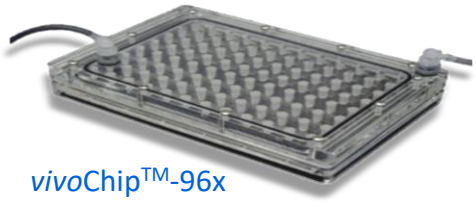
APPLICATION NOTE

High-throughput screening of protein aggregation model in *C. elegans*

Purpose

The study of age-dependent protein aggregation in *C. elegans* is enabled by 3-dimensional (3D), whole-body imaging of 4,000 animals, immobilized using the *vivo*Chip-96x. This kind of whole-animal imaging facilitates rapid analysis of polyglutamine-induced aggregation in the body wall muscle cells and can be used to identify new proteostasis regulators.



*vivo*Chip™-96x

Methods

- Up to 40 adult animals/well are immobilized and laterally oriented in the *vivo*Chip-96x within 3 min.
- Polyglutamine-induced aggregates in the body wall muscle cells are labelled with unc-54::Q35::YFP.
- 3D distribution of age-dependent polyglutamine aggregation is determined from the high-resolution whole-body images (Fig. 2b).
- Number of aggregates per unit animal length are quantified to determine the protein homeostasis.

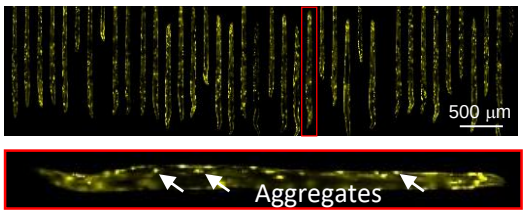
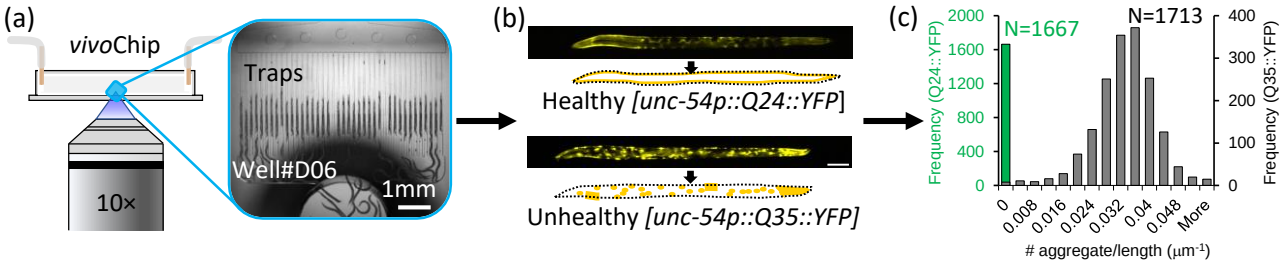


Figure 1. Images of unc-54::Q35::yfp animals immobilized in the *vivo*Chip-96x, showing the 3 – 5 µm size aggregates (white arrows).



Immobilize and image up to 4,000 animals in *vivo*Chip-96x

Identify aggregates using a customized analysis software

Aggregate number/length of healthy and unhealthy animals

Figure 2. (a) A high-throughput workflow for measuring protein aggregation in *C. elegans* using *vivo*Chip. (b) *C. elegans* with 35 glutamine repeats (Q35; beyond the threshold value) causes age-dependent aggregation despite being soluble during the early developmental stages. (c) The number of aggregates per unit length are distinctly different in the healthy (Q24; 24 glutamine repeats) and unhealthy (Q35) animals.

Conclusions

- The *vivo*Chip-96x enables rapid immobilization of 4,000 *C. elegans* from 96 different populations.
- Using 10×, 0.3NA objective and a large field-of-view camera, we can capture high-resolution whole body images with 1-micron resolution spanning fifteen z-stacks in 15 minutes (Fig. 2a).
- Parallel channel geometry of the *vivo*Chip simplifies image analysis of PolyQ::YFP animals using customized image-analysis software and allows aggregates (Fig. 2b) to be quantified in a high-throughput manner.
- Imaging 40 animals per population provides statistically significant data and can detect even the small changes in the number of aggregates during various treatment conditions (Fig. 2c).
- The ability to quantify the distribution of aggregates along the animal body can facilitate the investigation of proteostasis mechanisms and identify molecular and genetic interactors for protein homeostasis (Ref: Mondal S. *et al.* Nat. Commun. 2016).