

# Deep Learning-based Segmentation and Quantification of Nerve Fibers and Dendritic Cells in Confocal Microscopy of the Cornea

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## Purpose

In vivo confocal microscopy (IVCM) provides high resolution images of corneal nerve fibers (CNF) and dendritic cells (DC). Quantification of CNF and DC requires manual annotation or semi-automatic approaches, which are laborious, and non-reproducible. The purpose of this research was to develop deep learning-based models to segment and quantify CNF and DC automatically, therefore reducing inter- or intra-observer variability and processing time to analyze larger volumes of clinical images.

## Methods

- CNF segmentation was developed based on U-Net architecture and trained with 1036 and tested on 183 images.
- DC detection model was developed on a Mask R-CNN architecture and trained with 446 and tested on 50 images.
- All images are acquired by HRT-RCM (Heidelberg Engineering) and cover an area of  $400 \times 400 \mu\text{m}$  ( $384 \times 384$  pixels).
- Python-based software was written to compute number of nerves, branching points, nerves length (mm), density ( $\text{mm}/\text{mm}^2$ ), tortuosity (arc-cord ratio), and number of DC and size ( $\mu\text{m}^2$ ).

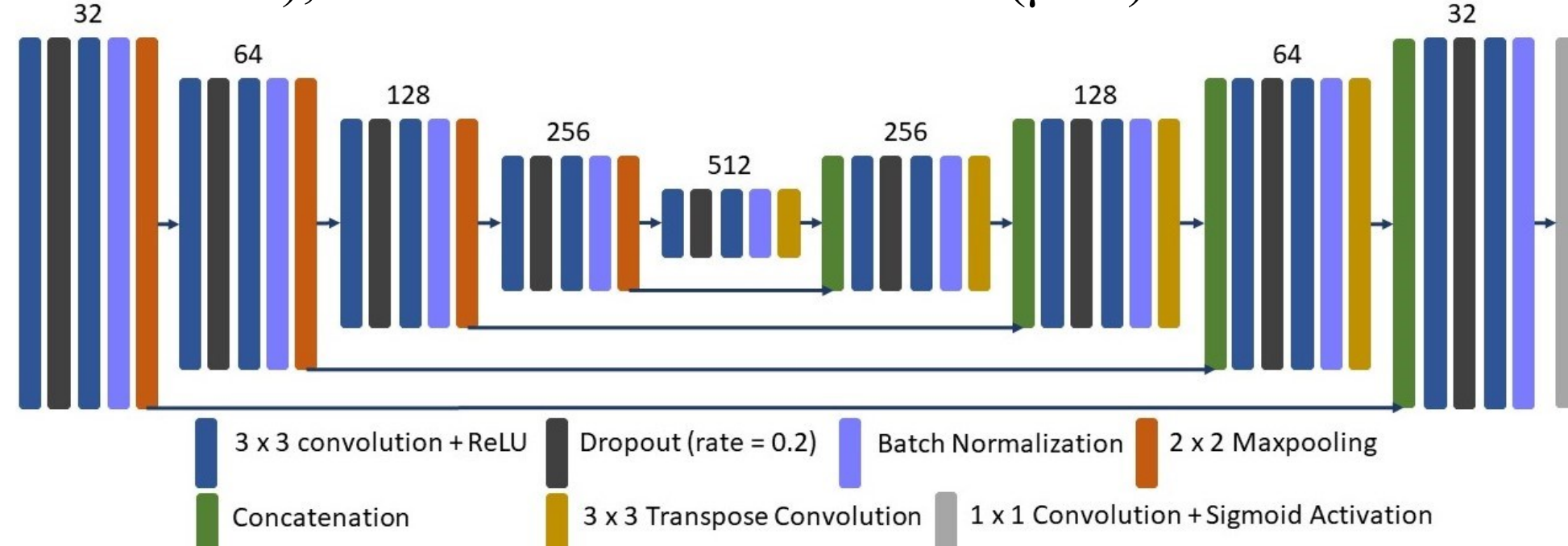


Figure 1. U-Net architecture.

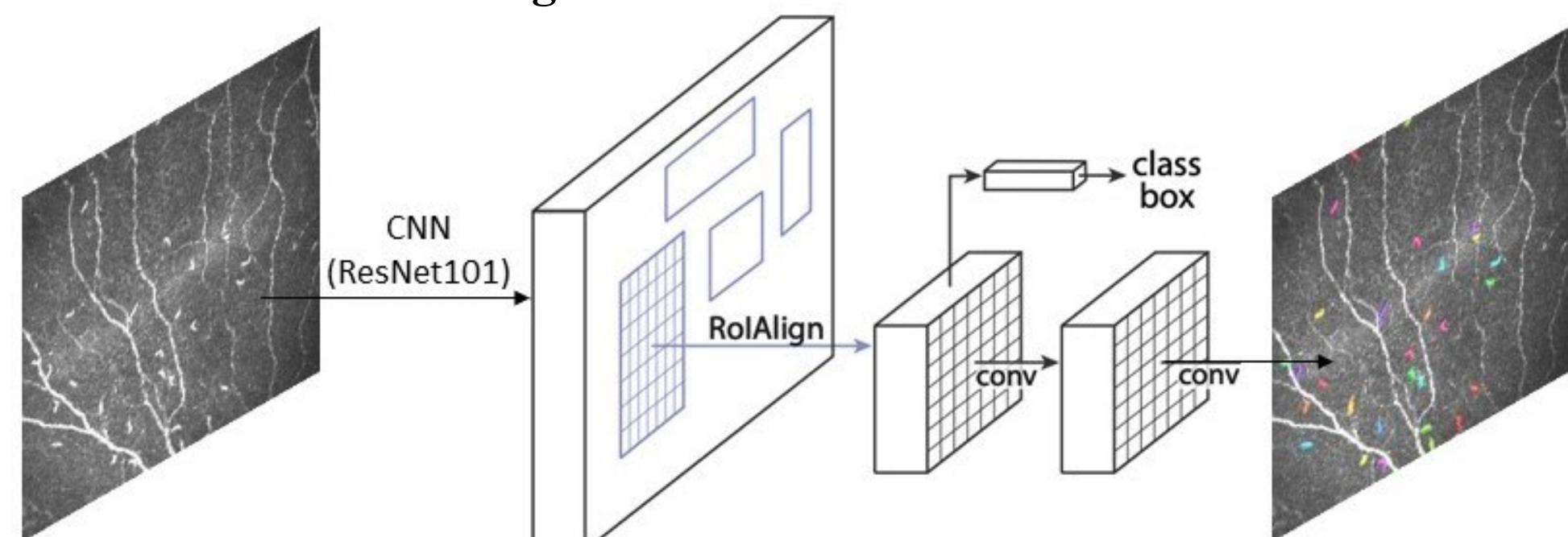


Figure 2. Mask R-CNN architecture.

## Results

### 1. Corneal nerve fiber segmentation and quantification

Table 1. CNF segmentation model training performance.

Accuracy	Sensitivity	Specificity	AUC based on ROC	Single image processing time
94%	85%	91%	0.88	4.5 sec

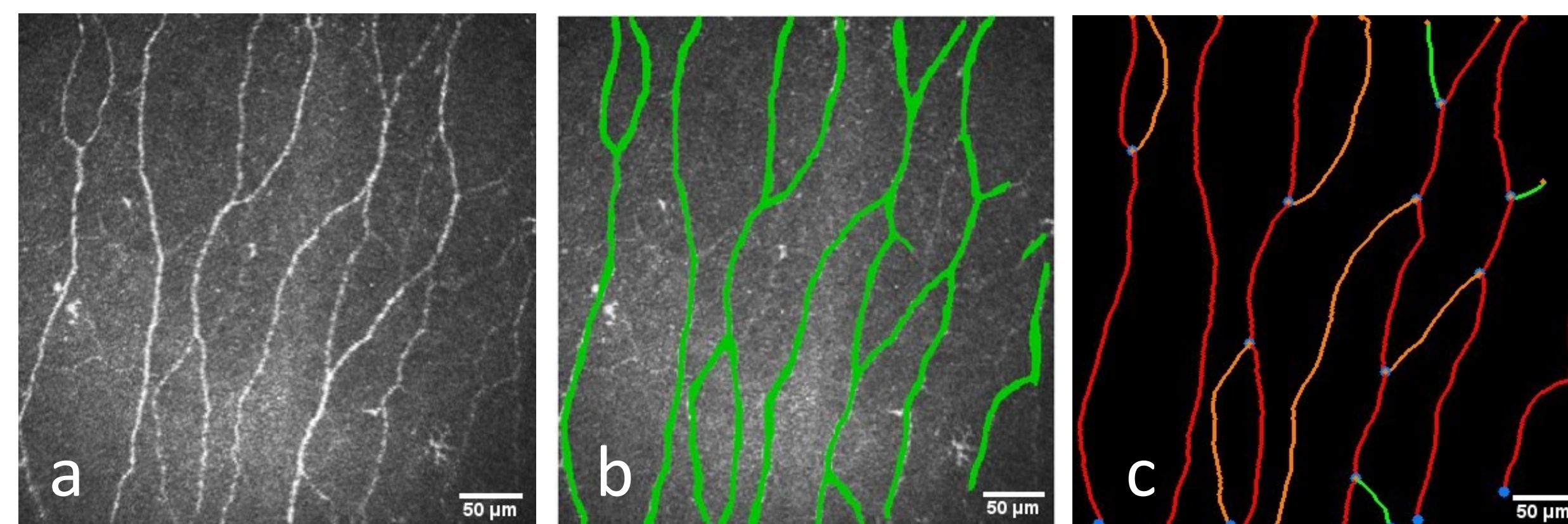


Figure 3: CNF segmentation and quantification. (a) Original image, (b) Overlay, and (c) Automatic quantification. Total CNF number (sum of red and yellow) are 11, total nerve length 3.59 mm (density  $22.43 \text{ mm}/\text{mm}^2$ ), branching points are 9, and average tortuosity is 1.18. The small branch nerves (green) whose length is less than 20% ( $80 \mu\text{m}$ ) of the image are not considered for nerve counting.

Table 2. Morphometric parameter analysis of corneal nerve fiber test dataset consists of diabetes, dry eye, and both dry eye and diabetes patients.

$p$ -value (paired t-test) is between manual annotation and automatic segmentation.

Parameters	Manual annotation	Automatic segmentation	$p$ -value
CNF number	$8.49 \pm 2.61$	$8.44 \pm 2.58$	0.57
CNF length (mm)	$3.45 \pm 3.49$	$3.29 \pm 3.60$	0.04
Branching points	$3.21 \pm 2.74$	$2.77 \pm 2.60$	0.01
Tortuosity	$1.43 \pm 0.47$	$1.44 \pm 0.51$	0.35

### Literature:

1. Wei et al. Trans Vis Sci Tech 2020
2. Setu et al. Sci Rep 2021
3. He K. et al. IEEE Int. Conf. Comput. Vis. 2017
4. Ronneberger et al. Lect. Notes Comput. Sci. 2015
5. Williams et al. Diabetologia 2020
6. Dabbah et al. Med Img Anal 2011

### 2. Dendritic cell detection and quantification

Table 3. DC detection model training performance.

Precision	Recall	F1 Score	ICC of DC number	ICC of DC size	Single image processing time
92%	95%	93%	0.98	0.92	3 sec

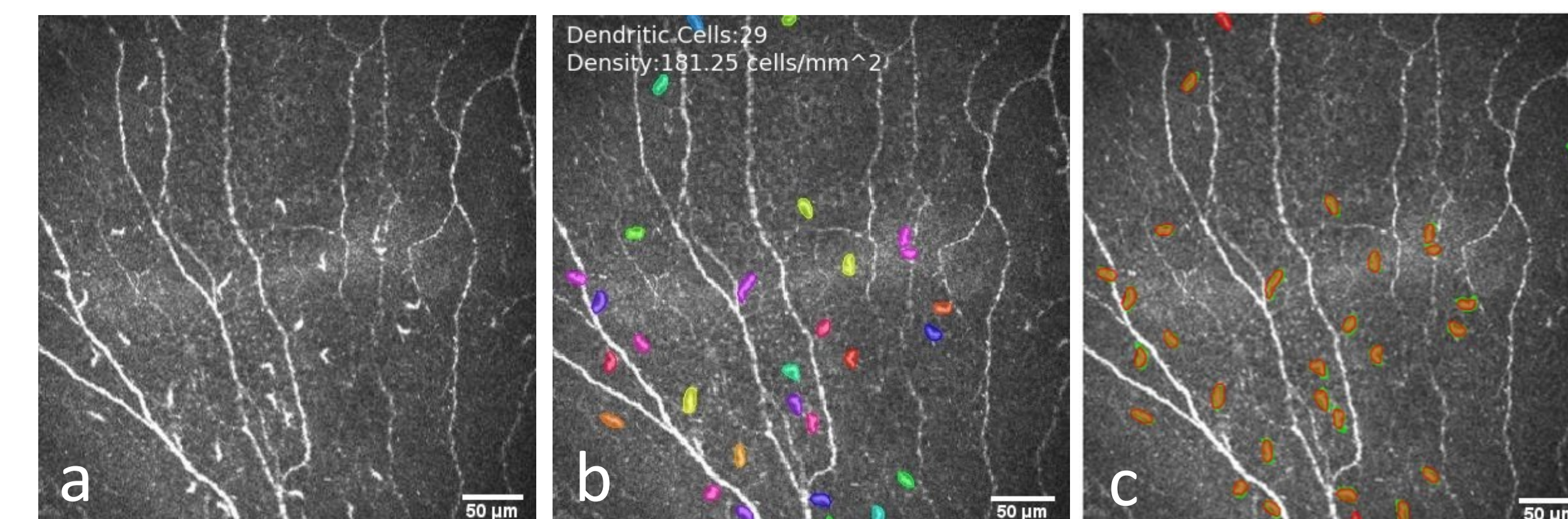


Figure 4: DC detection and quantification. (a) Original image, (b) Automatic detection, and (c) Overlay of manual annotation and predicted DC (Ground truth = green, Prediction = red).

Table 4. Morphometric parameter analysis of dendritic cell test dataset consists of dry eye, and neuropathic corneal pain patients.

$p$ -value (paired t-test) is between manual annotation and automatic segmentation.

Parameters	Manual annotation	Automatic segmentation	$p$ -value
DC number	$14.3 \pm 7.7$	$14.9 \pm 7.9$	0.70
DC size ( $\mu\text{m}^2$ )	$1842.47 \pm 936.76$	$1597.06 \pm 816.76$	0.20

## Conclusions

- Developed deep learning-based models demonstrate high consistency between automatic and manual segmentation with rapid speed.
- Proposed methods reduce inter/intra-observer variability and time to analyze a large volume of clinical images.
- The results show that the system has the potential to be implemented into clinical practice for CNF segmentation and DC detection in IVCM images.

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