Supplementary Information

Observation of mesenteric microcirculatory disturbance in rat by laser oblique scanning optical microscopy

Yichen Ding[1,§], Yu Zhang[2,§], Tong Peng[1], Yiqing Lu[4], Dayong Jin[4], Qiushi Ren[1],

Yuying Liu[2], Jingyan Han[2,3,*], Peng Xi[1,*]

[1]Department of Biomedical Engineering, College of Engineering, Peking University, No. 5 Yiheyuan Road, Beijing 100871, China

[2] Department of Integration of Chinese and Western Medicine, School of Basic Medical Science, Peking University, 38 Xueyuan Road, Beijing 100191, China

[3] Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing 100191, China

[4] Advanced Cytometry Labs, MQphotonics Research Centre, Macquarie University, NSW 2109, Sydney, Australia

*To whom correspondence should be addressed. Email: xipeng@pku.edu.cn (P. X.); hanjingyan@bjmu.edu.cn (J. H.).

This PDF file includes:

Figure S1
Figure S2
Figure S3

In Figure S1, with time lapse, it shows the number of counted leukocytes after processing in the I/R and sham groups. All the data are normalized to be within 150 μm venule.

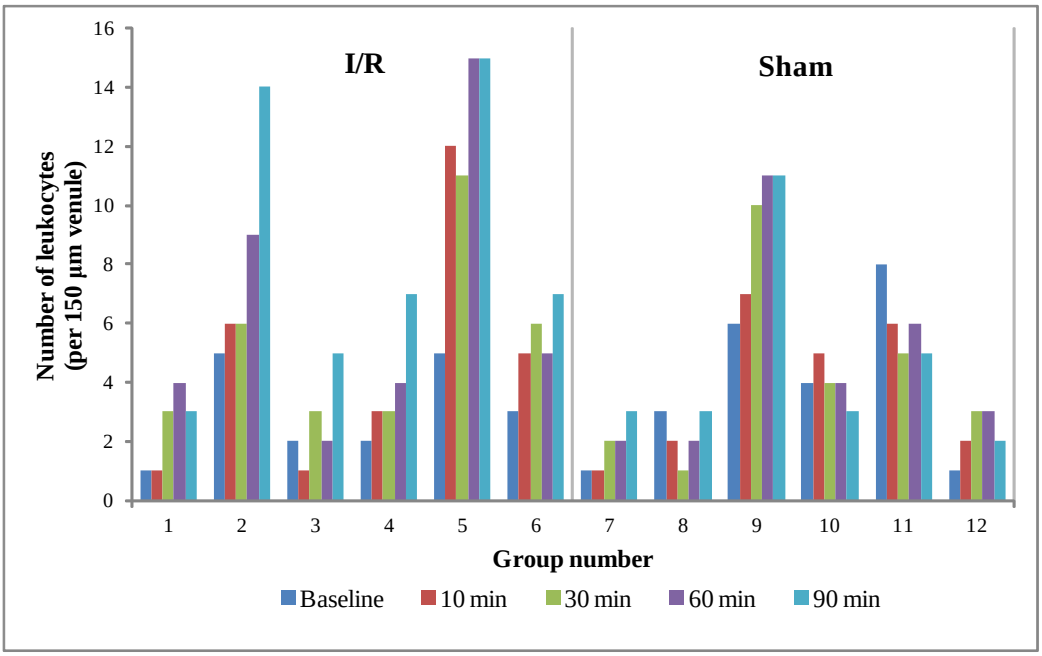


Figure S1 Raw data of the quantity of leukocyte in I/R and sham groups, at each time interval. Twelve rats were divided into two groups (n=6 per group). Horizontal axis indicates group number, and vertical axis is the number of leukocytes.

Human umbilical vein endothelial cells (HUVECs) were used to test the phototoxicity of 375 nm laser illumination. The cells were divided into two groups (n=6, per group). In Group 1, some domains were illuminated by 375 nm laser for 30 s (original condition), while as a control the other domains was not exposed to light so that the inherent cell death rate could be measured. In the other group (Group 2), except for changing the exposure time to 300 s (which was ten times longer than original case) for some domains, other conditions were the same as those in Group 1. After illumination at different timespans, cells of each group were labeled with Propidium Iodide, to test the cells viability.

Group 1 (illuminated for 30 s, and shown in Figure S2(a)), the cell death rate remained at 9%. Figure S2(b) shows the other domain without illuminated from the same group.

Group 2 (illuminated for 300 s, and shown in Figure S2(c)), the cell death rate increased to 25%. Figure S2(d) shows the other domain without illuminated from

same group.

Differences between the groups were analyzed with one-way ANOVA and Turkey post-test analysis. In Figure (e), there is no statistic difference between 30 s group (9%) and the control group (6%), while a significant difference ($p<0.001$) was found between the 300 s group (25%) and the control group (6%). Therefore, this result demonstrates that 375 nm excitation light applied in our experimental condition is relatively biological safe, while ten times of original dose was found to be phototoxic. Use of a longer wavelength with the LOSOM excitation laser can decrease its phototoxicity to cells and to other tissues.

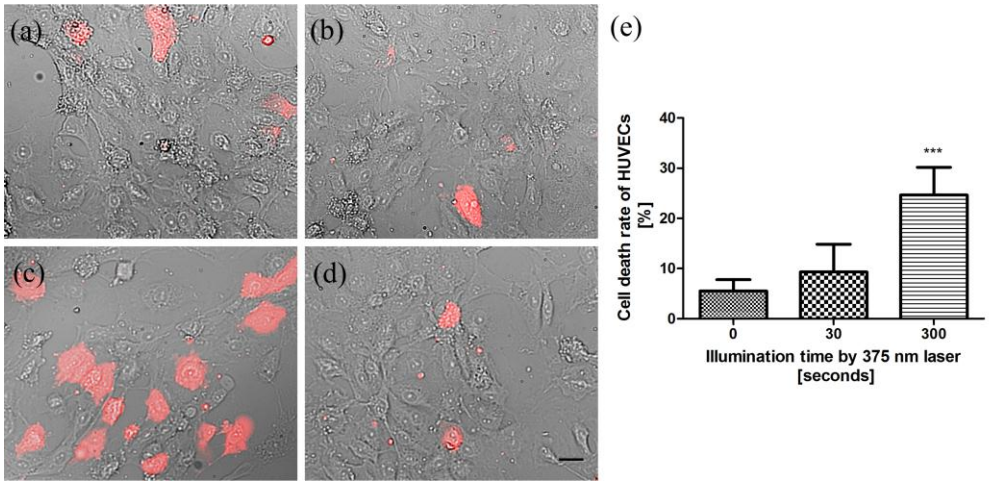
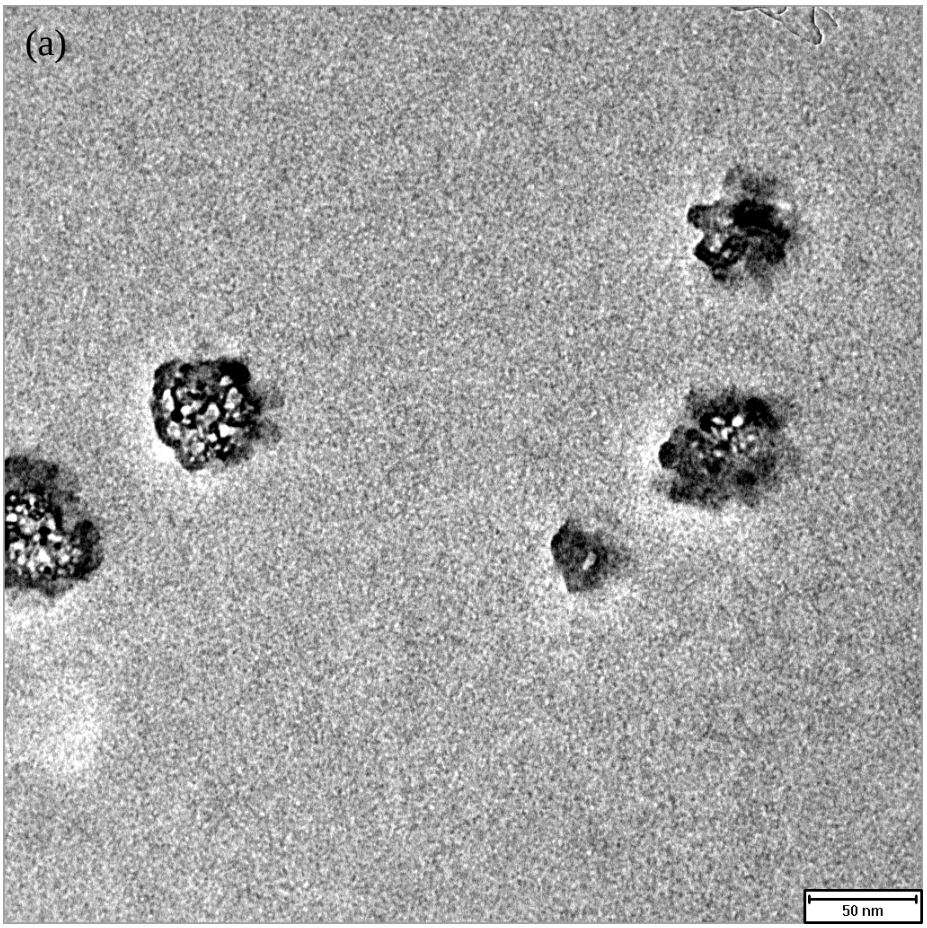
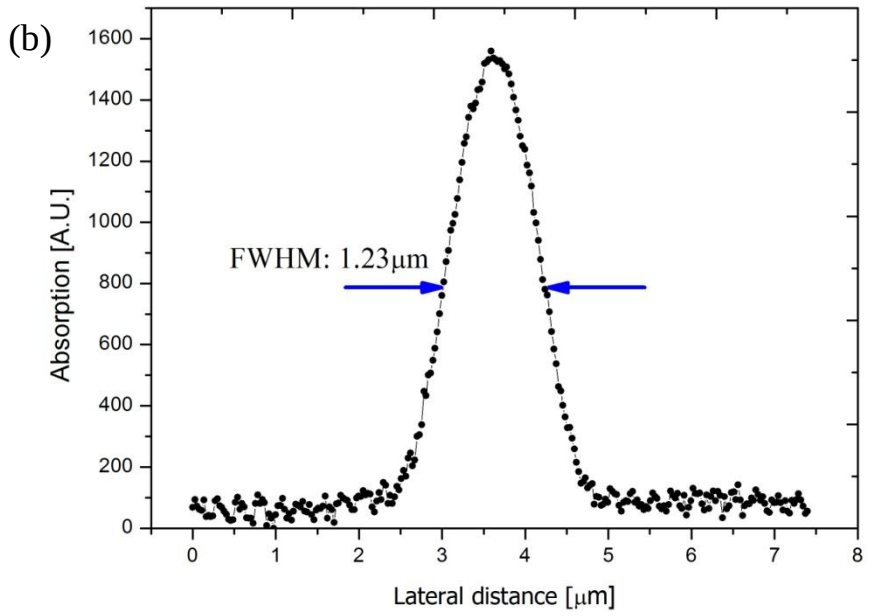


Figure S2 Imaging results of Propidium Iodide stained HUVECs, red areas mean injuries. (a) A 375 nm laser illuminating domain after 30 s and (b) the other domain in the same group, but without exposure to 375 nm light as in (a). (c) 375 nm laser illuminating domain after 300 s and (d) the other domain in the same group, but without exposure to the 375 nm light as in (c). Scale bar: 30 μm. (e) The result of an one-way ANOVA with Turkey post-test analysis of the treatment groups

In this study, we used a Nikon Plan Fluor objective with magnification of 10X, a numerical aperture (NA) of 0.3, and a working distance of 16 mm. The measurements of resolutions are based on the point spread function of a diffused nanoparticle in carbon black ink. The diameter of the carbon nanoparticle is measured to be ~40 nm by TEM (Hitachi H9000NAR, Japan). We defined Full Width at Half Maximum (FWHM) as its resolution. Thus, the lateral and axial resolutions were found to be 1.23 μm and 13.15μm, respectively.


(a)
50 nm


(b)
FWHM: 1.23μm
Absorption [A.U.]
Lateral distance [μm]
0
200
400
600
800
1000
1200
1400
1600
0
1
2
3
4
5
6
7
8

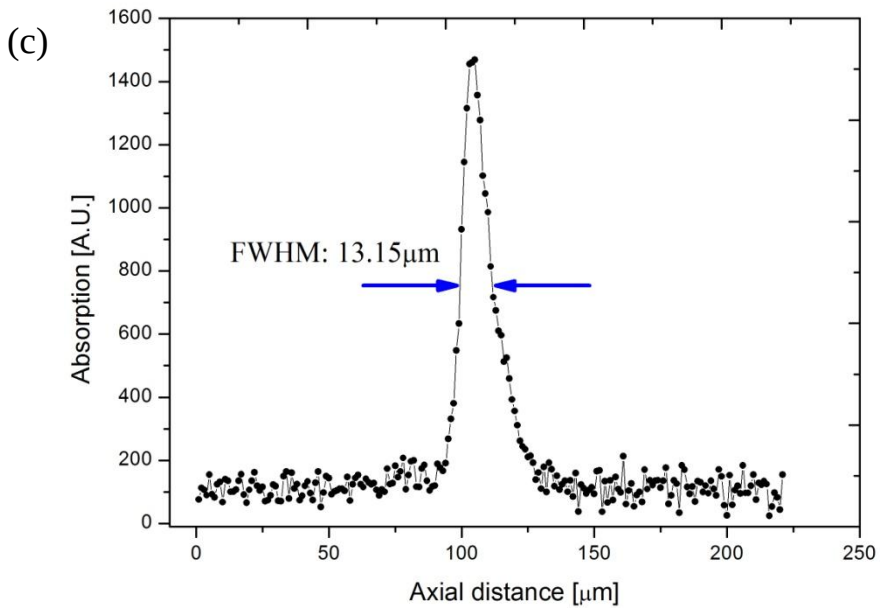


Figure S3 (a) TEM result of the nanoparticle. Scale bar: 50 nm. (b) Measured lateral resolution and axial resolution(c) of LOSOM.

Other Online Supporting Information for this manuscript includes the following:

Movie S1

Movie S2

Movie S3

Movie S4