Quantification of the 3D brain vasculature in zebrafish light sheet fluorescence microscopy data

Tutors: Elisabeth Kugler (kugler.elisabeth@gmail.com)

Session 1: 2020-11-30 18:00 UTC – 2020-11-30 22:00 UTC
Session 2: 2020-12-01 08:00 UTC – 2020-12-01 12:00 UTC

- **Information about the tutor[s].**

Elisabeth Kugler combines experimental sciences with computational modelling to examine vascular development and disease.
She conducted her BSc in Biology and MSc in Molecular Biology at the University of Innsbruck and interned at the Medical University of Innsbruck and EMBL Heidelberg.
For her PhD at the University of Sheffield, under the supervision of Dr Paul Armitage and Prof Dr Tim Chico, Elisabeth developed image analysis pipelines to quantify and describe the 3D zebrafish brain vasculature, as well as characterised the EC membrane behaviour in brain vessels, termed *kugeln*.
For her PostDoc as UCL Research Fellow with Dr. Ryan MacDonald, Prof. John Greenwood, and Prof. Christiana Ruhrberg, she aims to build an image-based model to describe neurovascular development in the zebrafish retina.

- **Title and abstract of the tutorial.**

Quantification of the 3D brain vasculature in zebrafish light sheet fluorescence microscopy data

Background: Zebrafish transgenic lines and light sheet fluorescence microscopy (LSFM) allow unrivalled insights into vascular development *in vivo* and 3D. The vascular architecture can be used to describe physiological status. However, assessment of the vasculature still relies on individual visual assessment rather than objective quantification. Thus, an image analysis pipeline is required to allow data assessment in 3D robustly and sensitively, while being able to handle LSFM data.
Kugler et al have produced an image analysis workflow to quantify the zebrafish brain vasculature in 3D (https://www.biorxiv.org/content/10.1101/2020.08.06.239905v2).

Aim: In this tutorial we will use the analysis workflow produced by Kugler et al to examine and quantify the zebrafish brain vasculature in 3D with a hands-on practical (https://github.com/ElisabethKugler/ZFVascularQuantification).

Methods: The open-source image analysis Fiji will be used to examine, enhance, segment, and quantify 3D data provided by the tutor. Briefly, data will be examined in 3D, image quality assessed using contrast-to-noise ratio (CNR) measurements, enhanced using Sato enhancement, segmented using Otsu thresholding, and quantified using the vascular skeleton and Euclidean distance map (EDM).

Learning outcomes: We will examine how to quantify the zebrafish brain vasculature in 3D in light sheet fluorescence microscopy data. We will examine data properties, image enhancement, segmentation, registration, and quantification. The presented processing methods are not only relevant to the vasculature, but will provide the attendees with a broader understanding of quantitative image analysis. Using reflection after each step of the tutorial, attendees will be able to assess the importance and generalization of the learning material.

- **A rough outline how the tutorial will be organized including technical requirements.**

Rough outline:

1. **Introduction of the topic, presented by the tutor (20min)**

2. **Introduction of each student and their background, presented by the students (2-5min each, 50min)**

3. **Practical work and training (2.5h)**

   a) **10 min Data download and checking all required software is installed**

Data for all steps of the processing will be provided (three 3dpf zebrafish) in case the attendees want to compare their processing outcomes or cannot perform one step or are falling behind schedule.
All data will be provided in a down-sampled format (original 1920x1920 ie 3.5Gb; down-sampled to 512x512)

Data download (approx. 18Gb)

Software check/download:

- Fiji (not older than 1year)
- Update site: **Neuroanatomy** for summarize skeleton function
- https://github.com/ElisabethKugler/ZFVascularQuantification

   b) **30 min: Understanding Zebrafish Vasculature LSFM data**

The data will be examined as stack, in 3D, and image properties (image and voxel: height, width, depth) assessed to allow familiarization with the data properties and structure.
Image quality will be quantified using contrast-to-noise ratio (CNR) measurements.

- What are the data we are working with?
- How do the data look like in 3D (3D viewer)?
- How can image quality be assessed (CNR)?

   c) **20 min: Image enhancement**

Enhanced data will be examined following Sato enhancement filter (provided data will be examined due to computational time requirement of Sato enhancement) as it enables vascular enhancement under the assumption that vessels are locally tubular.
Image quality will be compared visually between the “original” and “enhanced” data and CNR quantified to assess quality quantitatively.

- What is image enhancement?
- What is Sato enhancement?
- How does image enhancement impact image quality?

   d) **30 min: Image segmentation**

Image segmentation will be performed using Otsu Thresholding. To visualize the impact of image enhancement, segmentation will be conducted on both, “original” and “enhanced” data and data outputs compared.

- What is image segmentation?
- What is Otsu thresholding?

- How does image enhancement impact segmentation outcomes?

e) 30 min: Image Quantification

In this step, vascular length, branching points, and diameter will be automatically quantified in the "original" and "enhanced" segmented data.

- What is image quantification?
- What are parameters that you could quantify in other data?

f) 30min: Data visualization

Data outputs will be plotted using software such as Excel or Graphpad Prism.
Image analysis steps will be visualized using software such as Powerpoint, Inkscape, or Photoshop.

4. Summary and questions (1h)

- **Q&A**
- **Attendee reflection:**
  - Summarize what we have done in this tutorial and why is it important?
  - What have you learned and how could this be useful for your work?
  - What did you find particularly challenging and why?
  - What did you enjoy most about this practical?

Technical Requirements:

- Data for all steps of the processing will be provided in case the attendees want to compare their processing outcomes to provided data or are falling behind schedule (e.g. technical issues or computational requirements).
- All data will be provided in a down-sampled format (original 1920x1920 ie 3.5Gb; down-sampled to 512x512 approx 1.5 Gb; total approx 18Gb)
- Software:
  - Fiji (not older than 1year)
  - Update site: Neuroanatomy for summarize skeleton function
  - https://github.com/ElisabethKugler/ZFVascularQuantification
  - Powerpoint, Inkscape, or Photoshop (software that can make figures)
  - Excel or Graphpad Prism (software that can plot data)

- **At what time of the day you will be able to host the tutorials.**

UK time 8am to 10pm