Supporting information

Label-free Imaging of Heme Dynamics in Living Organisms by Transient Absorption Microscopy

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Abstract

In this support information, firstly, a diagram of TA microscopy is presented. Secondly, we observed that heme exists as granules and diffused form in the intestine cells of *C. elegans*. Here we presented TA images showing that in pharynx and hypodermis, heme exists as diffused form. Thirdly, we demonstrated that TA microscopy can image heme in worms kept in conditions where heme concentrations were artificially defined. Here we present data showing that in standard culture condition, TA microscopy has the sensitivity to image heme as well. fourthly, we presented the lateral and axial intensity profiles of heme granule, based on which the lateral and axial resolutions were calculated. Lastly, we put the captions for supporting videos at the end.

SUPPLEMENTAL FIGURES AND CAPTIONS

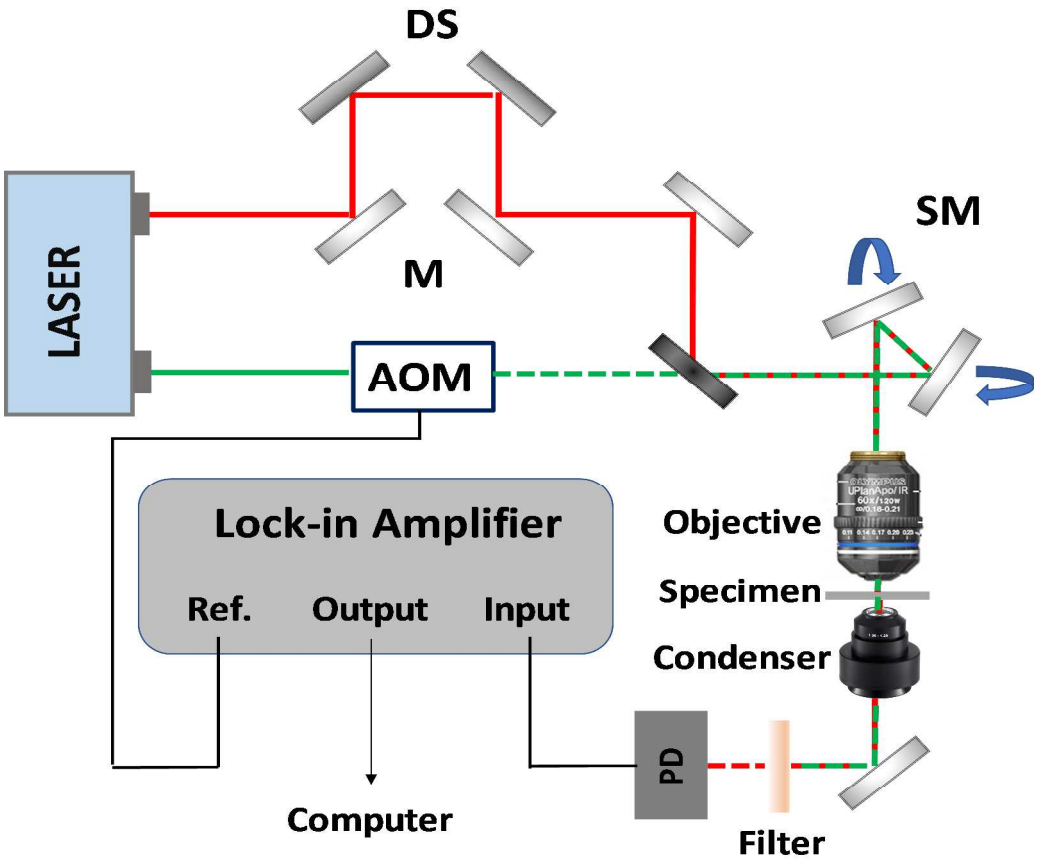

Figure S1. Scheme of TA microscope. DS: delay stage; M: mirror; SM: scanning mirror; PD: photo-diode; Ref: reference.

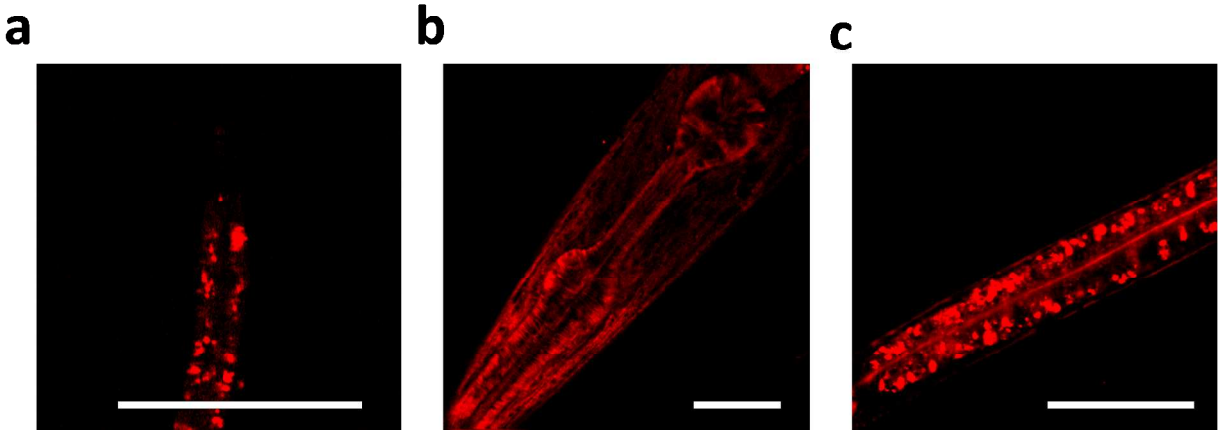

Figure S2. Diffused heme observed in intestine cell, pharynx and hypodermis region. a. Diffused heme was observed in intestine cells in worm fed with 20 μM hemin, displaying dynamic range: 0.32-1.17; **b.** Heme in pharynx was observed in worms fed with 100 μM hemin, displaying dynamic range: 1.3-6.89; **c.** Heme was observed hypodermis region in worms fed with 80 μM hemin; contrast was enhanced to make hypodermis region display evidently; displaying dynamic range: 1.11-5.18. Scale bar: 40 μm.

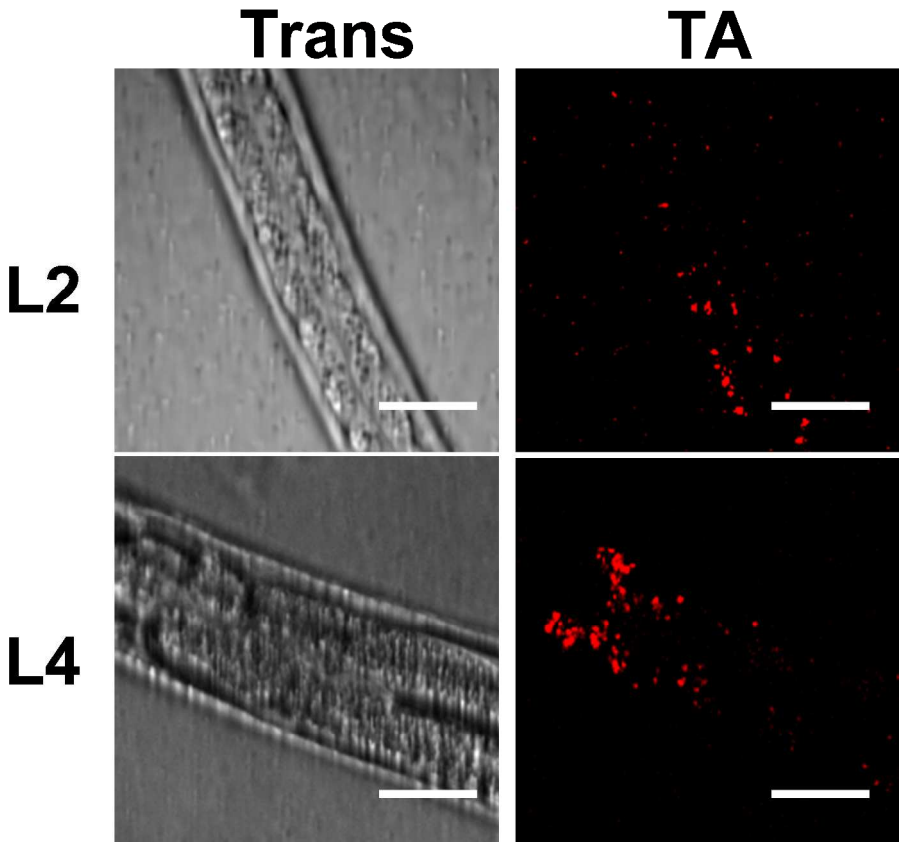

Figure S3. Transient absorption microscopy has the sensitivity to image endogenous heme in *C. elegans*. Wild type L2 and L4 worms kept on NGM plates were imaged using TA microscopy and transmission imaging; scale bar: 15 μm.

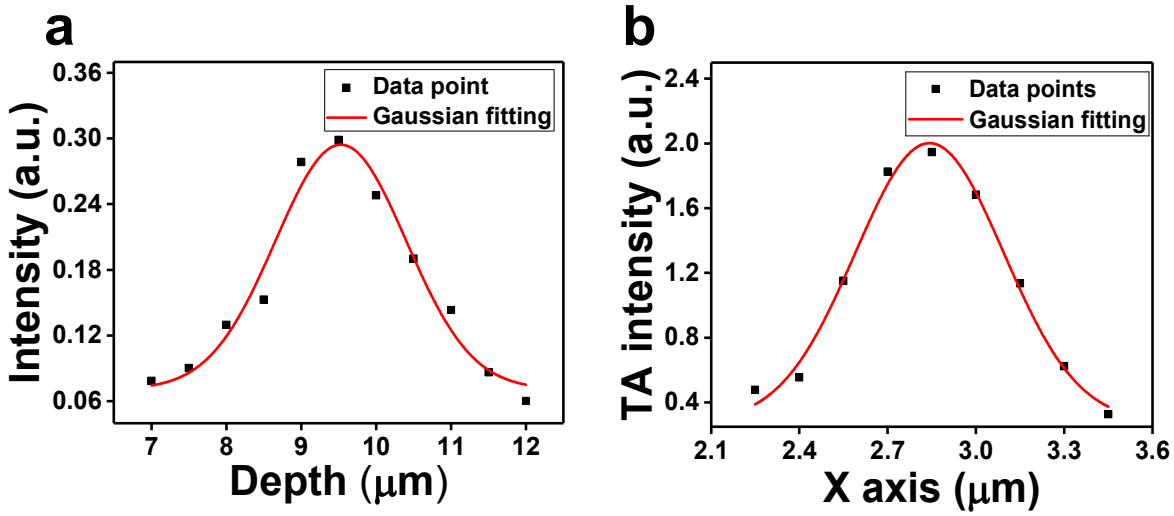

Figure S4. Axial and lateral resolutions of TA microscopy. a. Intensity profile of heme granule along z direction was plotted, curve was fitted using Gaussian function; **b**. Intensity profile of heme granule on lateral dimension was plotted and fitted using Gaussian function.

SUPPORTING VIDEO CAPTIONS:

Supporting Video 1. 3D view of heme revealed by 3D TA imaging Wild type. *C. elegans* kept in CeHR medium (+5 μM hemin) was imaged using 3D TA microscopy. 3D image was reconstructed from image stacks and rendered using ImageJ. Scale bar: 30 μm.

Supporting Video 2. Dynamic TA imaging of heme granules in intestine cells. Wild type worms were fed with 200 μM hemin for 1 hour, then 100 frames of TA images were recorded continuously. Frame size: 400X400 pixel, dwell time: 10 μs. Scale bar: 40 μm. Intestine and worm was outlined in red and blue, respectively. The granules whose breakdown was recorded was indicated by white arrows.