One-Step Recognition of Living *C. elegans* in 384-well Plate Cultures using Linear Scale Space Mathematics

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Introduction

Automated recognition of C. elegans in 384-well plates by conventional image analysis algorithms is difficult.

Many discrete events in the culture well lead to a high variability of the primary image quality, even under optimal microscopic conditions. Meniscus effects due to the small well size, compound induced changes on E. coli concentration, changes in composition of the nematode population, superimposed ('crossing') nematodes (Fig. 1.).

Scale space theory provides a powerful framework for one-step feature extraction in 'high-complexity' microscopy images (ref.1). Differential geometry is applied in image analysis by convolving the image with Gaussian derivative kernels of the appropriate scale (σ) for the objects of interest. Applying these principles to microscopy of C. elegans allows for rapid development of image analysis algorithms very well suited for robust high volume image analysis, such as in drug screening.

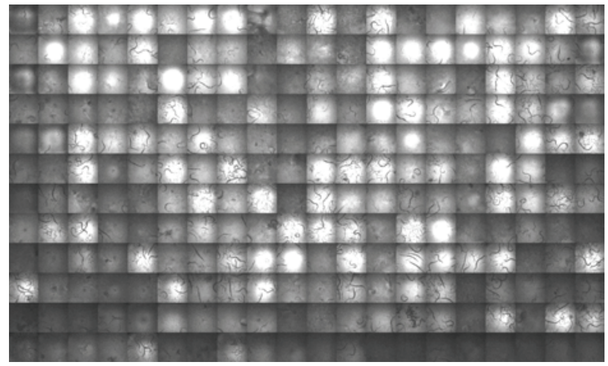

Fig. 1. Tiled 'super-image' of a 384-well plate showing the variability of imaging conditions depending on culture and compound treatment.

Results

Feature detectors can be constructed based on differential invariants, which are relatively insensitive to changes in illumination condition and signal-to-noise ratio, which is an important feature in automated microscopy (Fig. 2.).
An example of a detector for ridges is shown, the scale (σ) of the Gaussian kernel is adapted to the size of the structure to be detected. L denotes the image itself, Lx and Lxx the first and second Gaussian derivative in the x direction of the image.

$$Lxx + Lyy - \frac{1}{2}\sqrt{(Lxx - Lyy)^2 + 4Lxy^2} > 0$$

This filter adequately detects elongated structures, in microscopic images of living wild type C. elegans in 384-well plates with liquid E. coli culture within a wide spectrum of background conditions.
Including directionality into the algorithm by introducing anisotropic Gaussian filtering, makes it even more discriminative to curled-elongated structures such as C. elegans, the filter response is optimized to the underlying elongated structure (ref. 2, fig. 3 top). There is no need for prior background subtraction or modeling, to eliminate the background variation (fig. 3 top), although background correction will improve the performance of the algorithm (compare images of Fig. 3.).

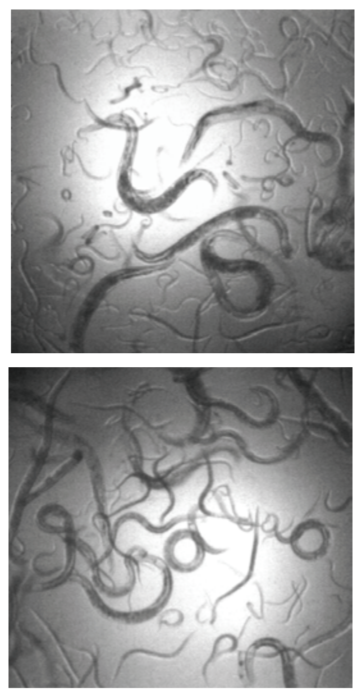

Fig. 2. Two C. Elegans cultures in 384-well plate, 5X magnification B/W camera and with very unequal background intensity from centre to border.

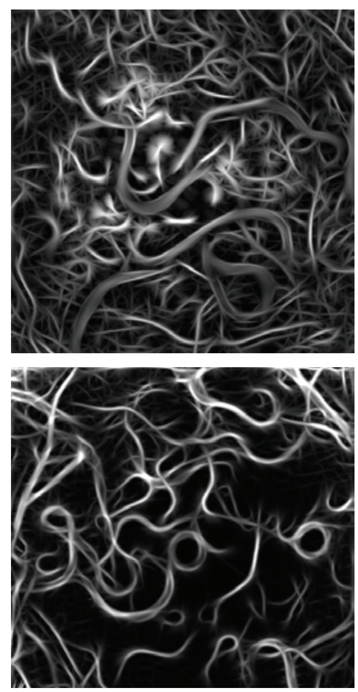

Fig. 3. The result of applying an anisotropic directional Gaussian filter. Top image algorithm only. Bottom image algorithm after parabolic background correction.

Conclusion

Scale space filtering can robustly convert grayscale images of viable *C. elegans* microcultures in 384-well plates with varying population density, *E. coli* concentrations, background intensity one-step into binary images for downstream image analysis of population composition.

References

1. Koenderink, *1984*, Biol, Cybern.: 50, 363
2. Seinstra et al., *2001*, *In*: Eds. Sakellariou, *et al*., 'Euro-Par 2001 Parallel Processing', p 653-662, Springer-Verlag (LNCS 2150)