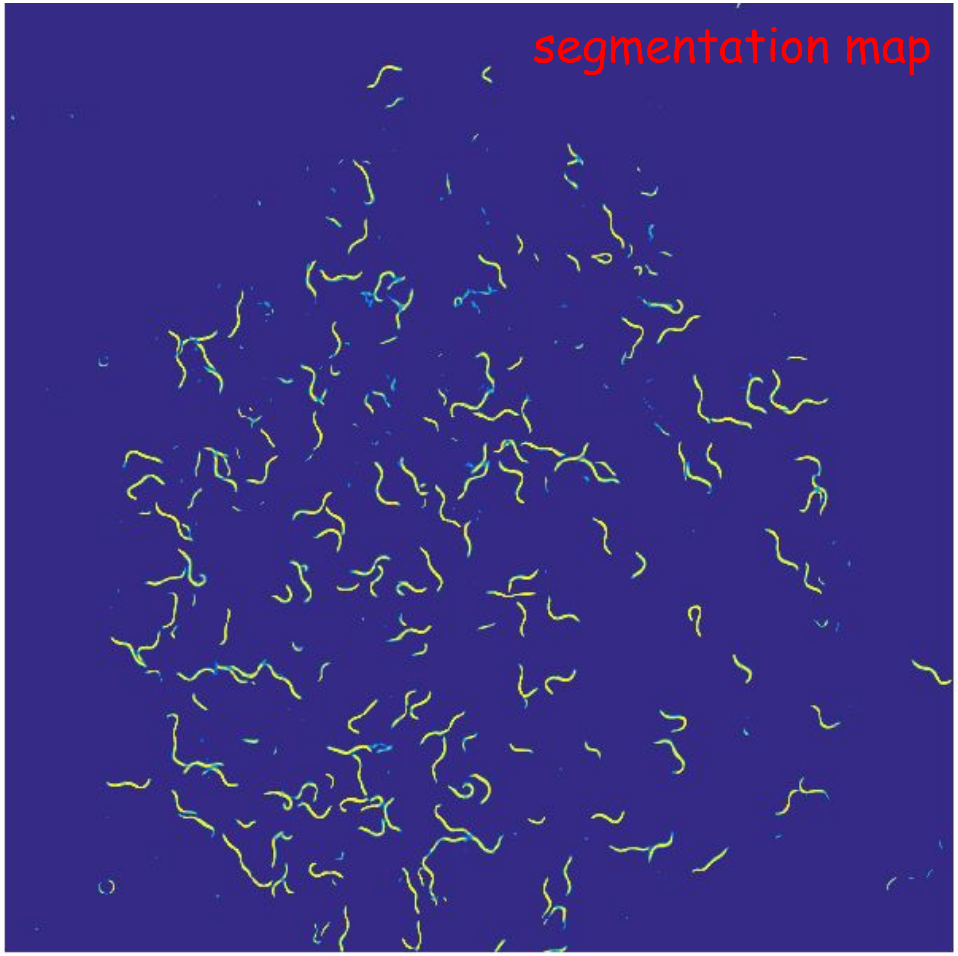




Result

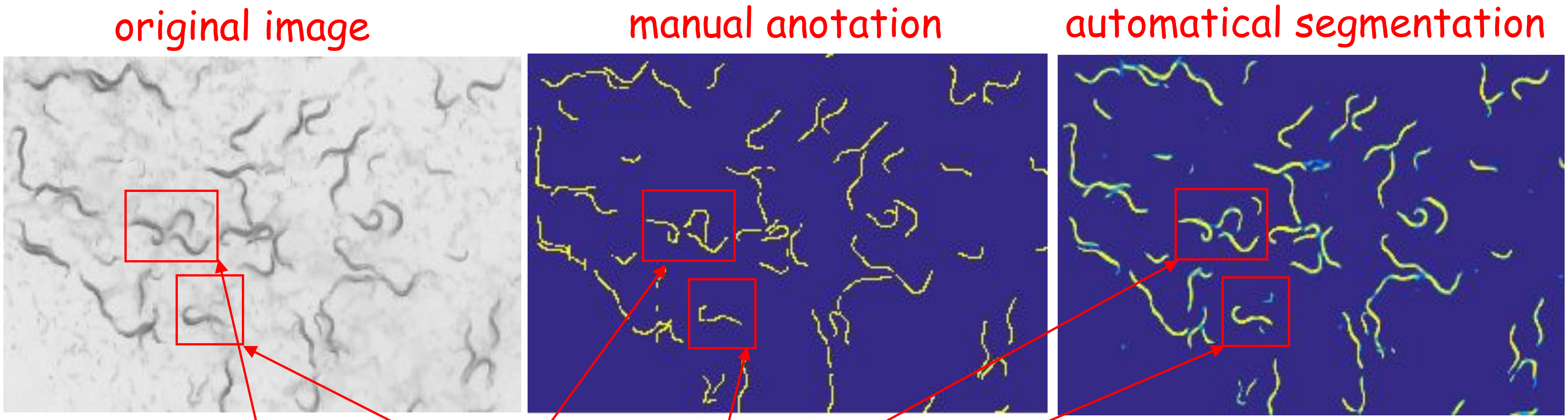
With the trained model, we can segment the worms.





Result

Let's zoom in to have a look at it...



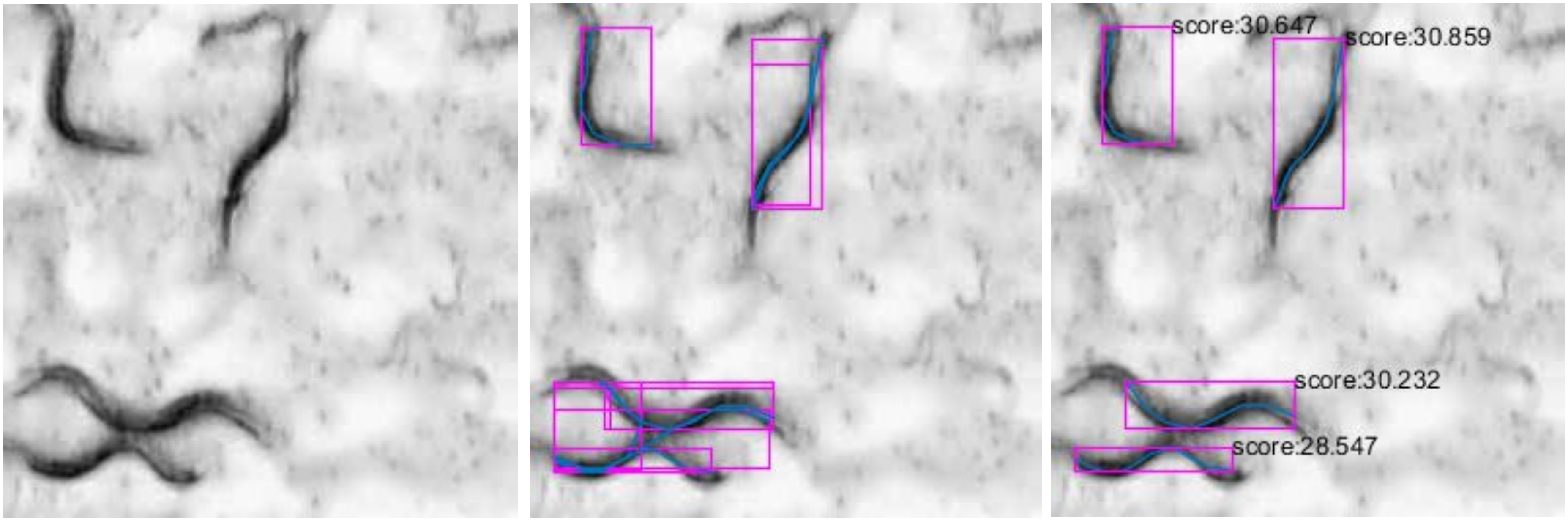
Two interesting observations:

1. Even though many larvae are not annotated in the training images, the model can find them.

2. Even though the annotated worms consist of line segments, meaning there are sharp angles between two consecutive segments, the segmented worms are smooth.

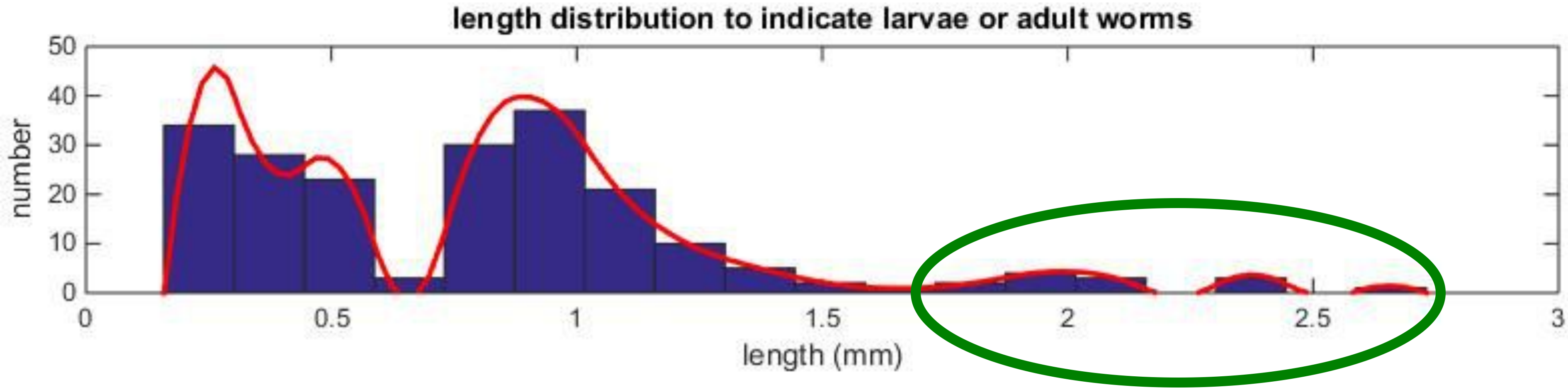
Result

- detect every single worm appearing in the image
- a deformable part based chain model to detect worms



Result

- count the number of larvae and adult worms
- obtain their aging stage distribution according to their body length



Thank you

Thank you!