Supporting Information

Gold nanorod enhanced Photoacoustic Microscopy and Optical Coherence Tomography of Choroidal Neovascularization

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Supplementary Information

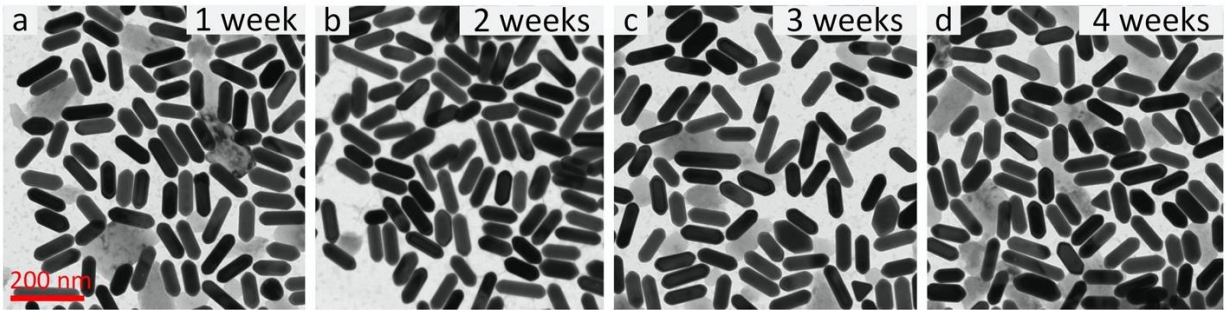

Figure S1. TEM of GNR-RGD obtained at different time points: (a) 1 week, (b) 2 weeks, (c) 3 weeks, and (d) 4 weeks.

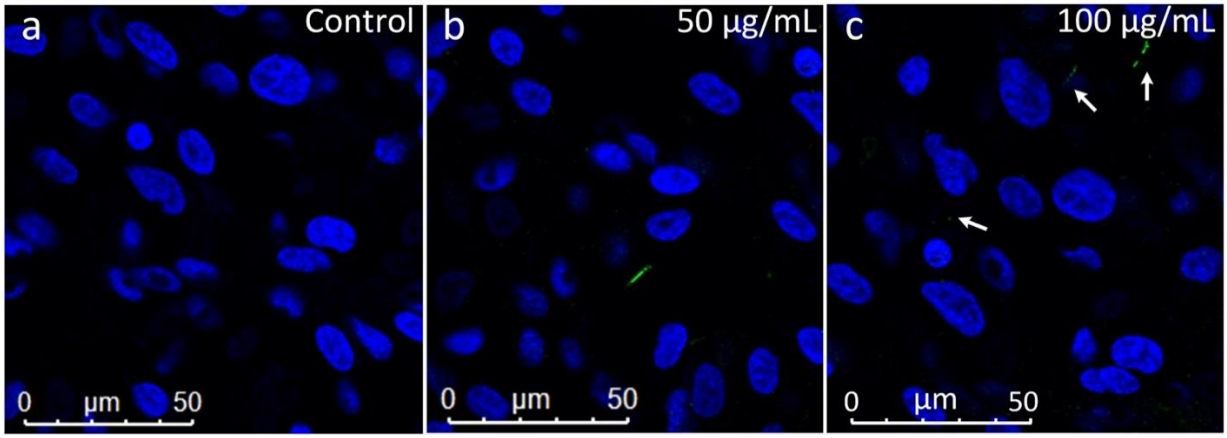

Figure S2. Confocal laser scanning microscopic image of the cells: (a) Cells treated without GNRs (control). (b-c) Cells treated with GNRs at concentrations of 50 and 100 µg/mL. There was

no fluorescent emission from the GNRs, confirming that GNRs without conjugation with RGD were not able to target the cells. Blue fluorescent signal indicates the cell nuclei stained by DAPI.

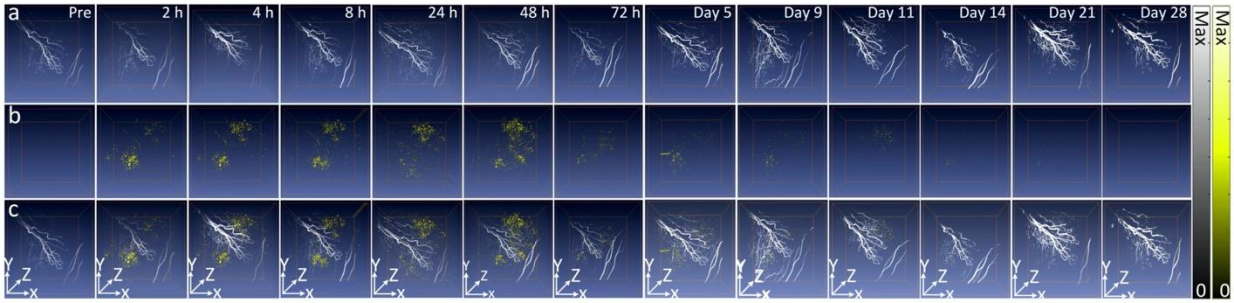

Figure S3. Longitudinal *in vivo* PAM visualization of CNV before and after administration of GNR-RGD: (a) selected PAM images obtained using an excitation wavelength of 578 nm. The PAM images show clearly retinal vessels, choroidal vessels, and capillaries with high resolution due to strong optical absorption of hemoglobin within vessels. (b) PAM images acquired at 700 nm show distribution of GNR-RGD targeted at CNV (pseudo-yellow color). (c) Overlay PAM images.

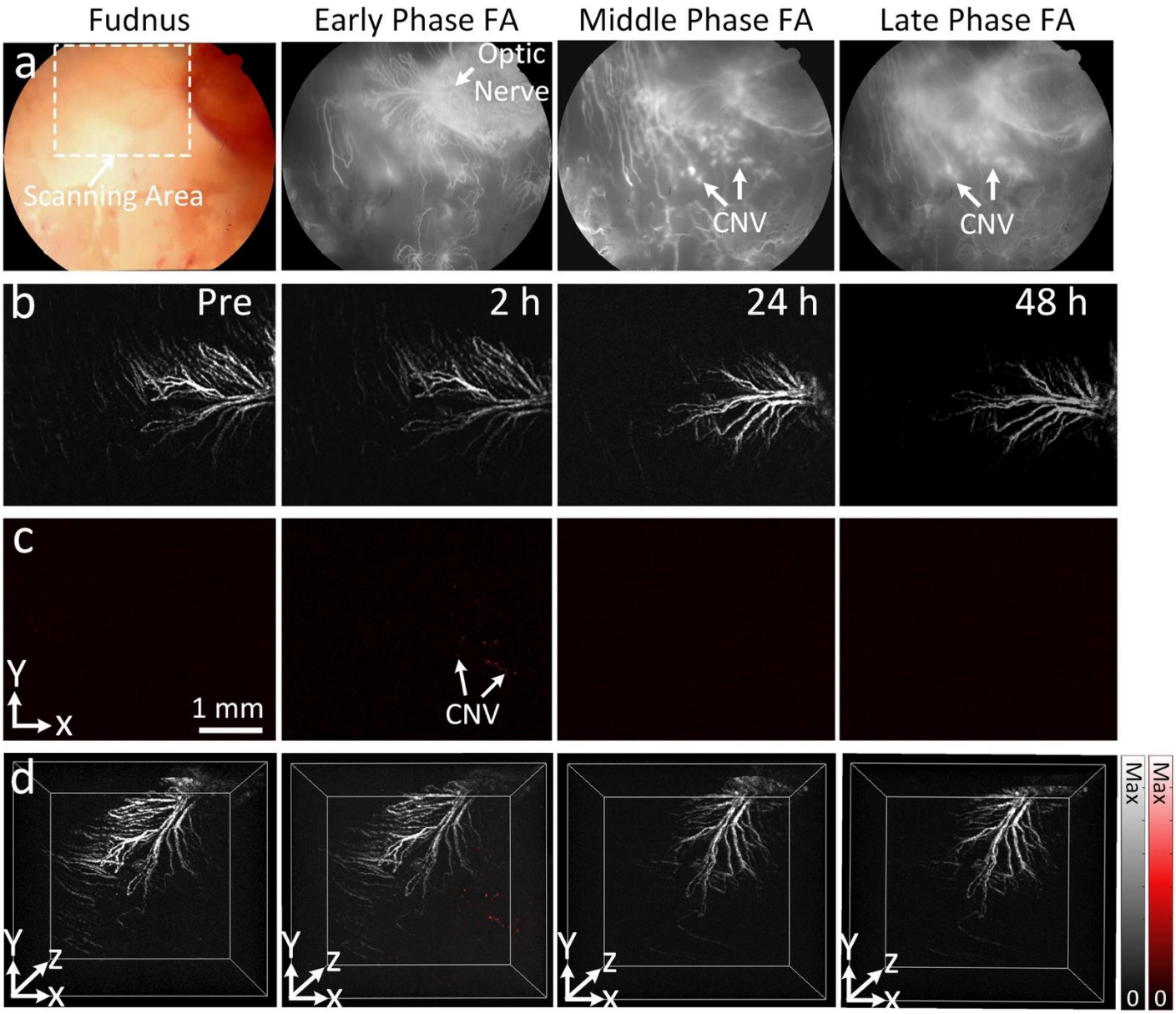

Figure S4. *In vivo* PAM visualization of CNV pre and post administration of GNR without conjugation with RGD peptide. (a) Color fundus photography of rabbit with retinal vein occlusion and fluorescein angiography (FA) acquired at various time points: early phase, middle phase, and late phase. White dotted rectangle represents the selected scanning area. FA image illustrates major retinal vessels and optic nerve (white arrow). Newly developed choroidal neovascularization (CNV) was observed on the middle and late phase FA (white arrows). (b–c) Maximum intensity projection (MIP) PAM images of CNV obtained using two different excitation wavelengths: 578

(Fig. S1a) and 650 nm (Fig. S1b) before and after injection of 400 μL GNR at concentration of 5 mg/mL at 2, 24, and 48 h (N=3). Minimal PA signal was observed on the PAM image acquired at 700 nm before the injection. On the other hand, the location of CNV were visualized on the PAM at 2 h after the injection of nanoparticles (white arrows). However, the margin of CNV was not clearly observed due to lack of targeting peptides. The detected PA signal obtained at 2 h post-injection may be caused by the extravasation of GNR at CNV as a result of the EPR effect. (d) Overlay 3D PAM images. Pseudo-red color indicates the location of GNR at CNV

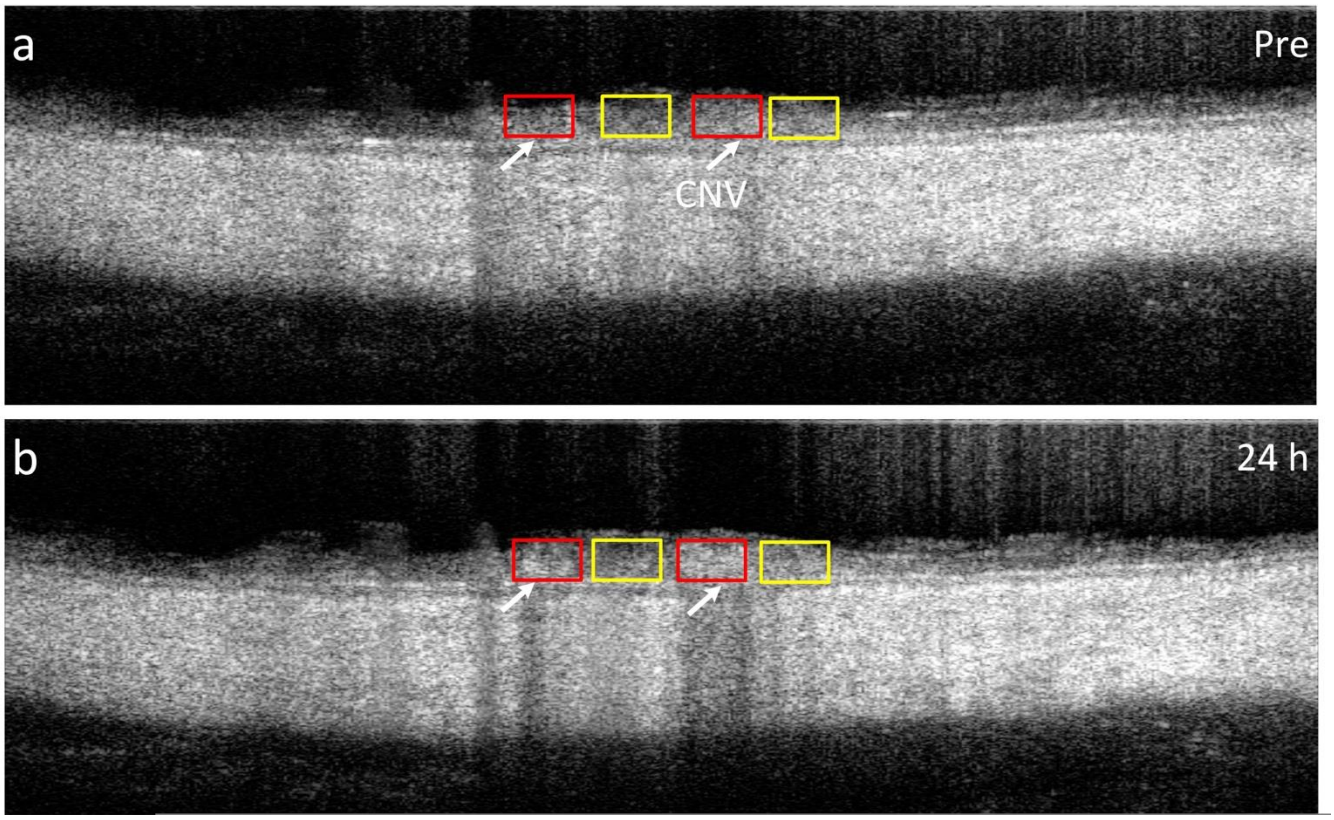

Figure S5. Region of interest (ROI) to determine average OCT signal intensity. ROIs were selected at the position of CNV (red rectangles) and adjacent tissues (yellow rectangles) before (a) and after administration of GNS (b).

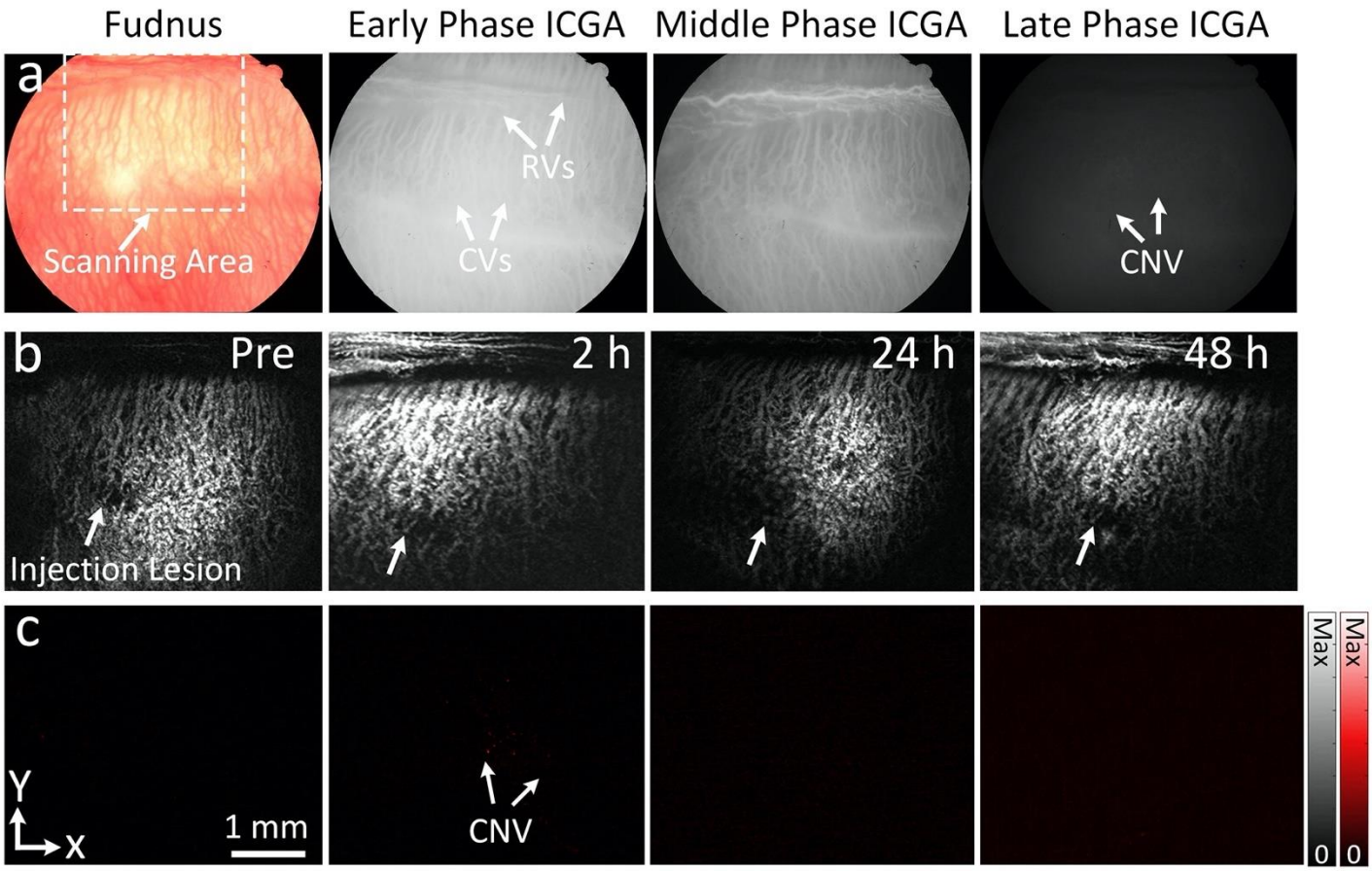

Figure S6. *In vivo* PAM images of untargeted GNRs in a CNV rabbit model using subretinal injection of VEGF-165: (a) Color fundus photograph of rabbit eye after subretinal injection of VEGF at day 7 before the injection of GNRs (left), early, middle, and late phase indocyanine green angiography (right). Early phase ICGA image shows the retinal and choroidal vessels network whereas late phase ICGA demonstrates the developed CNV (white arrows). (b–c) MIP PAM images acquired at multiple optical wavelengths of 578 and 700 nm at different times points: pre, 2 h, 24 h and 48h post-injection of GNRs (0.4 mL, 5 mg/mL) (N=3).

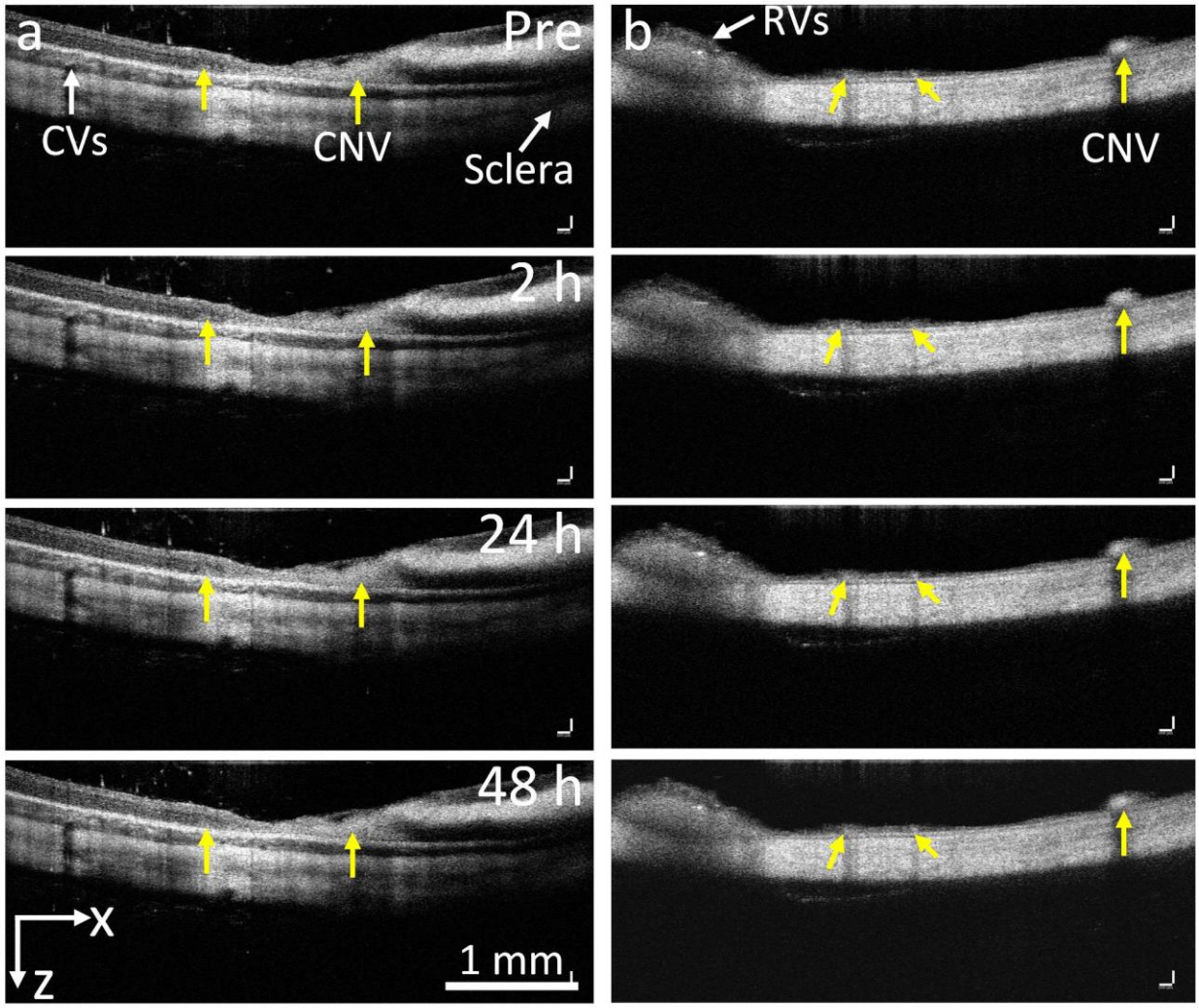

Figure S7. *In vivo* OCT images of untargeted GNRs in rabbit models. (a) Cross-sectional 2D OCT images of a CNV rabbit model using subretinal injection of VEGF-165 obtained before and after injection of nanoparticles (0.4 mL, 5 mg/mL) at 2 h, 24 h and 48h (N=3). (b) OCT images of a CNV model using laser-photocoagulation. Yellow arrows indicate the position of newly developed CNV.

Media:

Visualization 1: 3D visualization of internalized GNRs uptake by cells

Visualization 2: 3D volumetric rendering of choroidal vessels and choroidal neovascularization 24 hours after the injection of GNRs.