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

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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, illumining 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 LImb 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

i. Automatic generation of training samples

Video frame → Background Subtraction → Edge Operation → Intersection → Skeletonisation → High confidence Leg training samples: Positive (Red) and Negative (Blue)

ii. Supervised segmentation classifier training: Training set augmented at each iteration

| Image channels | Example filter/kernel | Result of filter/kernel |
|----------------|-----------------------|-------------------------|
| Original Image Channel | ![19 x 19 px](image) | ![Result of filter/kernel](image) |
| Silhouette Channel | ![9 x 9 px](image) | ![Result of filter/kernel](image) |

Learning filters/kernels

Learning decision trees as weak classifiers

B

i. Elev-Gal4>SCA3fliQ27WT

ii. Elev-Gal4>SCA3fliQ84mut

iii. Elev-Gal4>SCNA

iv. parkin'

C

- Mutant vs Control
- i) Elev-Gal4>SCA3-fliQ27
- ii) Elev-Gal4>SCNA
- iii) parkin'

- Body veining
- Footprint regularity Mid legs
- Footprint regularity Hind legs
- Domain length Mid legs
- Domain length Hind legs
- Hind vs Mid Domain length
- Domain overlap
- Stride length Mid legs
- Stride length Hind legs
- Hind vs Mid Stride length

- Cliff's Delta

- P < 0.05
- P > 0.05
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.