sliding movement to the stick, which is provided in front with a special holder for the filter.

An 8 cm long graduated metal ruler is attached alongside the sliding stick and just above, aligned with it, at the end of the supporting bar, a vernier is also attached. A 'center finder' in front of the stick makes it possible to take more accurate measurements. It consists of a small rectangular plexiglas plate provided with a small pin-like screw which, when the plate is in the up-position, is aligned with the source of light and faces the bevelled tip of the needle. When light transmission measurements are taken the plate is rotated down so as not to interfere with the beam of light. When the needle is inserted, for the sliding action of the stick, the head of the pin can be made to touch the surface of the tissue and subsequently the bevelled tip of the needle, when the latter is withdrawn. The difference between the 2 distances, read on the graded ruler with the help of the vernier, represents the thickness of the tissue or the depth reached by the tip of the needle.

Measurements of light transmission are taken by inserting the bevelled tip of the optic probe into the tissue or cavity or by holding the tissue so that it lies between the tip of the optic probe and the source of light. A first intensity is read with the light impinging directly on the tip of the optic probe going to the photometer. A second intensity is then read with the interposition of the tissue. From these 2 values the percentage light transmission is calculated as previously reported.

The acoustic method described in the first part of this study can also be used with these 2 instruments, provided a rheostat is connected with the power supply in order to regulate the intensity of light. The procedure would be basically the same. The variations in light intensities, to elicit the peep-like sound, would be brought about by rhythmically intercepting the beam of light.

1 Acknowledgments. The author would like to thank Dr Leonard Salzarulo, chairman of the Physics Department of the New Jersey Institute of Technology for the critical reading of the paper. Special thanks are extended to Mr Simon H. Gregory of the Valtec Corp. for supplying the fibreoptics and for his helpful suggestions in how to handle them. Appreciations are also extended to Prof. Giuseppe Battendieri of the 'Liceo Scientifico Enrico Fermi' in Cosenza, Italy, for the schematic reproduction of the instruments.

2 Istituto di Fisiologia Umana, II Facoltà di Medicina e Chirurgia della Università, via S. Pansini 5, I-80131 Napoli (Italy).

3 G.E. Friedrich, R.H. Kaufman, P.J. Lynch and D. Woodruff, Obstet. Gynec. *48*, 564 (1976).
4 T.R.C. Sisson, N. Kendal, S.G. Glauser, S. Knutson and E. Bunyaviroch, J. Pediat. *79*, 904 (1971).
5 J.F. Kelly, M.E. Shell and M.C. Berenbaum, Br. J. Cancer *31*, 237 (1975).
6 S.G. Granelli, I. Diamond, A.F. McDonagh, C.B. Wilson and S.L. Nielsen, Cancer Res. *35*, 2567 (1975).
7 E.E. VanBrunt, M.D. Shepherd, J.R. Wall, W.F. Ganong and M.T. Clegg, Ann. N.Y. Acad. Sci. *117*, 217 (1964).
8 E. Viggiani, M. Ciesla and O.L. Russo, Boll. Soc. ital. Biol. sper. *46*, 470 (1970).

A demonstration of the resolution of NMR imaging in biological systems[1]

W.S. Hinshaw[2], P.A. Bottomley[3] and G.N. Holland

Department of Physics, University of Nottingham, Nottingham NG7 2RD (England), 2 October 1978

Summary. A proton NMR imaging study of several fruit specimens demonstrates the integrity and resolution of this new imaging method.

Macroscopic thin-section images of heteregeneous systems can be produced by modification of the NMR technique[4-6]. The application of NMR imaging methods to the investigation of living intact organisms is of potential value since it is non-invasive, has no known hazard[7], and the imaging parameter is a powerful measure of molecular level structure and motion, reflecting also variations in biological tissue age, origin and health[8]. We demonstrate here the spatial resolution, image parameter contrast, and geometric accuracy obtainable by the new 'multiple sensitive point' method[9,10] of NMR image formation by reference to 3 fruit specimens. The fruit specimens possess a higher degree of tissue heterogeneity and geometric complexity than can be imitated by artificial phantoms, thus enabling reasonable extrapolation of the results to complex animal structures.

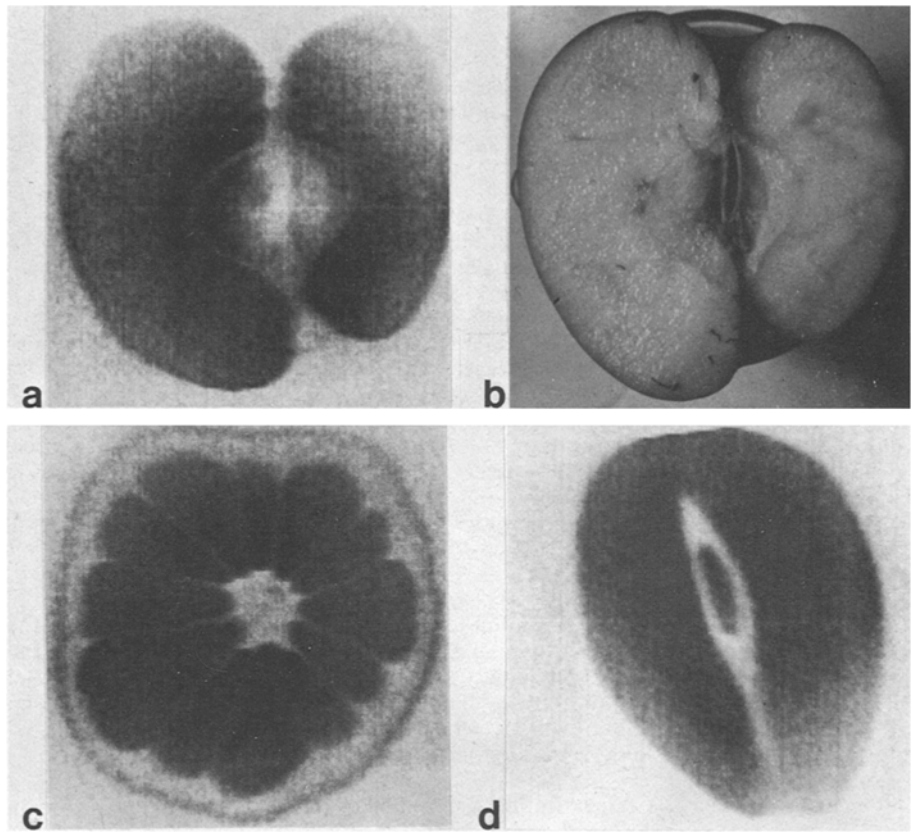
Experimental. In multiple sensitive point imaging, the NMR spectrometer sensitivity is restricted to a thin 'sensitive line' within the sample by application of 2 orthogonal time-dependent magnetic field gradients[9]. A third, static magnetic field gradient, applied in the direction of the sensitive line, enables the distribution of the NMR signal along the line to be determined after time-averaging and Fourier transformation[5]. Scanning the sensitiveline slowly across the imaging plane generates a complete thin cross-sectional image.

Our experiments employed a home-built NMR spectrometer operating at a 30 MHz proton resonance frequency, and a Varian V-7300 electromagnet with 13 cm pole gap. The sample chamber diameter was 8 cm. Image data were stored on computer cassette tape and displayed as beam intensity on a storage oscilloscope screen. Regions of high proton NMR signal level in the sample are thus rendered as areas of high intensity on the displayed image.

All images were obtained under similar spectrometer operating conditions and consist of 128×128 independent picture points. The cross-sectional shape of the sensitive line, which is determined by the magnetic field gradient strengths, was roughly 3×0.5 mm. The spatial resolution in the 3rd direction was also 0.5 mm. Each of the 128 sensitive lines was averaged for 3 sec, and the total imaging time was 380 sec. The proton resonance was excited by a steady-state free precession pulse sequence.

Results and discussion. NMR images of an intact apple, satsuma and plum are shown in figures a, c and d. A subsequent section at the level of the imaging plane of the apple, is pictured in figure b. All images demonstrate accurate structure reproduction with spatial resolution approaching, and in some cases equal to the picture element spacing. Regions such as pith, skin, core, pips and flesh are defined and contrasted by intensity variation within the images. Worthy of particular note are the fine septa of average thickness 0.5 mm which define the flesh segments in the satsuma and the approximately 1 mm thick outline of the seed core of the apple, clearly discernible in these images. Note also the differentiation of the oily surface layer of the satsuma skin which possesses a higher proton NMR signal level than the drier sub-surface pithy layer covering the flesh.

These results illustrate the integrity of NMR imaging as a new non-invasive diagnostic tool whereby high resolution



a, c and *d* show thin section NMR images of intact apple, satsuma and plum respectively. *b* is a subsequent section taken at the level of the imaging plane of the apple. These images demonstrate the resolving power of NMR imaging when applied to biological systems.

images of biological objects are produced in a reasonable time. Tissue contrast is clearly demonstrated and can be attributed to variations in water content and NMR relaxation times, signal amplitude increasing as the mobility of the system increases[11]. Further exploitation of the method could involve such intriguing possibilities as the measurement of NMR relaxation times, diffusion coefficients, flow, chemical shifts[12], and the distribution of other resonant nuclei[13,14] at localized regions within intact heterogeneous systems.

1 Acknowledgment. We thank Professor E.R. Andrew, Dr W.S. Moore and Miss C. Simaroj for their contributions. The work was supported by MRC grant G975-102.
2 Present address: Department of Radiology, Massachusetts General Hospital, Boston (Massachusetts 02114, USA).
3 Present address: Department of Physiological Chemistry, Johns Hopkins University School of Medicine, 725 N Wolfe Street, Baltimore (Maryland 21205, USA).

4 P.C. Lauterbur, Pure appl. Chem. *40,* 149 (1974).
5 W.S. Hinshaw, P.A. Bottomley and G.N. Holland, Nature *270,* 722 (1977).
6 P. Mansfield and I.L. Pykett, J. Mag. Res. *29,* 355 (1978).
7 P.A. Bottomley and E.R. Andrew, Physics Med. Biol. *23,* 630 (1978).
8 W.R. Inch, J.A. McCredie, R.R. Knispel, R.T. Thompson and M.M. Pintar, J. nat. Cancer Inst. *52,* 353 (1974); J.C. Eggleston, L.A. Saryan and D.P. Hollis, Cancer Res *35,* 1326 (1975).
9 W.S. Hinshaw, J appl. Phys. *47,* 3709 (1976).
10 E.R. Andrew, P.A. Bottomley, W.S. Hinshaw, G.N. Holland, W.S. Moore and C. Simaroj, Physics Med. Biol. *22,* 971 (1977).
11 W.S. Hinshaw, E.R. Andrew, P.A. Bottomley, G.N. Holland, W.S. Moore and B.S. Worthington, Br. J. Radiol. *51,* 273 (1978).
12 P.C. Lauterbur, D.M. Kramer, W.V. House and C.N. Chen, J. Am. chem. Soc. *97,* 6866 (1975).
13 D.I. Hoult, J. Mag. Res. *26,* 165 (1977).
14 G.N. Holland, P.A. Bottomley and W.S. Hinshaw, J. Mag. Res. *28,* 133 (1977).

Methods of experimental yolk removal from *Brachydanio rerio* eggs

Eva-Maria Langer

Zoologisches Institut der Westfälischen Wilhelms-Universität Münster, Hüfferstrasse 1, D-4400 Münster (Federal Republic of Germany), 27 November 1978

Summary. The yolk of *Brachydanio rerio* eggs was removed by pipettation, bursting and cutting off with a scalpel. The total yolk removal before the 8-cell stage led to a germ with irregular groups of cells, at the 8-cell stage to a topologically irregular differentiation, at the 64-cell stage to a nearly normal embryo, which is not viable.

The influence of yolk on the development of the extreme yolky teleostean egg with partial egg-cleavage of *Brachydanio rerio* can be investigated. 3 methods of experimental yolk removal will be demonstrated. The standard development of the zebrafish was described in detail by Hisaoka and Battle[1]. Experimental yolk removal from other teleostean eggs was carried out by Oppenheimer[2] and Trinkaus and Drake[3] in *Fundulus,* Tung and Tung[4] in *Carassius,*