

Update on the AOSLO: first images and image processing procedures

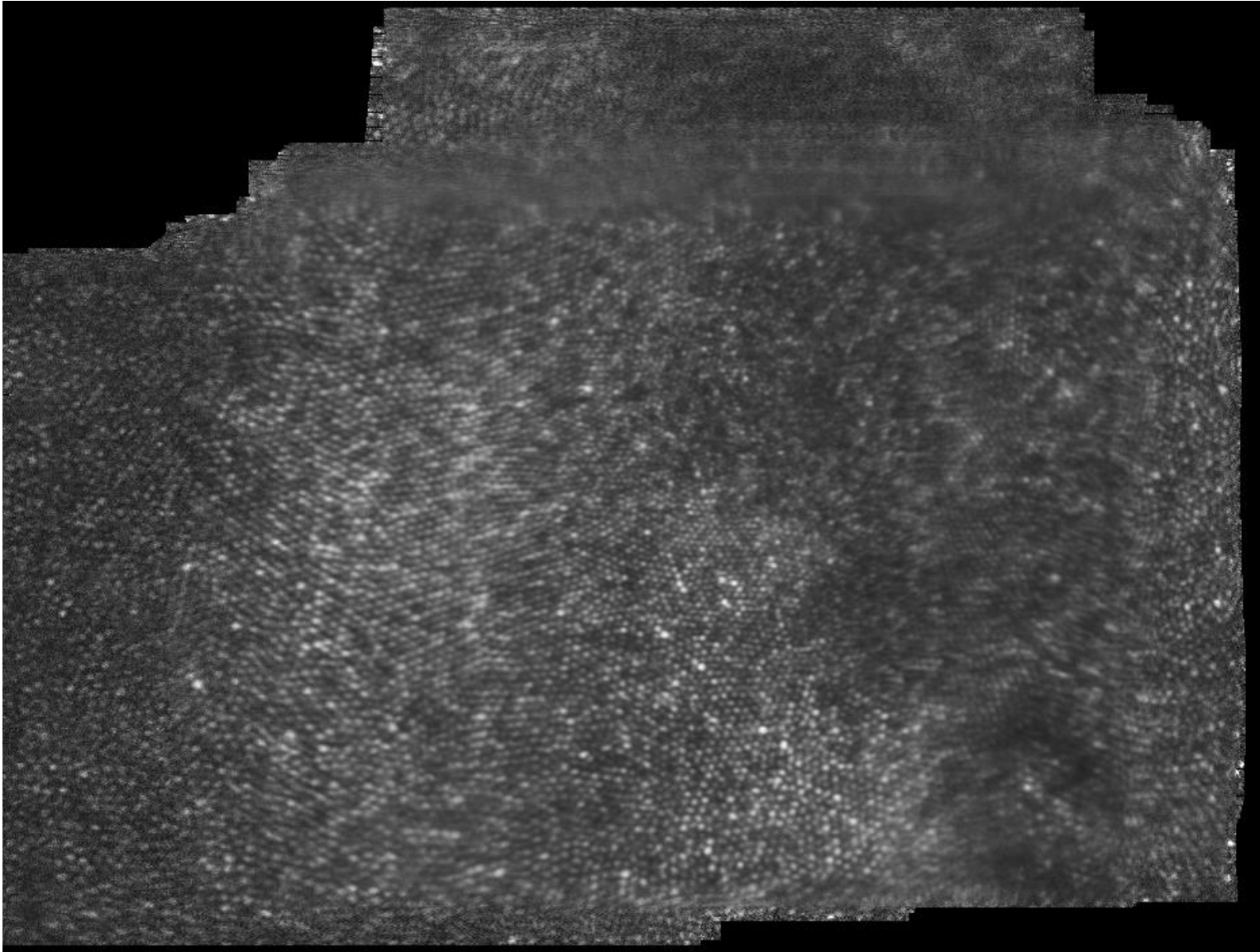
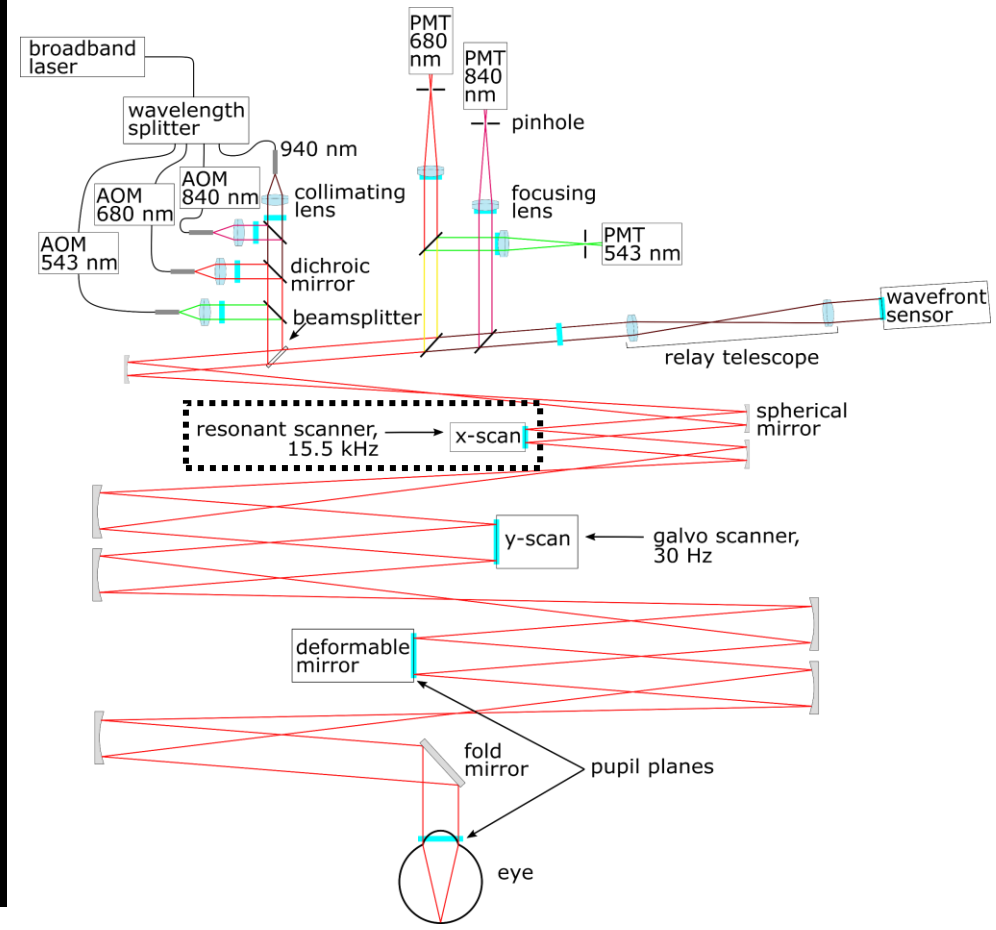
Ben Moon

AP Lab Meeting

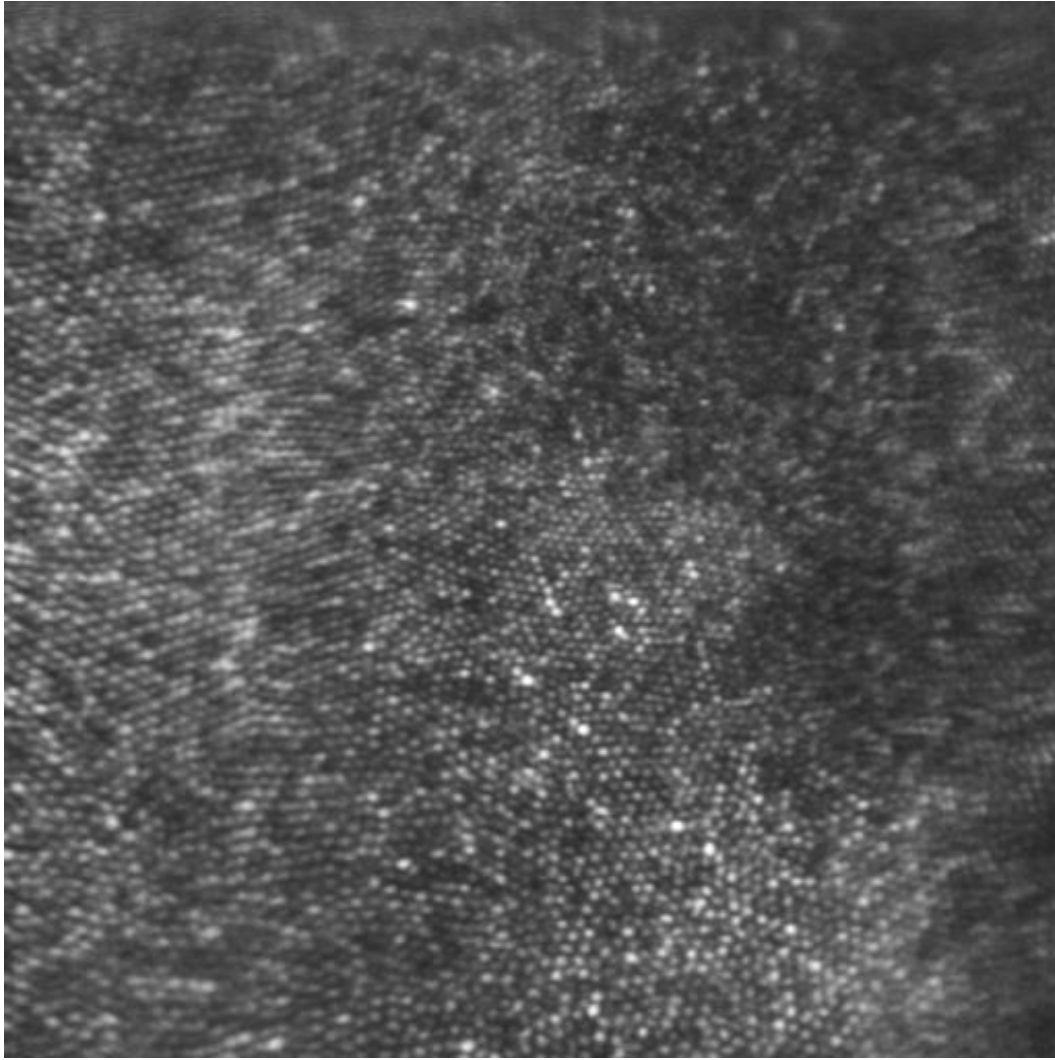
Tuesday, March 22, 2022

Images from the first session in early February were negatively impacted by residual sinusoidal distortion

Sinusoidal motion of the resonant scanner was not properly compensated, causing blurring of the images after frame averaging



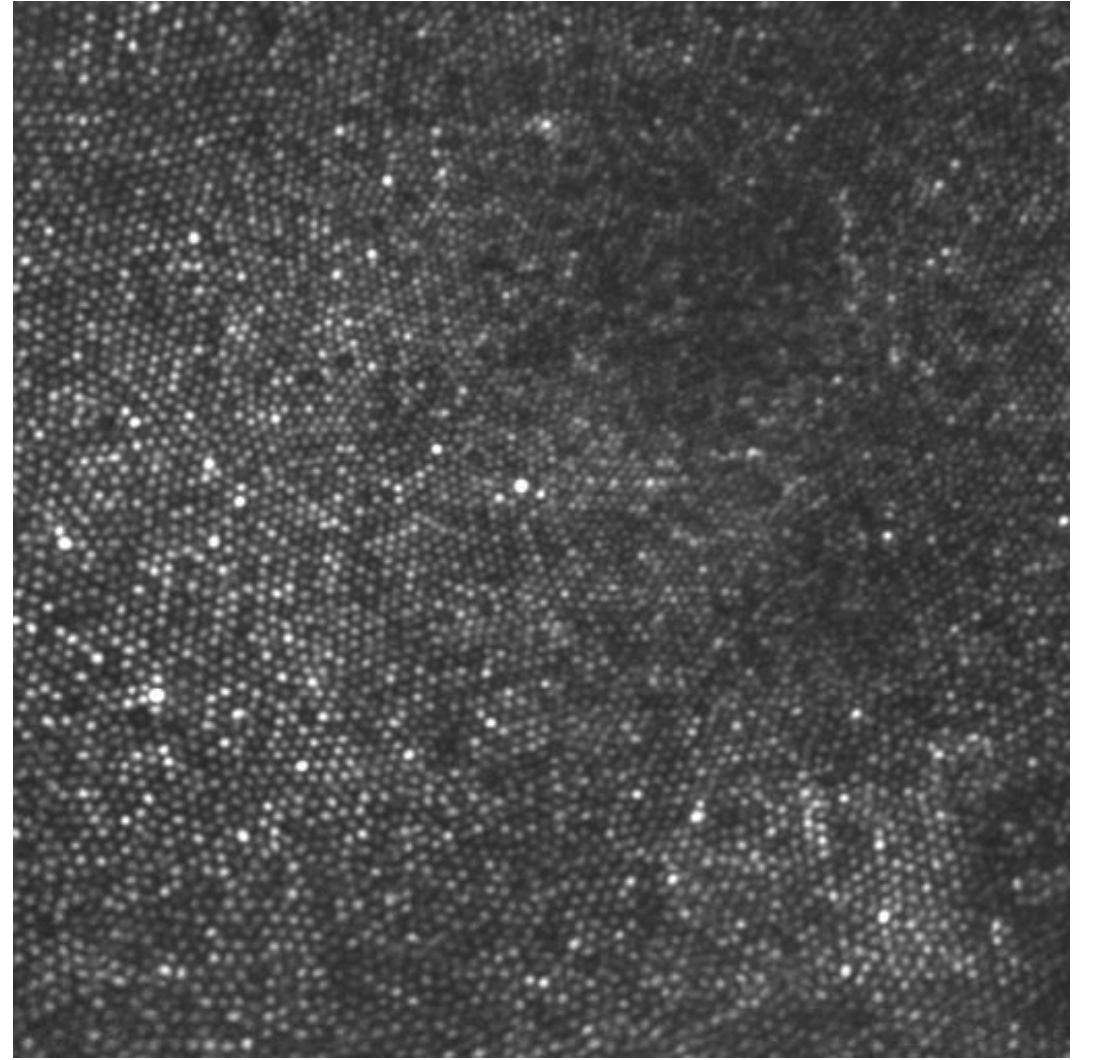
Generating a new lookup table for desinusoidalizing the images solved the blurring issue



From old video (2/2), taken with old lookup table

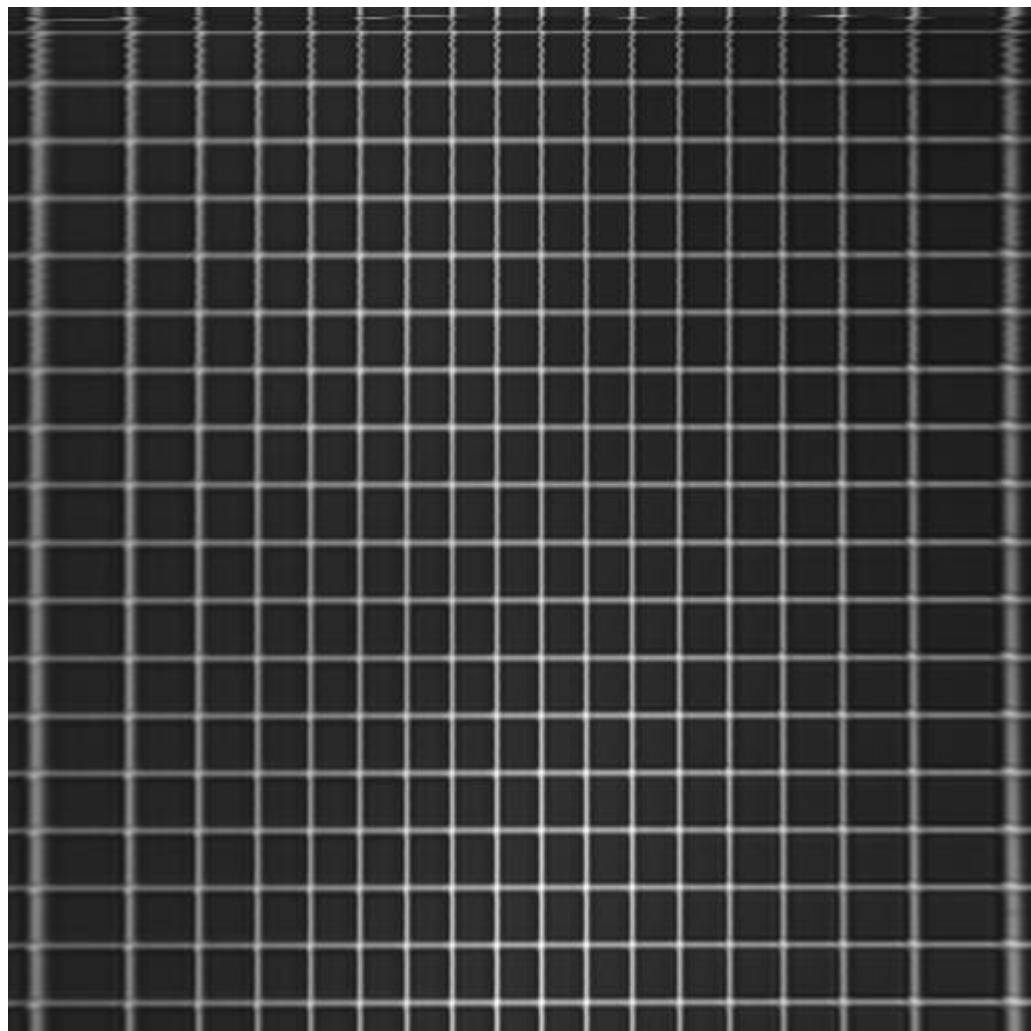


1°

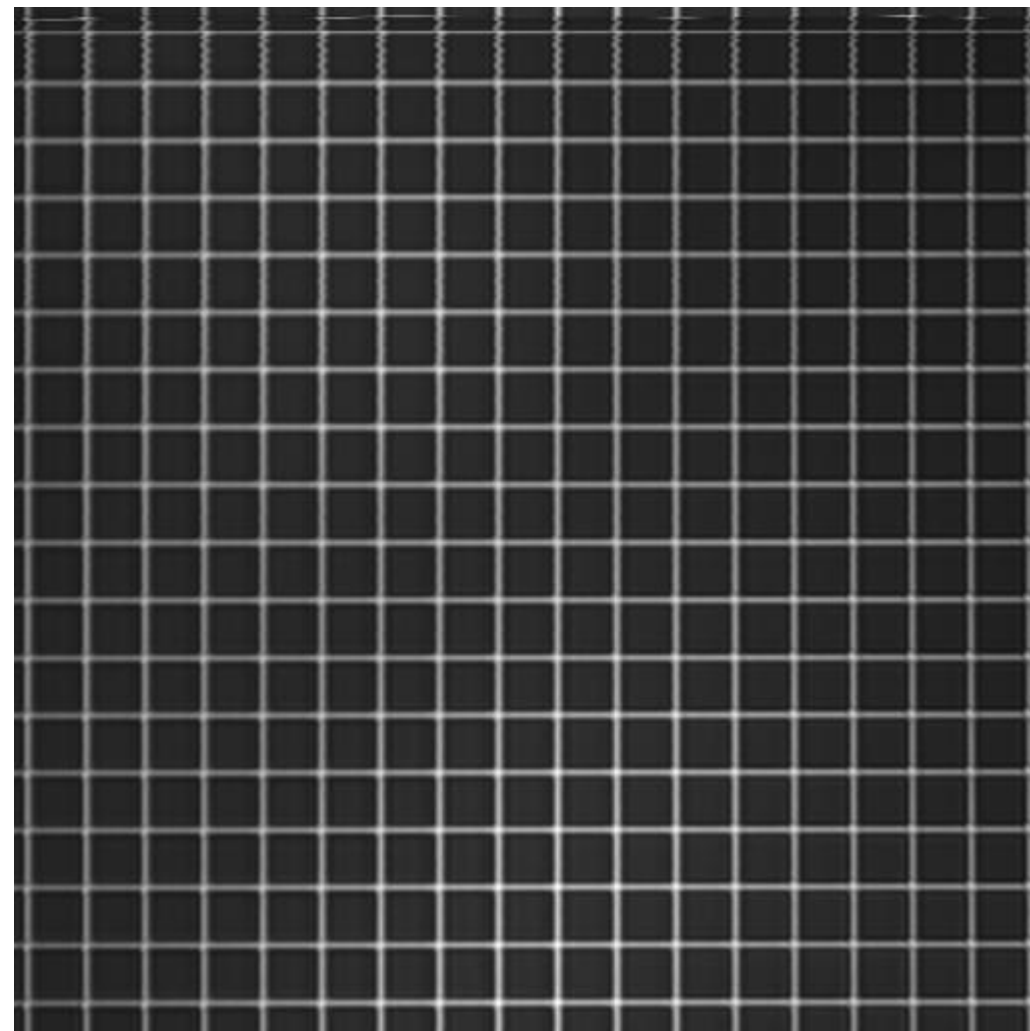


From new video (3/1), taken with new lookup table

Our procedures now include generating a new lookup table for each imaging session to avoid this issue

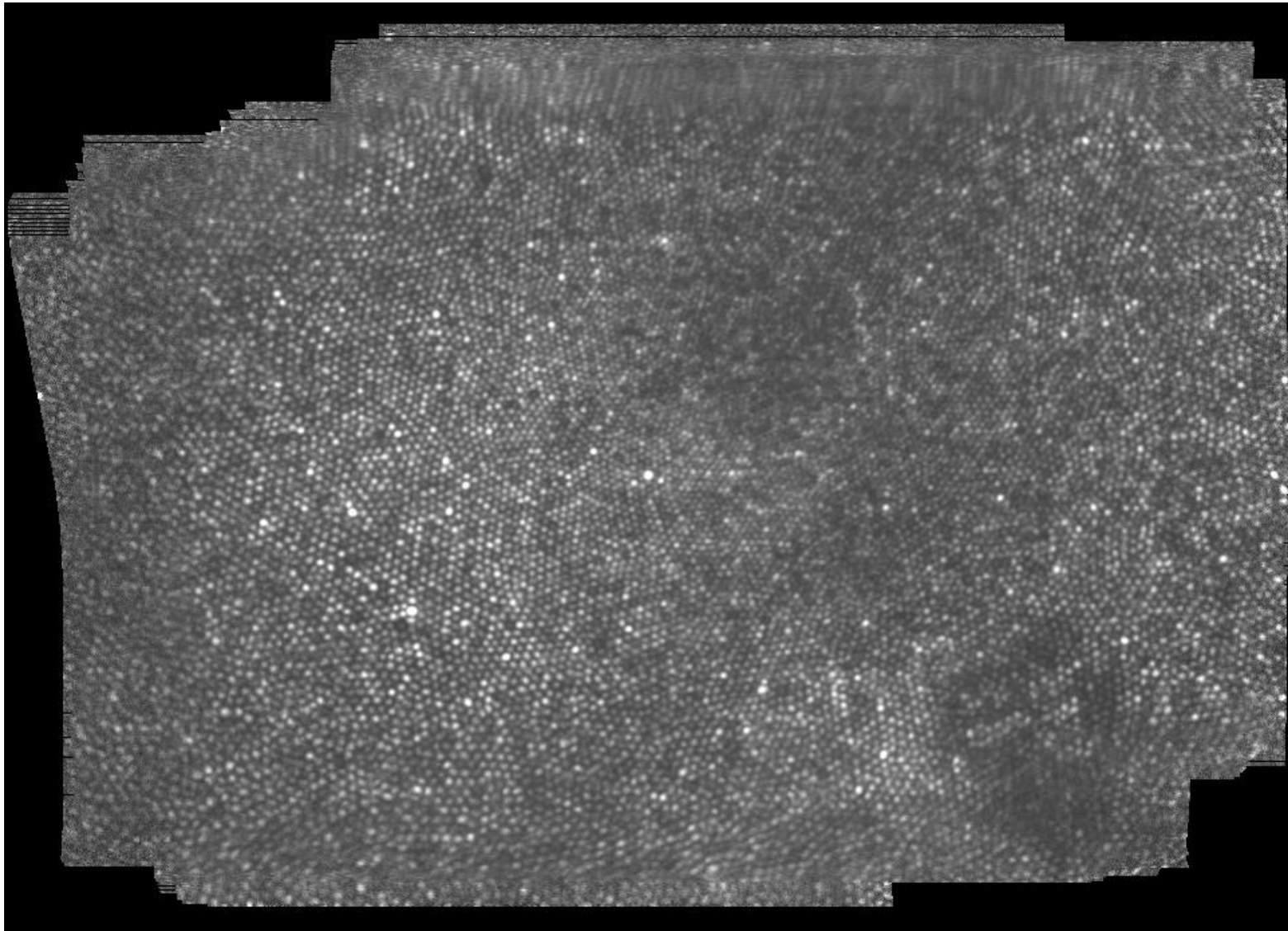


Raw grid pattern image without desinusoiding



Grid pattern after generating the lookup table and desinusoiding

Using the correct lookup table ensures that video frames can be successfully registered and averaged



1.4°

- This image does not have the blurring artifact of the initial images (except at the top, which is unavoidable)
- Average of ~ 300 frames after strip-based image registration
- Image is from the central fixation point (fixating on a 4.7 arcmin square within a 1° field of view)

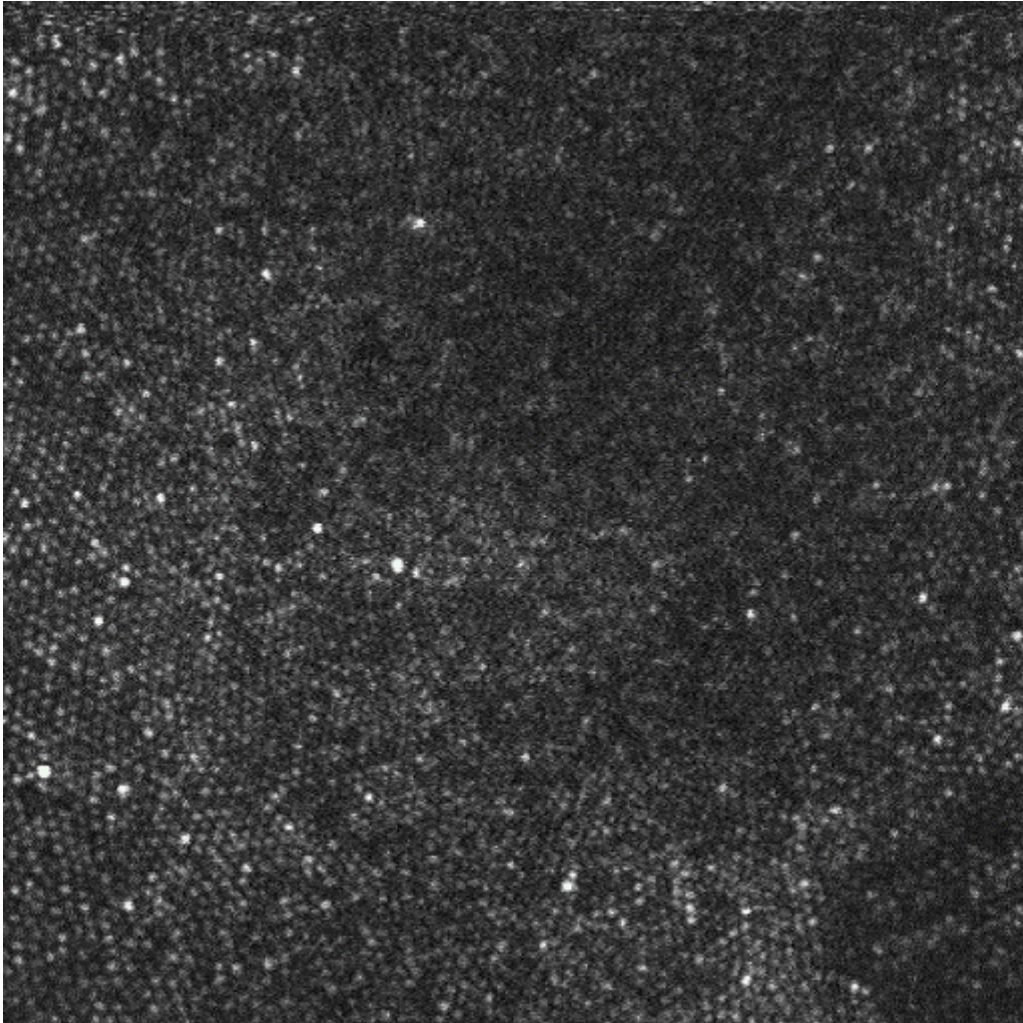
Outline for the rest of the presentation

- Video processing steps
- Image processing steps
- Creating a montage of retinal images
- Future analyses:
 - Cone detection and marking
 - Cone density calculation
 - Determining the PRL
 - Eye movement traces

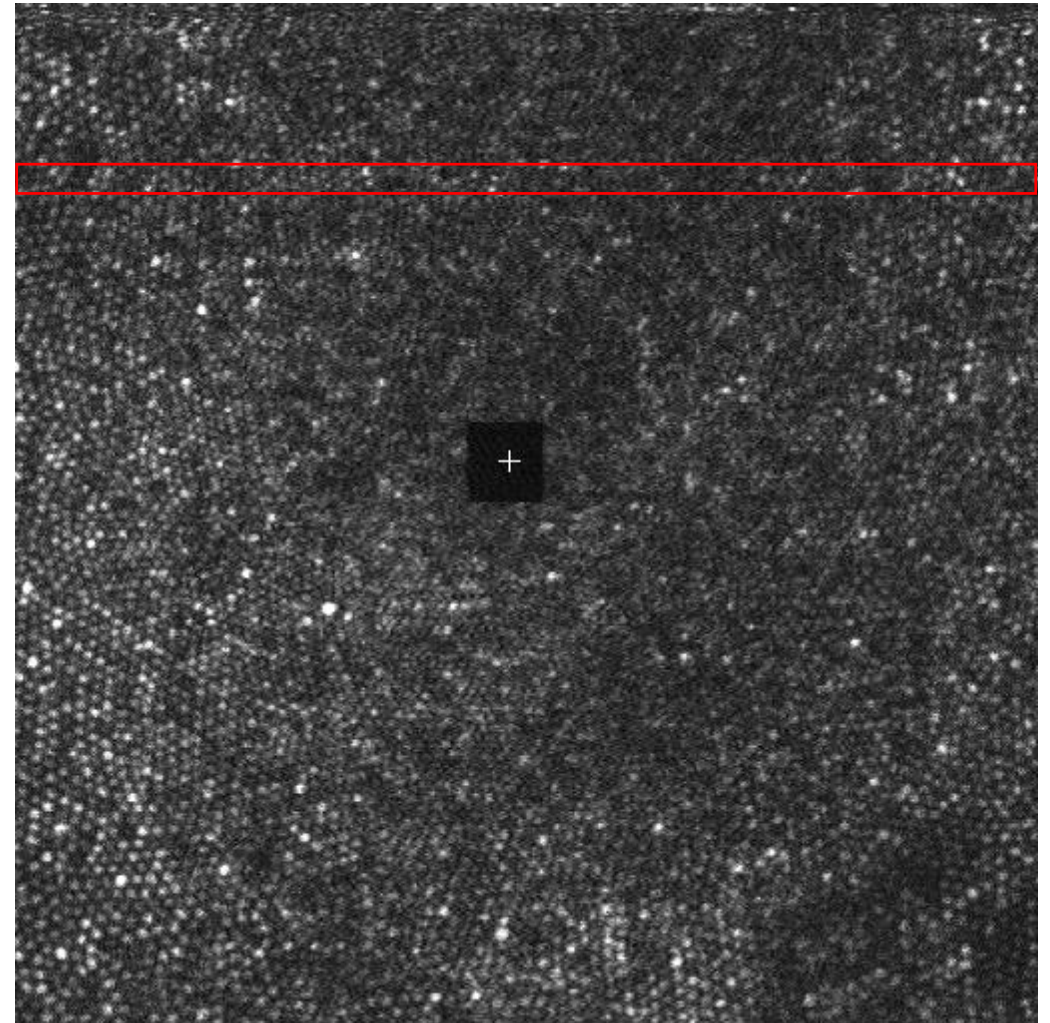
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Raw videos need to be registered to compensate for retinal image motion during the recording

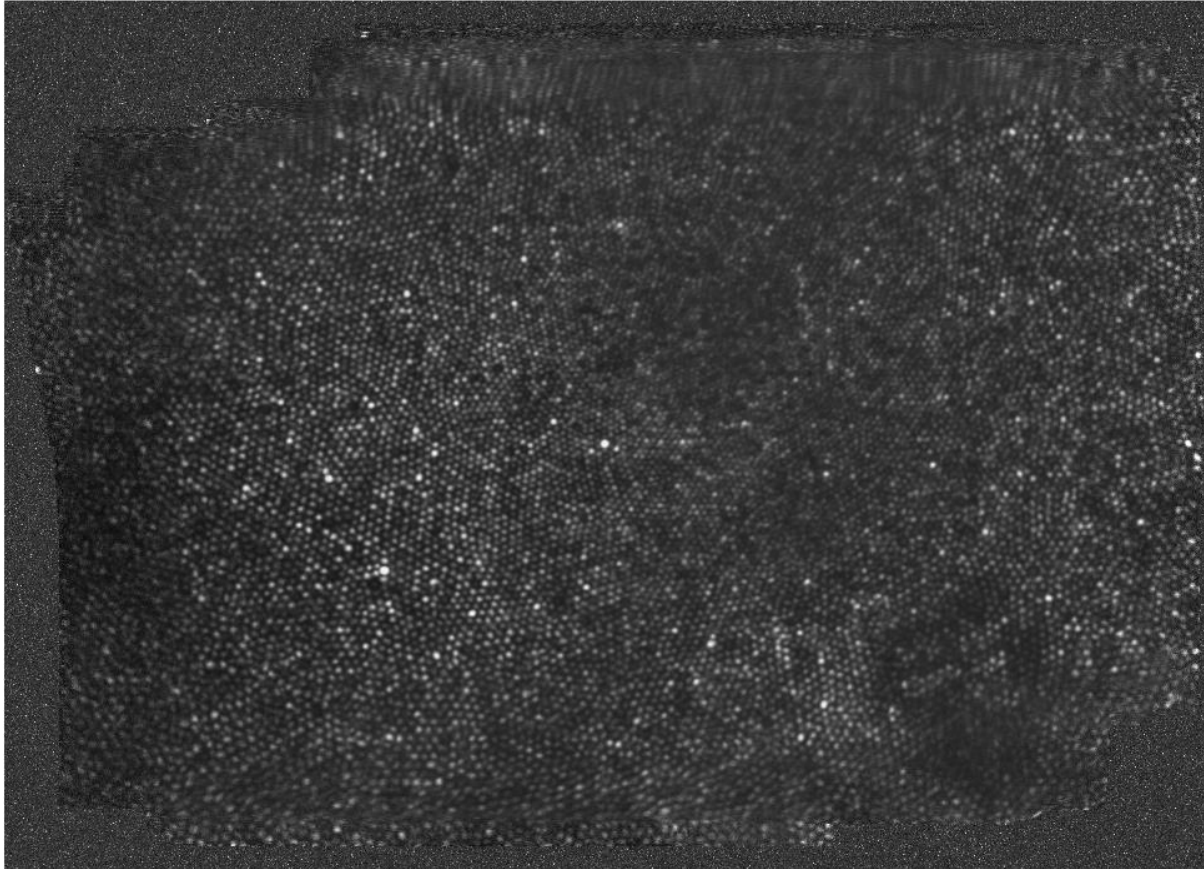


Raw video showing the retinal image motion

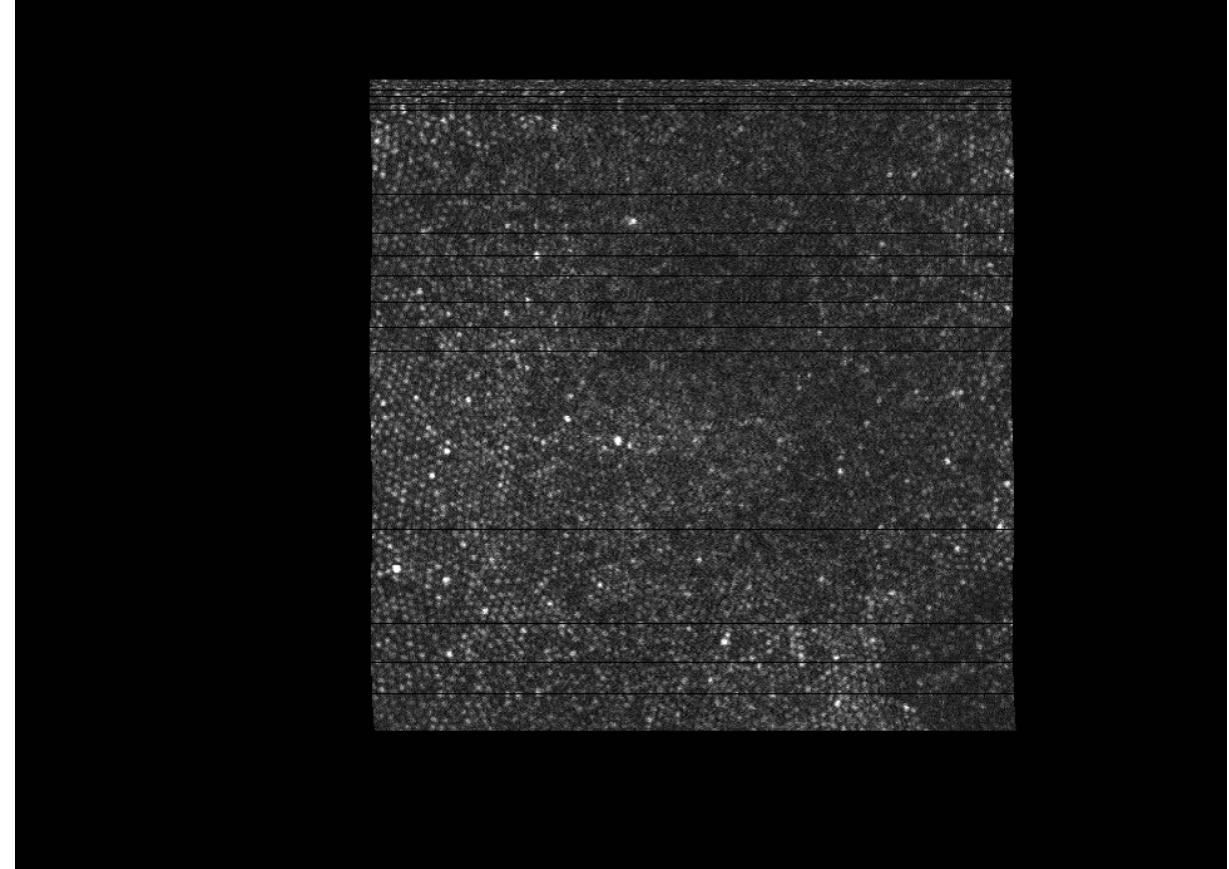


Single frame showing a narrow strip (16 pixels in height) used for strip-based image registration

A reference frame is automatically generated, and each video frame is aligned to the reference frame



Reference frame generated by the stabilization software and used for making the stabilized video



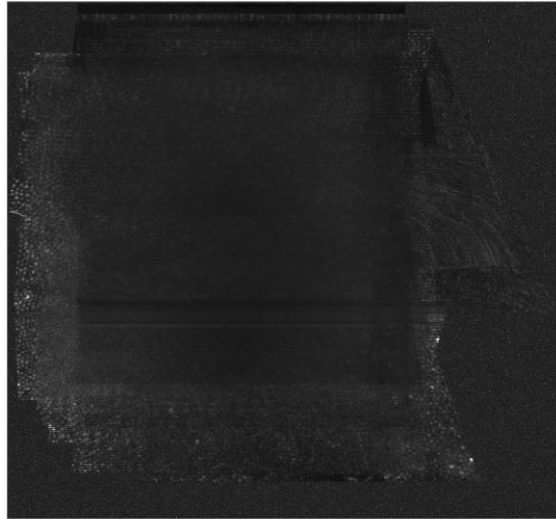
Stabilized video produced by the software from Austin Roorda. Blink frames are replaced with a blank image. Black horizontal lines are added when vertical drift occurs to prevent warping of the stabilized image.

For generating a stabilized image, it is efficient to trim the video before running the stabilization code

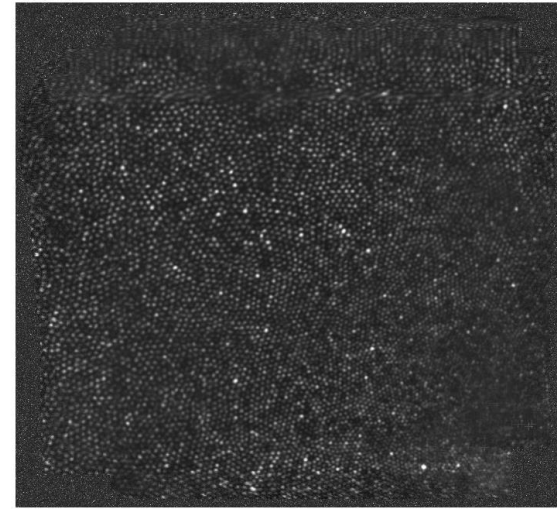
- Best 60-120 frames (2-4 seconds) can be selected for further analysis

- Image brightness and sharpness are prioritized
- Blinks and large eye movements are avoided

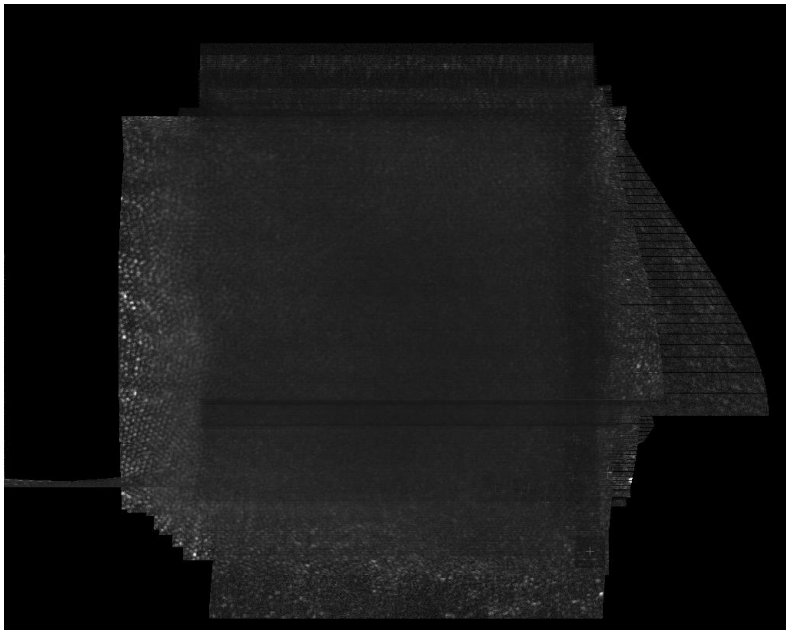
- If a video starts with a blink, it must be trimmed to avoid bad reference frame selection



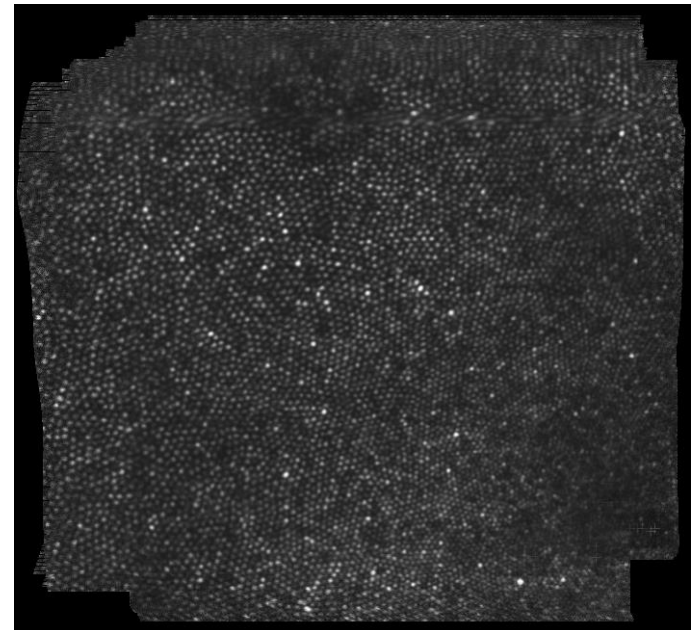
original
reference
frame



new reference
frame (trimmed
video)

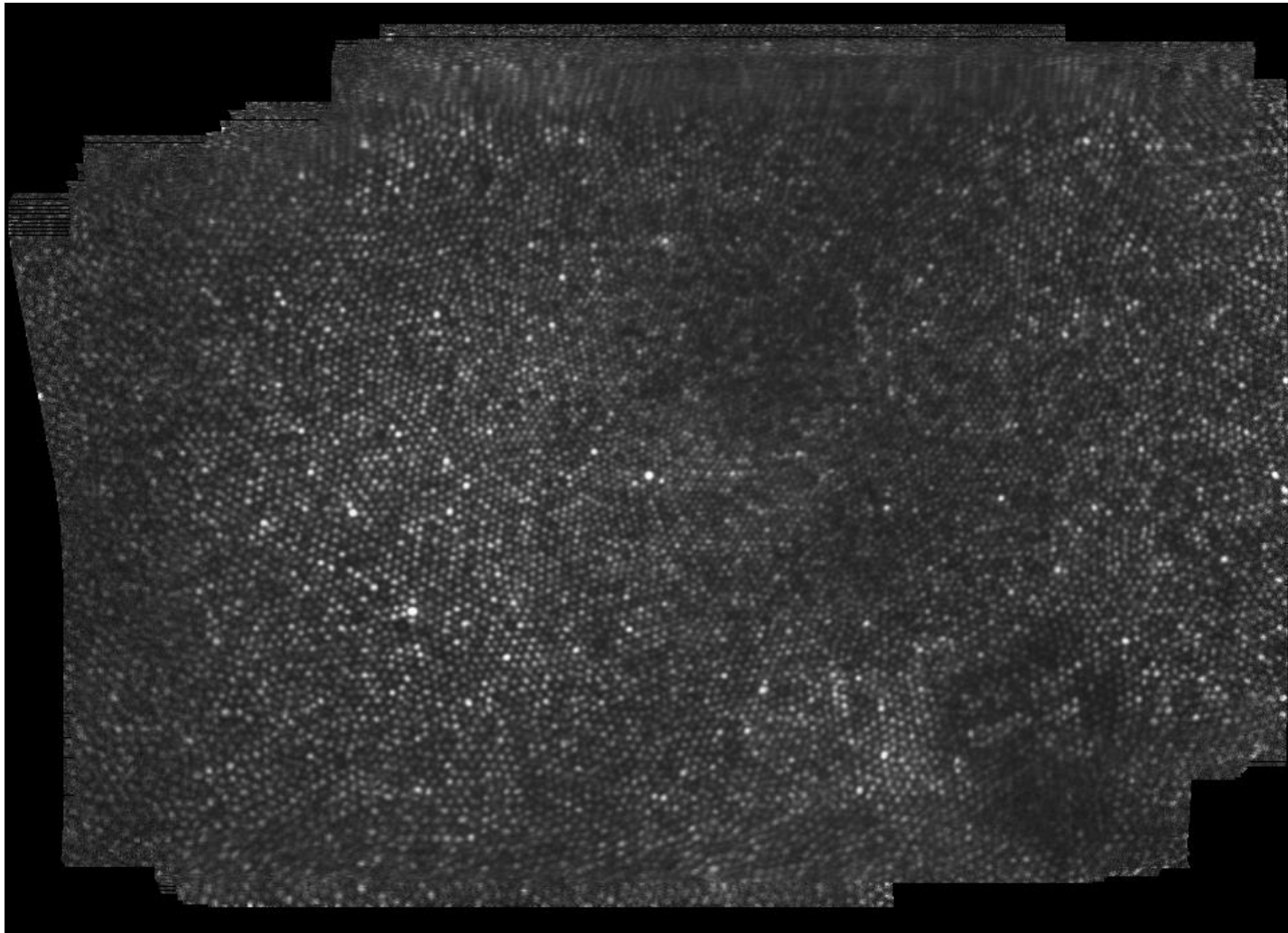


original
stabilized
frame



new
stabilized
frame
(trimmed
video)

The outputs from the stabilization code are a video, a frame-averaged image, and a MATLAB workspace



Frame-averaged image from the stabilized video

Workspace	
Name ^	Value
<input type="checkbox"/> analysedframes	268x1 double
<input type="checkbox"/> badframesandstrips	8896x5 double
<input type="checkbox"/> badstripthreshold	0.6000
<input type="checkbox"/> blinkframes	22x1 double
<input type="checkbox"/> coarsereferimage	629x768 dou...
<input type="checkbox"/> defaultsearchzone_ho...	384
<input type="checkbox"/> defaultsearchzone_ver...	55
<input checked="" type="checkbox"/> dep	0
<input type="checkbox"/> droppedframes	[28;34;53;118...
<input type="checkbox"/> endframe	300
<input type="checkbox"/> finereferimage	663x915 dou...
<input type="checkbox"/> frameheight	512
<input type="checkbox"/> frameshifts_strips	8576x2 double
<input type="checkbox"/> frameshifts_strips_spli...	8576x2 double
<input type="checkbox"/> frameshifts_thumbnails	278x2 double
<input type="checkbox"/> framewidth	512
<input type="checkbox"/> goodframesegmentin...	10x3 double
<input type="checkbox"/> largemovementframes	25x1 double
<input type="checkbox"/> maxvals_strips	8576x1 double
<input type="checkbox"/> maxvals_thumbnails	278x1 double
<input type="checkbox"/> minpercentofgoodstri...	0.4000
<input type="checkbox"/> noises_strips	8576x1 double
<input type="checkbox"/> noises_thumbnails	278x1 double
<input type="checkbox"/> numbervideoframes	300

- stabilized video saved as [vName]_stab.avi
- Frame-averaged image saved as [vName].tiff
- MATLAB workspace saved as [vName]_meanrem_960_hz_[xxxx].mat

Variables saved to the MATLAB workspace.

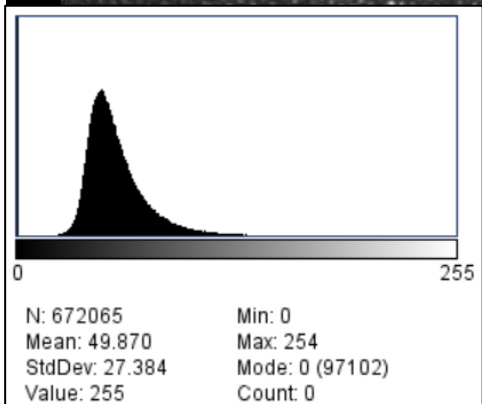
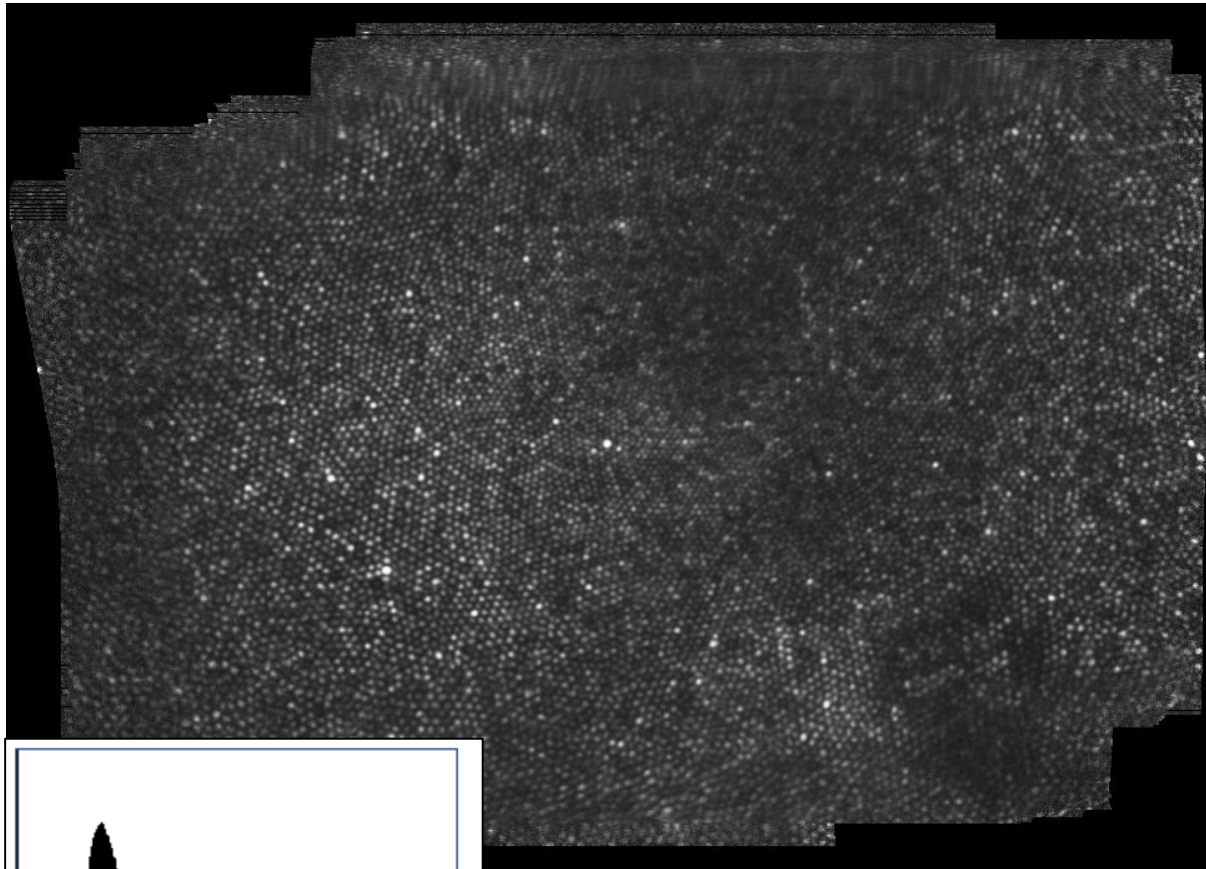
Summary of video processing steps

1. Trim the video to select the best 60-120 frames (only required if video starts with a blink, but makes processing much faster overall)
2. Run the MATLAB code from Austin Roorda:
`stabilizefromraw_multiple.m`
3. View the output files to verify that good stabilization is achieved (use ImageJ or VirtualDub for frame-by-frame viewing of the stabilized video)
4. Organize the output files and proceed to the next video (or batch of videos)

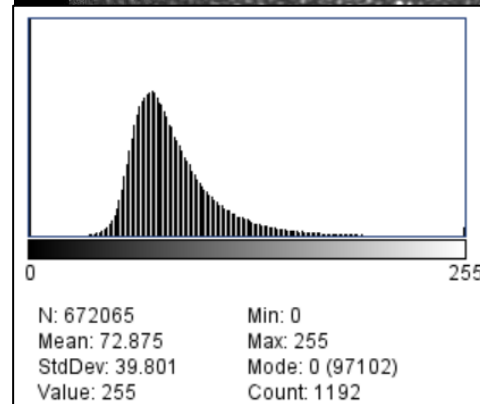
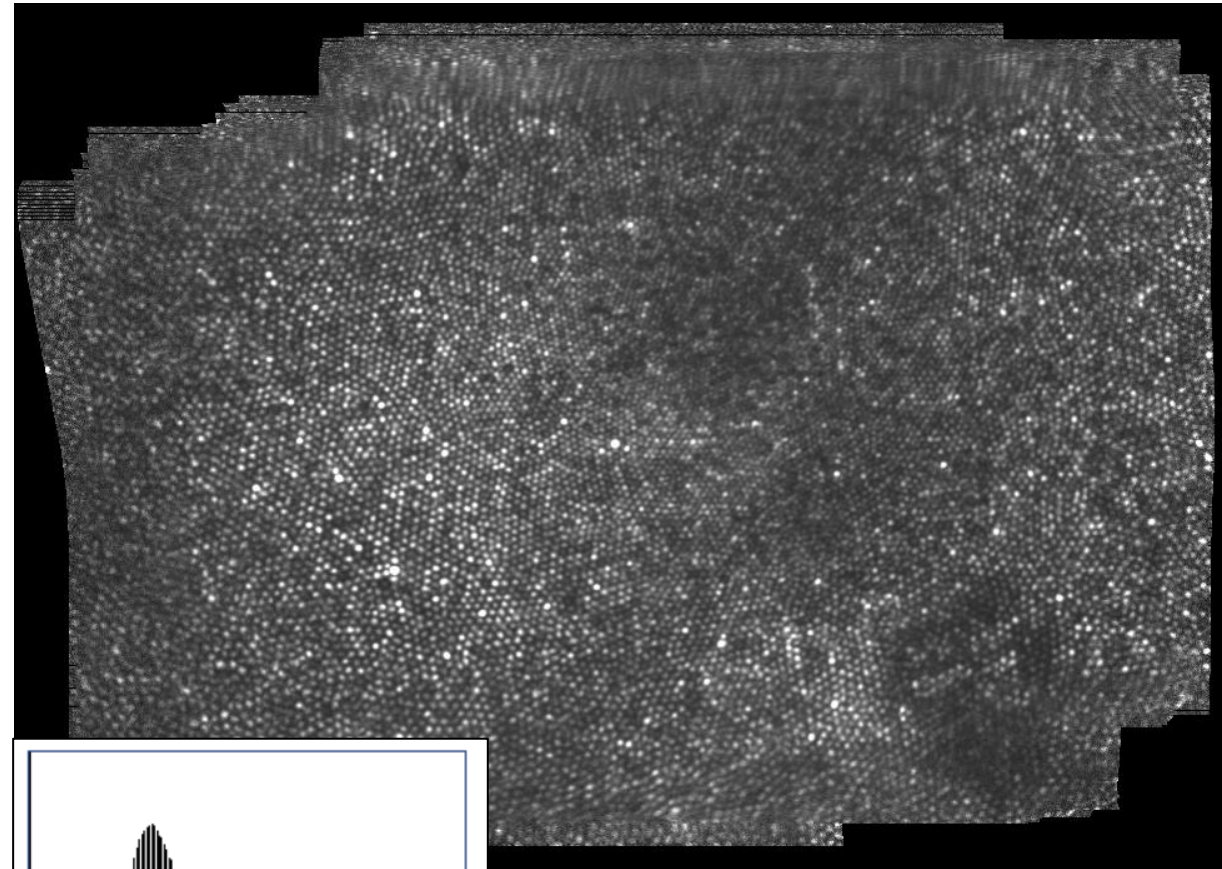
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Conduct a linear rescaling of the pixel values to optimize the contrast



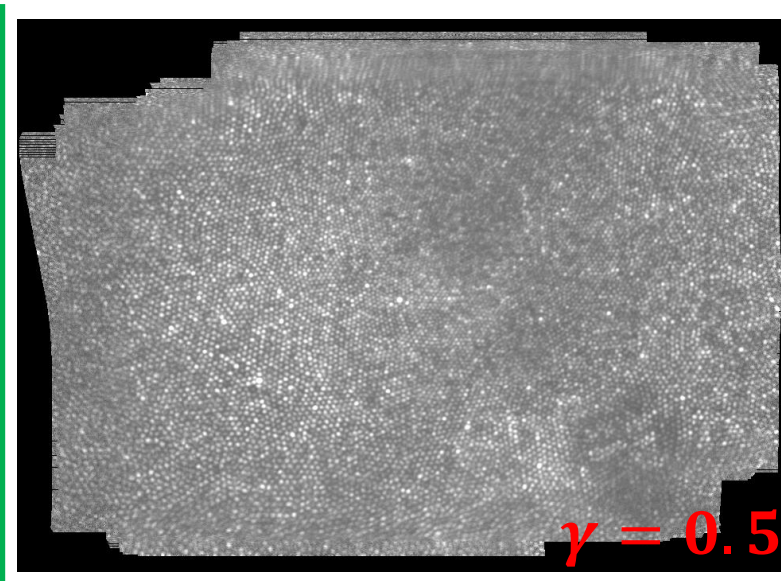
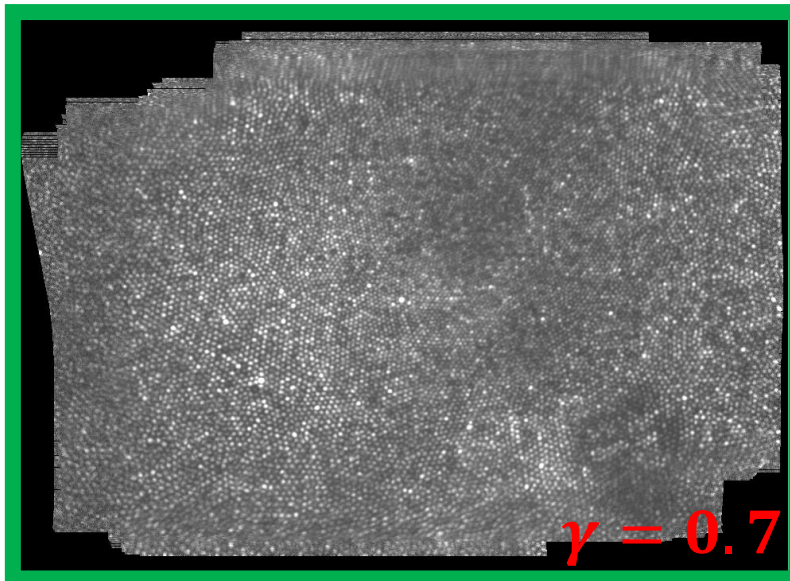
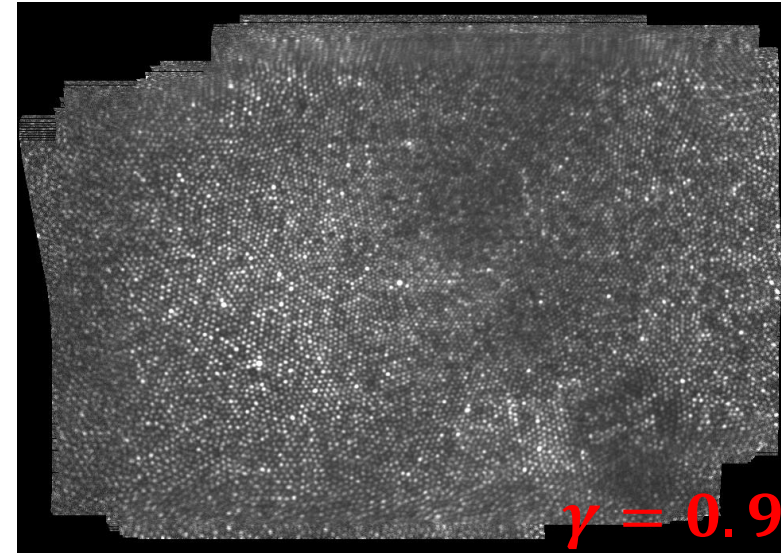
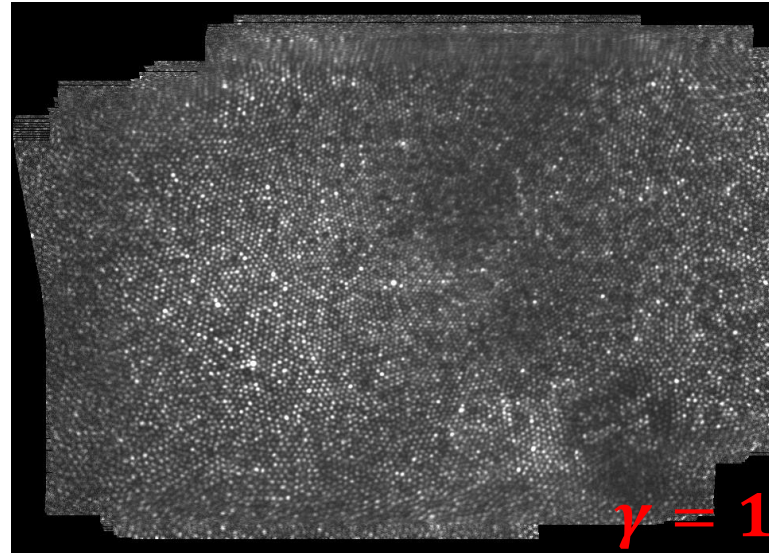
Original stabilized image



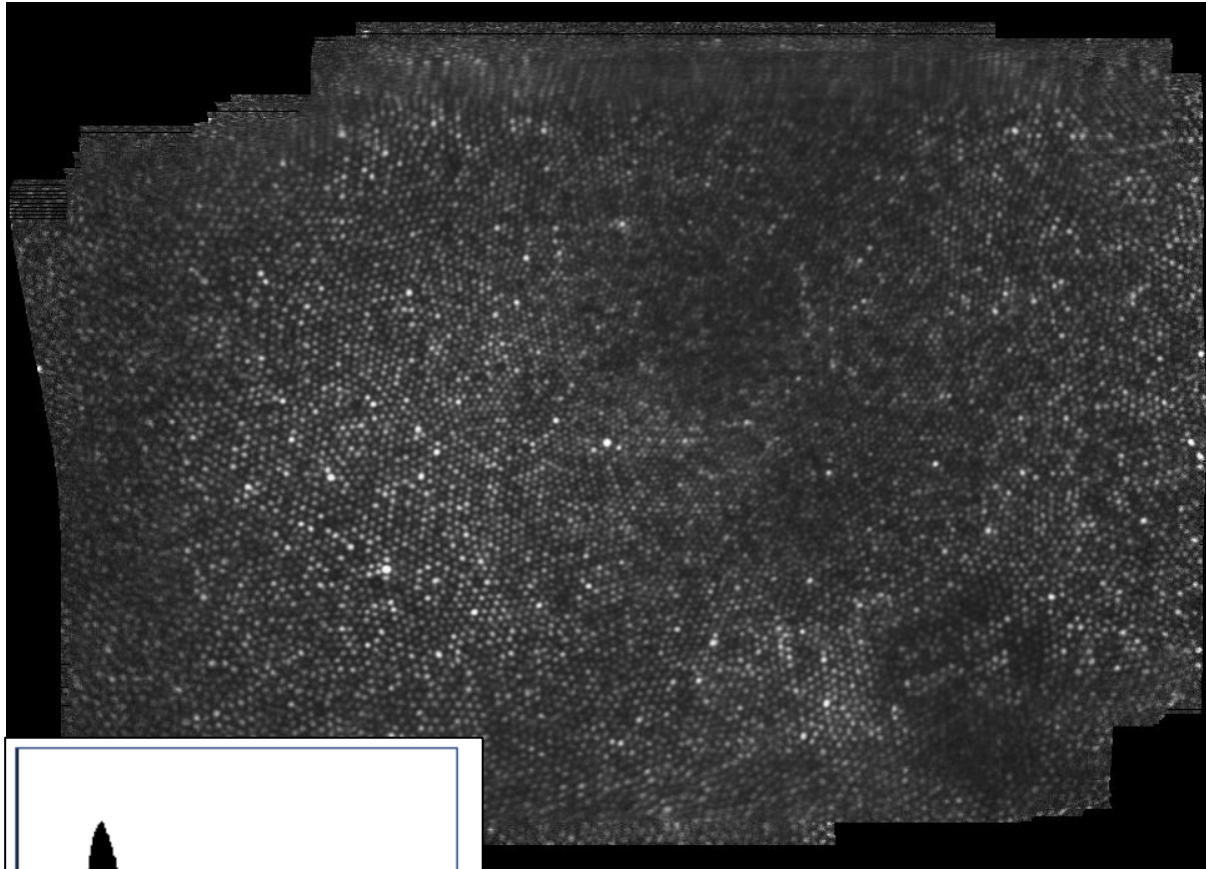
Contrast-enhanced image with 0.4% of the pixels (~1200) saturated. The mean pixel value has increased from 50 to 73.

Gamma correction improves the appearance of the image through nonlinear rescaling of pixel values

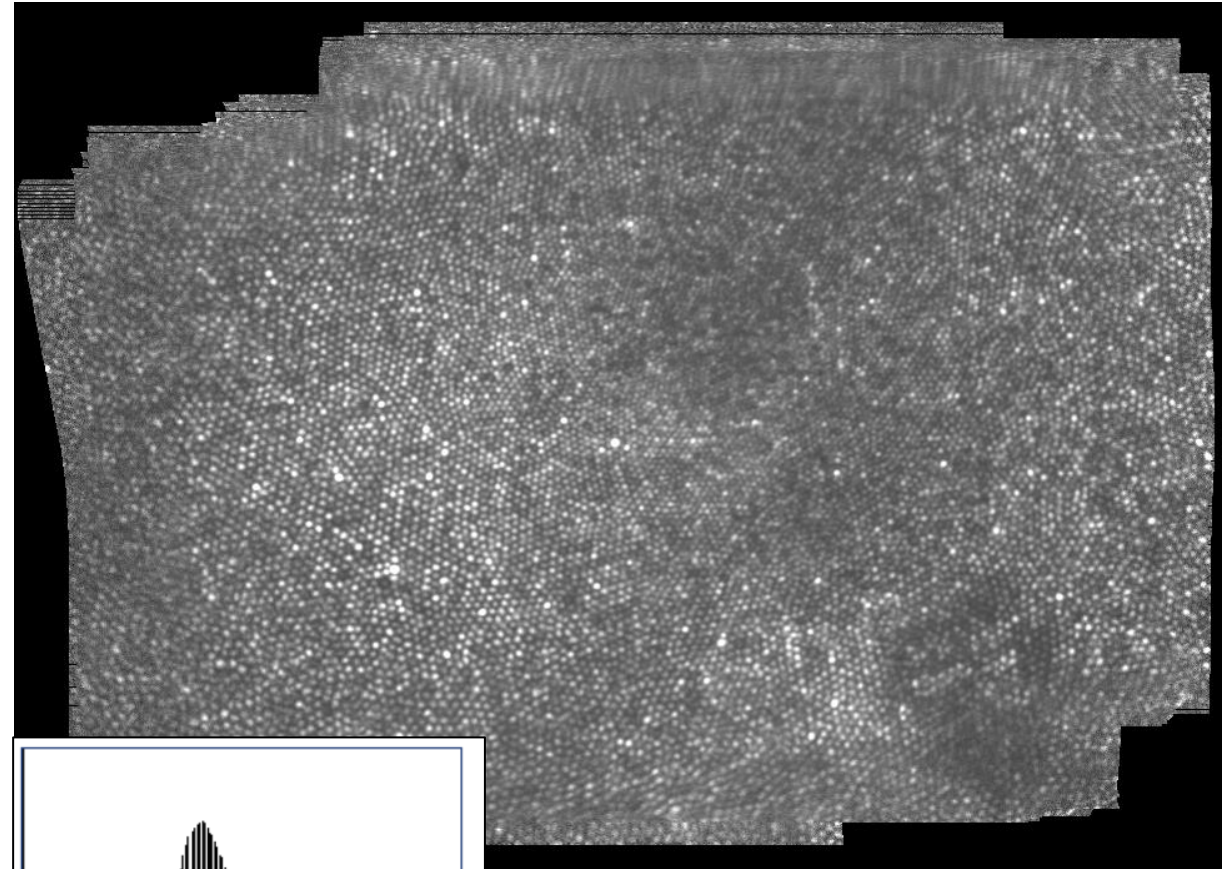
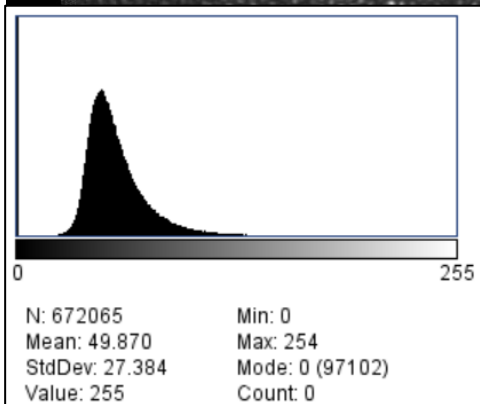
$$V_{out} = AV_{in}^{\gamma}$$



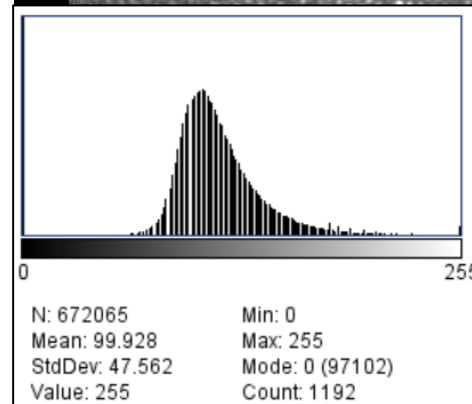
Side-by-side comparison of the original image and the contrast-enhanced, gamma-corrected image



Original stabilized image



Gamma-corrected image with $\gamma = 0.7$. The mean pixel value is 100 (compared with 50 for the original and 73 for the contrast-enhanced).



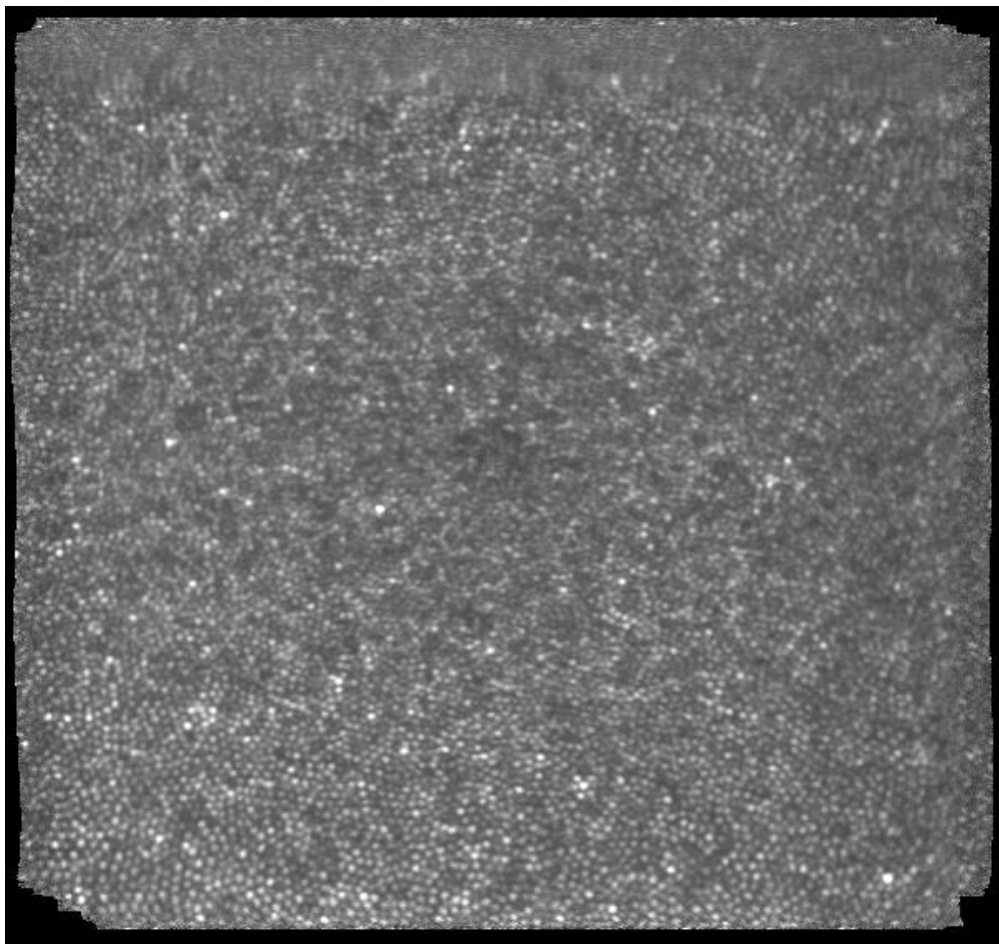
Open questions related to image processing

- Although contrast enhancement and gamma correction is good for displaying images, should we still use the raw images for analysis (such as cone detection)?
- Are there other rescaling or contrast-enhancing techniques that we may want to use for generating nice images for display or analysis?
- I have been using ImageJ for this image processing, which seems to work well. Does anyone have experience with other software which might be useful for image processing?

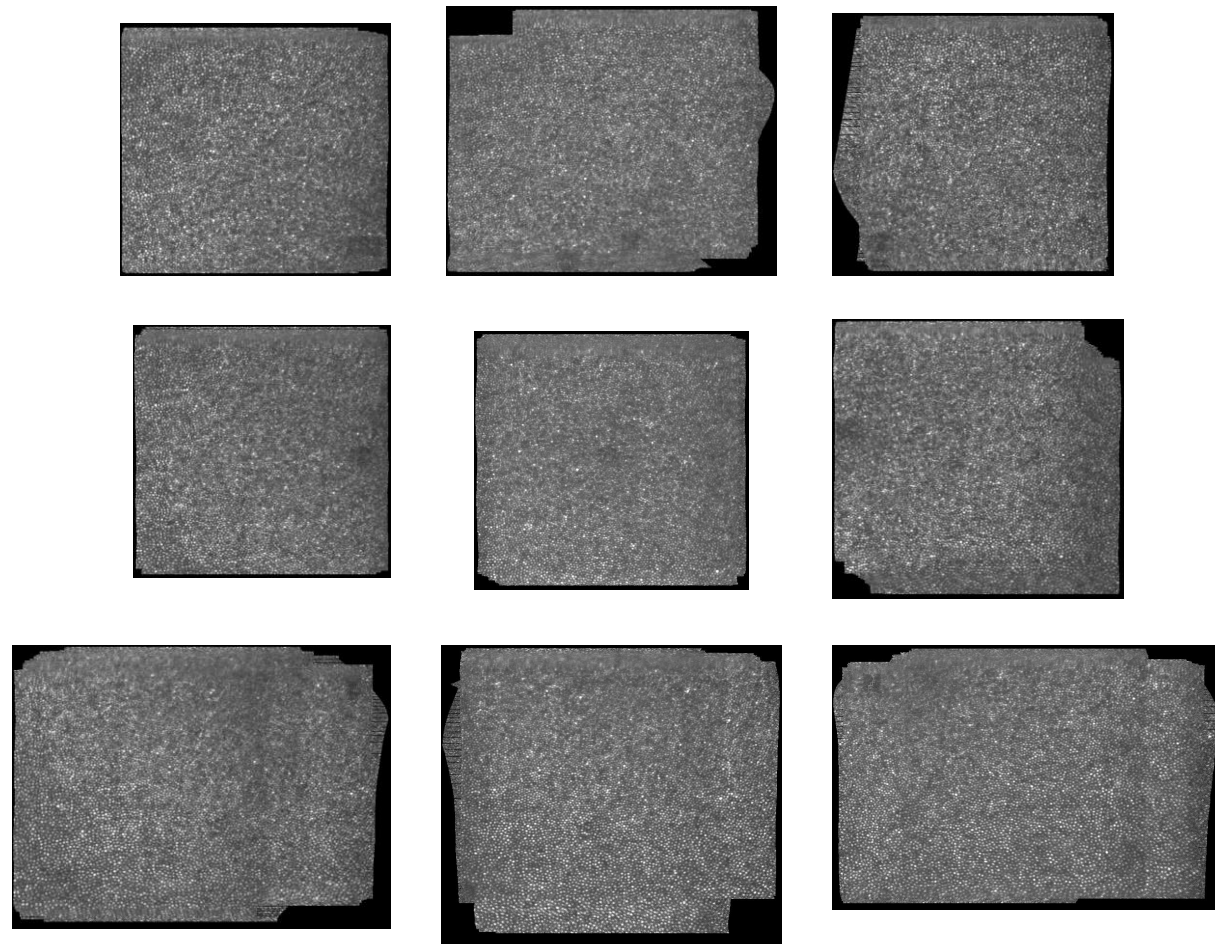
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Nine fixation points were used during the imaging session, resulting in nine stabilized images

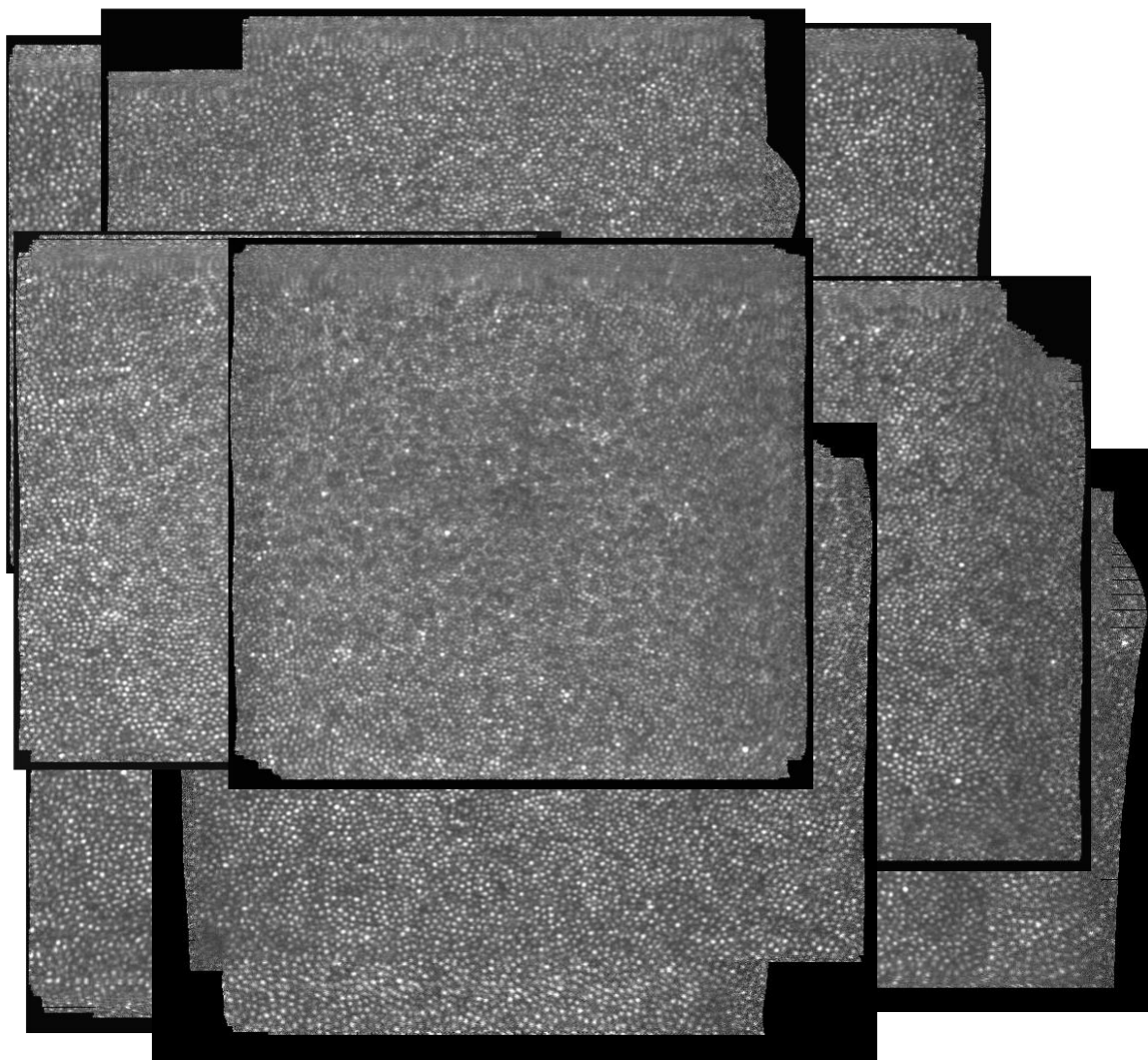


Stabilized frame from central fixation point for Z055

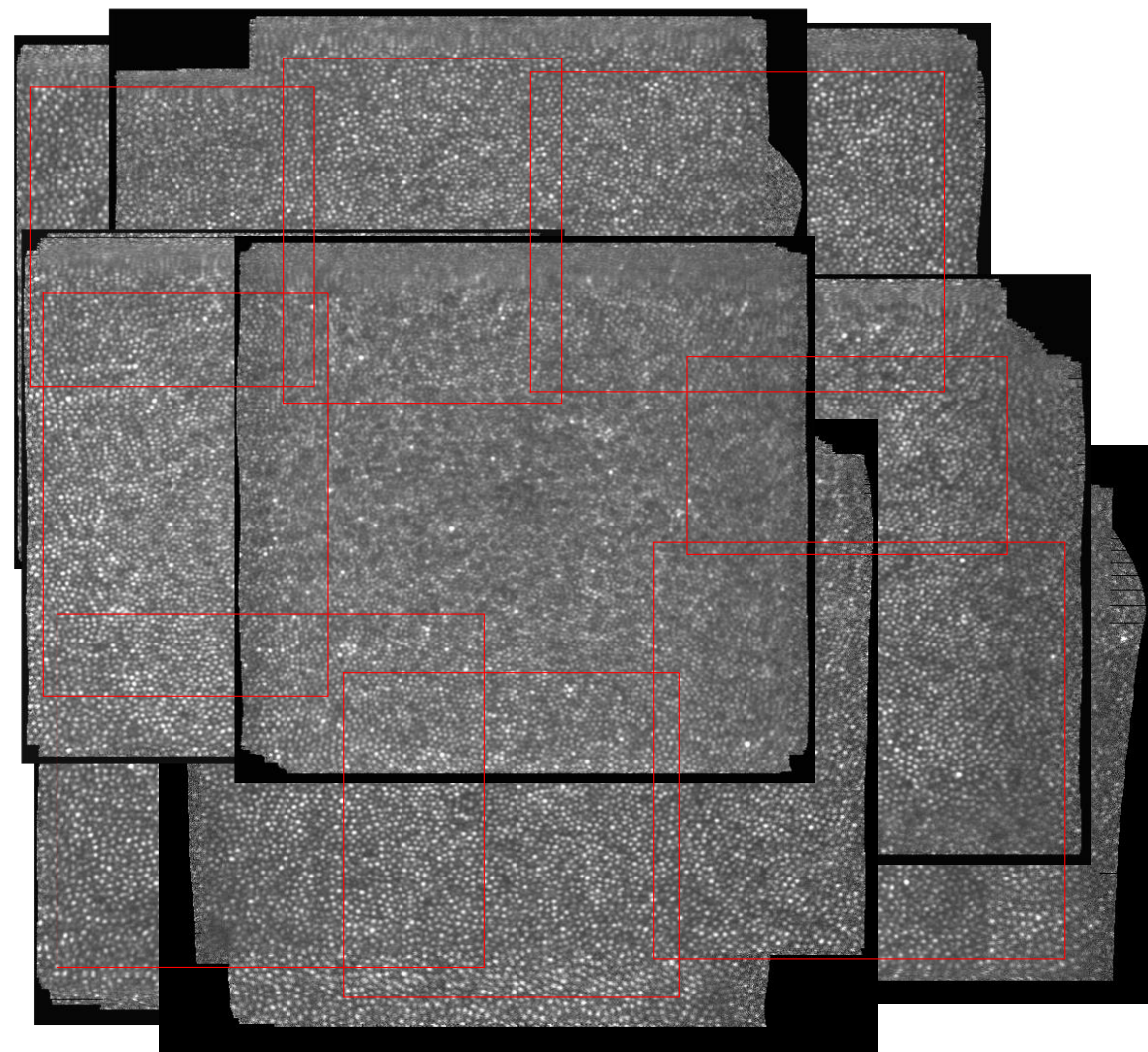


Grid of 9 stabilized images at the beginning of the montage procedure

Images were manually aligned in a vector graphics program by making one image partially opaque

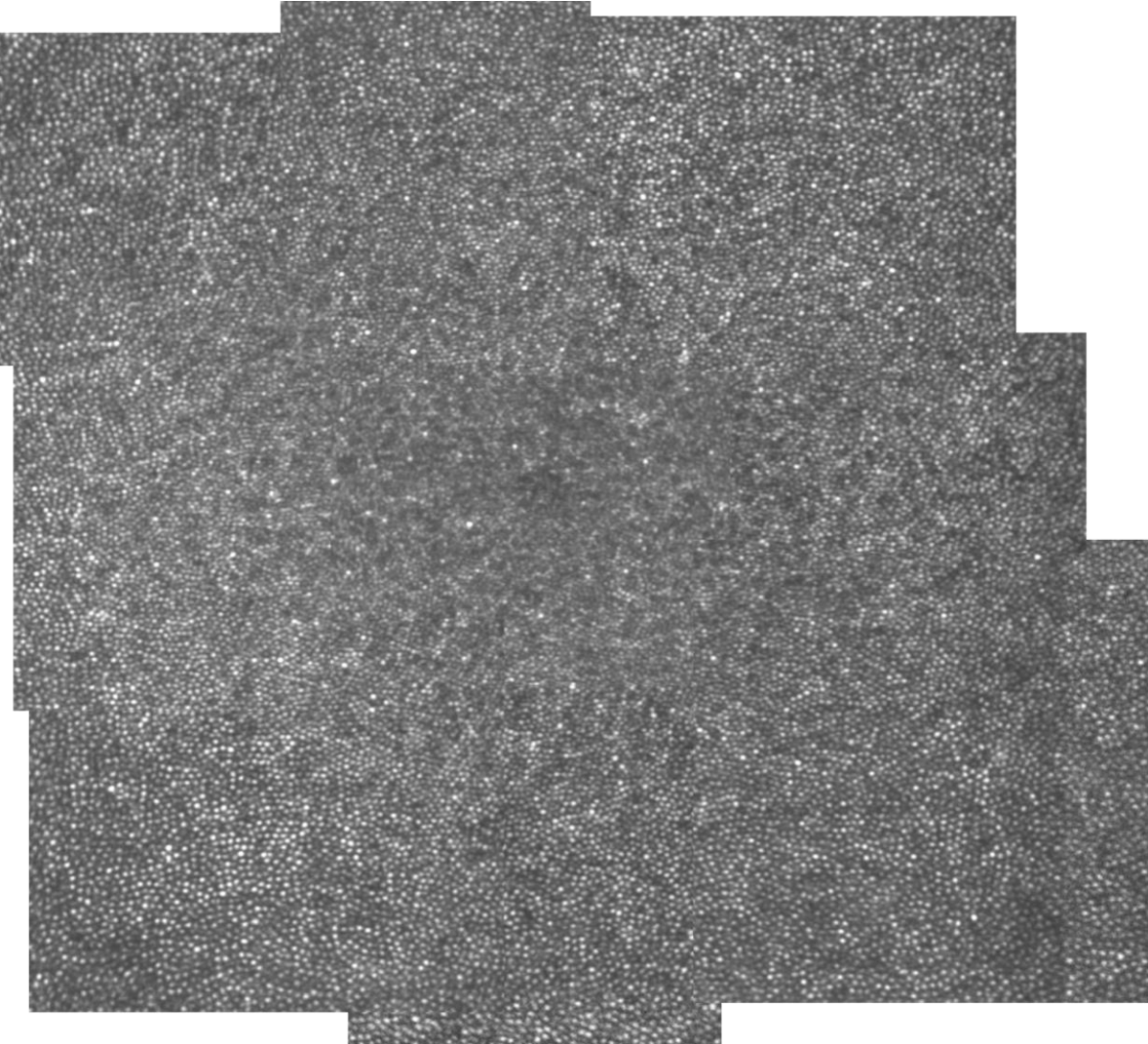


9 images after being aligned to a common reference frame

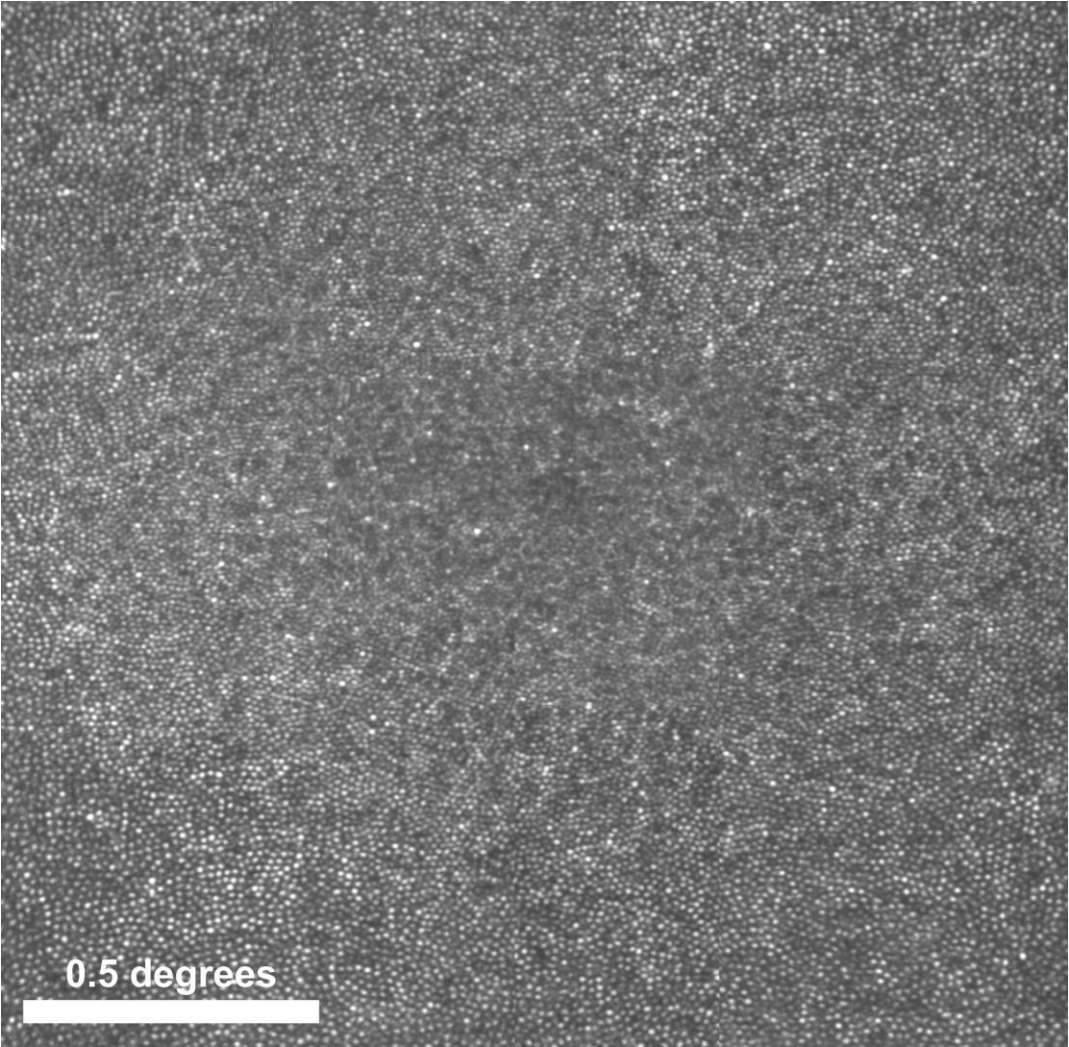


crop regions selected

After cropping and layering each image, brightness is fine-tuned to minimize the image borders

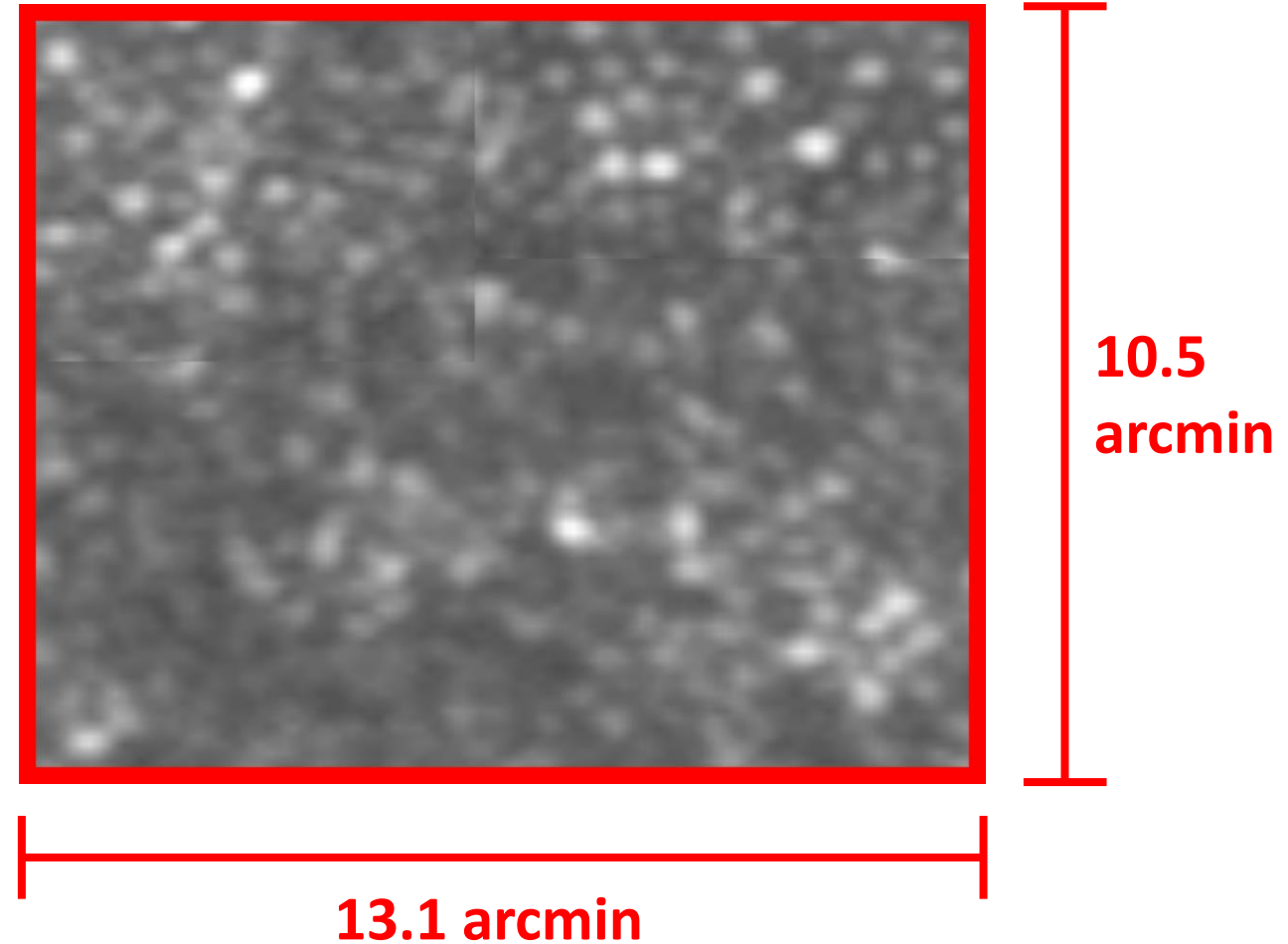


retinal montage after cropping and fine-tuning the brightness

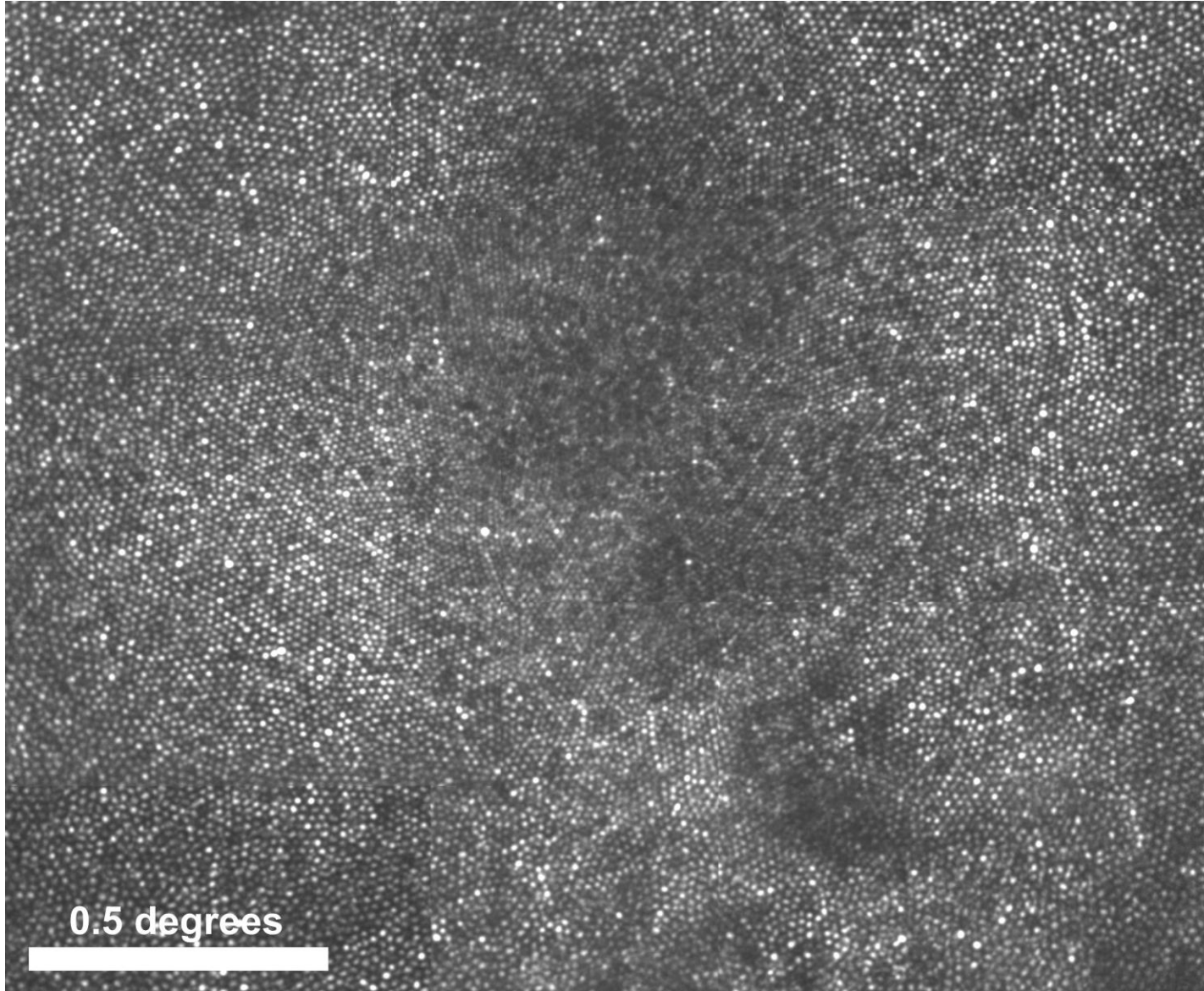


retinal montage after final cropping, with scale bar

Stitching lines are visible when zooming in on the boundaries between the different images



Retinal montage for subject Z160



Summary from retinal montage investigation

- Manual alignment of the different images is feasible
- Stitching lines are visible when zooming in, but this does not seem like a significant issue
- The program I used for doing the montage does not support uncompressed .tiff output, but CorelDRAW has this feature
- We will also explore automated montage techniques

Outline

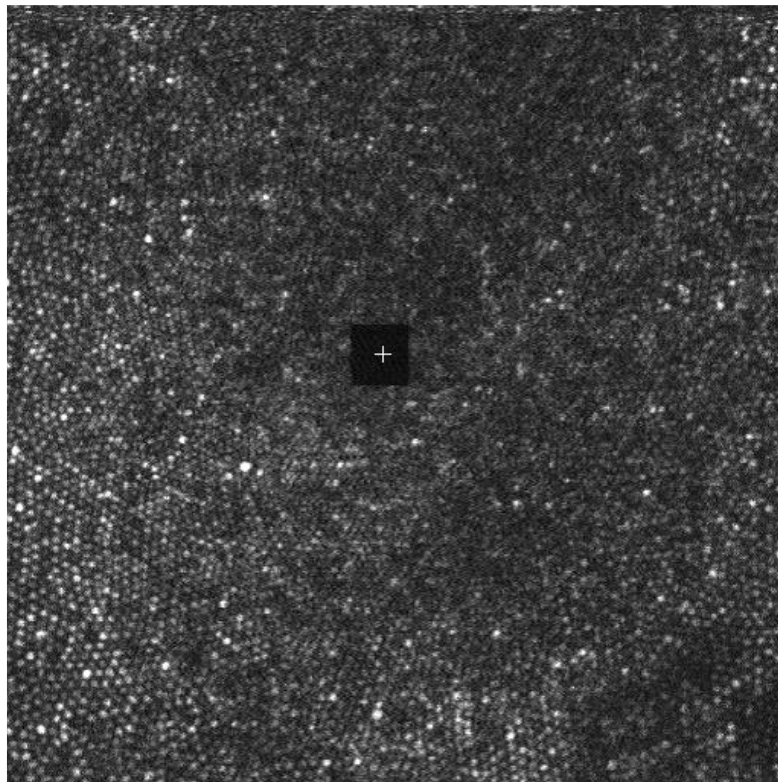
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Cone detection and cone density computation

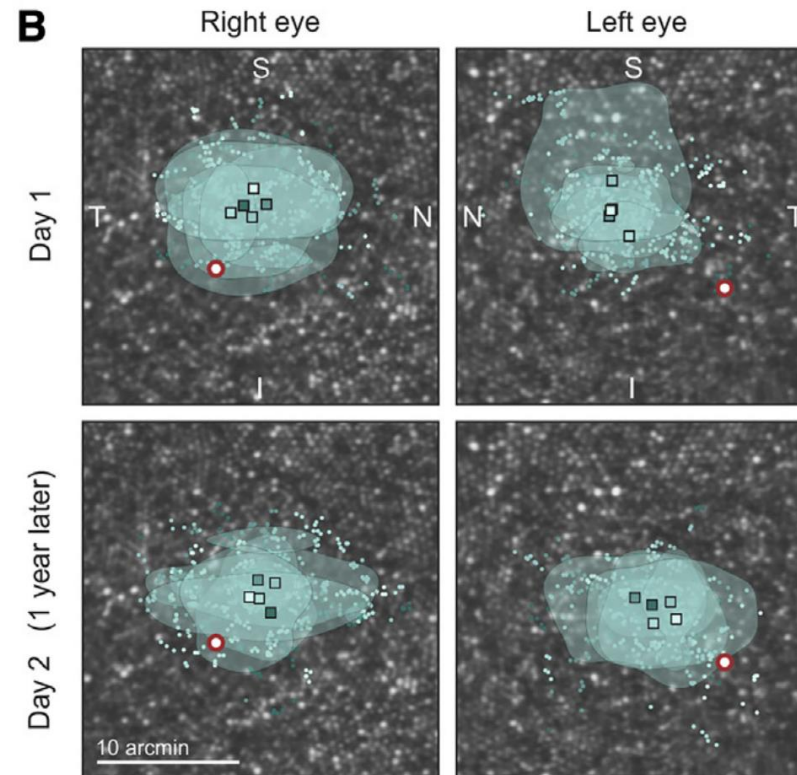
- Sanjana is investigating a cone detection algorithm that uses a computational neural network for semi-automated detection, based on Cunefare et al., Sci. Rep. **7** (2017).
- We also have a custom cone marking program from Austin Roorda for manual tagging of cones (which will likely be necessary at the center of the fovea). This is based on Li and Roorda, J. Opt. Soc. Am. A **24** (2007).
- Sam is developing a procedure for computing the cone density once we have the cone locations marked

We are planning to determine the PRL based on where the stimulus is displayed in each frame

- From the video of the retina, we know where the stimulus was presented for each frame, with 30 frames per second over about 10 seconds of fixation
- Using the stimulus presentation location will allow us to determine the PRL

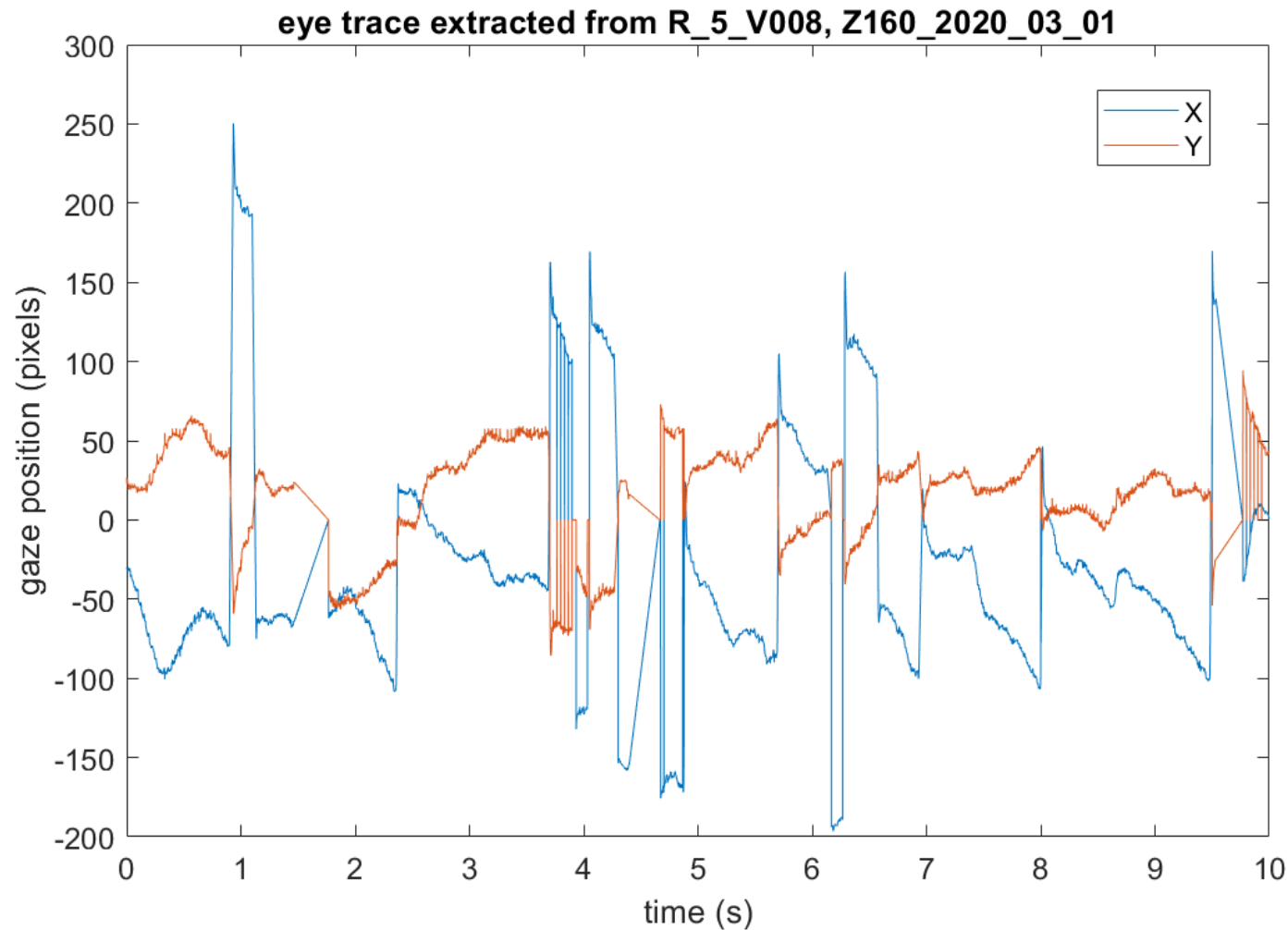


single frame, showing the stimulus location on the retina



- This approach was used by Reiniger et al. to determine the PRL
- Dots represent stimulus locations during fixation trials

The image registration code provides eye movement data as an output, so we can start analyzing this data



- Need to add tags for blinks and dropped frames (currently just interpolated with straight lines)
- Our system has 8.53 pixels per arcmin (y-axis range is from -23.4 arcmin to 35.2 arcmin)
- We have this data for each stabilized video, so someone could do some analysis on these eye traces

Conclusion

- The AOSLO is working and can resolve the smallest cones in the central fovea for the two subjects we have imaged
- We have a working video and image processing pipeline, but we may want to refine some of the steps
- We have additional data analysis steps planned to investigate foveal cone density, the PRL, and eye movements
- I need to finish writing up some procedures for running the system, but we are almost ready to train other users and begin running naive subjects
- Thanks to everyone who has helped with this project (Ashley, Sanjana, Sam, Edith Hartmann, Glory Linebach, Soh Hang Liu, Martina, Michele, Jannick Rolland, Austin Roorda, Pavan Tiruveedhula)!