

# Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy

Raphaël Candelier, Thomas Panier, Sebastián Romano, Raphaël Olive,  
Thomas Pietri, Germán Sumbre, Georges Debrégeas

► **To cite this version:**

Raphaël Candelier, Thomas Panier, Sebastián Romano, Raphaël Olive, Thomas Pietri, et al.. Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy. Gennady S Cymbalyuk and Astrid A Prinz. the Twenty Second Annual Computational Neuroscience Meeting: CNS\*2013, Jul 2013, paris, France. Biomed Central, 14 (Suppl 1), pp.P97, 2013. <inserm-00842318>

**HAL Id: inserm-00842318**

**<http://www.hal.inserm.fr/inserm-00842318>**

Submitted on 8 Jul 2013

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

POSTER PRESENTATION

Open Access

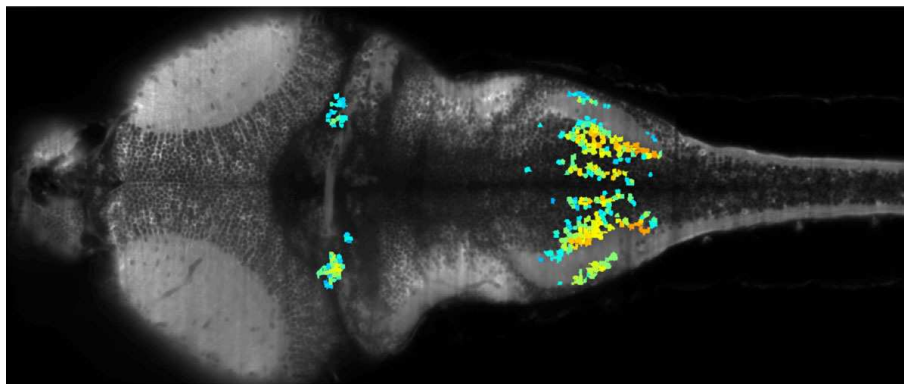
# Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy

Raphaël Candelier<sup>1\*</sup>, Thomas Panier<sup>1</sup>, Sebastián Romano<sup>2,3,4,5</sup>, Raphaël Olive<sup>1</sup>, Thomas Pietri<sup>2,3,4,5</sup>, Germán Sumbre<sup>2,3,4,5</sup>, Georges Debrégeas<sup>1</sup>

From Twenty Second Annual Computational Neuroscience Meeting: CNS\*2013  
Paris, France. 13-18 July 2013

The optical transparency and the small dimensions of zebrafish at the larval stage make it a vertebrate model of choice for brain-wide *in-vivo* functional imaging. However, current point-scanning imaging techniques, such as two-photon or confocal microscopy, impose a strong limit on acquisition speed which in turn sets the number of neurons that can be simultaneously recorded [1]. At 5 Hz, this number is of the order of one thousand, *i.e.* approximately 1-2% of the brain. We demonstrate that this limitation can be greatly overcome by using Selective-Plane Illumination Microscopy (SPIM) [2-4]. Zebrafish larvae expressing the genetically encoded calcium indicator GCaMP3 were illuminated with a scanned laser

sheet and imaged with a camera whose optical axis was oriented orthogonally to the illumination plane. This optical sectioning approach was shown to permit functional imaging of most of the brain volume of 5-9 day old larvae with single-cell resolution. The spontaneous activity of up to 5000 neurons was recorded at 20 Hz for 20-60 min. By rapidly scanning the specimen in the axial direction, the activity of 25000 individual neurons from 5 different z-planes (approximately 30% of the entire brain) could be simultaneously monitored at 4 Hz. Compared to point-scanning techniques, this imaging strategy thus yields a ~20-fold increase in data throughput (number of recorded neurons times acquisition rate) without



**Figure 1** Image of the brain of a 6 day-old GCaMP3 zebrafish obtained by SPIM. Colored neurons indicate a set of neurons showing correlated activity.

\* Correspondence: raphael.candelier@upmc.fr

<sup>1</sup>CNRS / UPMC Univ. Paris 06, FRE 3231, Laboratoire Jean Perrin LJP, F-75005, Paris, France

Full list of author information is available at the end of the article

compromising the signal-to-noise ratio. The extended field of view offered by the SPIM method allowed us to directly identify large scale ensembles of neurons, spanning several brain regions (see Figure 1), that displayed correlated activity and were thus likely to participate in common neural processes.

#### Author details

<sup>1</sup>CNRS / UPMC Univ. Paris 06, FRE 3231, Laboratoire Jean Perrin LJP, F-75005, Paris, France. <sup>2</sup>Ecole Normale Supérieure, Institut de Biologie de l'ENS, IBENS, Paris, F-75005 France. <sup>3</sup>Inserm, U1024, Paris, F-75005 France. <sup>4</sup>CNRS, UMR 8197, Paris, F-75005 France. <sup>5</sup>IBENS, ENS, Paris, France.

Published: 8 July 2013

#### References

1. Christine Grienberger, Arthur Konnerth: **Imaging calcium in neurons.** *Neuron* 2012, **73**(5):862-885.
2. Michael Weber, Jan Huisken: **Light sheet microscopy for real-time developmental biology.** *Curr Opin Genet Dev* 2011, **21**(5):566-572.
3. Jerome Mertz: **Optical sectioning microscopy with planar or structured illumination.** *Nature Methods* 2011, **8**(10):811-819, October.
4. Raju Tomer, Khaled Khairy, Philipp JKeller: **Shedding light on the system: studying embryonic development with light sheet microscopy.** *Curr Opin Genet Dev* 2011, **21**(5):558-565.

doi:10.1186/1471-2202-14-S1-P97

**Cite this article as:** Candelier *et al.*: Fast functional imaging of multiple brain regions in intact zebrafish larvae using Selective Plane Illumination Microscopy. *BMC Neuroscience* 2013 **14**(Suppl 1):P97.

Submit your next manuscript to BioMed Central  
and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit

