

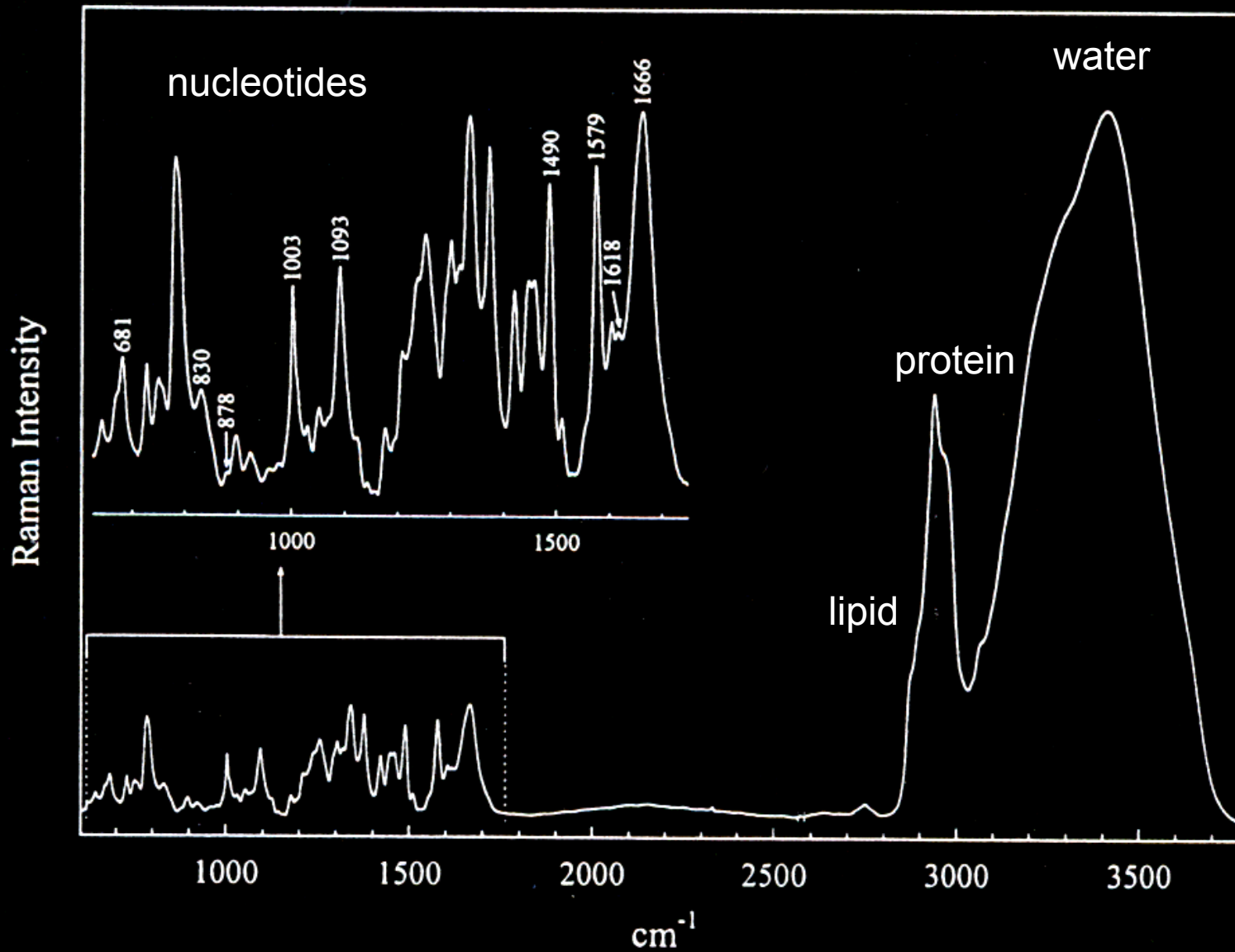
A CARS microscopy image showing several cells. The cells are primarily colored in shades of red and orange, with some blue and cyan highlights, indicating chemical selectivity in the imaging process. The background is dark, making the cellular structures stand out.

# Chemical selective imaging of disease with of CARS microscopy

Eric O. Potma, Beckman Laser Institute

Bruce Tromberg, Zhonging Chen, James Jester, Joerg Meyer

# Vibrational imaging



# Not enough!



C.V. Raman in 1928:

“..that the effect is a true scattering and not a fluorescence is indicated in the first place by its feebleness in comparison with the ordinary scattering.”

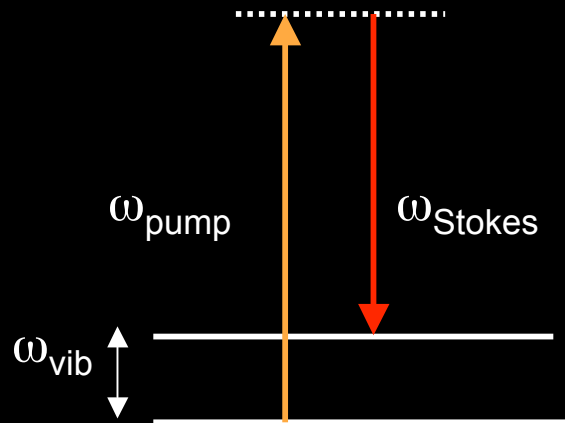


Nicolaas Bloembergen in 1978:

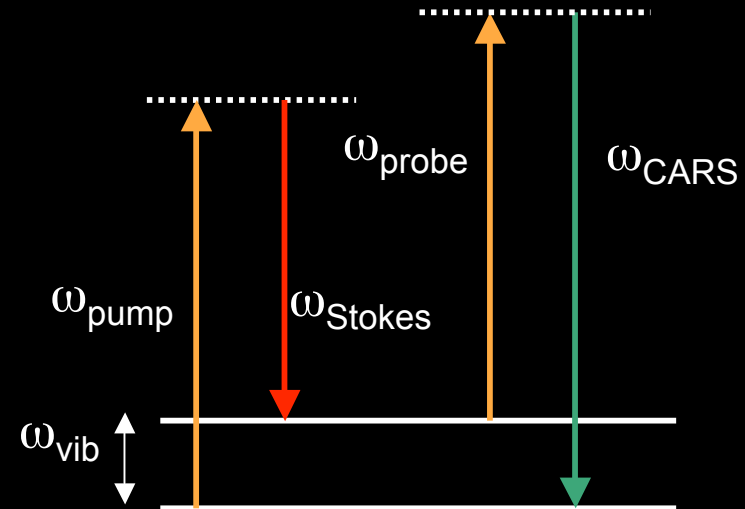
“As spontaneous Raman spectroscopy has blossomed and grown during one half-century, it may be predicted with some confidence that coherent nonlinear Raman spectroscopy will yield many new results in the next half-century .”

# CARS

## Raman



## CARS



# first CARS experiments



PHYSICAL REVIEW

VOLUME 137, NUMBER 3A

1 FEBRUARY 1965

## Study of Optical Effects Due to an Induced Polarization Third Order in the Electric Field Strength

?

P. D. MAKER AND R. W. TERHUNE

*Scientific Laboratory, Ford Motor Company, Dearborn, Michigan*

(Received 19 August 1964)

This paper presents the results of a series of experiments in which a giant pulsed ruby laser is used to study several different nonlinear optical effects arising from an induced optical polarization third order in the electric field strength. The various phenomena studied are special cases of either frequency mixing or intensity-dependent changes in the complex refractive index, including Raman laser action at a focus. A wide range of crystalline and isotropic materials was studied. The theory for these effects is extended to cover resonant interactions. The experimental results are interpreted in terms of simplified models, and quantitative values for the nonlinear polarizability coefficients are given. The rather large experimental uncertainties in these coefficients are discussed.

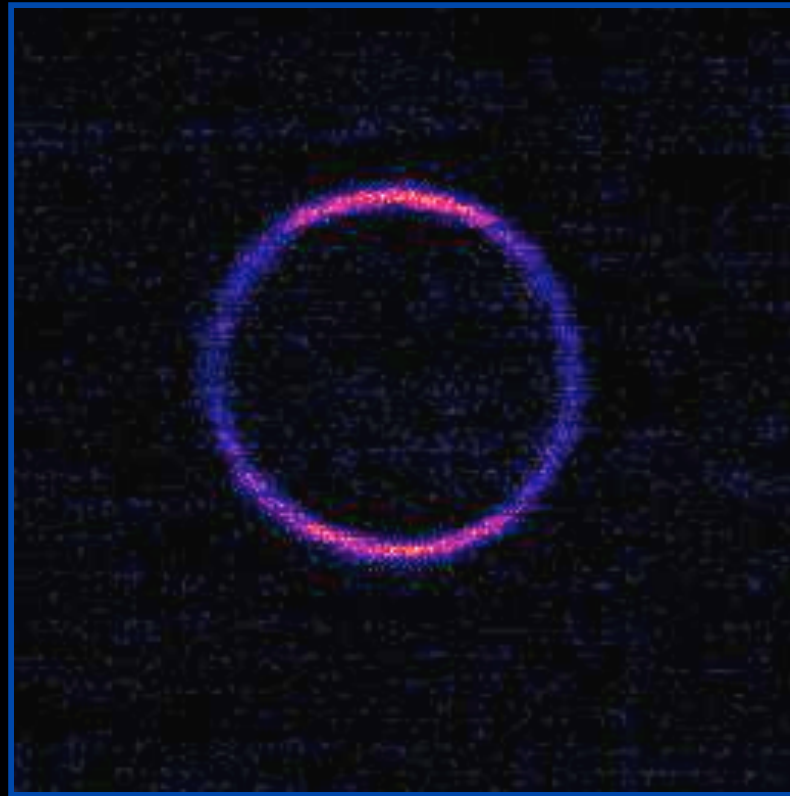
# CARS contrast in tissues

Lipid ( $\text{CH}_2$  stretch vibration)

Protein ( $\text{CH}_3$  stretch vibration)

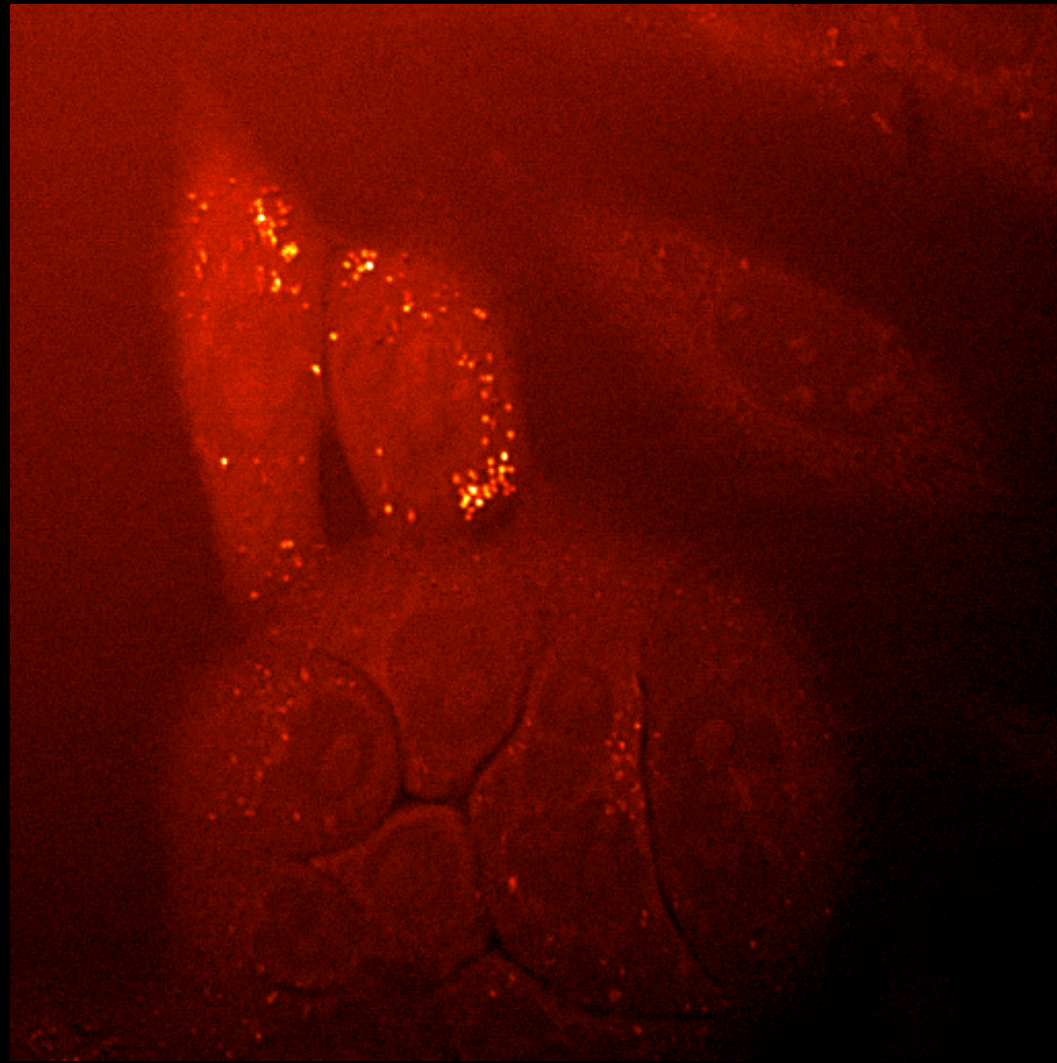
Water (OH stretch vibration)

# What CARS can do



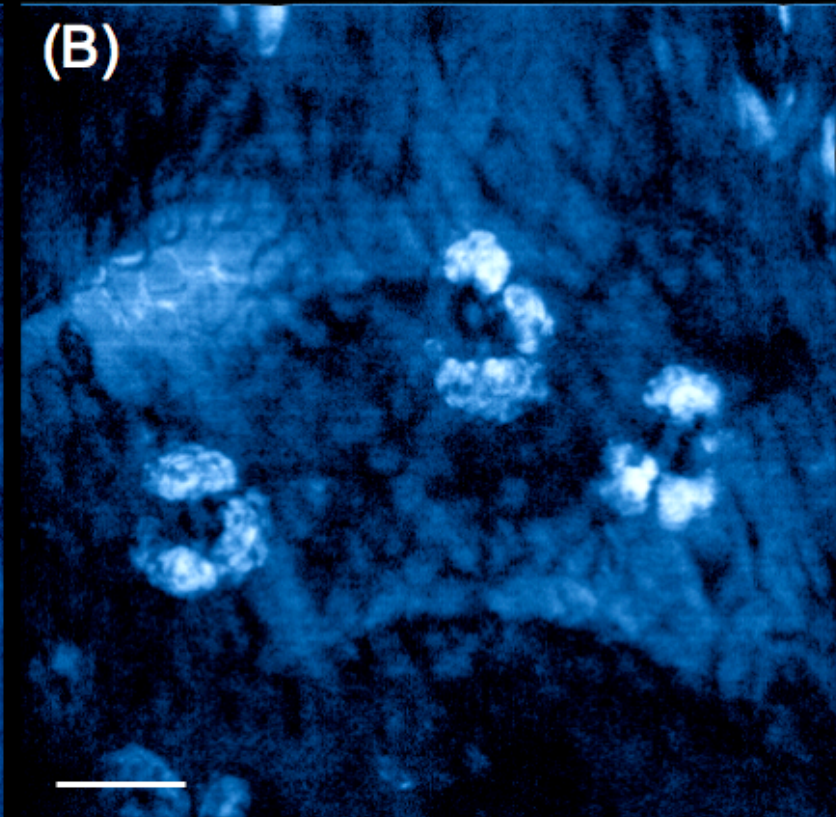
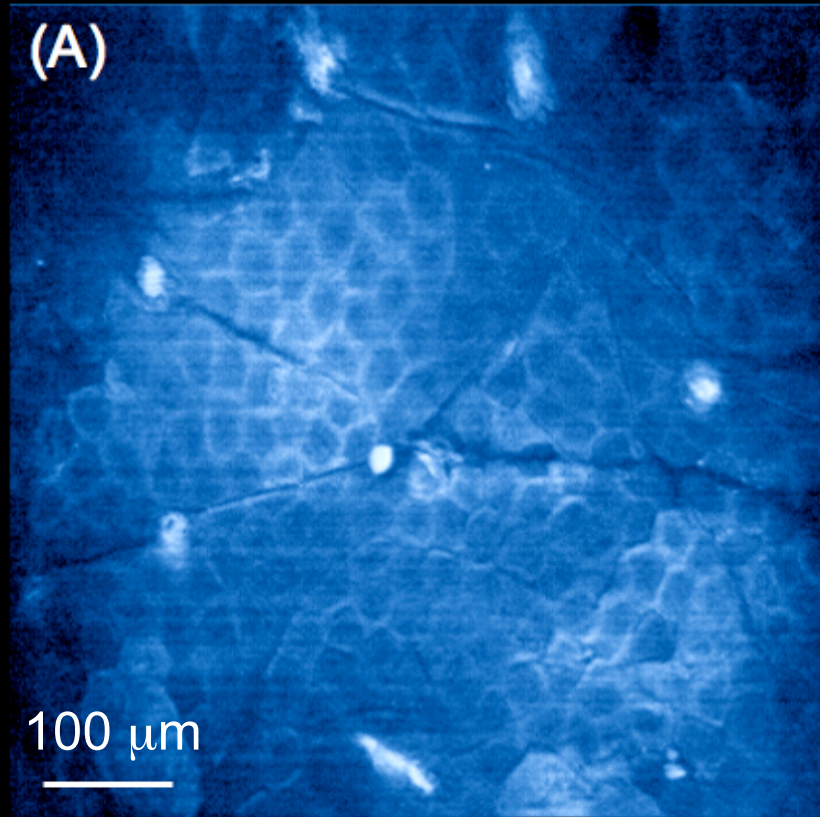
CH<sub>2</sub> stretch

# Cellular imaging

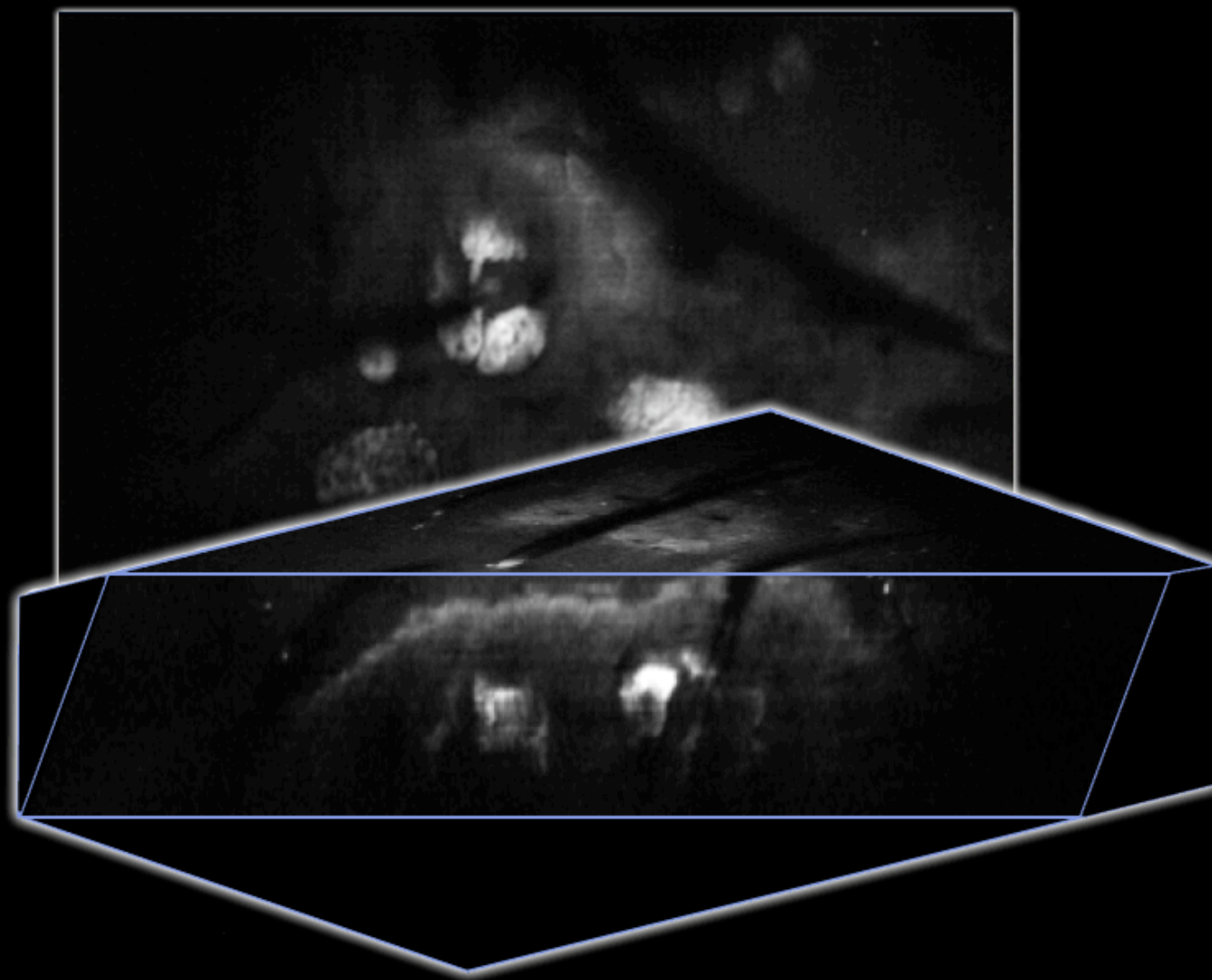


CH<sub>2</sub> stretch

# *in vivo* tissue imaging



# 3D in vivo imaging



# ps-CARS microscopy

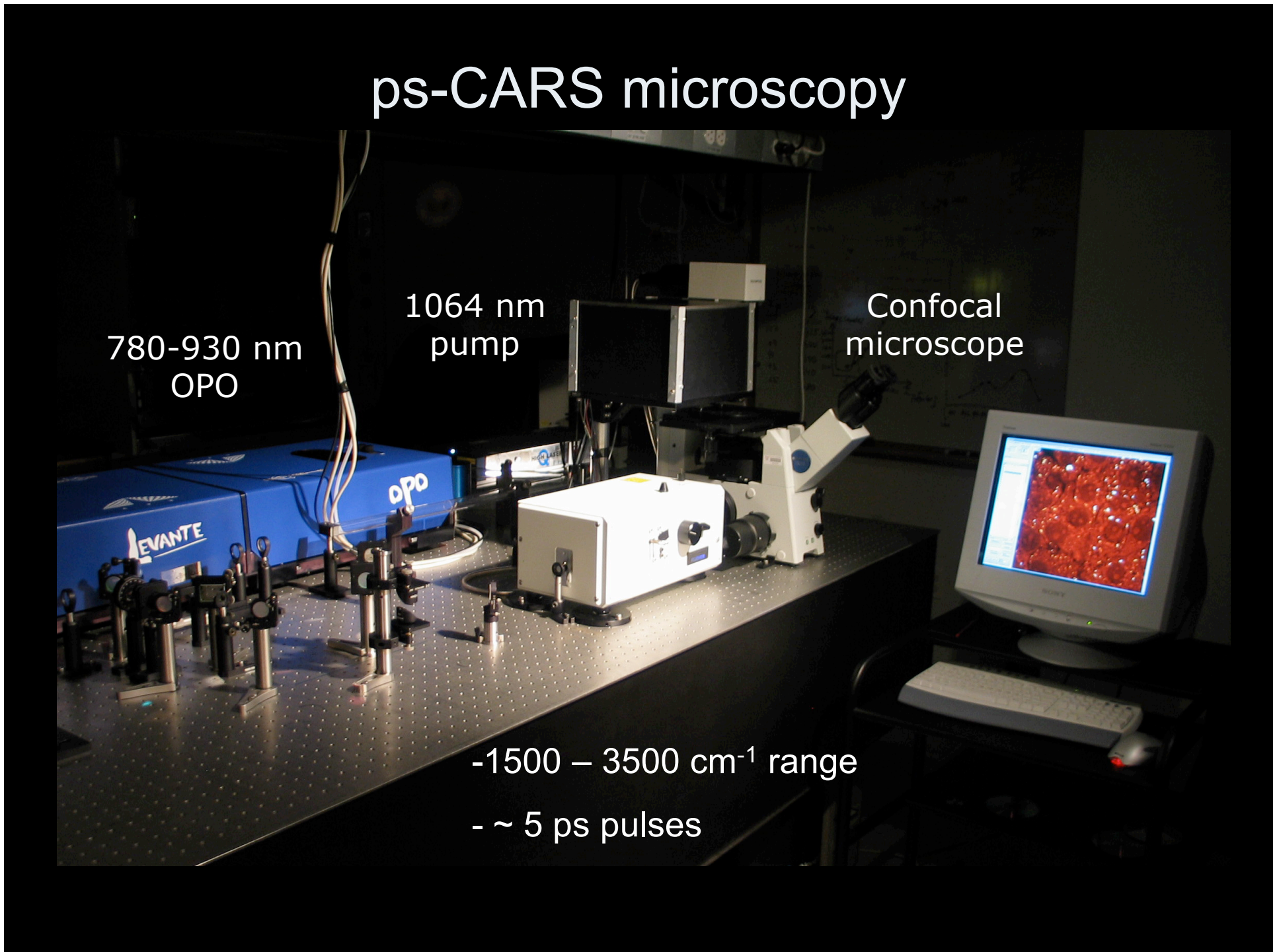
780-930 nm  
OPO

1064 nm  
pump

Confocal  
microscope

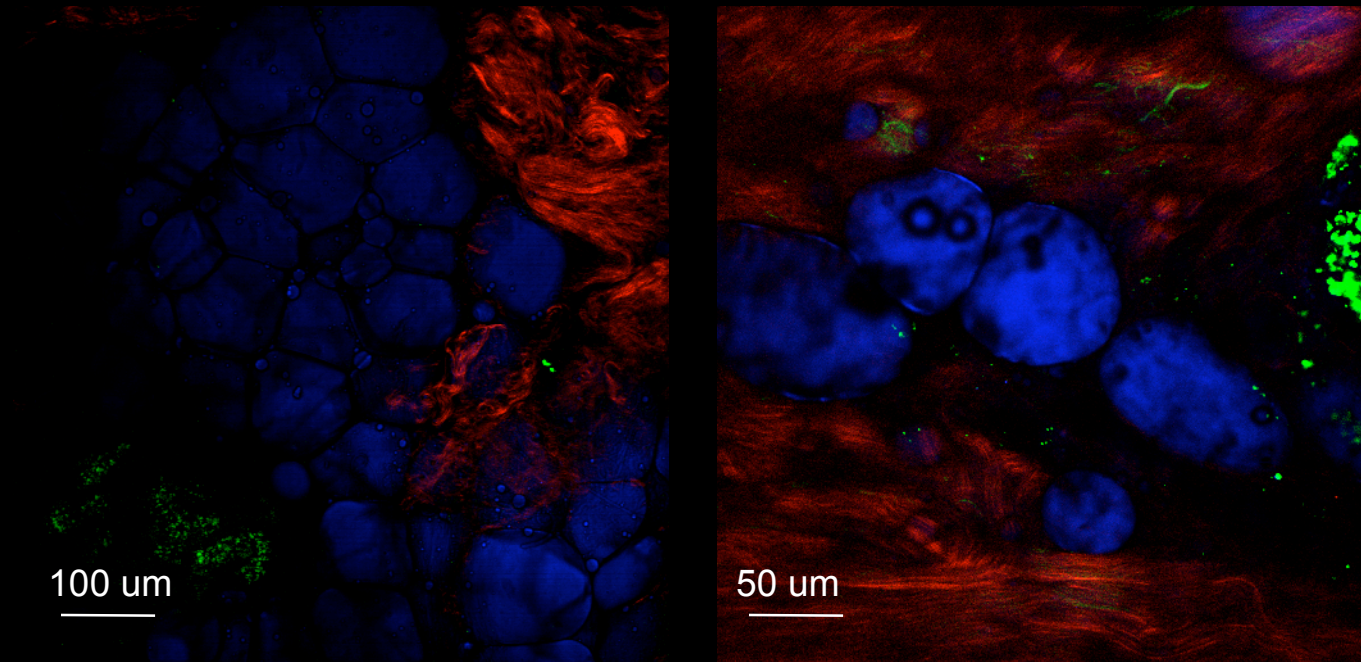
-1500 – 3500  $\text{cm}^{-1}$  range

- ~ 5 ps pulses



# CARS combined with TPEF and SHG

Nonlinear imaging of human skin

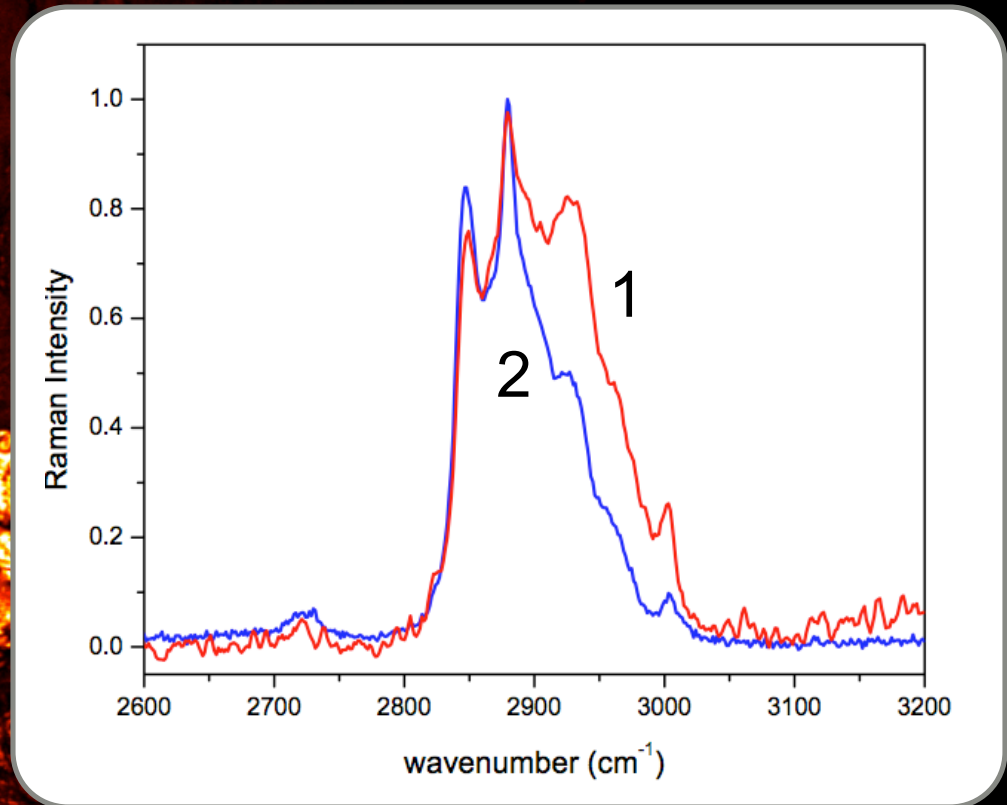
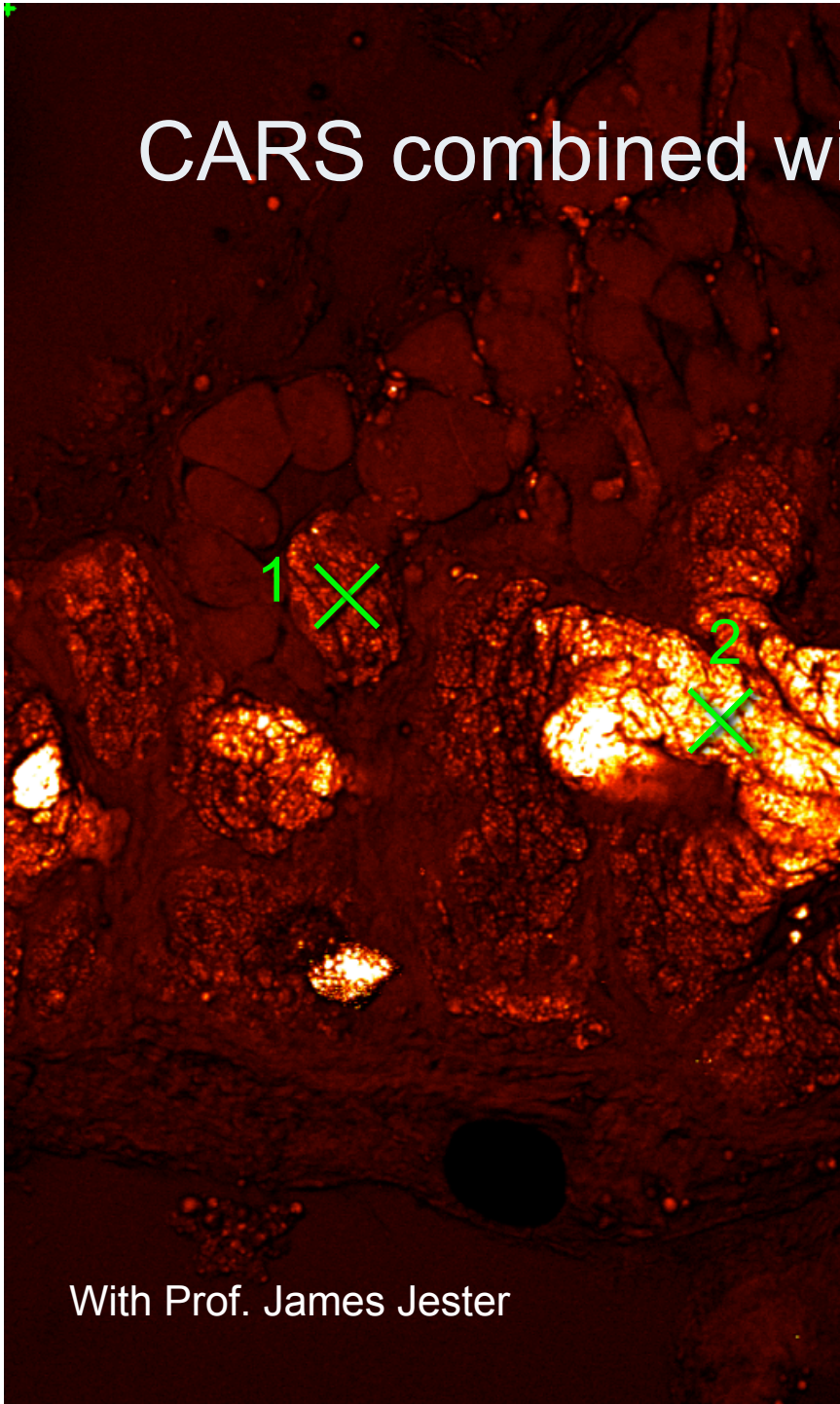


Blue: CARS, lipid

Red: SHG, collagen

Green: TPEF, elastin

# CARS combined with Raman spectroscopy

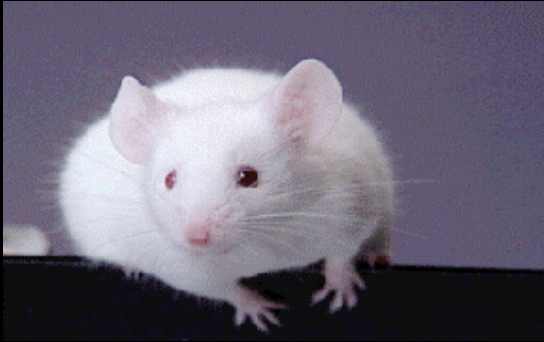


With Prof. James Jester

# CARS imaging of disease

# Atherosclerosis

ApoE deficient mouse

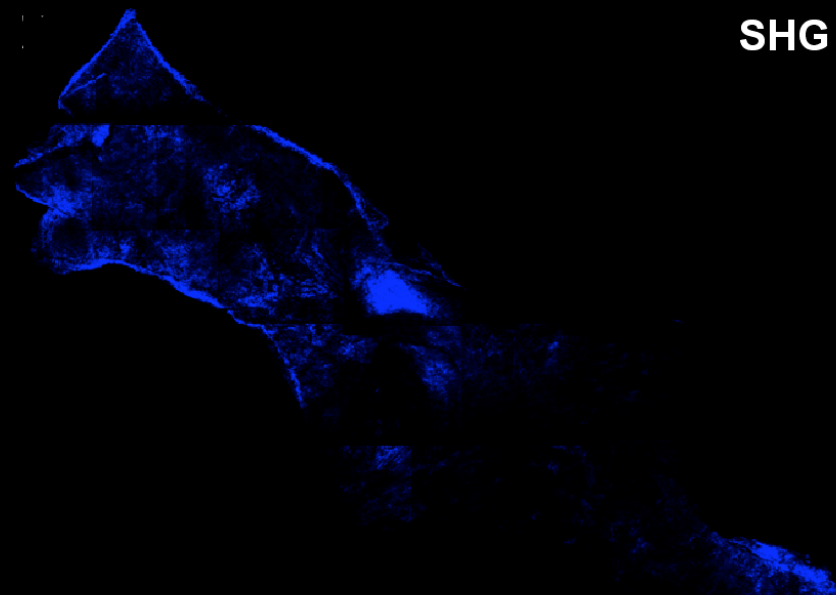
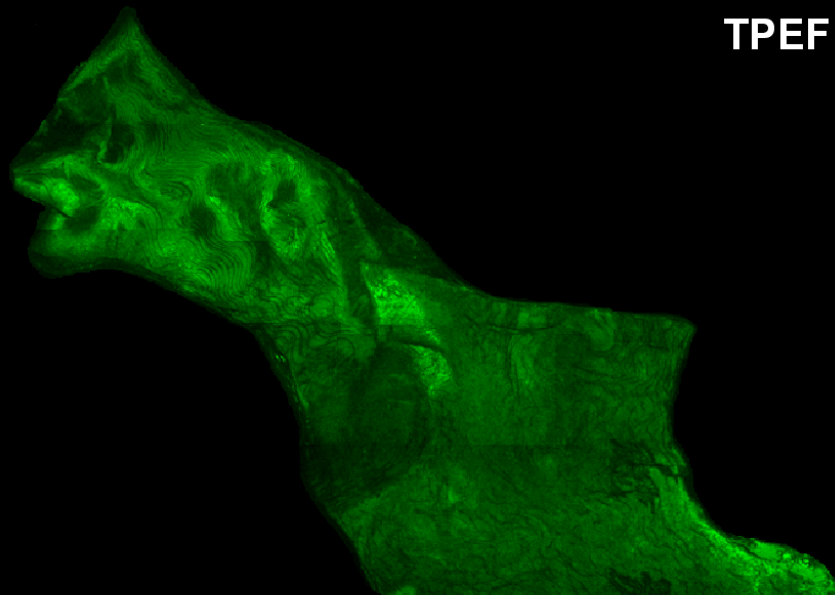


With Prof. Bruce Tromberg

# Multimodal contrast

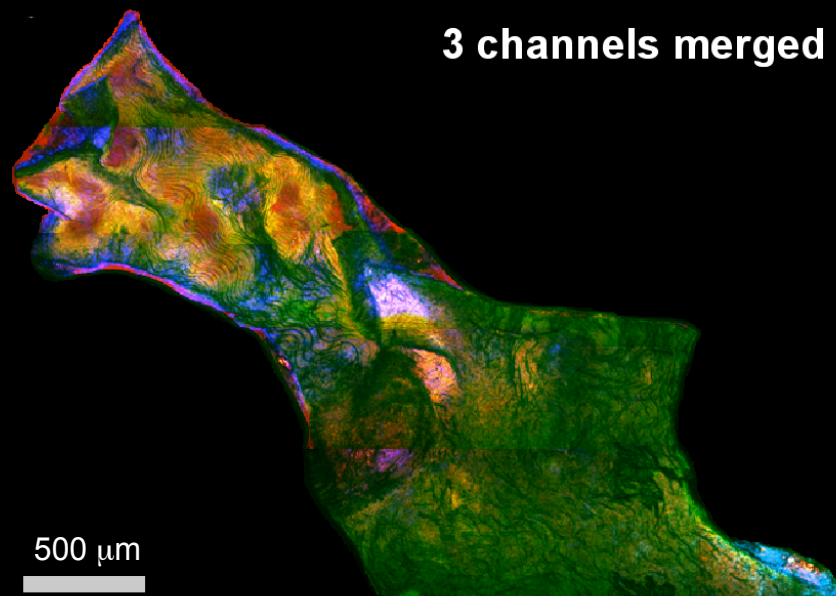
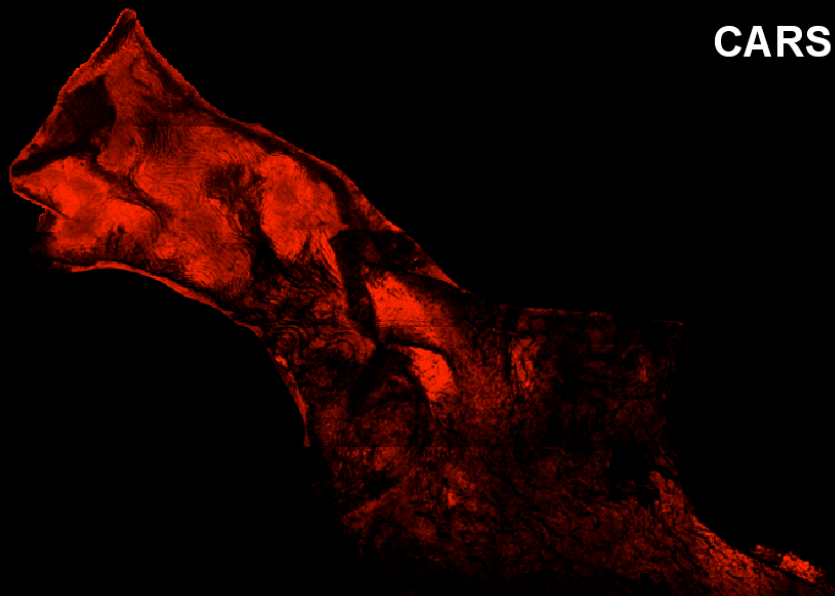
TPEF

SHG



CARS

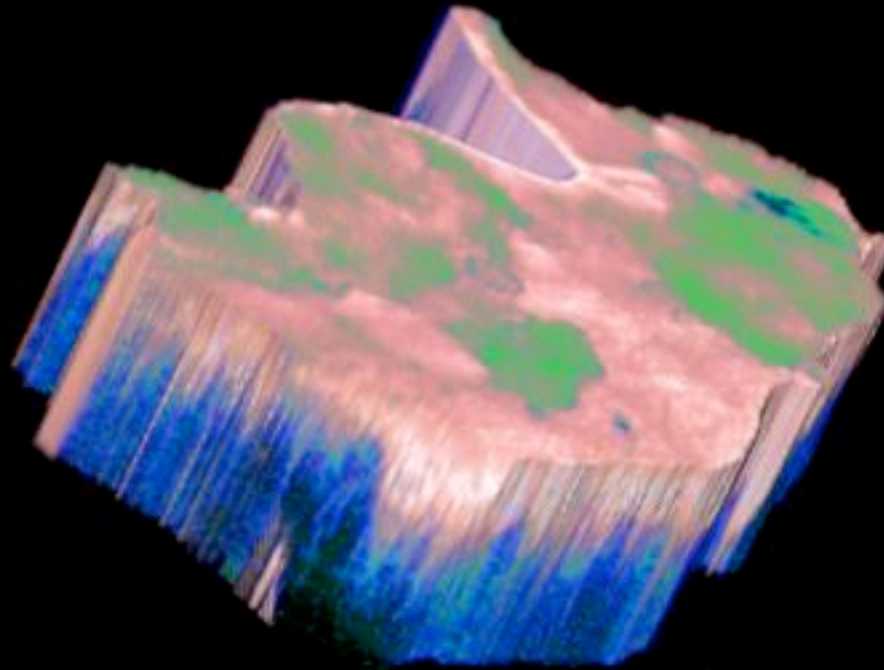
3 channels merged



500  $\mu\text{m}$



# Automated stitching and analysis

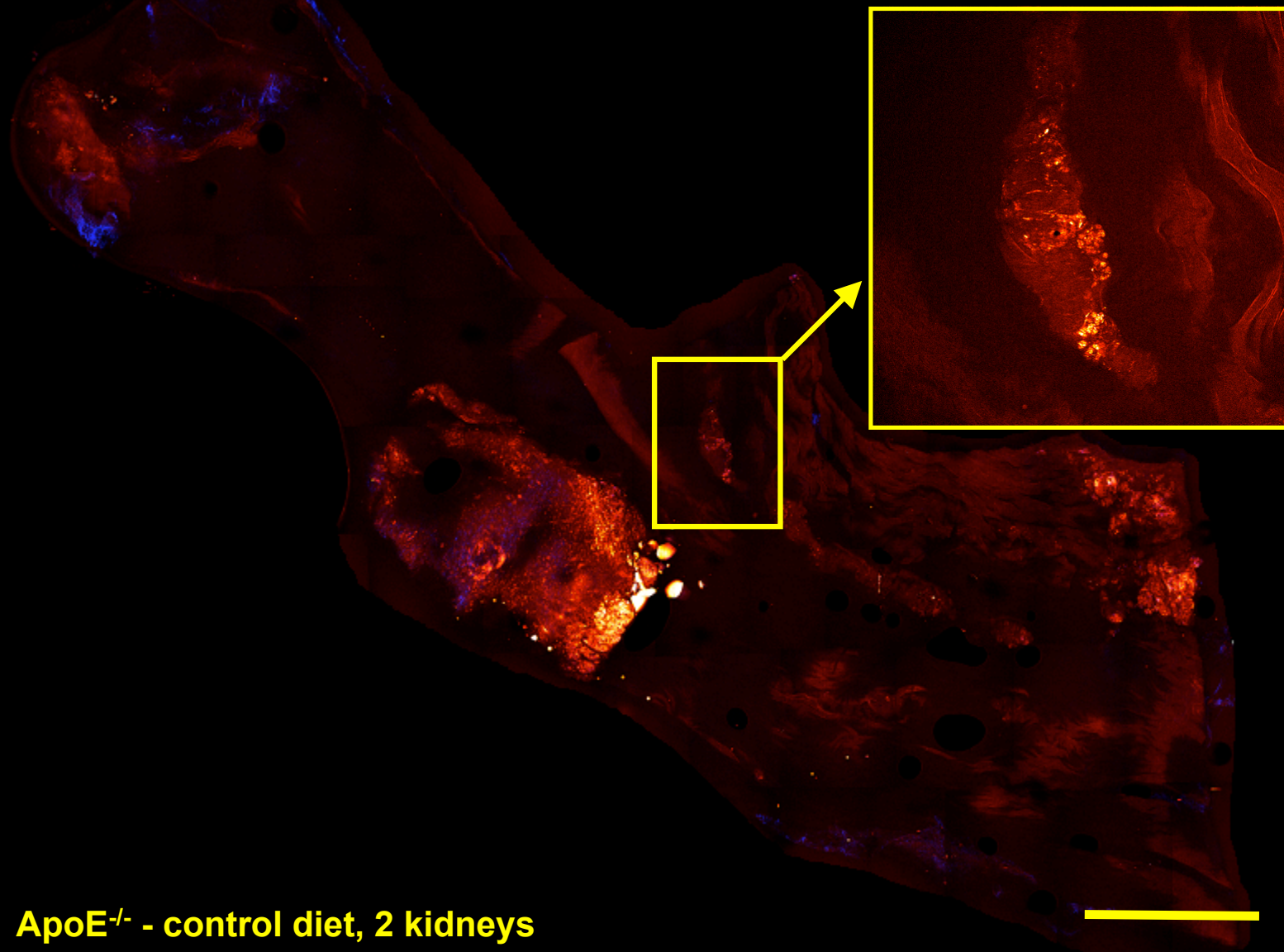


$5 \times 3 \times 0.1 \text{ mm}^3$

$\sim 1 \text{ Gpx}$

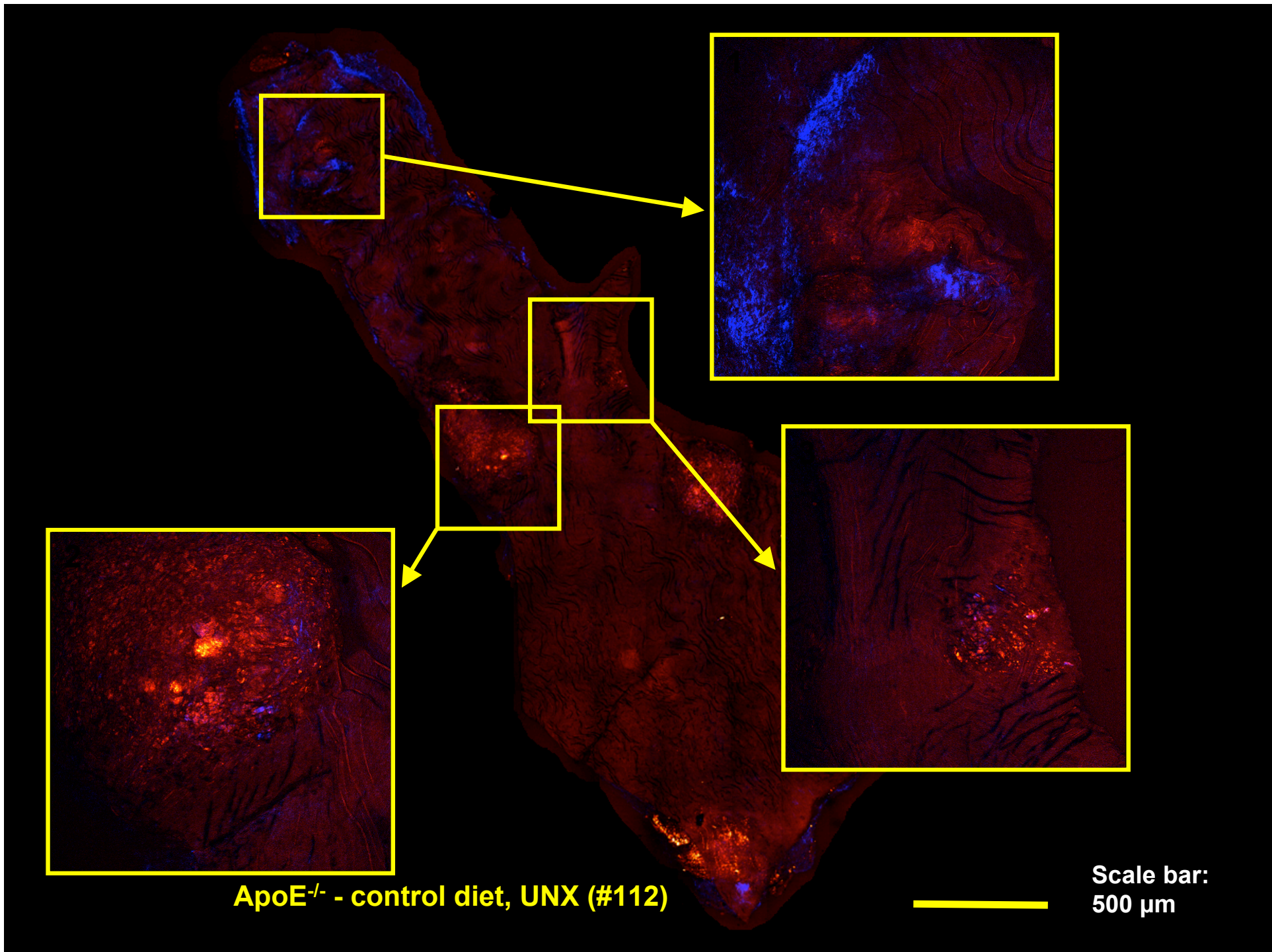
With Prof. Joerg Meyer

# Lipid deposits in aortic arch



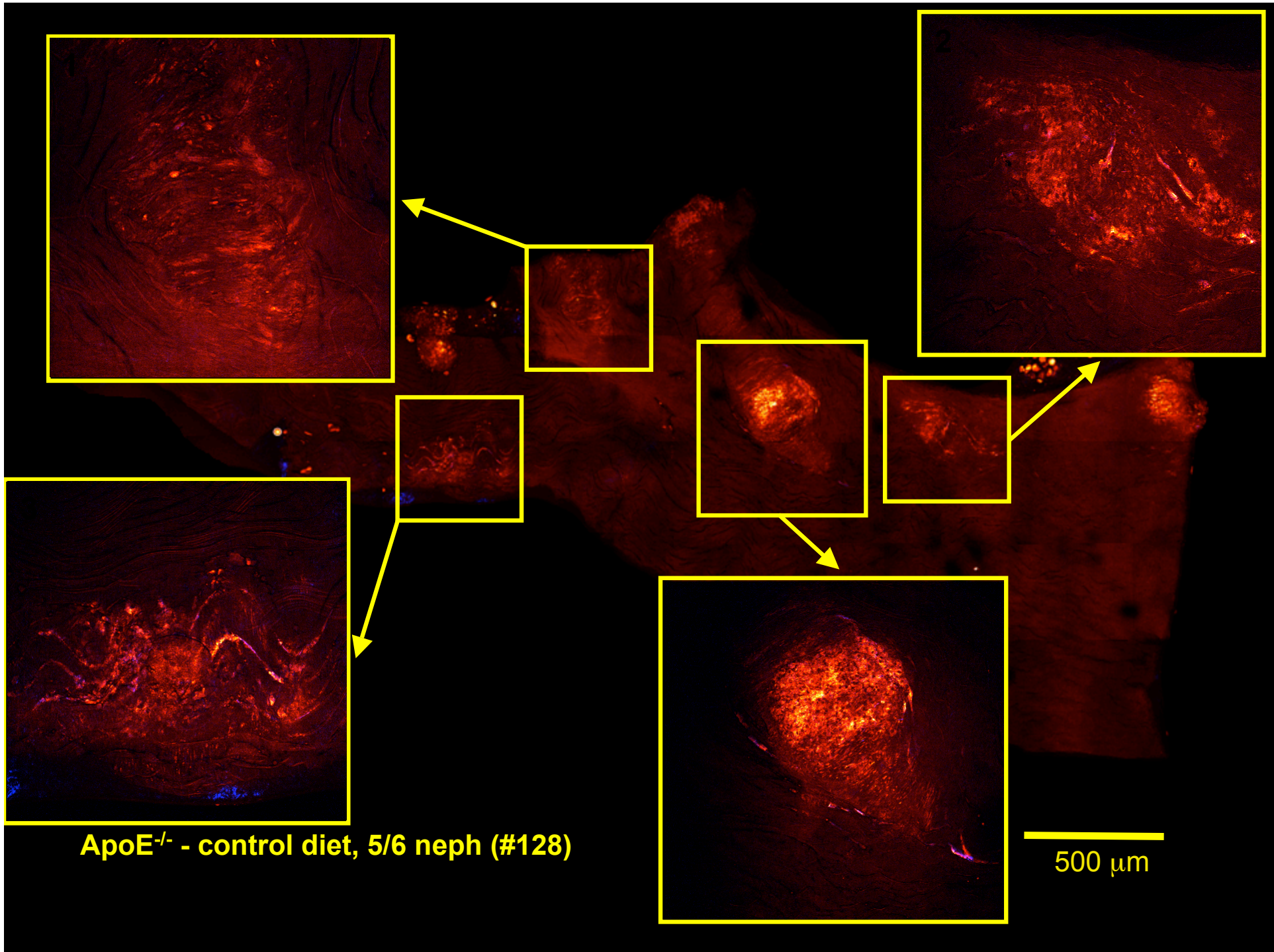
**ApoE<sup>-/-</sup> - control diet, 2 kidneys**

Scale bar: 500  $\mu$ m

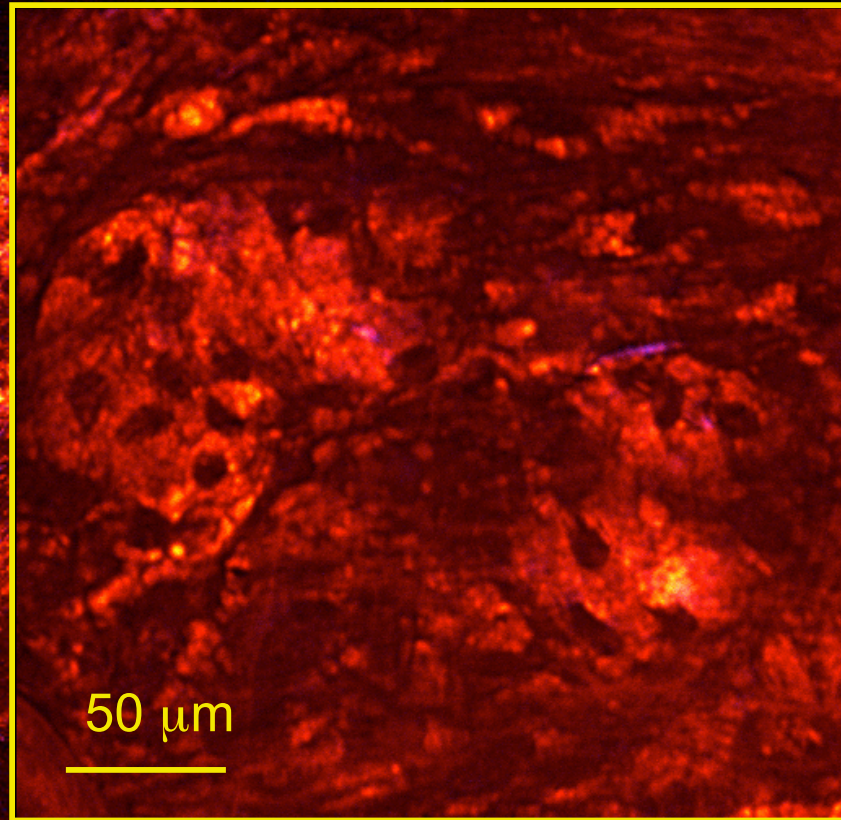


**ApoE<sup>-/-</sup> - control diet, UNX (#112)**

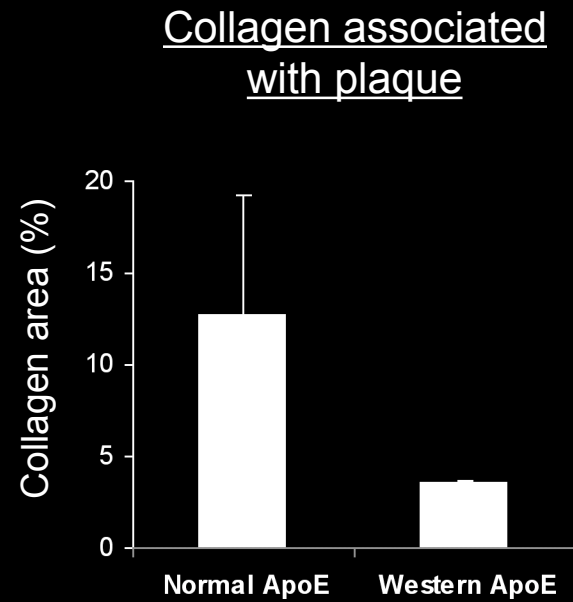
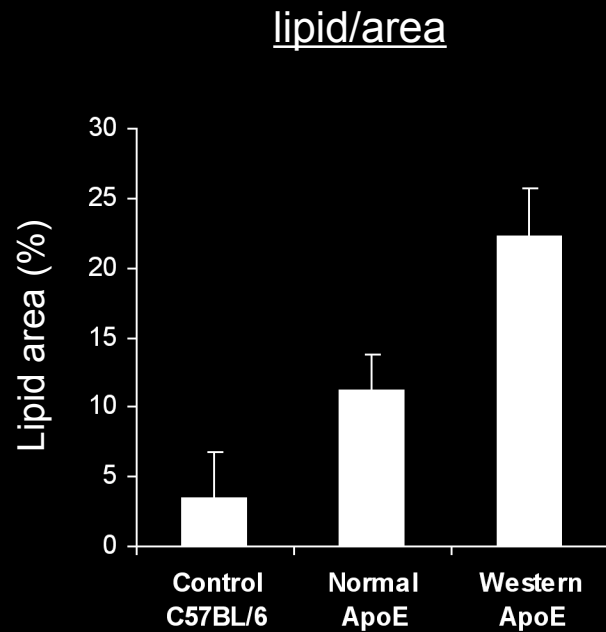
Scale bar:  
500 μm



# Seeing individual macrophages

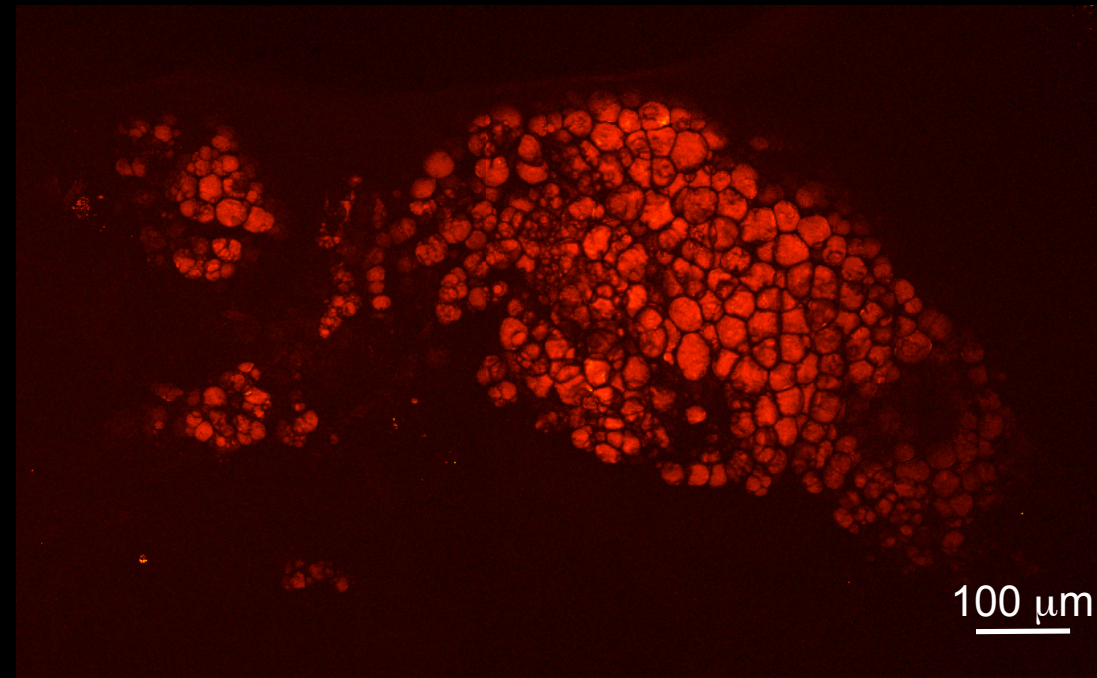


# Diet dependence

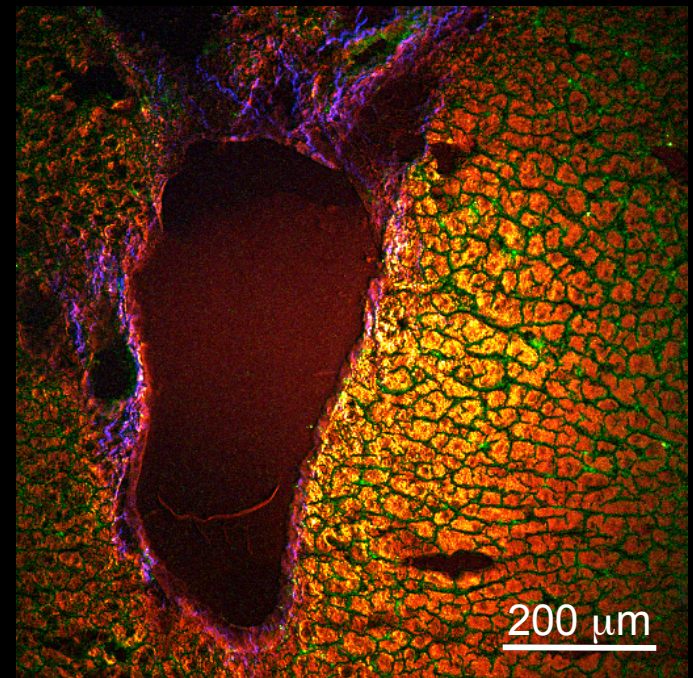


# Western diet and atherosclerosis

Lipid deposition in the heart



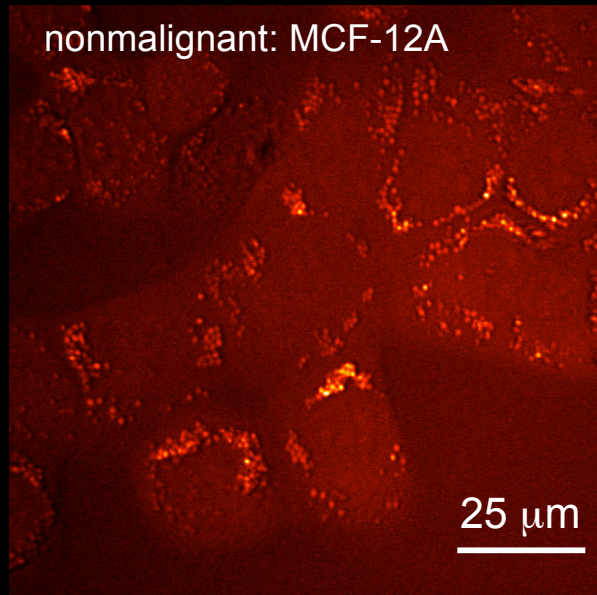
Lipid deposition in the liver



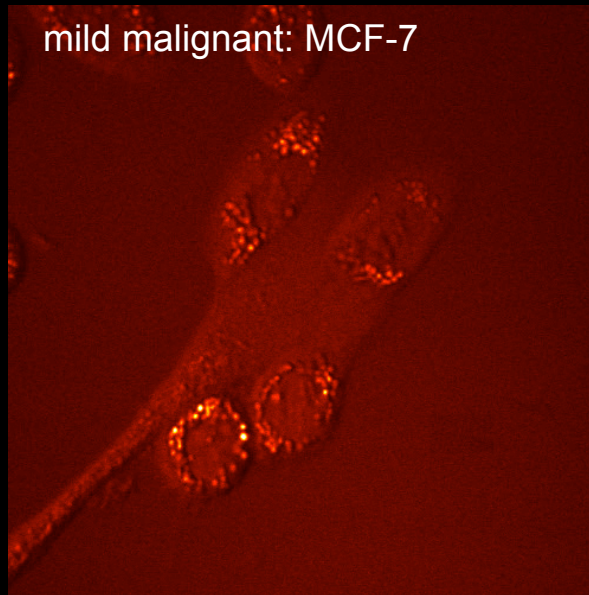
# Breast cancer and lipid droplets

human epithelial breast cells

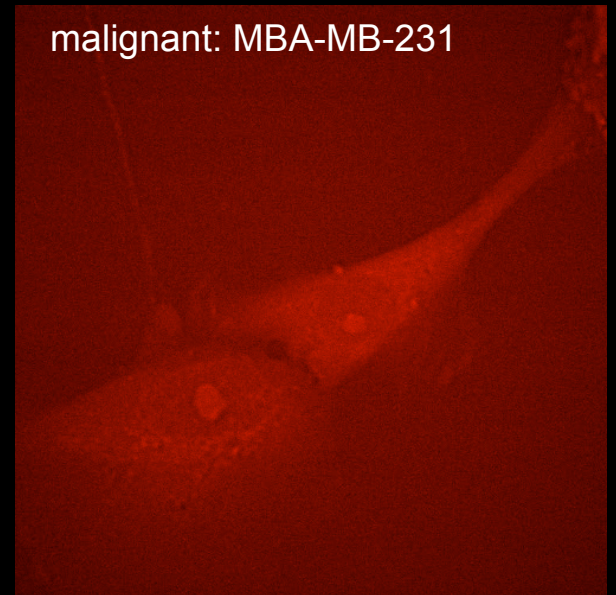
nonmalignant: MCF-12A



mild malignant: MCF-7

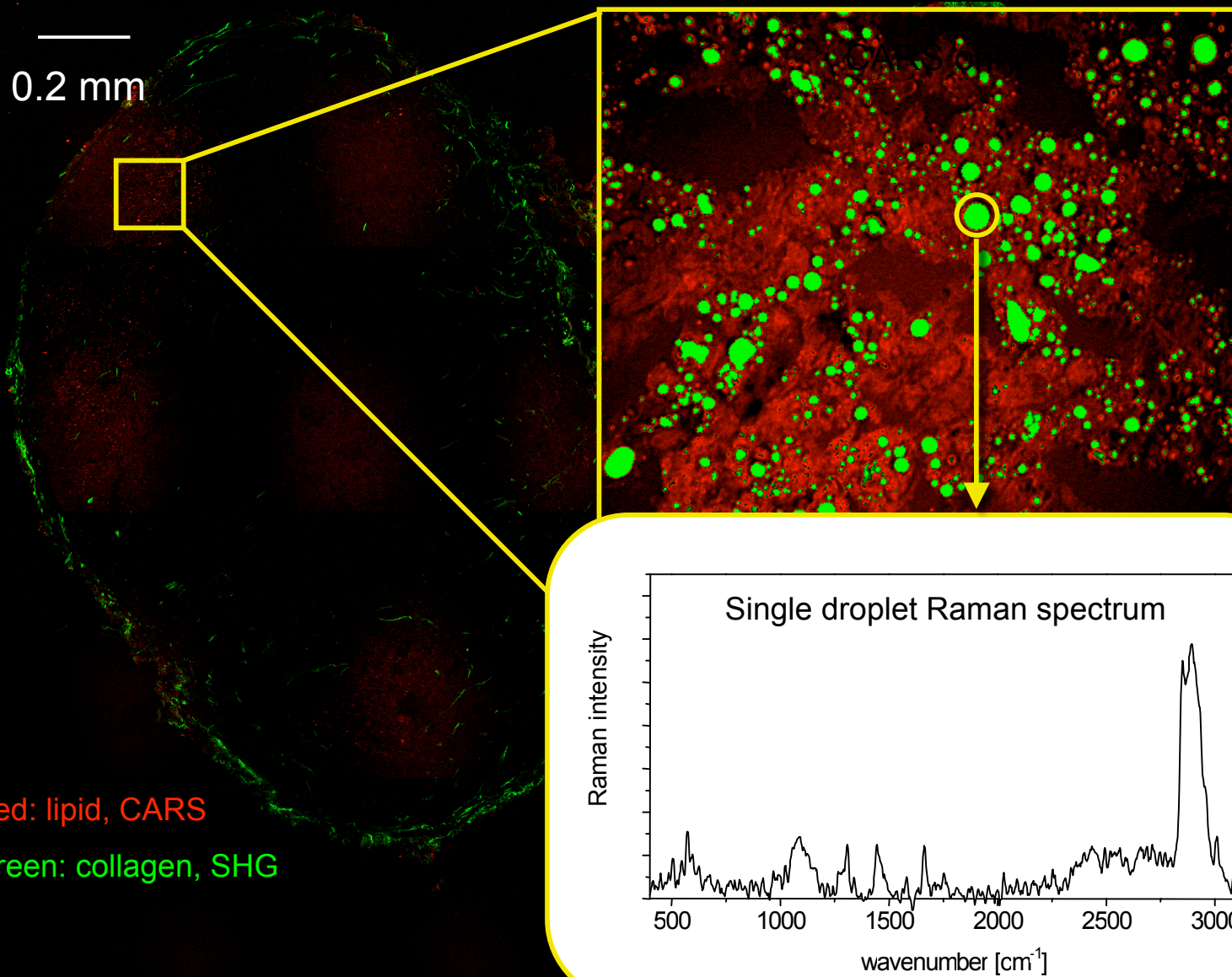


malignant: MBA-MB-231

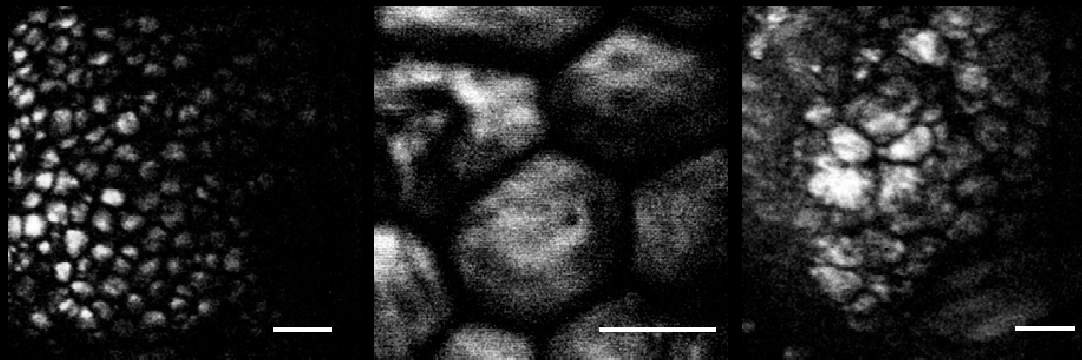
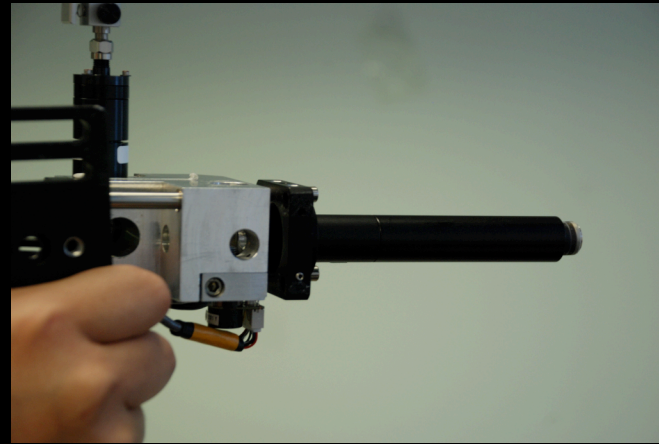


With Prof. Bruce Tromberg

# Breast cancer and lipid droplets



# From benchtop to bedside



with Prof. Zhongping Chen

# Take home messages

CARS provides chemical selectivity without labels

CARS is ideal for rapid noninvasive imaging of superficial tissues

CARS is finding its way into the clinic

Thanks!

Funding: ACS PRF grant, NSF IDBI grant