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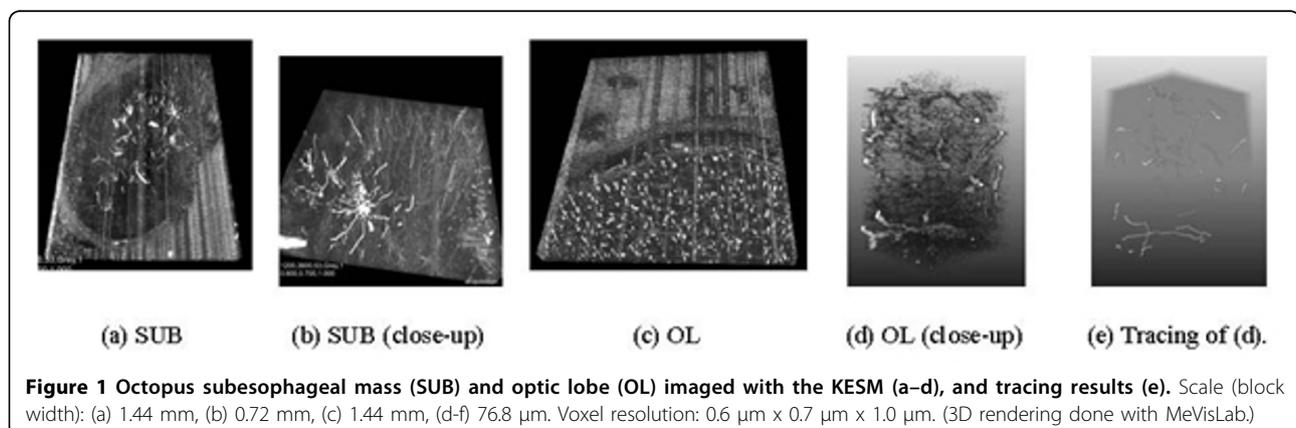
Charting out the octopus connectome at submicron resolution using the knife-edge scanning microscope

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The common octopus, or *Octopus vulgaris*, has the largest nervous system of any invertebrate, and has been shown to possess learning and memory capabilities that in many ways rival those of some vertebrates [1]. Nevertheless, the neural architecture of this cephalopod mollusk differs markedly from that of any vertebrate. Investigating the differences and similarities between the neural architecture—or connectome—of the octopus and mammals, such as the mouse, may lead to deep insights into the computational principles underlying animal cognition. The octopus brain provides some unique advantages for anatomical research, since its axons are generally thick and unmyelinated, allowing traditional staining methods, such as Golgi, to be used effectively. With this in mind, we first imaged the brain

using the Knife-Edge Scanning Microscope [2], a custom serial sectioning microscope that can image large blocks of tissue (1 cm³) at sub-micrometer resolution. We imaged large portions of the octopus subesophageal mass (SUB) and the optic lobe (OL) which were stained using Golgi. In order to extract the geometry of the neuronal morphology, we used our Maximum Intensity Projection (MIP)-based tracing algorithm [3]. The imaging results are shown in Figure 1(a-d), and tracing results are shown in 1(e). Although quite preliminary, to our knowledge this is the first time large volumes of the octopus brain have been imaged at sub-micrometer resolution, allowing us to resolve many of the processes that make up the neural network. We expect that this pilot study and the more detailed investigations to



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follow will allow fruitful comparisons of the neural circuitries of individual octopuses with different ecological life histories, as well as of animals that have been exposed to a variety of neurodegenerative insults. Moreover, such explorations will engender a greater understanding of how functional neural architecture is altered by learning in invertebrates such as the octopus and vertebrates such as the mouse. In sum, this approach should contribute greatly to our understanding of the computational architecture of invertebrates and ultimately provide insights into the differences between invertebrate and vertebrate cognitive capabilities.

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