

Fully automated leg tracking of *Drosophila* neurodegeneration models reveals distinct conserved movement signatures

Tuesday, 09 Jul 2019



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Published in ***PLOS Biology*** on June 27, 2019

<https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.3000346>

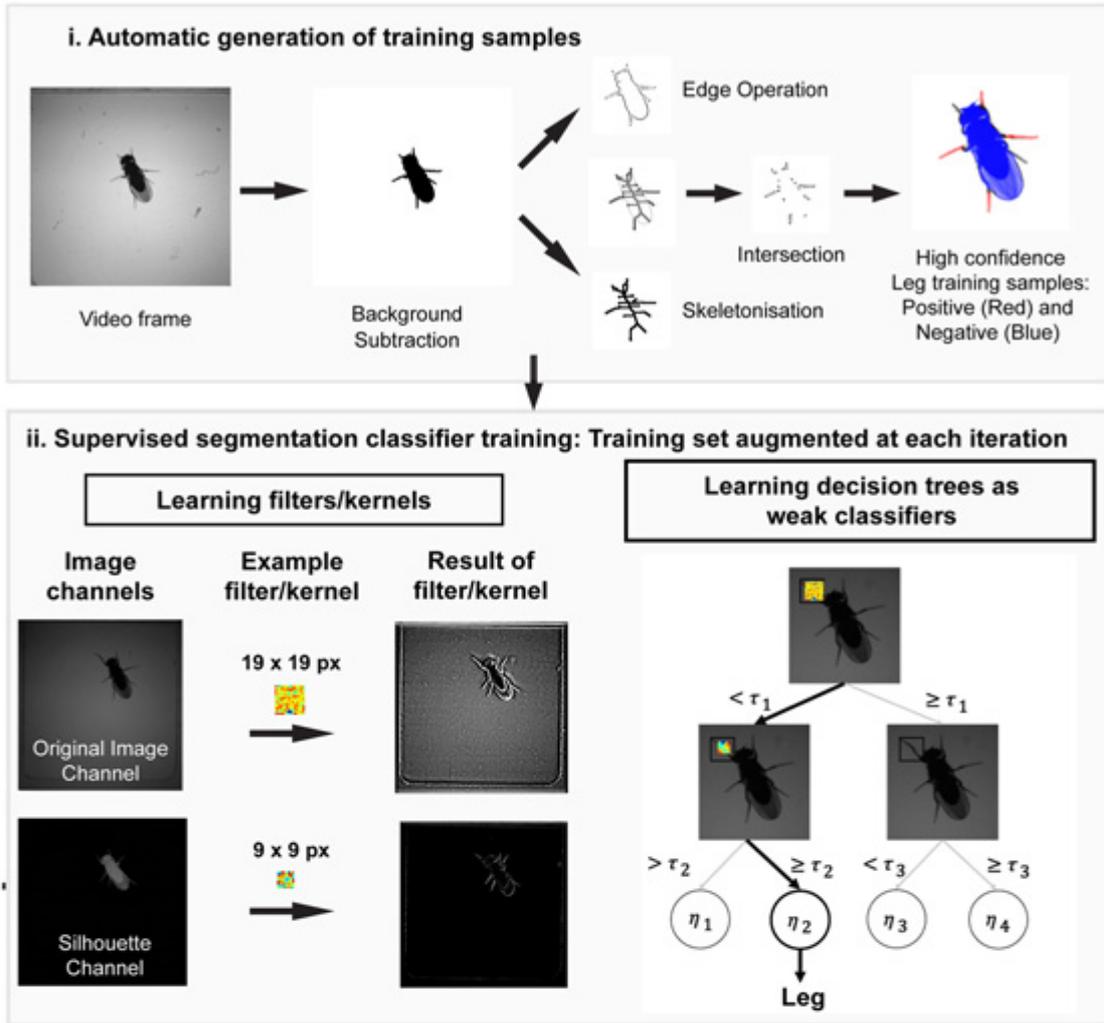
Abstract

In patients with movement disorders, gait and tremor symptoms ("phenotypes") often reflect the underlying disease etiology (genotypes & mechanisms). These symptoms are important in clinical diagnosis. For example, Parkinson's Disease (PD) patients exhibit a stiff, rigid gait and resting tremor (hypokinesia), while patients with Spinocerebellar ataxia 3 (SCA3) exhibit lurching, irregular movements and action tremor (hyperkinesia). Do PD flies walk differently from SCA3 flies? While fly models of neurodegeneration have been studied for more than two decades, illuminating our understanding of the molecular and cellular mechanisms of human disease, it was not known whether these models show specific movement dysfunctions, or whether these resemble the human diseases. To answer this question, we developed a machine-learning image-analysis program, Feature Learning-based Lmb segmentation and Tracking (FLLIT), that automatically tracks leg claw positions of freely moving flies recorded on high-speed video, producing a series of gait parameters. Notably, unlike most other machine-learning methods, FLLIT is fully automated & generates its own training sets without need for user-annotation. This was achieved using morphological parameters inbuilt into the learning algorithm. This enables FLLIT to be very accurate for our particular task, which is important for measuring movements as small and rapid as tremors (high recording speeds resulted in large numbers of video frames to track).

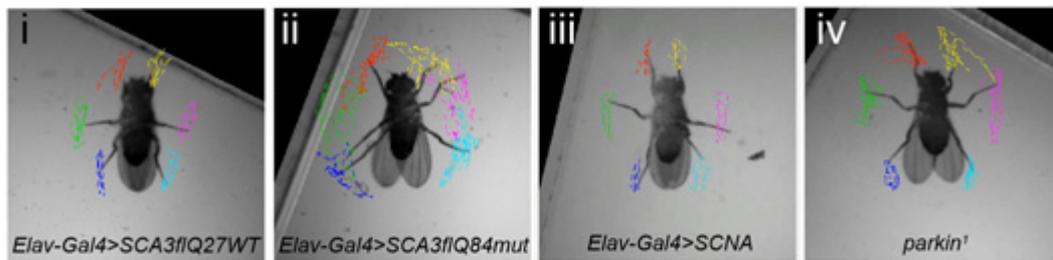
Using FLLIT, we carried out high-throughput and high-resolution analysis of gait and tremor features in *Drosophila* neurodegeneration mutants for the first time. We found that fly models of PD and SCA3 exhibited markedly different walking signatures, which recapitulated characteristics of the respective human diseases (rigid vs ataxic). Surprisingly, two different PD models, *Elav-Gal4>UAS-alpha-synuclein*, and *parkin[1]* mutants, showed strikingly similar gait signatures despite being of completely different genetic backgrounds. Selective expression of mutant SCA3 in dopaminergic neurons led to a gait signature that more closely resembled those of PD flies. These data suggest that rigid gait is a signature of dopaminergic dysfunction, and that the behavioral phenotype depends on the neurons affected, rather than the specific nature of the mutation. In addition, gait analysis could distinguish between flies that performed similarly in a climbing assay, and flies that climbed well could also show gait dysfunctions. This demonstrates that gait analysis is a sensitive method by which to examine locomotor dysfunction.

Using FLLIT, we also automatically measured tremor in flies for the first time. Different mutations produced tremors in distinct leg pairs, indicating that different motor circuits were affected. Using this approach, fly models can be used to dissect the neurogenetic mechanisms that underlie movement disorders.

A



B



C

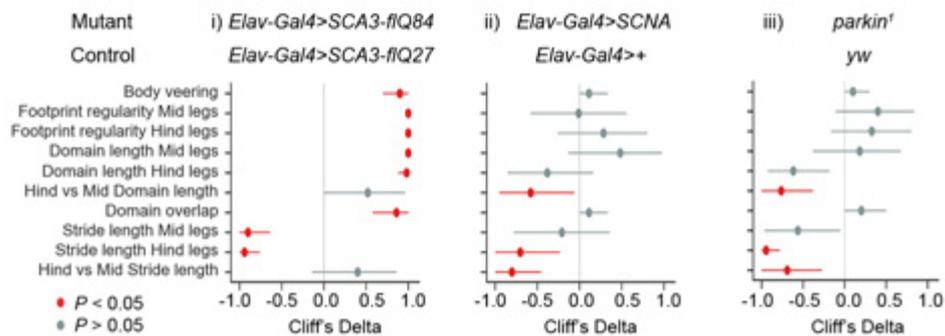


Figure Legend: **A.** The FLLIT (Feature Learning-based Limb segmentation and Tracking) programme tracks insect leg movements from high-speed video in a fully automated manner, producing 20 gait parameters, 5 plots and a tracked video for analysis. **B.** Representative FLLIT-derived walking leg traces of control, Spinocerebellar ataxia 3 (SCA3) disease model and Parkinson's Disease (PD) model flies. **C.** Cliff's Delta indices of effect sizes of SCA3 and PD-relevant gait parameters reveal distinct movement signatures and a signature rigid gait in PD flies.