

Longitudinal analysis of AF in NHP baseline *in vivo* retinal images

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Summary of data & findings

- 13 NHP in vivo datasets/eye samples from 12 subjects over 2 visits 6 months apart
- 6-8 images time-lapse images per sample, half of them acquired prior to dye injection
- 10 of 13 eyes exhibit no auto-fluorescent (AF) blobs (could match based on vessel patterns)
- Visit 1: 5 AF blobs and 7 infrared (IR) channel blobs detected
- Visit 2: 3 AF blobs detected (visibly better image acquisition quality)
- Multiple parameters to optimize:
 1. Detection -> contrast threshold, edge threshold, #layers in SIFT octave, Gauss filter sigma
 2. Matching -> method, match threshold, max. ratio, distance metric, uniqueness
- The largest baseline AF blob has a peak intensity 40 [A.U.], perimeter 200 um, area .003 mm²
- Intensity range is 0 to 255 [A.U.]; 1 image pixel = 7 um in sample

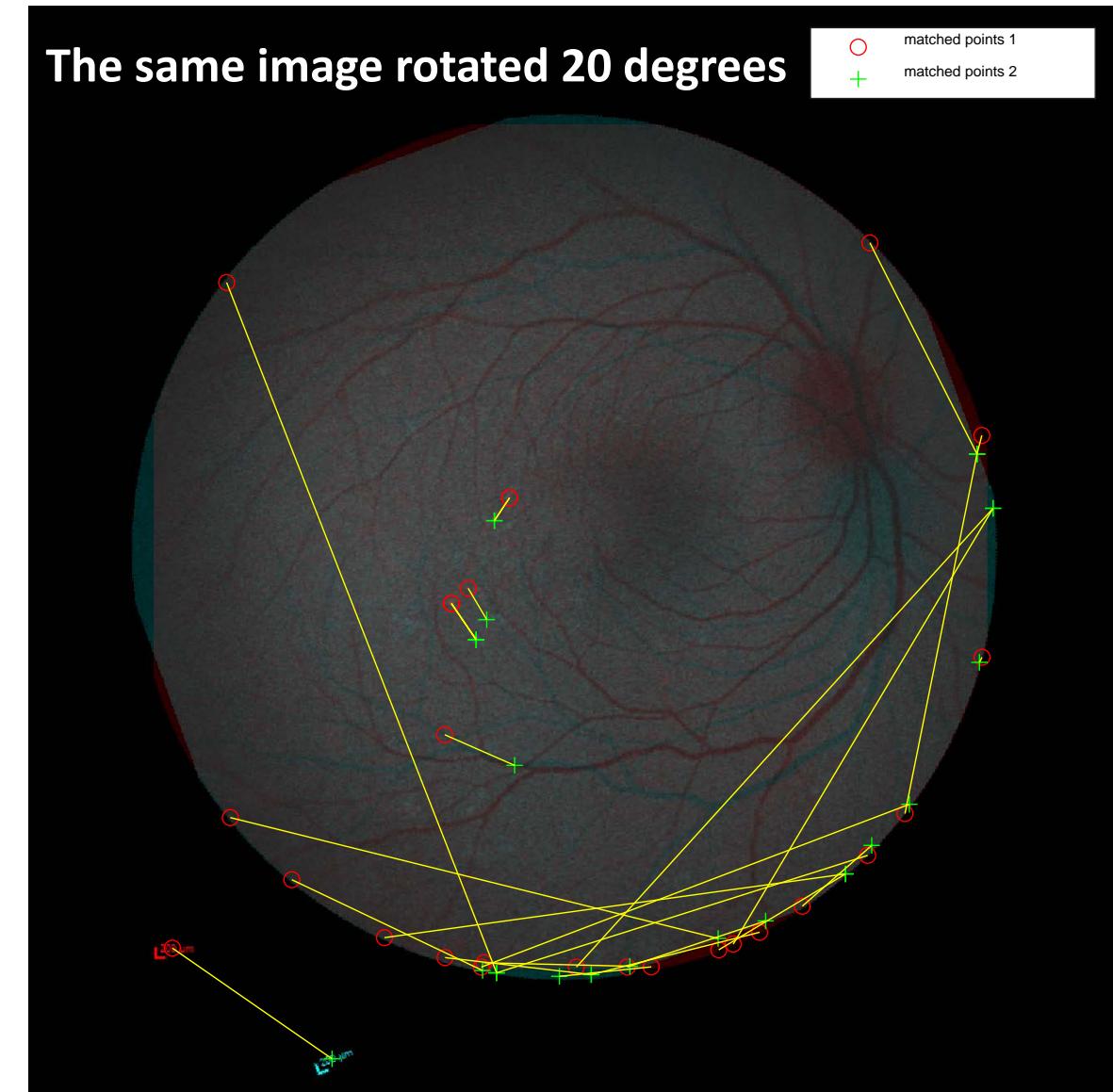
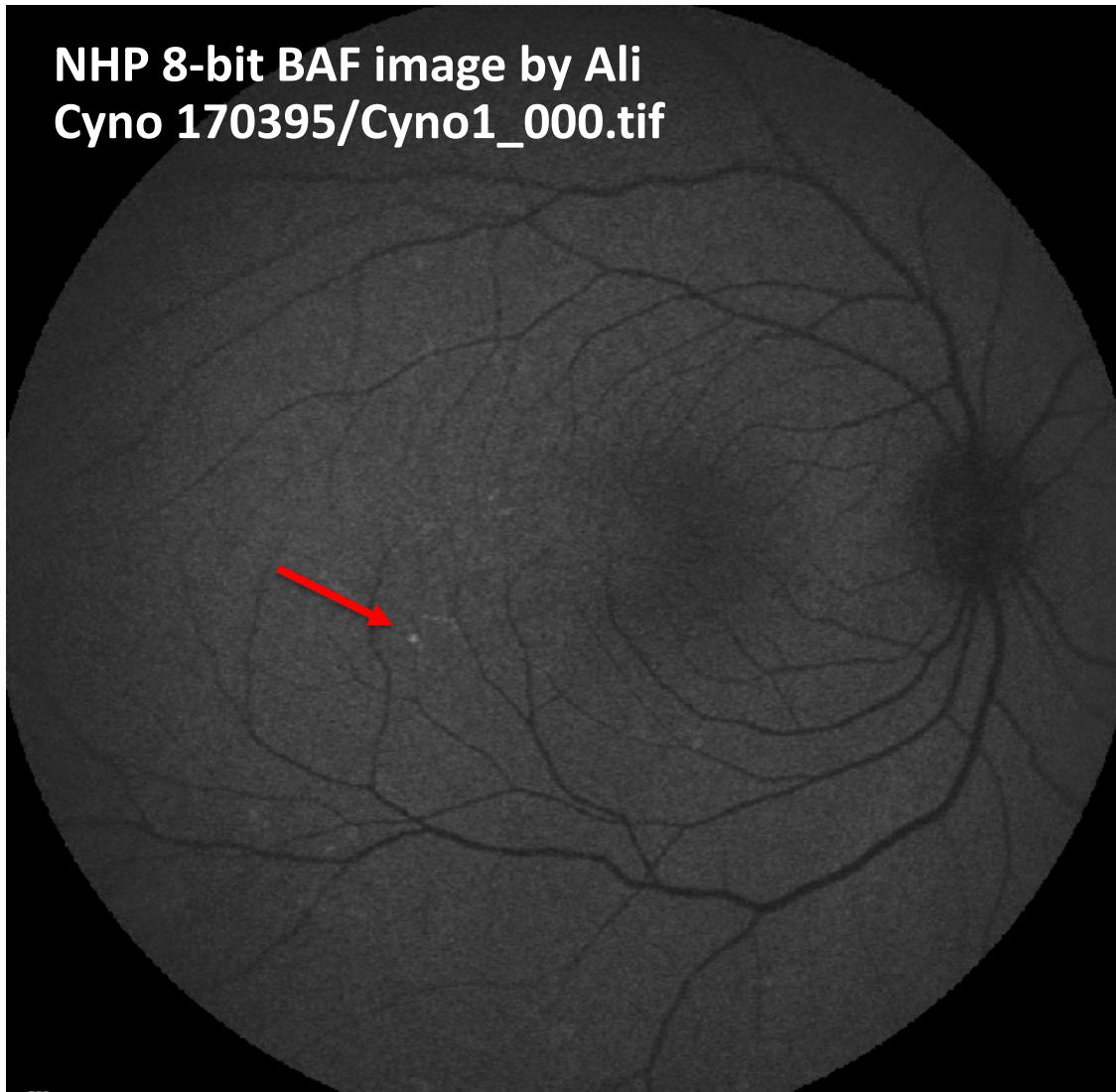
Note: Figures w SIFT detections show the strongest 50, 125 or 250 points

Number of detected AF blobs per eye

		Visit 1	Visit 2	AF Match
Cyno 170395	-	1	0	--
Cyno 180424	-	0	0	--
Cyno 191797	-	0 (1xIR)	0	
Cyno 191800	-	0 (2xIR)	0	
Cyno 191815	-	0	0	--
Cyno 191817	-	0 (1xIR)	0	
Cyno 191823	-	0	0	
Cyno 200188	-	0	n.a.	
<u>Cyno2 200188</u>	-	<u>3 (1xIR)</u>	<u>2</u>	<u>SURF/SIFT</u>
Cyno 200190	-	0	0	
Cyno 200193	-	0	0	
Cyno 200217	-	0 (2xIR)	0	
<u>Cyno 200251</u>	-	<u>1 (nerve)</u>	<u>1</u>	<u>SIFT</u>

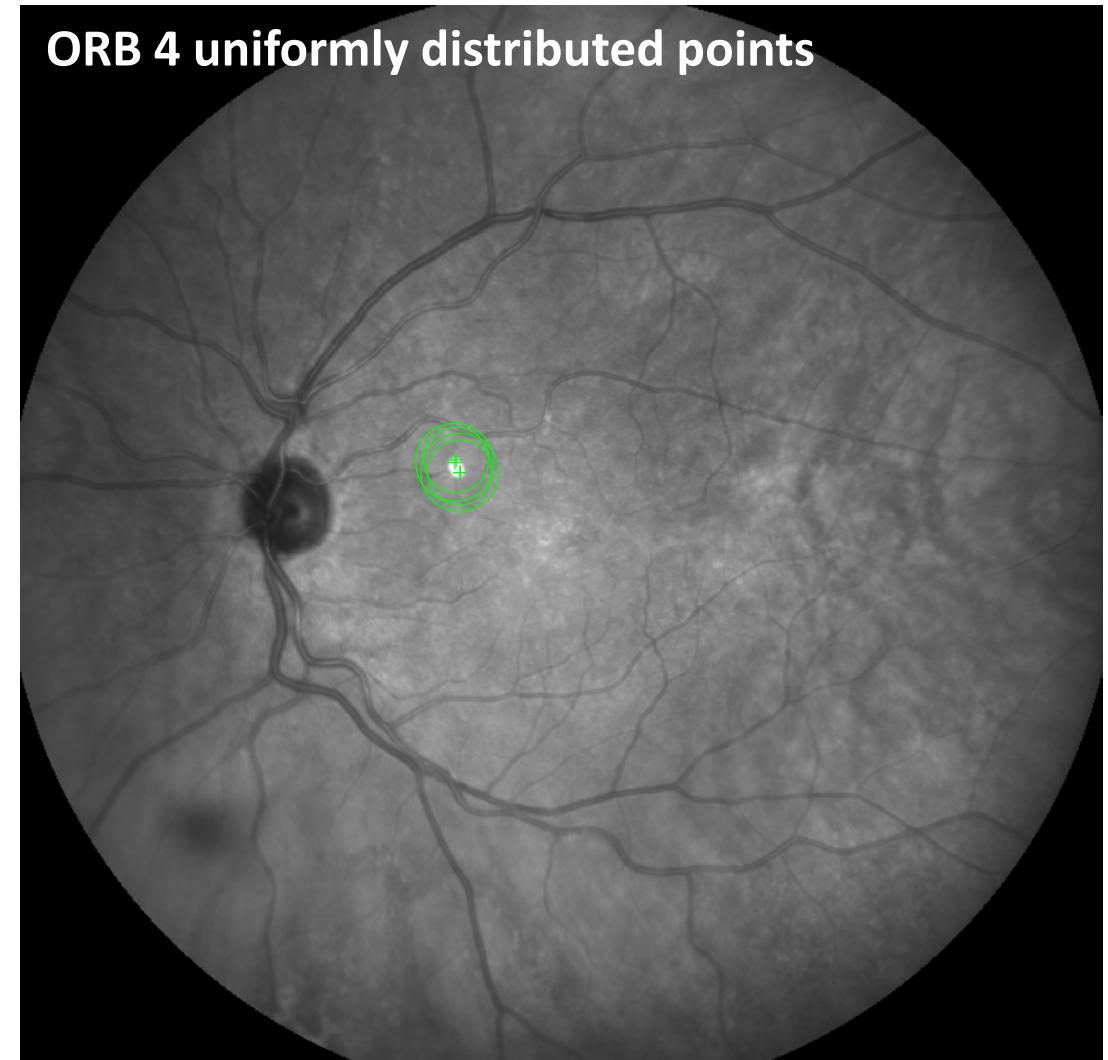
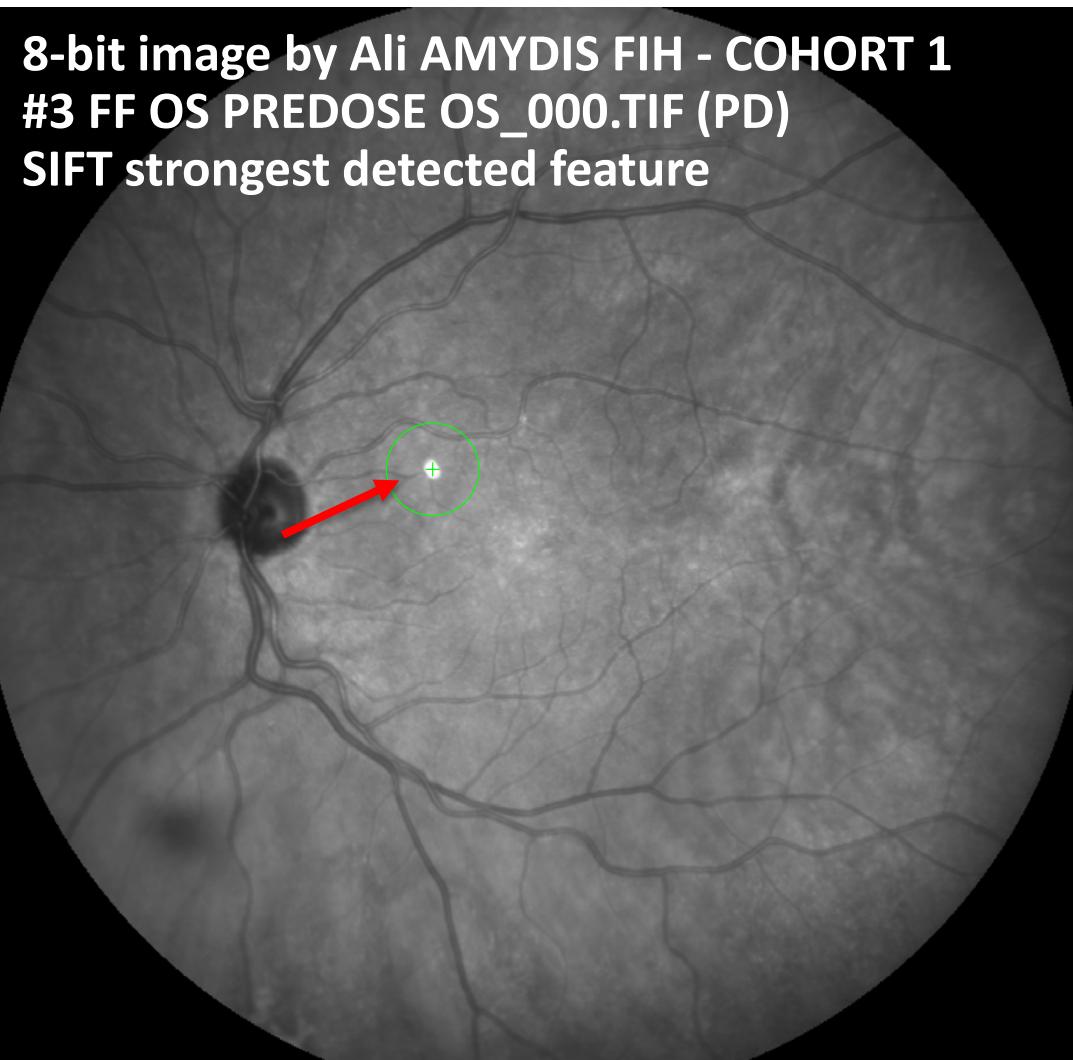
When AF (or IR) blobs are present, images can be tracked & matched in longitudinal datasets

Example of AF matching with the SIFT detector



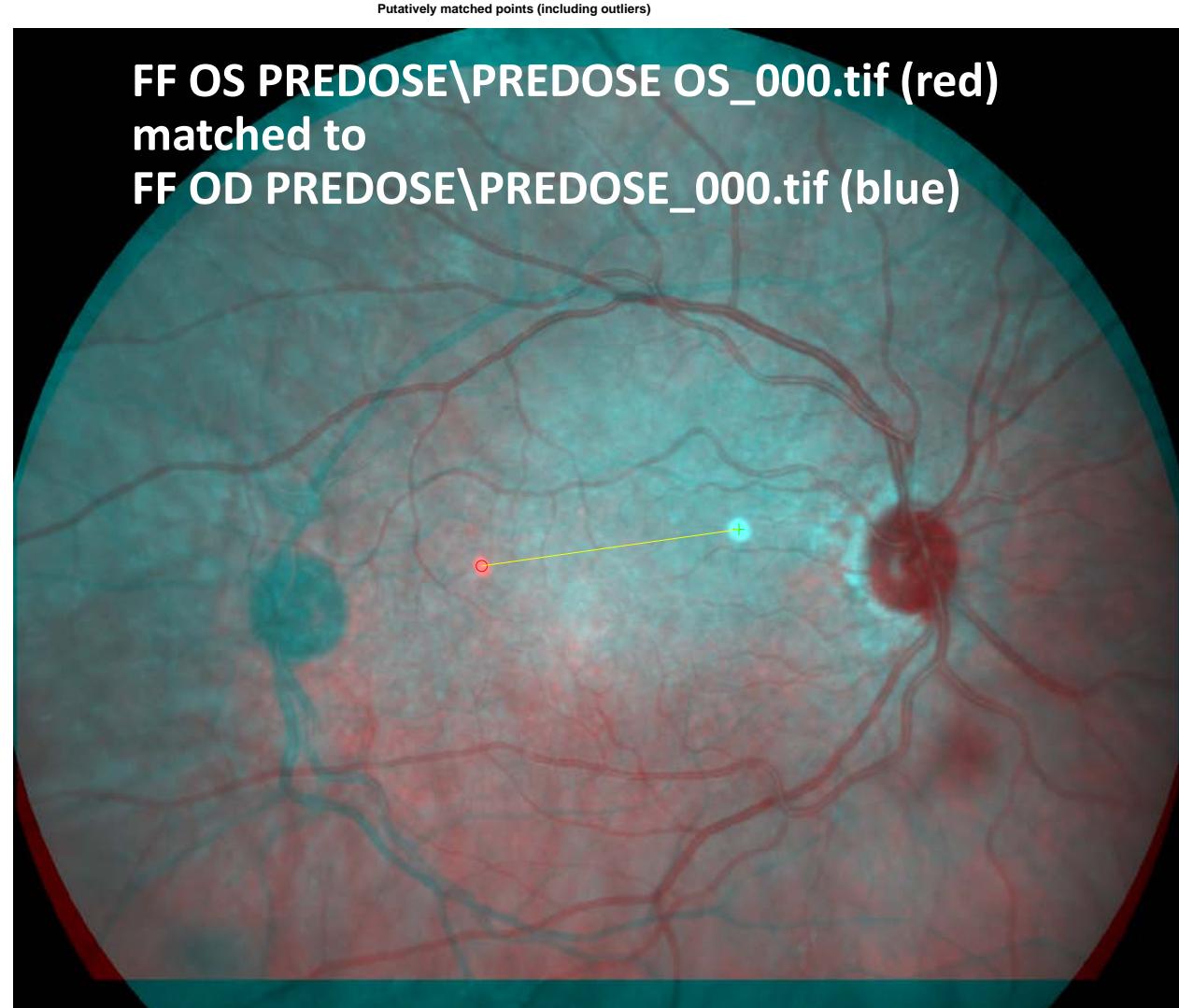
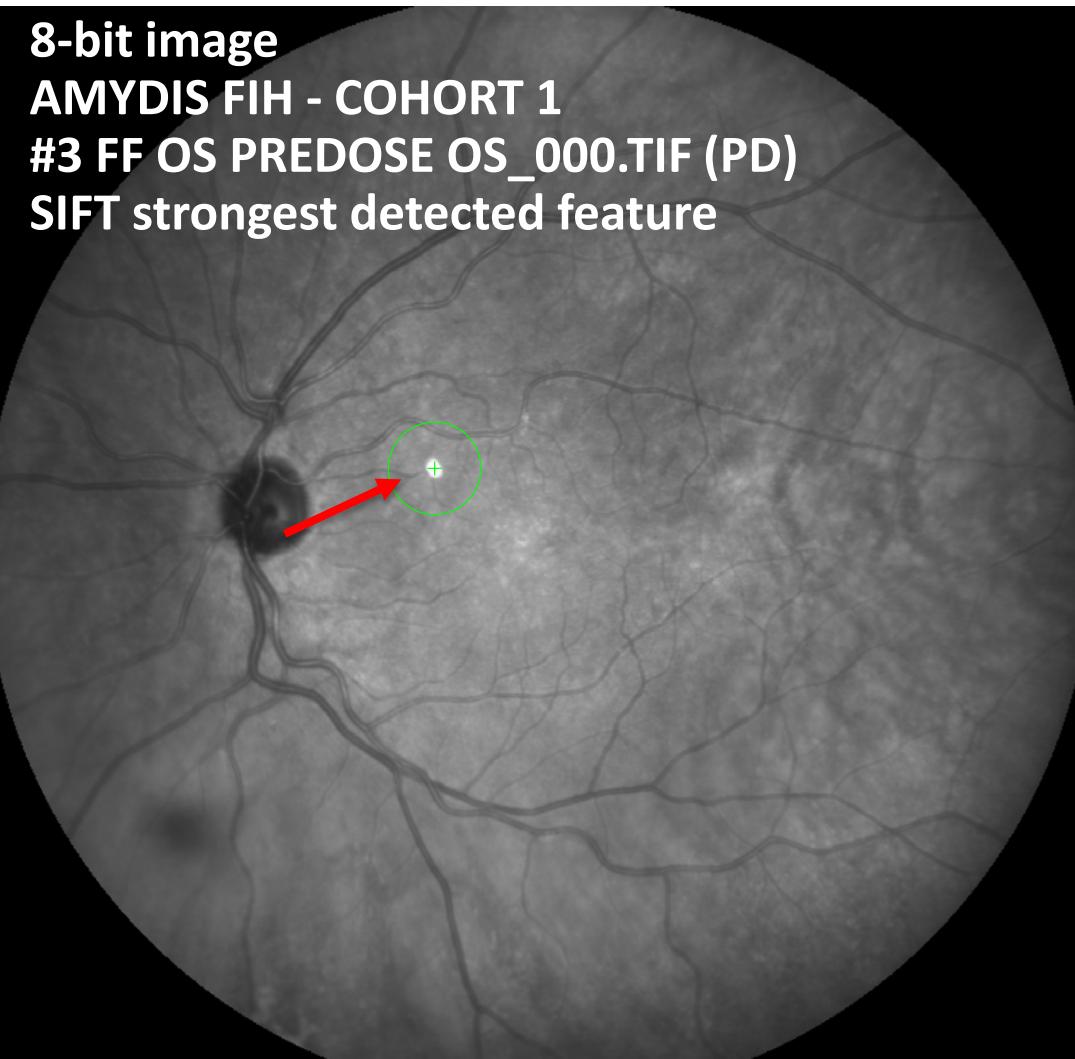
Input parameters are intentionally not stringent, allowing for some wrong links

Example from the IR channel: Pre-dose (no labeling) aggregates in in vivo human PD



SIFT & ORB test on in vivo PD image data pre-injection – perhaps could be used in a classification test as positive samples

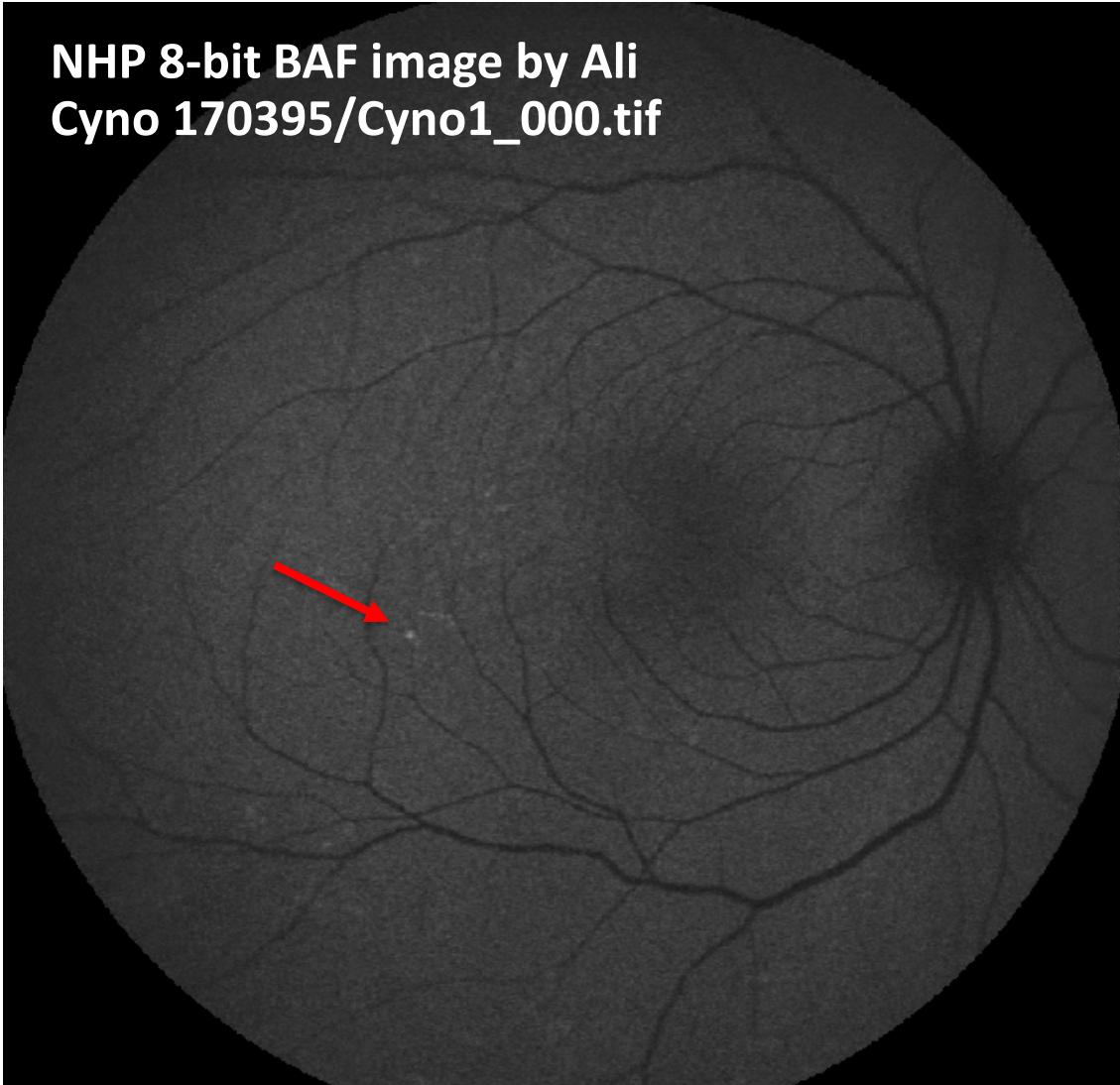
Example of IR matching left and right eye with the SURF detector



Most PD eyes do not exhibit such visible aggregates in the IR channel

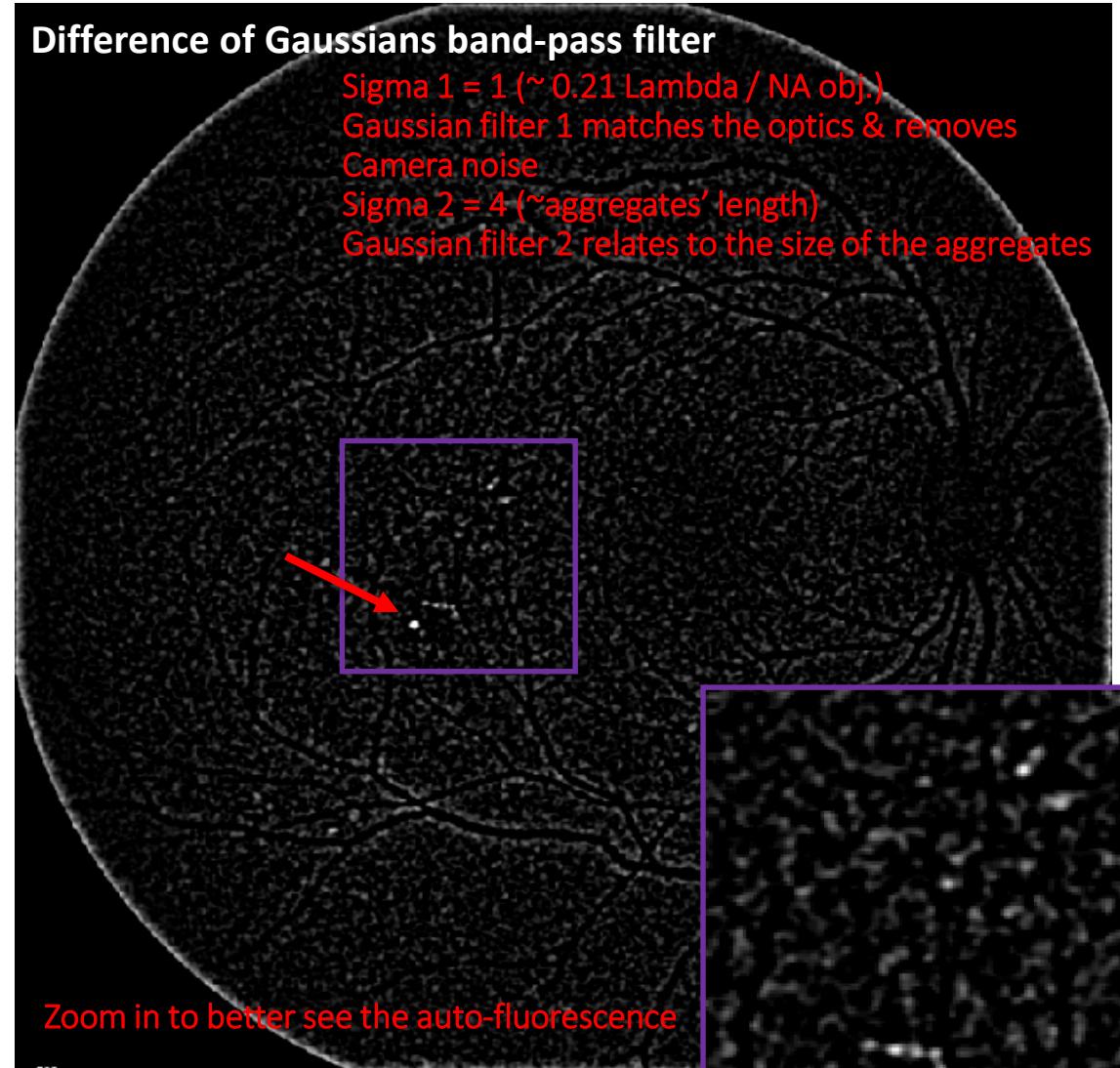
Subject 1 Visit 1 AF channel

NHP 8-bit BAF image by Ali
Cyno 170395/Cyno1_000.tif



Difference of Gaussians band-pass filter

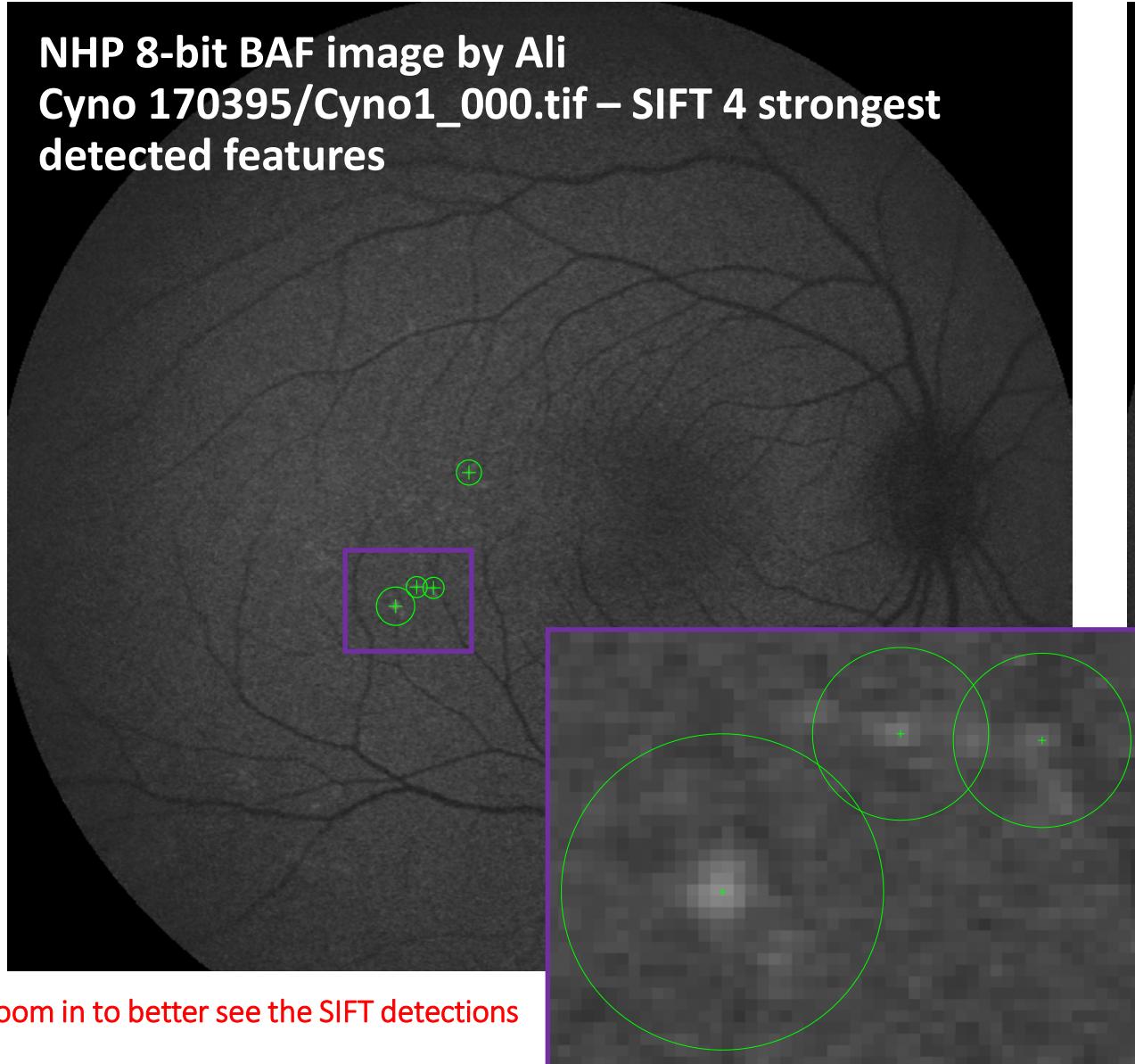
Sigma 1 = 1 (~ 0.21 Lambda / NA obj.)
Gaussian filter 1 matches the optics & removes
Camera noise
Sigma 2 = 4 (~aggregates' length)
Gaussian filter 2 relates to the size of the aggregates



Zoom in to better see the auto-fluorescence

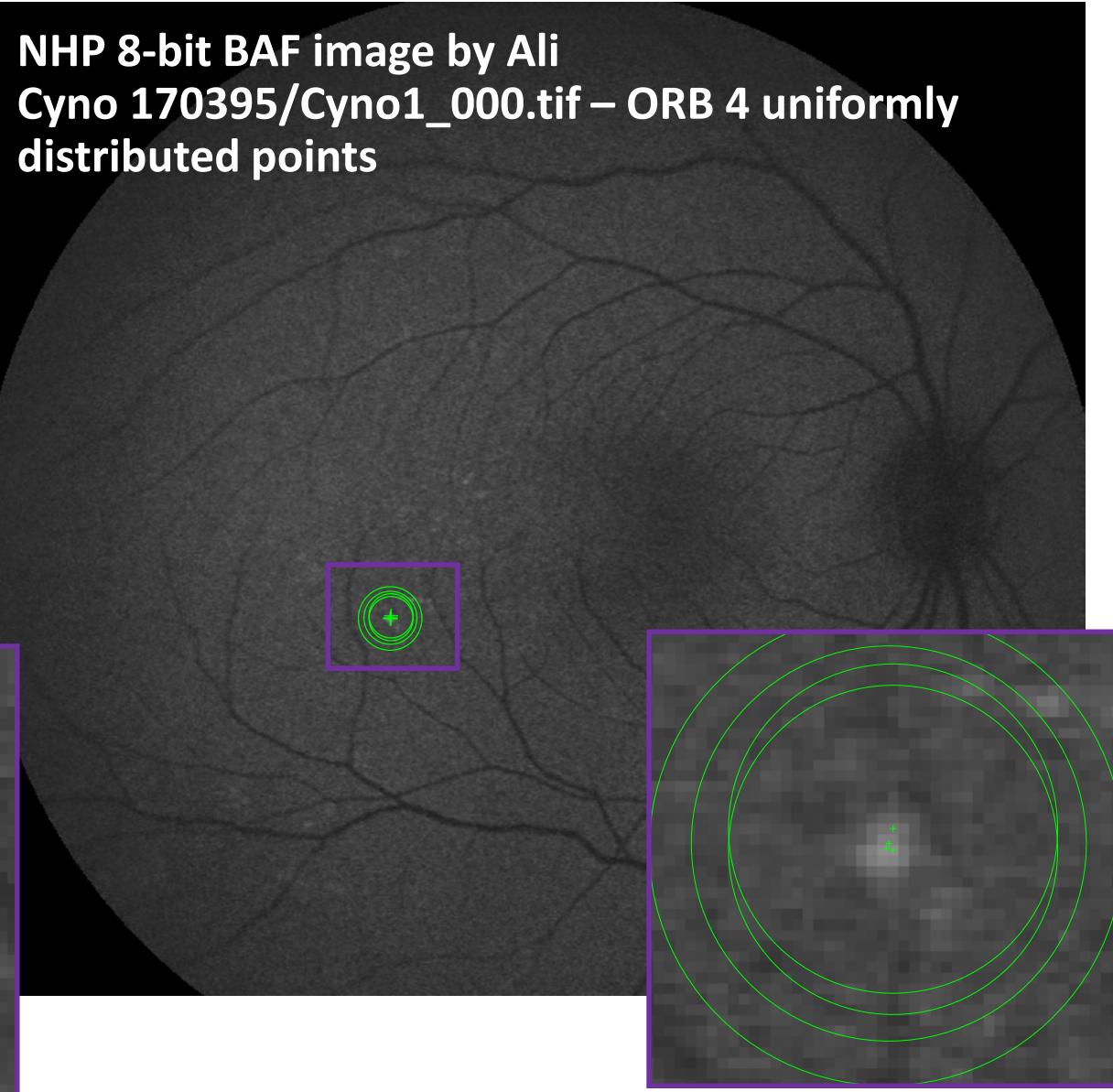
Subject 1 Visit 1 SIFT detector and ORB analysis

NHP 8-bit BAF image by Ali
Cyno 170395/Cyno1_000.tif – SIFT 4 strongest
detected features



Zoom in to better see the SIFT detections

NHP 8-bit BAF image by Ali
Cyno 170395/Cyno1_000.tif – ORB 4 uniformly
distributed points



Automated metrics of the AF blob based on DoG transformed followed by pixel intensity analysis

perimeter in microns (red), area in square microns (green), max. intensity in arbitrary units (yellow)

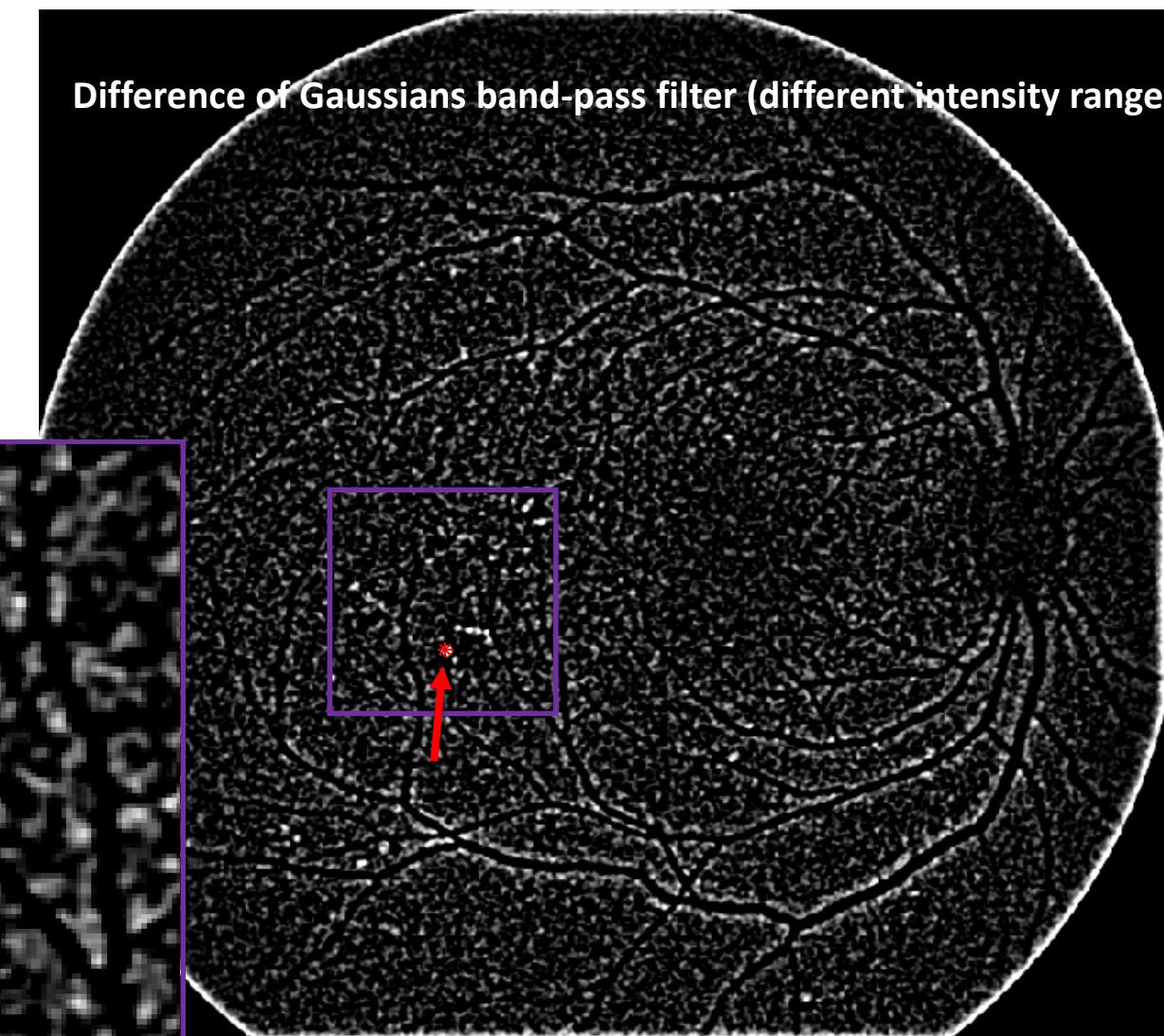
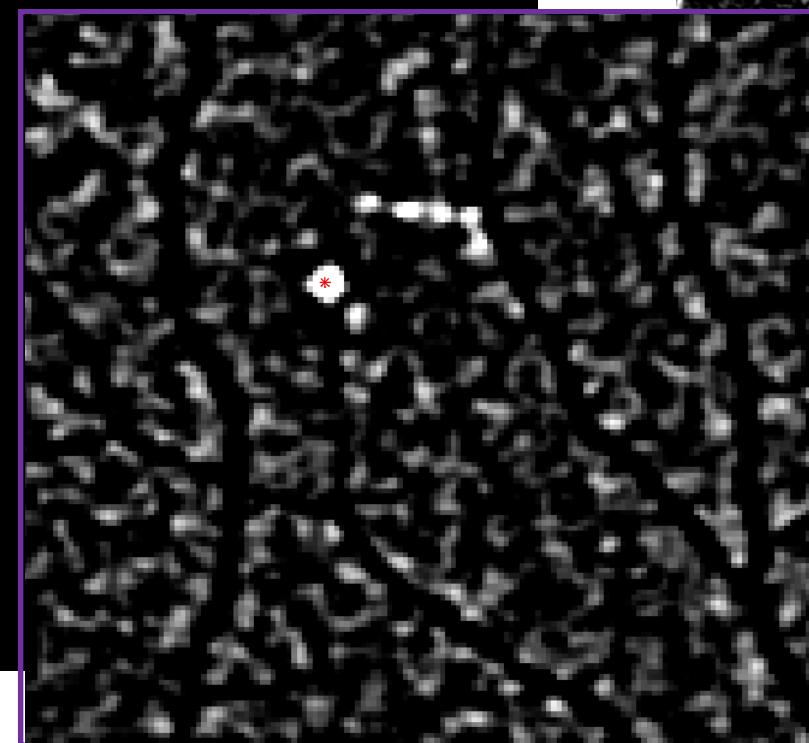
NHP 8-bit Post-injection image by Ali

Cyno 170395/Cyno1_000.tif – Segmentation:

- 1) Clip (set to 0) pixels <50% of max. intensity of DoG
- 2) Select blob w max. value for peak intensity

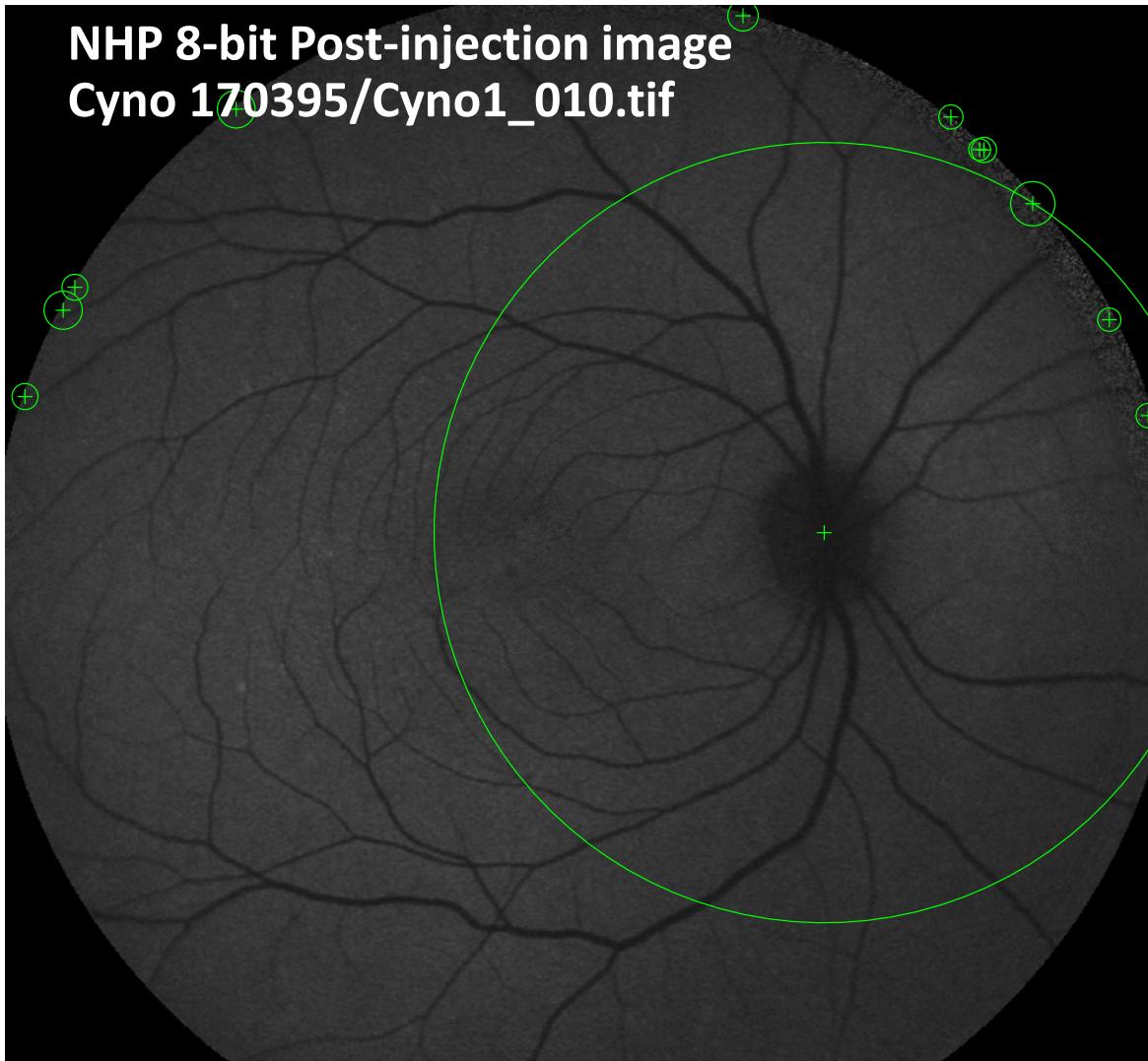
177
2646
41.6973

0.002646 square millimeters



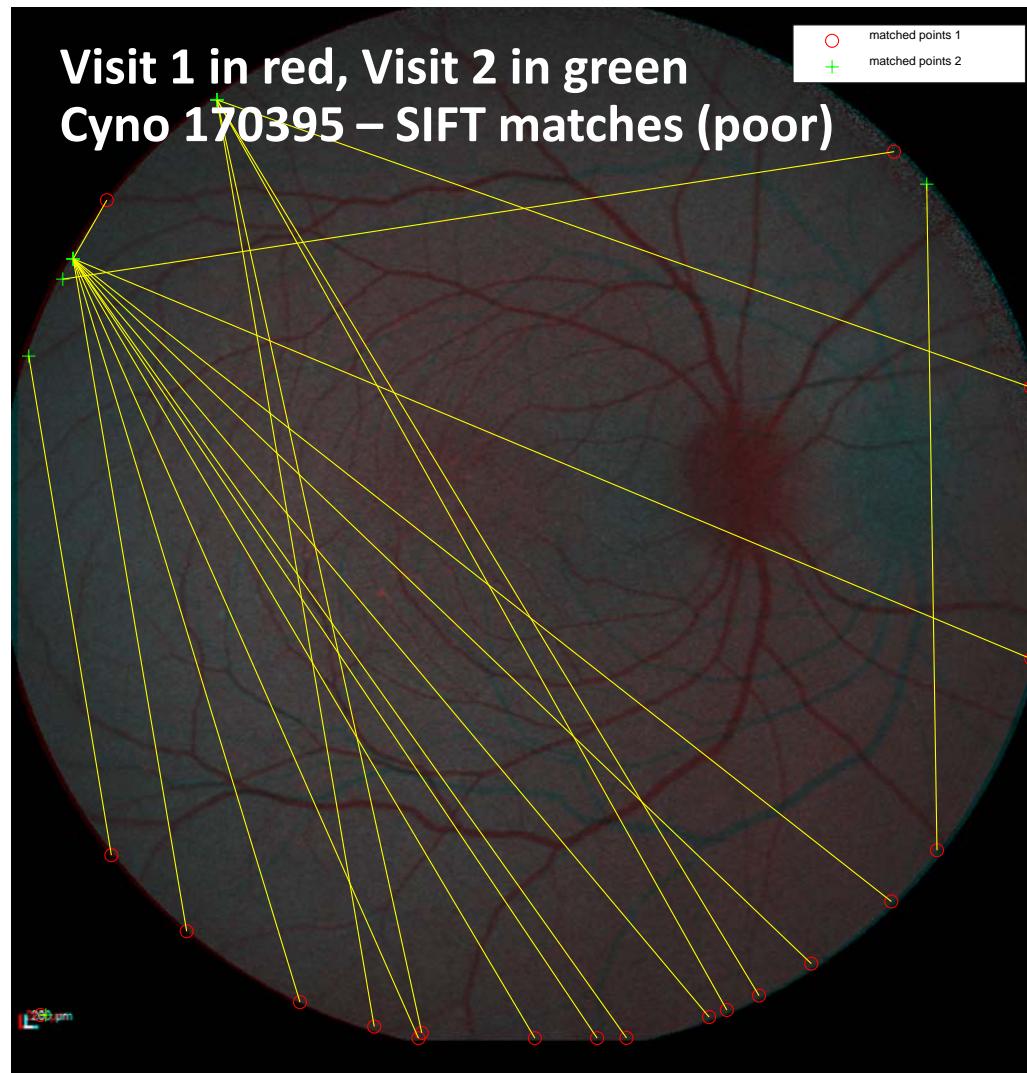
Zoom in to better see the auto-fluorescence

Subject 1 Visit 2 – no fluorescent keypoints within the eye detected



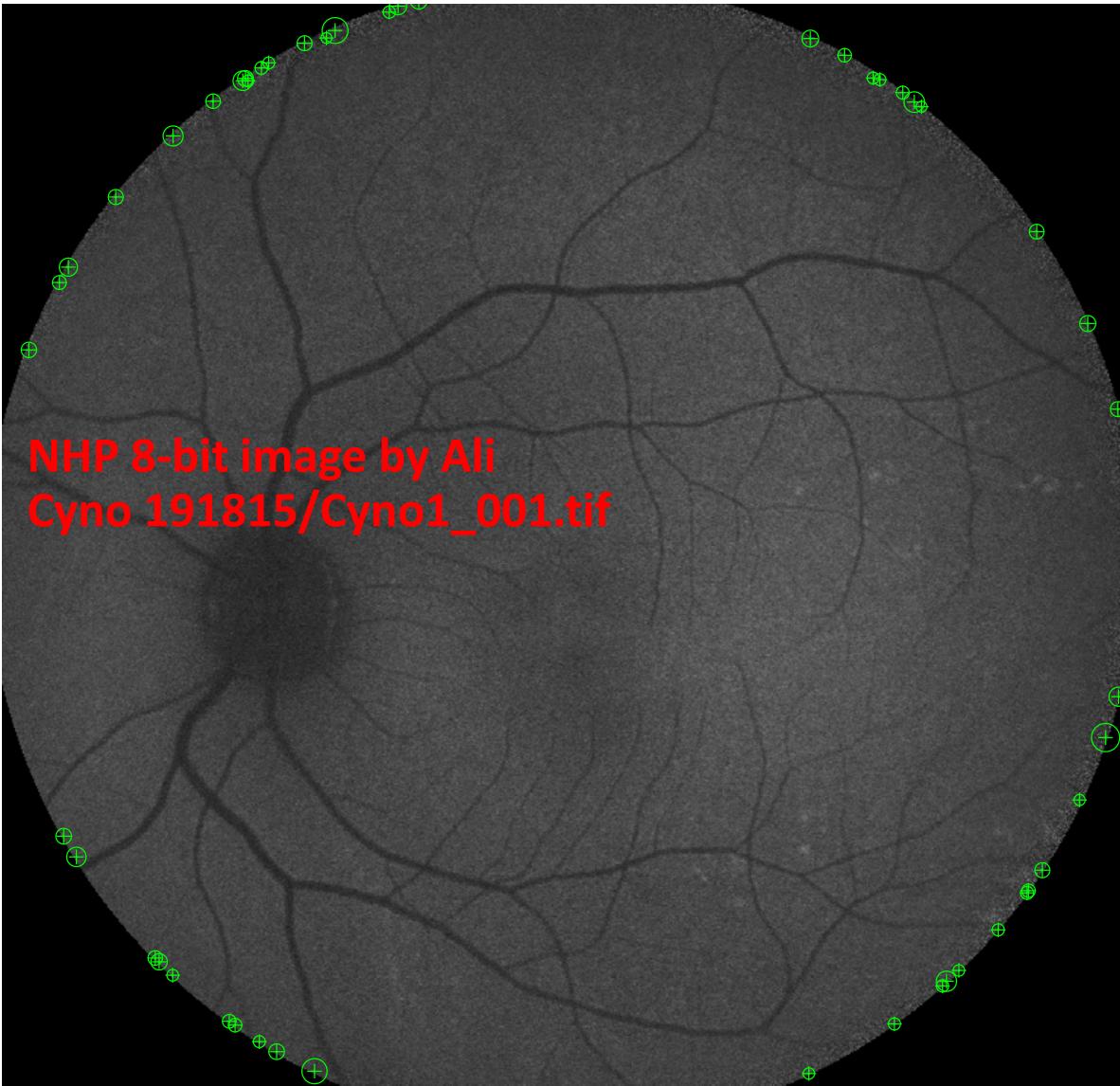
Most significant SIFT points are at the edge; ORB points are the branching point of the vessels, i.e. identifies no fluorescent keypoints in these baseline images

No good matching between visits 1 and 2 based on preliminary analysis with SIFT and SURF

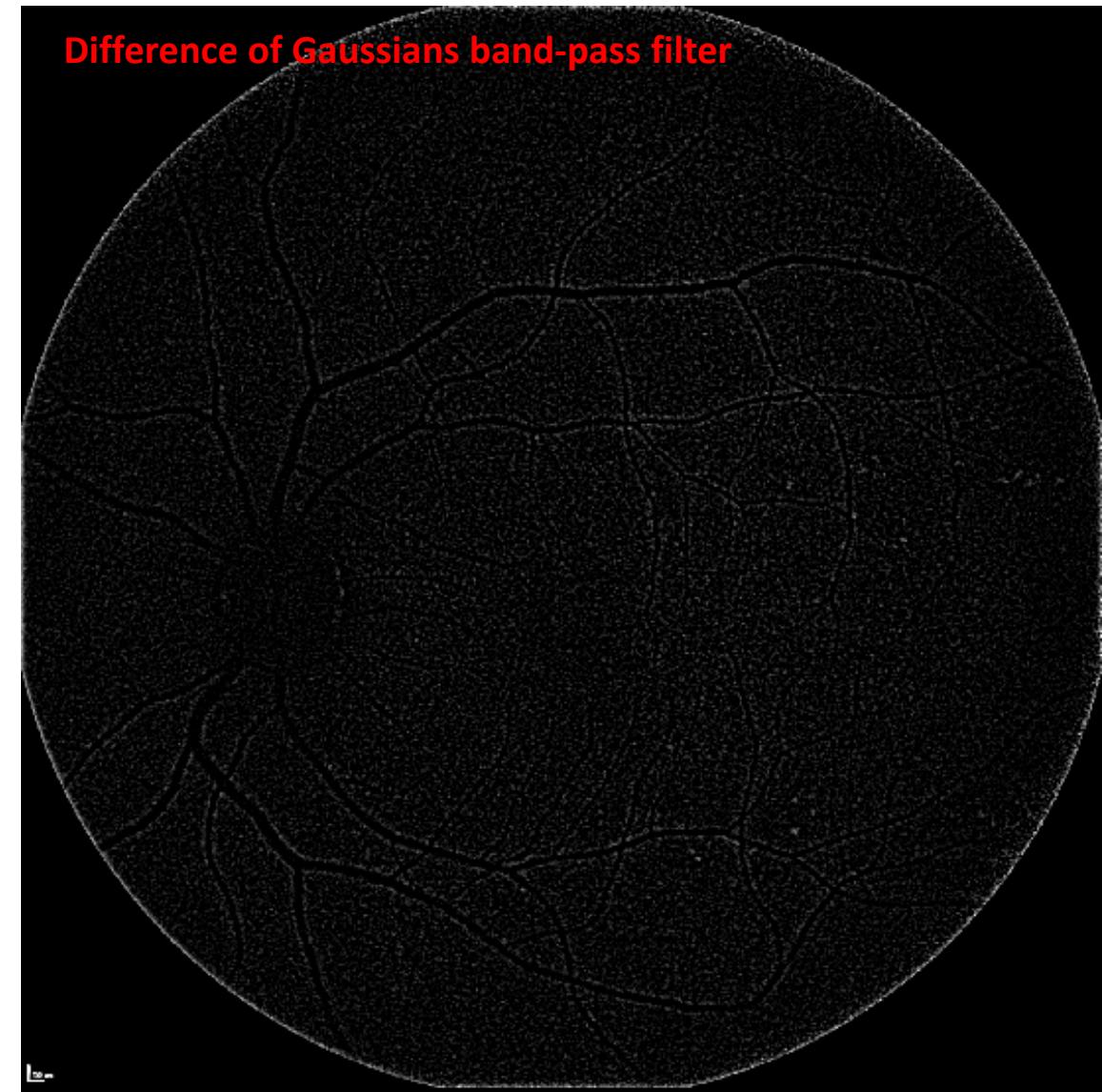


The visit 2 data only has SIFT keypoints at the edges, which might be artifact of high contrast; the default SURF analysis does not match the two images

Subject 5 Visit 1 – no fluorescent keypoints within the eye detected



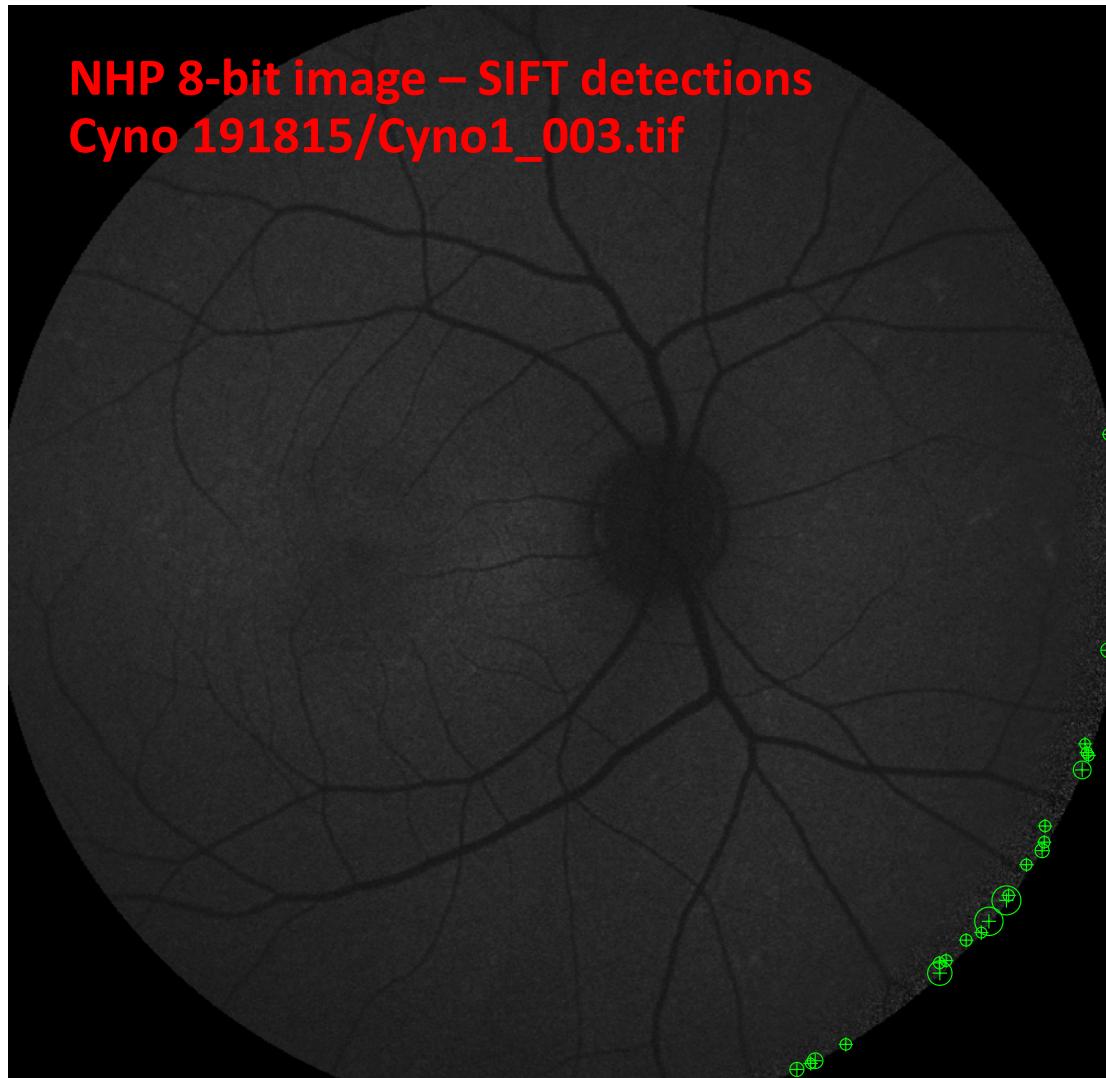
NHP 8-bit image by Ali
Cyno 191815/Cyno1_001.tif



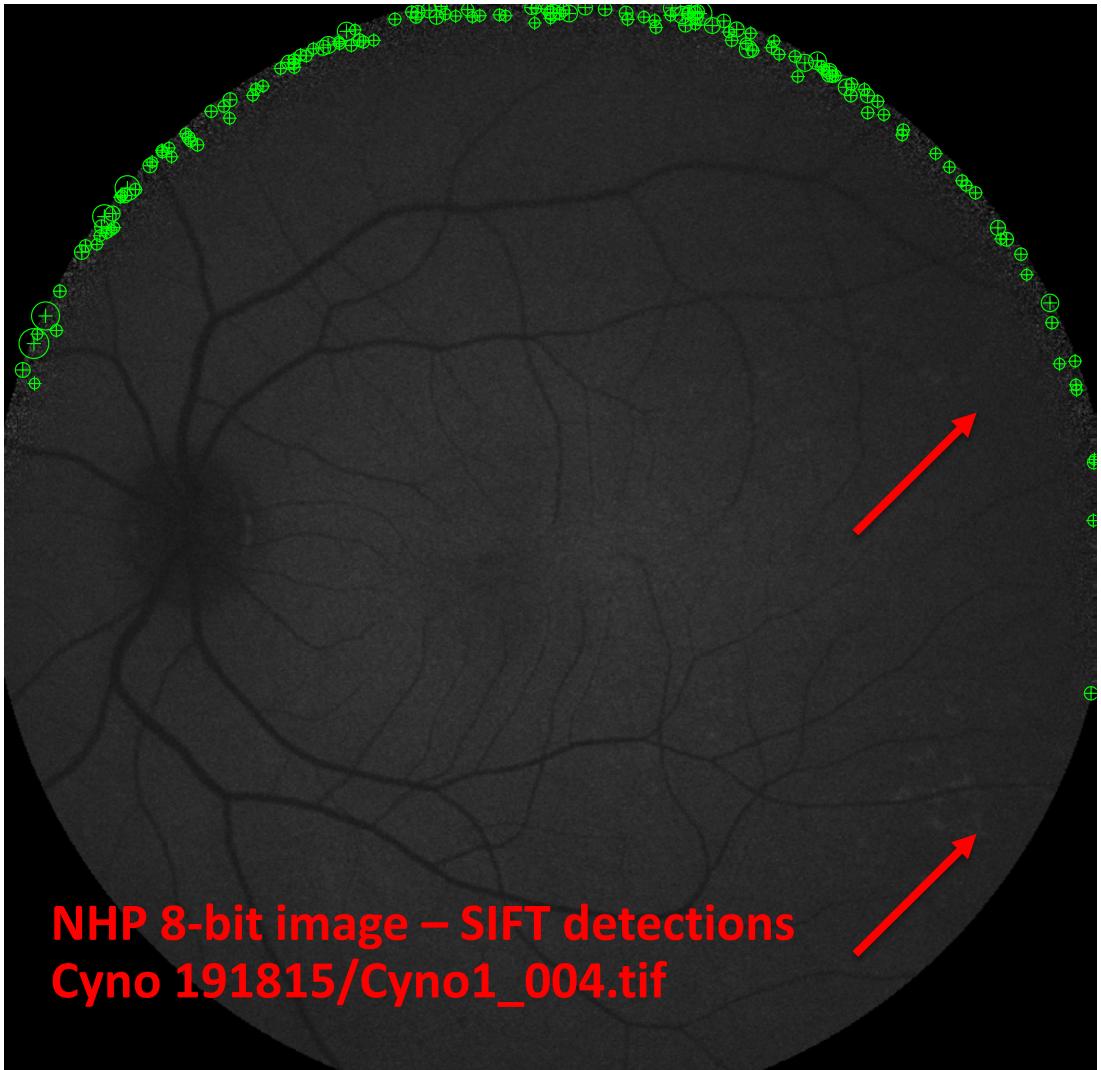
Difference of Gaussians band-pass filter

Features at the edge are false positive, appear because of the sudden change in contrast

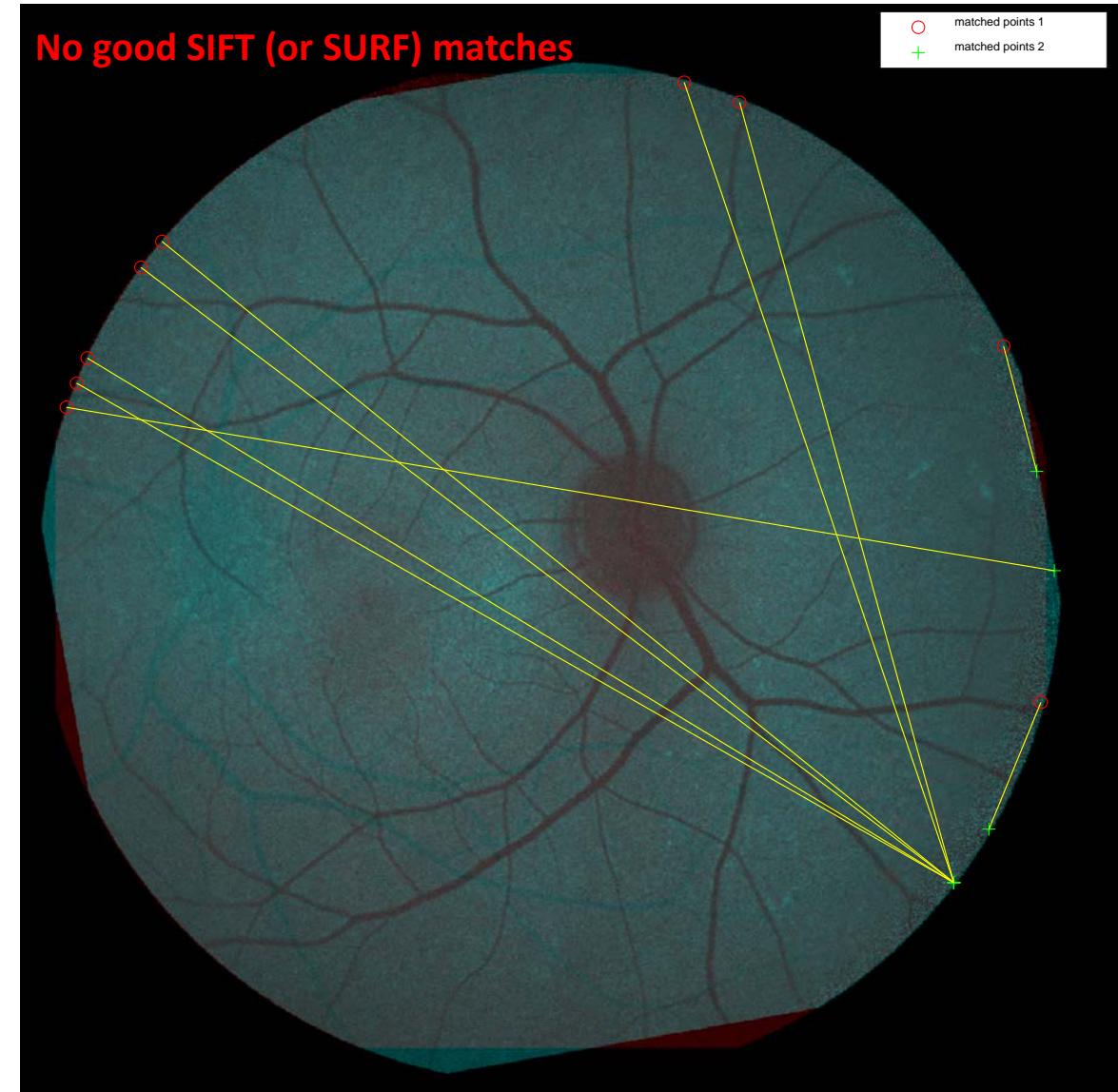
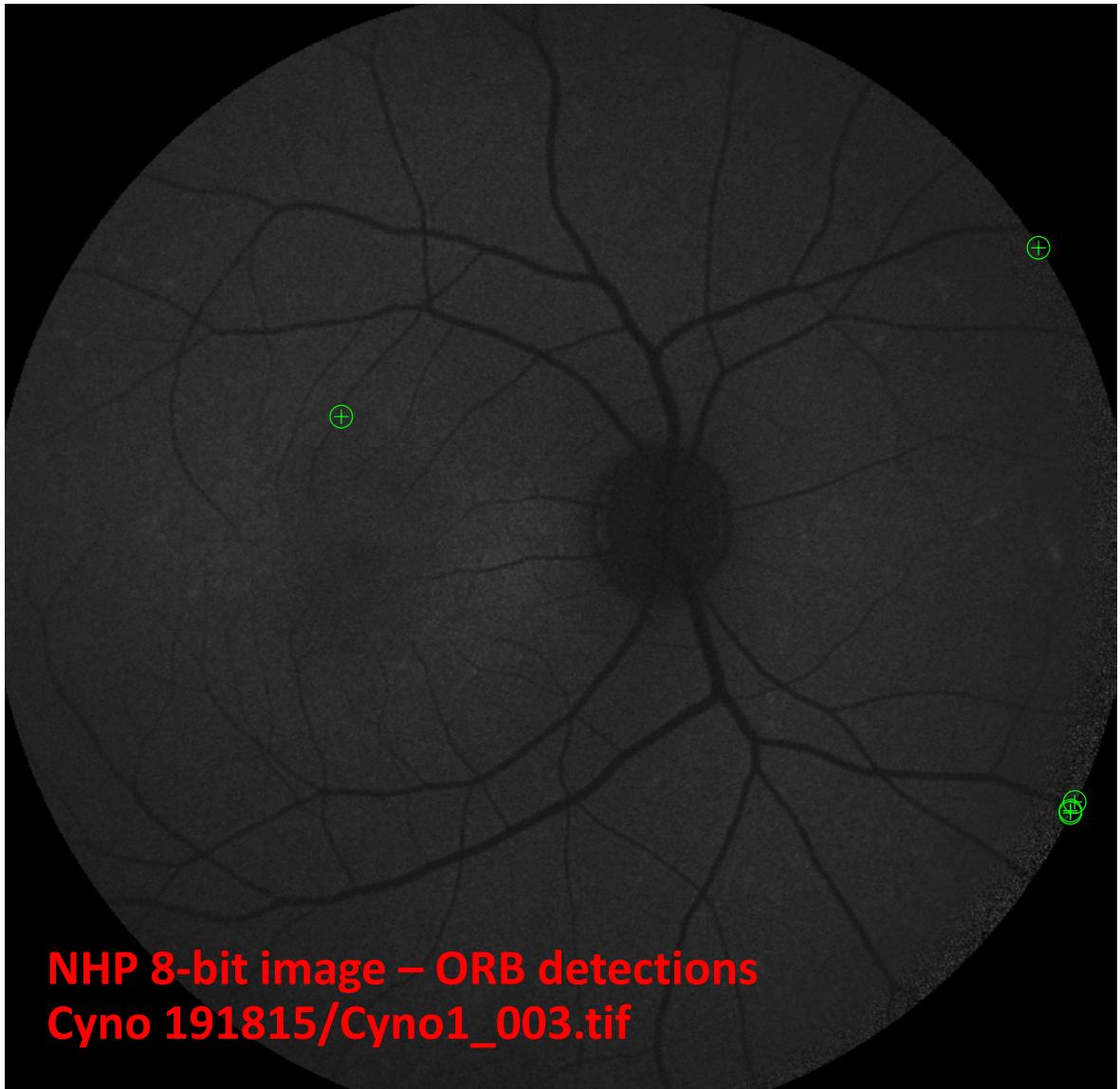
Subject 5 Visit 2



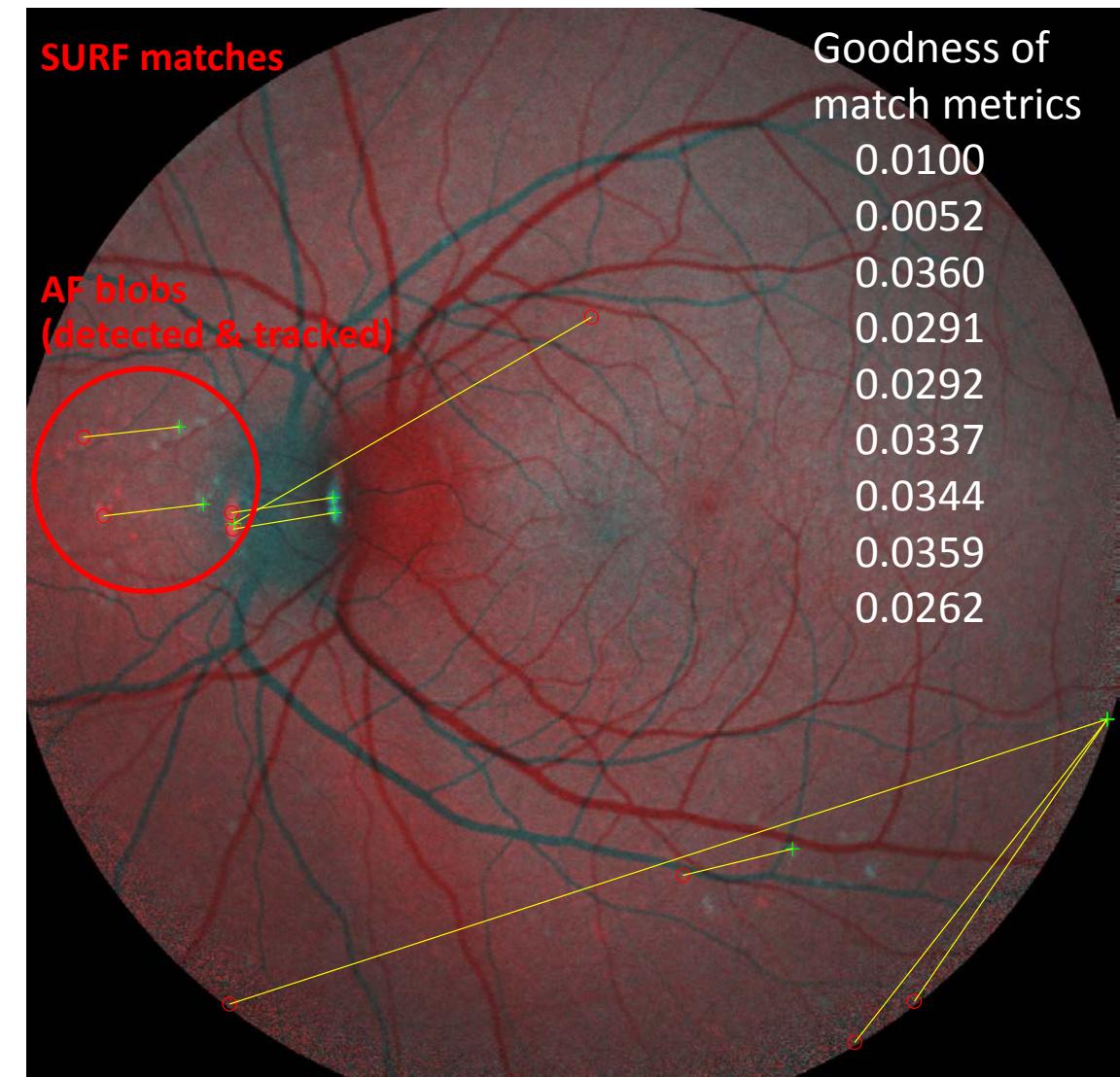
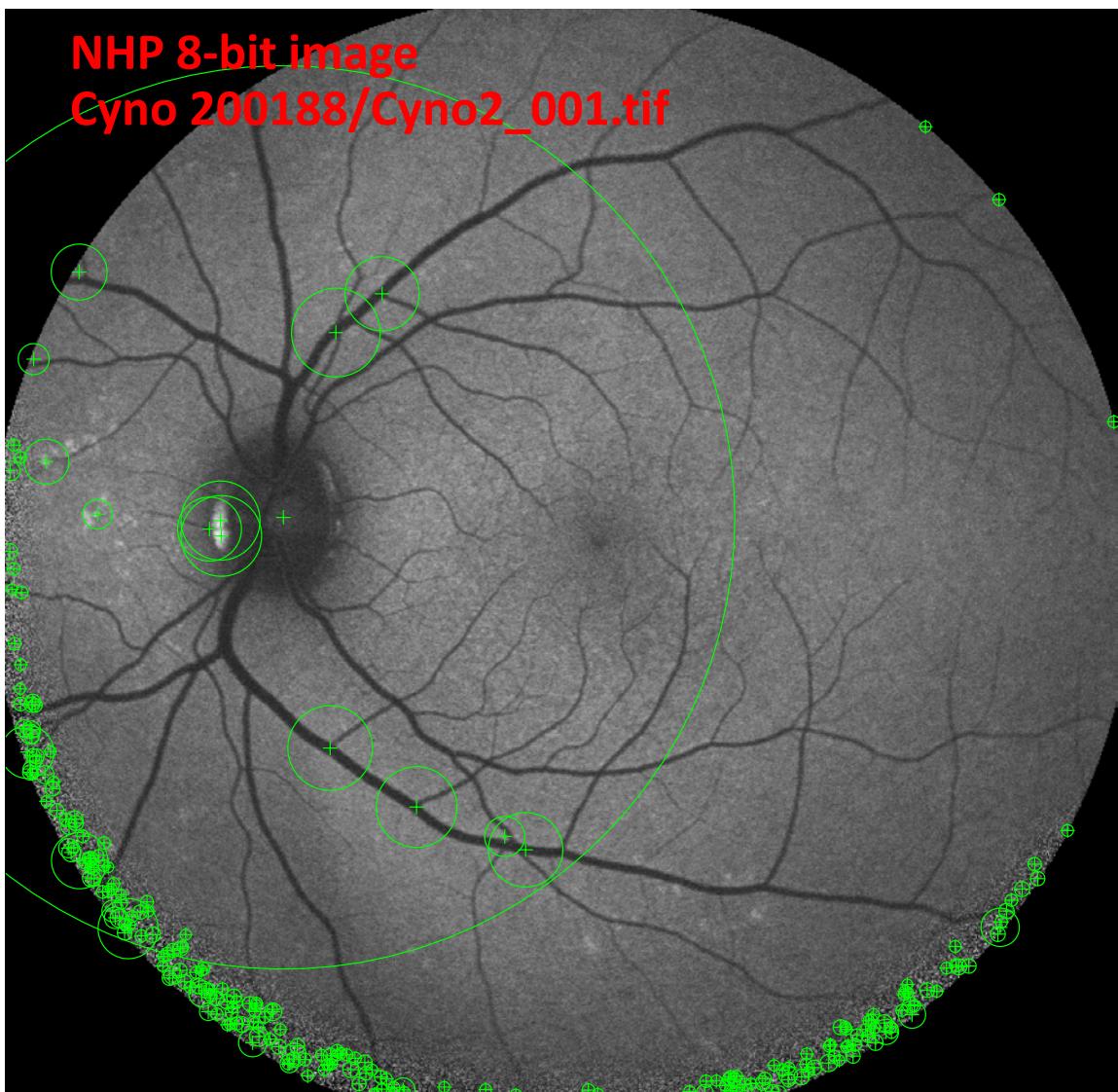
Subject 5 Visit 1



Subject 5 Visit 2

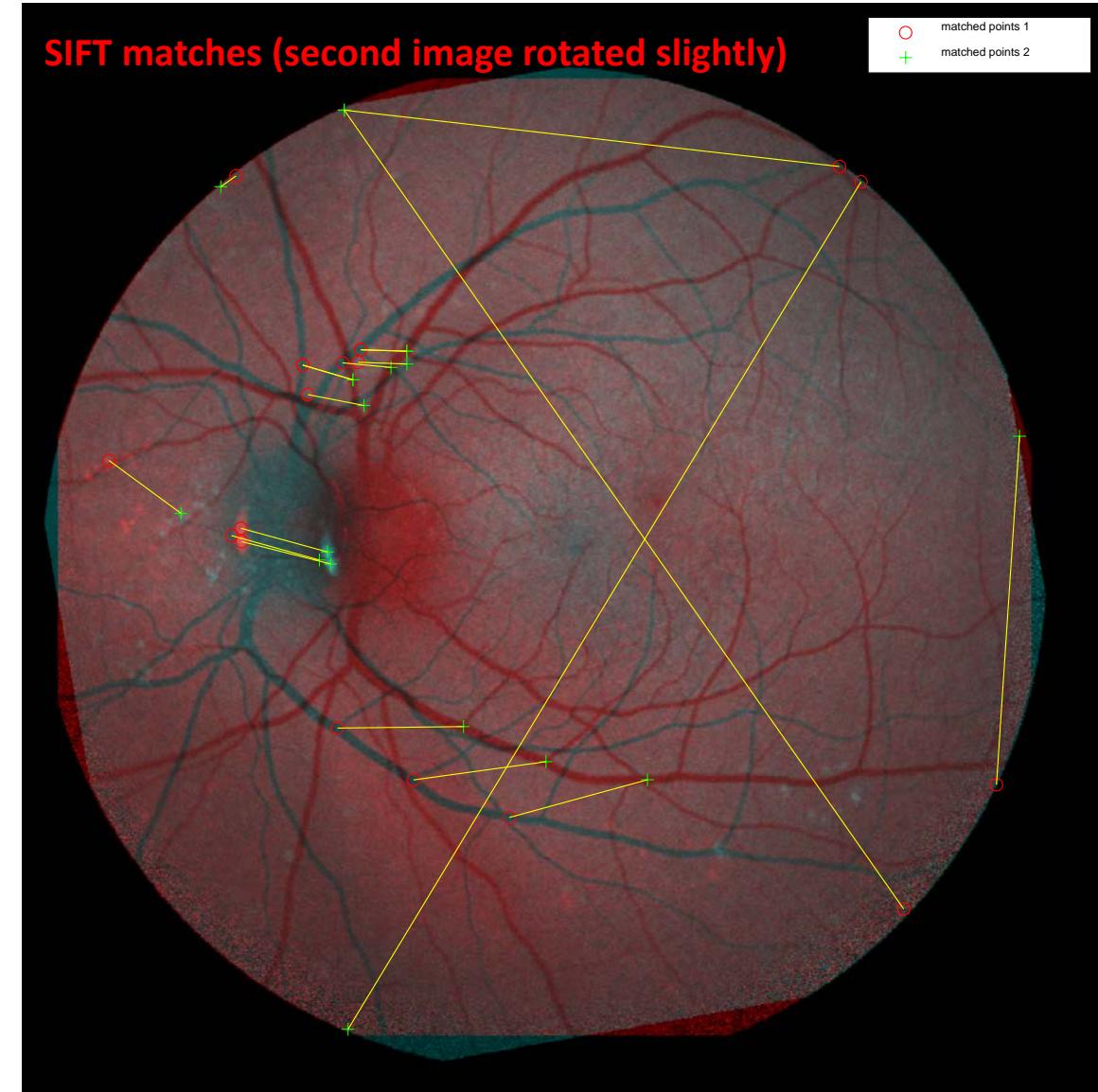
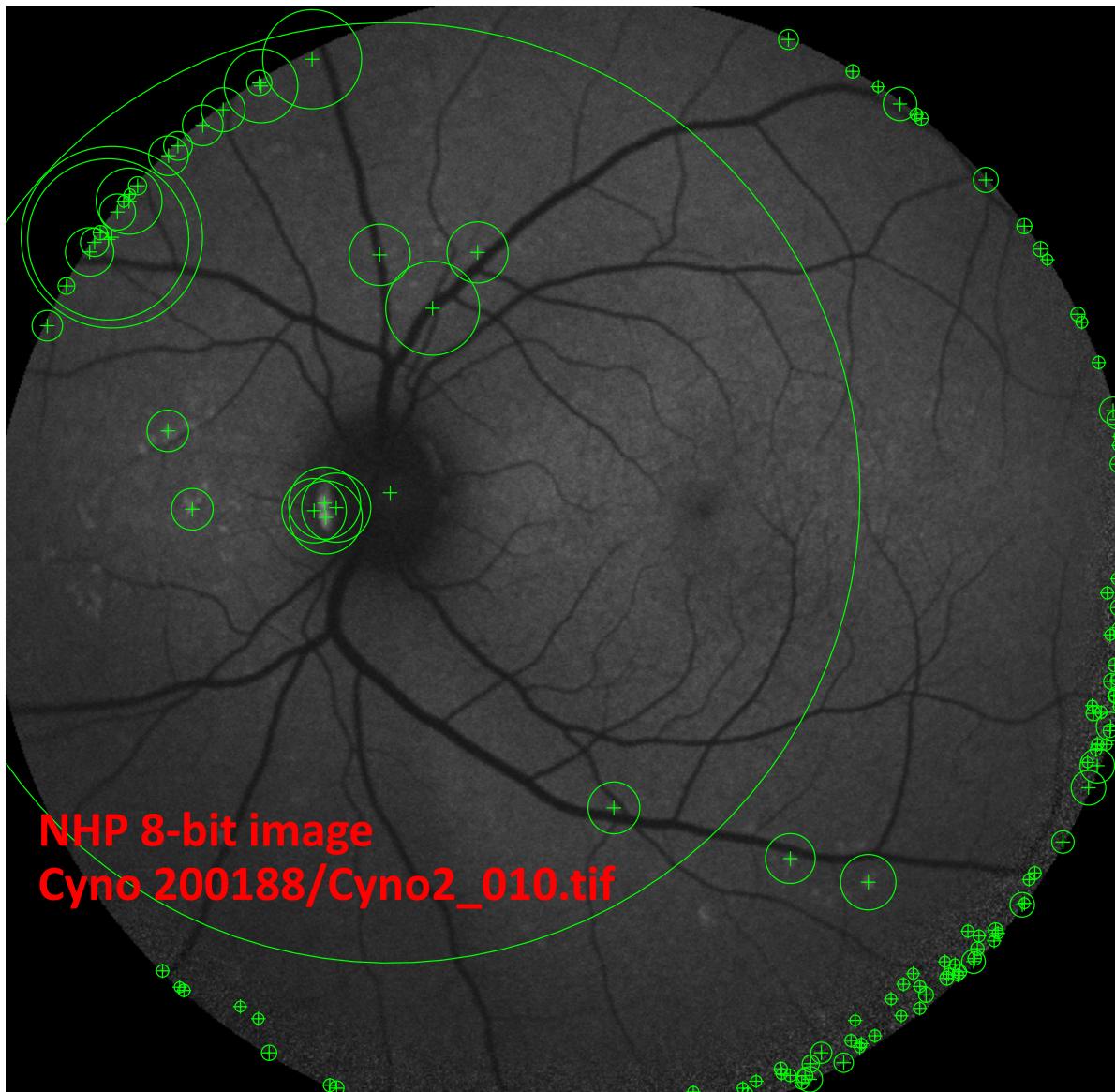


Subject 8 Visit 1

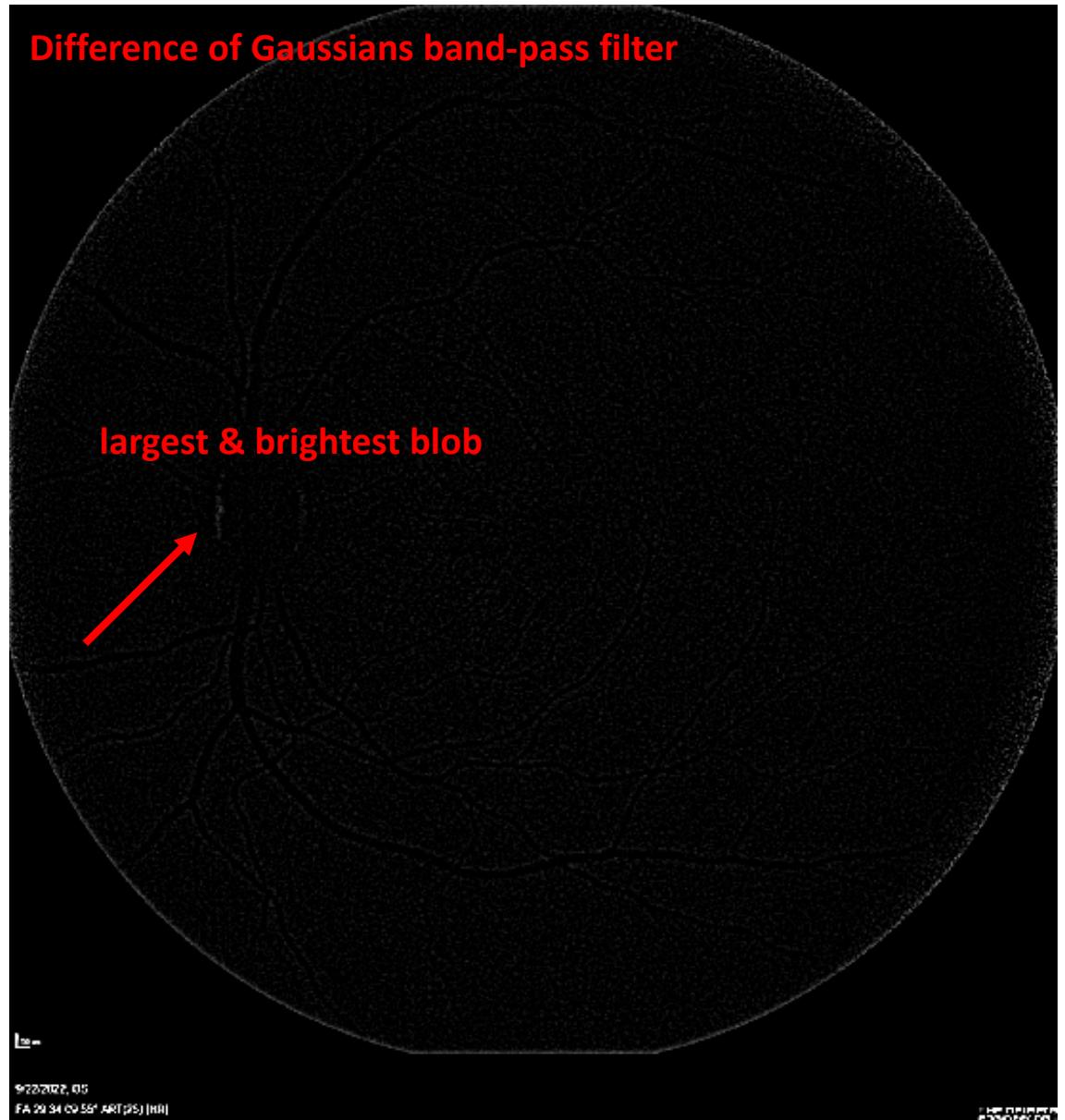
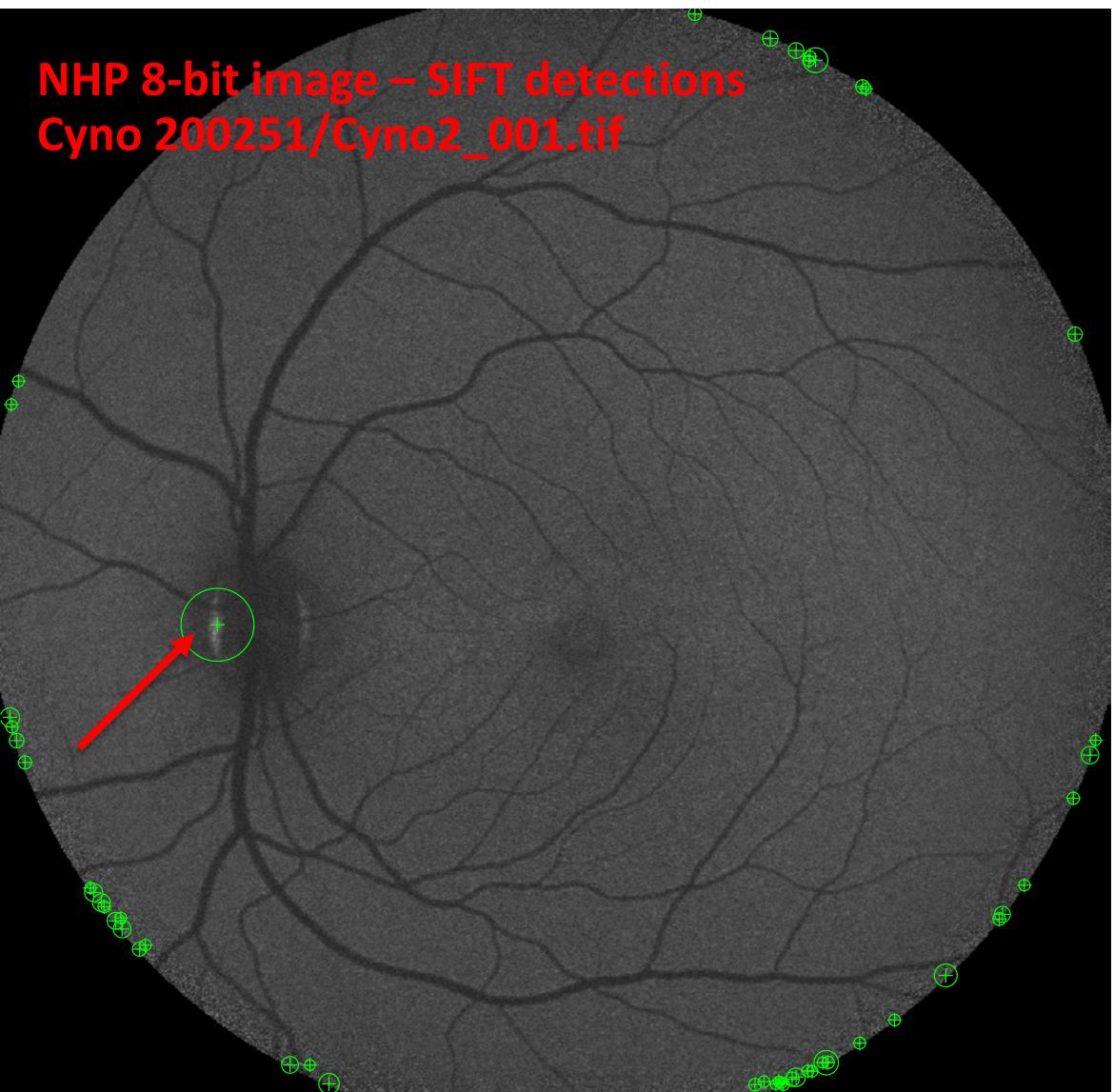


Additional keypoints detected at the optical nerve and branching vessels

Subject 8 Visit 2



Subject 12 Visit 1



Subject 12 Visit 2

