NMR spectroscopy of subnanoliter ova with ultra-compact inductive probes

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Due to sensitivity limitations, NMR spectroscopy of single cells was previously reported down to a minimum volume of 10 nl [1], a volume scale where only a few microscopic biological entities exist in nature. In this study we employed an ultra-compact 1 mm^2 single-chip high sensitivity probe (Fig. 1A) to sense endogenous compounds in intact single ova (Fig. 1B) having subnanoliter volumes down to 0.1 nl. At this volume scale, life development begins for a broad variety of animals, humans included (Fig. 1C). Despite a relatively low spectral resolution of about 0.3 ppm, in our experimental conditions a single scan limit of detection (LOD) of about 300 pmol of ^{1}H nuclei within a sensitive volume of about 250 pl is achieved [2,3]. 1D ^{1}H spectra of single ova of tardigrade *Richtersius coronifer* (*Rc*, 0.5 nl) and nematode *Heligmosomoides polygyrus bakeri* (*Hp*, 0.1 nl) are obtained, here shown after averaging times of respectively 12 and 36 hours (Figs. 1D and 1E). With a LOD of about 2 pmol of ^{1}H nuclei, lipids and other unassigned endogenous compounds are detected. Repeated experiments of eight *Rc* ova, and a study of spectra reproducibility, also suggest that *Rc* ova have visibly heterogeneous spectra. These results seem to indicate that miniaturized inductive high sensitivity probes are a promising candidate for the NMR-based analysis of single microscopic biological entities such as mammalian zygotes.

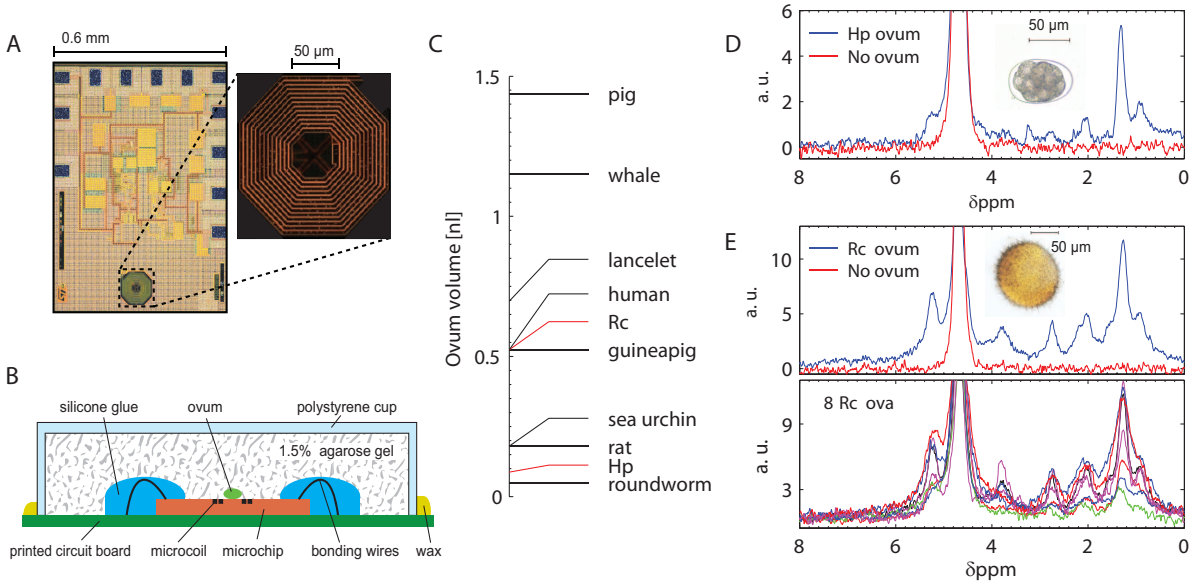

Figure 1: (A) Photographs of the single-chip probe. (B) Single ovum probe in section view. (C) Approximate volumes of ova of selected animals. (D) Spectrum and photograph of a Hp ovum (0.1 nl). (E) Spectrum and photograph of a Rc ovum (0.5 nl), and spectra of eight different single Rc ova.

[1] S. C. Grant, et al., NMR spectroscopy of single neurons. *Magnetic resonance in medicine*, 2000, **44(1)**, 19-22. [2] M. Grisi, G. Gualco and G. Boero, *Review of Scientific Instruments*, 2015, **86**, 044703. [3] M. Grisi, B. Volpe, R. Guidetti, N. Harris and G. Boero, *arXiv:1511.06719*, 2015.