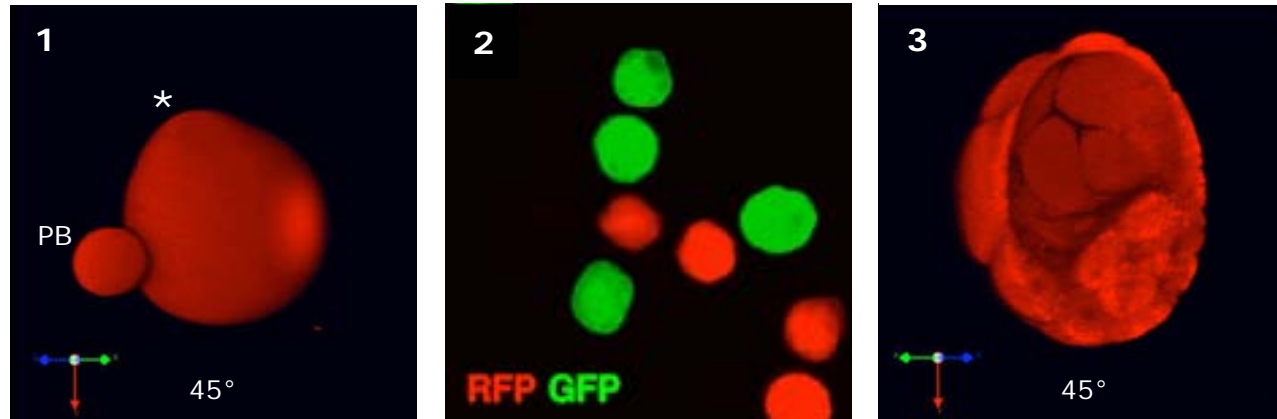




3D *In Vivo* Imaging: mRFP1 as a Genetically and Spectrally Distinct Alternative to GFP



RFP (DsRed) expression in CAG::mRFP1 preimplantation stage embryos

Figure 1: 3D reconstruction rendered in **Volocity Visualization** of a single CAG::mRFP1 Tg/+ zygote including the second polar body (PB), rotated by 45° counter clockwise. The zygote is not spherical as it has a short and long axis and a clearly visible protrusion (asterix). **Figure 2:** a red and green fluorescence channel overlay of CAG::EGFP Tg/+ and CAG::mRFP1 Tg/+ embryos representing compacted morulae through to blastocyst stages. **Figure 3:** 3D reconstruction generated in **Volocity Visualization** of a single CAG::mRFP1 blastocyst, computationally bisected and rotated by 45° counter clockwise.

The RGB colored vector on the bottom left of the 3D reconstructions depicts the x-axis in green, y-axis in red and z-axis in blue.

Dr Hadjantonakis and colleagues from the Sloan-Kettering Institute investigate the red fluorescent protein isolated from *Discosoma sp.* corals (DsRed RFP) as an alternative to green fluorescent protein (GFP) for application in mice. Not only do DsRed RFPs provide a genetically and spectrally distinct addition to the available repertoire of autofluorescent proteins, but by virtue of their spectral properties, they permit deeper tissue imaging, which is particularly suitable for 3D imaging.

The group investigated the expression and germline transmission of mRFP1 (monomeric RFP1) in embryonic stem (ES) cells and mice. Images of the expressed transgene reporter in preimplanted mouse embryos hemizygous (Tg/+) for the CAG::mRFP1 transgene were acquired with both wide field and confocal microscopes. This non-invasive technique to visualize living cells allowed the researchers to acquire high-magnification, sequential z-stacks that were 3D rendered. **Figure 1** shows 3D reconstructions of a single CAG::mRFP1 Tg/+ zygote including the second polar body, generated in **Volocity Visualization**. **Figure 2** shows CAG::eGFP Tg/+ and CAG::mRFP1 Tg/+ embryos representing compacted morulae through to blastocyst stages. **Figure 3** shows 3D reconstructions of a single CAG::mRFP1 Tg/+ blastocyst generated in **Volocity Visualization**. The **QuickTime Pro movie** at <http://www.biomedcentral.com/content/supplementary/1472-6750-5-20-S2.mov> shows **Volocity** rendered images being rotated around each axes (x, y and z).

This work illustrates a robust, homogenous and widespread expression of mRFP1, and demonstrates that DsRed-based RFPs provide a genetically and spectrally distinct addition to the available repertoire of autofluorescent proteins like GFP. Because of their spectral properties RFPs can be used as subcellular-localized tags for high-resolution live imaging *in vivo*. The ongoing development of mouse strains expressing subcellularly-localized protein fusions incorporating RFPs, and contrasting with GFP-variants play an essential role for visualizing 4-dimensional (3D over time) anatomy and tracking cell position, morphology and behavior *in vivo*.