
# TD-NMR and Body Composition Analysis for Lab Animals

## 時域磁共振及實驗鼠體內組分的測量

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*Bruker Optics, Inc.*



# Key Topic

## Body Composition Analysis (BCA) on lab animals with NMR

## 采用核磁共振分析實驗鼠的體內組分

## BCA on Mice and Rats 實驗鼠體內組分分析

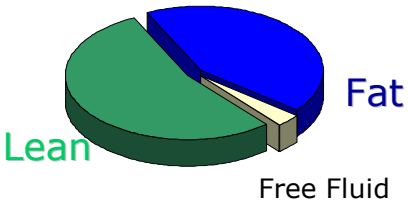
Body Composition is a **BioMarker** (body weight often not)

Animal Models (Mice & Rats) for:

- Diabetes & Obesity Research
- Dietary Analysis
- Gene Knock-Out Mice

**Requirements:**

- Routine & Quick Method
- Compatible to Animal House
- Low animal health risk (longitudinal studies)
- Method for Mice & Rats






## Current Methods for BCA on Mice and Rats
## 實驗鼠體內組分分析現行方法

**Dissection 解剖**

**DEXA (Dual Energy X-Ray Absorption)**
**雙能量X光吸收**

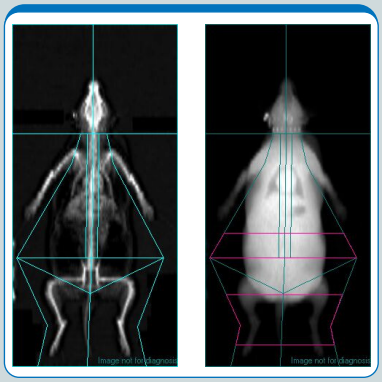
**NMR/MRI 磁共振或磁共振成像**

**TD-NMR 時域核磁共振**

## DEXA Method方法

Dual Energy X-ray Absorption
(also DXA)

- Harmful radiation
- Requires Anesthesia
- Animals don't eat following scan
- Only 1 scan per week (rats)
- overlap bone, tissue (2D scan)
- ID Chips not possible



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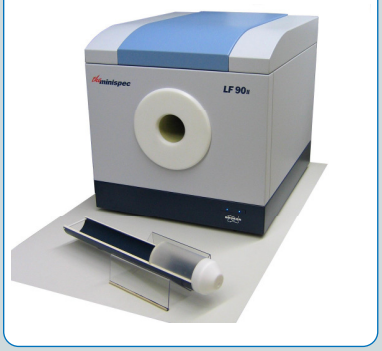
## TD-NMR Method 時域核磁共振方法

1. Weighing

2. Loading

3. Inserting

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## TD-NMR BCA History




First Mouse Systems Introduced in USA in 2001 (mq-7.5, LF50)

First Rat Systems Introduced in USA in 2004 (LF90)

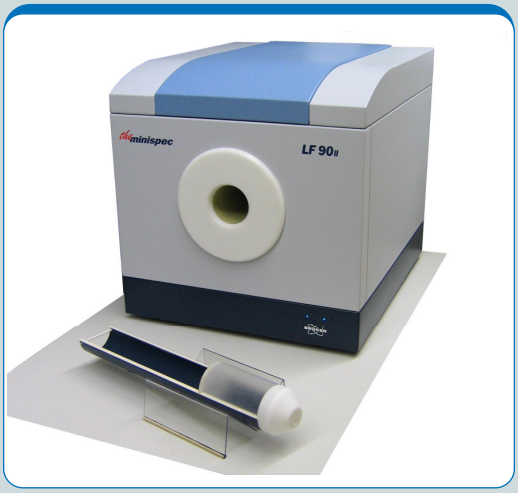


## The 2nd Generation BCA Analyzer: the minispec LF90II

Introduced ISMRM May 2007

Press Release Pittcon 2008

(with capability of measuring rats up to 850g body weight)

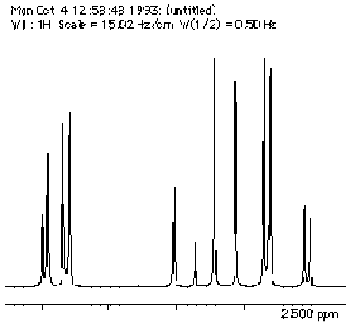


# BCA by TD-NMR

Body Composition Analysis (BCA)
with the minispec
bench-top TD-NMR



## When we talk about N.M.R.




**…NMR spectrum or MR Image will come to our mind.**
**But TD-NMR does not deal with the above**

## What is TD-NMR?

**T**ime **D**omain **N**uclear **M**agnetic **R**esonance;

Benchtop NMR;

Low Resolution NMR;

Low Field NMR;

QC type NMR analyzer;

Non-spectroscopic NMR;

Relaxometry;



## TD-NMR History

2nd generation (1972)

3rd generation (1980)

4th generation (1983)

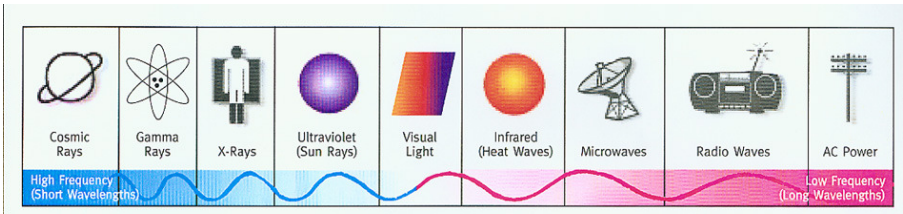
5th generation (1993)

6th generation (1999)

New Additions (2008)

## NMR Nutshell

NMR requires only very low energy radiation



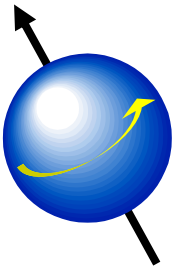

the minispec 6.2 MHz up to 60 MHz



## Where NMR signals come from?

Any NMR-sensitive nucleus (such as hydrogen) has a positive electric charge and a nuclear spin. Together, these give the nucleus a magnetic moment, so that it behaves like a small magnet.

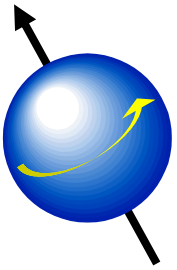
A hydrogen nucleus is a small magnet



## 核磁共振信號的產生

原子核，如氫核，即質子，具有兩個很基本的物理屬性—帶有正電荷以及原子核自旋。由于這兩個屬性，原子核就具有磁矩。因此，原子核的行爲在某種程度上就象小磁體。

**一個氫核就是一個小磁體**



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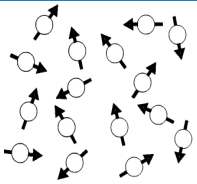
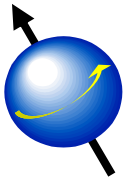
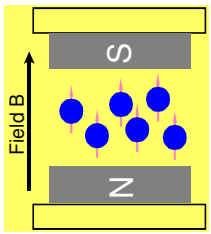
## 核磁共振信號的產生

在自由空間中，原子核小磁體的指向是隨機和無規則的。





**當處于外加磁場中，原子核要受外場磁力的作用，重新分布小磁體的指向。并且，由于受外加磁力矩的作用，原子核在自旋的同時，還繞着外加磁場的方向作進動。**




**核磁共振** $\omega = \gamma B_0$

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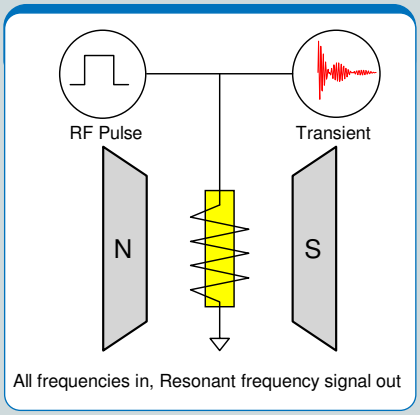
## Where NMR signals come from?




Conditions for observing NMR signals:

- A fix external magnet;
- Devices for transmitting and receiving RF;
- A sample.

A RF pulse changes direction of spins in the sample inside a detecting coil;

This leads to a change in magnetic flux in the detecting coil;

Inducted current, i.e. NMR signal, is then produced in the detecting coil

**similar to an electric generator**



## 核磁共振信號的產生

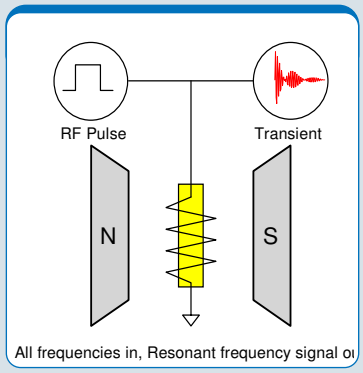
**獲得核磁共振信號的條件：**

- 固定的外加磁場；
- 發射和接收射頻的裝置；
- 樣品

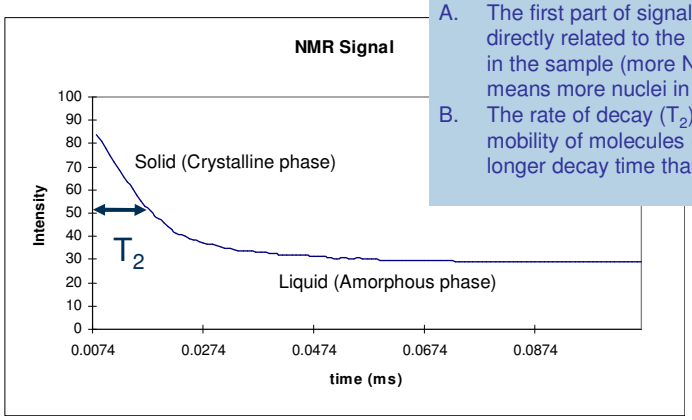
**核磁共振信號產生的原理**

- 射頻脉衝通過綫圈改變核磁矩的方向；
- 轉動的核磁矩作切割磁力綫運動，使綫圈中的磁通量發生變化；
- 磁通量的變化，在綫圈中產生感應電流，即核磁共振信號。

**與發電機工作原理相似**



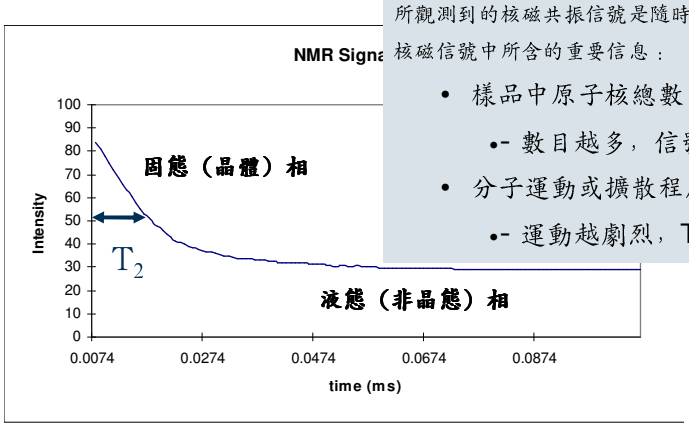
# TD-NMR Signal




A. The first part of signal (intensity) is directly related to the number of nuclei in the sample (more NMR signal means more nuclei in the sample);
B. The rate of decay ($T_2$) is related to mobility of molecules (liquids have longer decay time than solids).



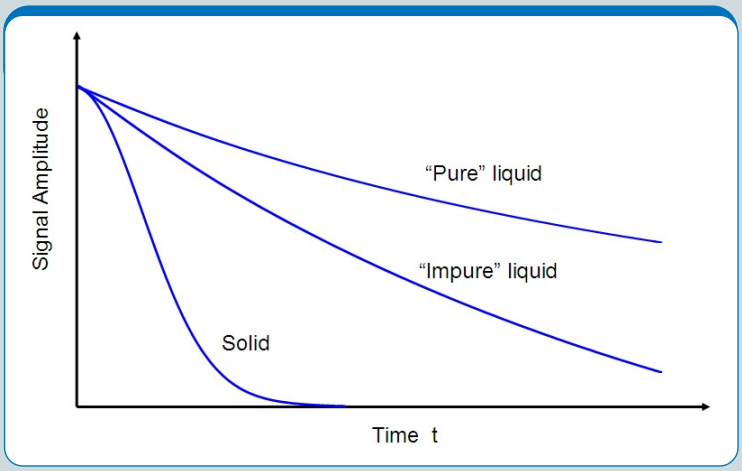
# TD-NMR Signal




所觀測到的核磁共振信號是隨時間衰減的信號；
核磁信號中所含的重要信息：

- 樣品中原子核總數目
  - 數目越多，信號越强
- 分子運動或擴散程度
  - 運動越劇烈，T2越長

## TD-NMR Signals

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## 利用小核磁作測量的特點

樣品直接測量，無需任何處理

分析迅速(在數秒至數分鐘內完成)

樣品分析無損無傷，可進行重復測量

整體樣品分析，無表面效應，顏色效應

定標過程和模型簡單

操作簡單，結果不受操作人員的水平影響

有大量被驗證過的工業應用

有相應國際測量標準作後盾

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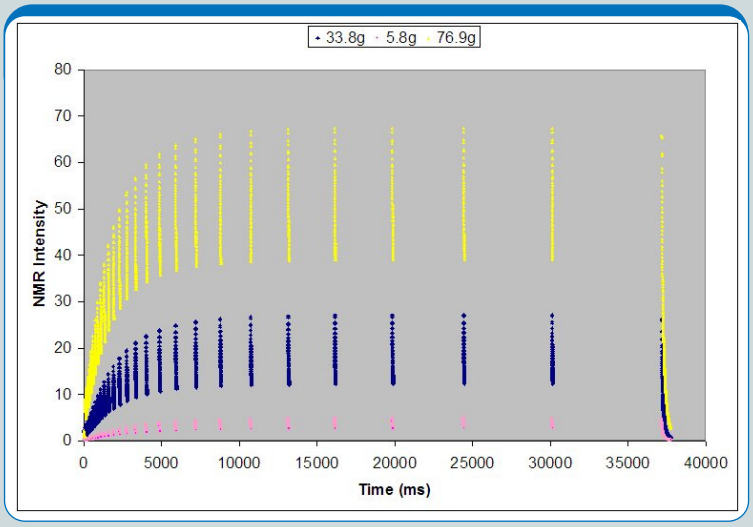
## TD-NMR Signals from mice

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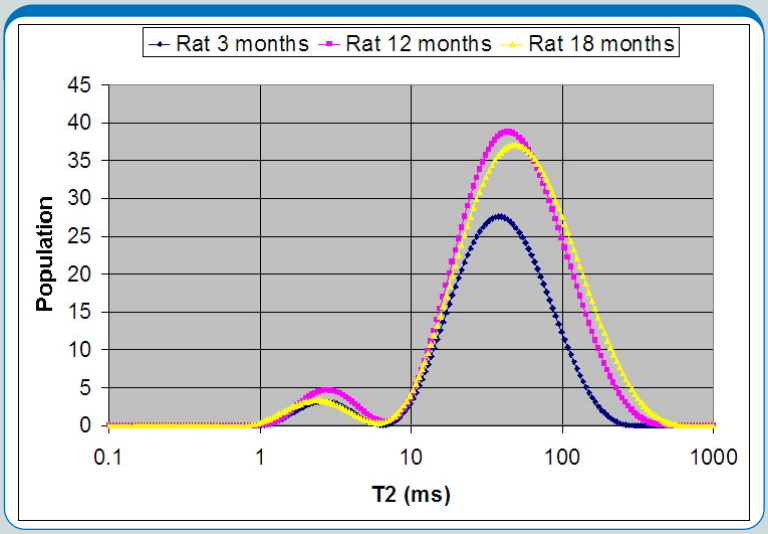
## TD-NMR Signals from mice

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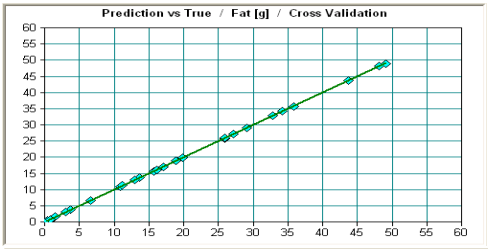
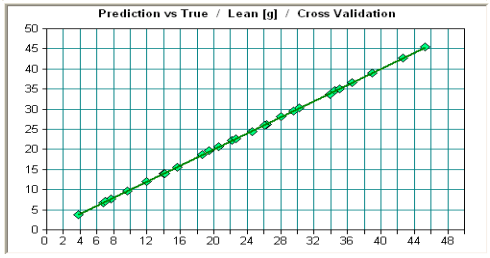
## BCA Calibration




TD-NMR is a proven technique for the determination of body fat and lean in live mice and rats.

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## Measuring a rat


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# BCA by NMR: Accuracy & Precision

| No | Sample ID | Date Time | Fat (g) | Lean (g) | Fluid (g) |
|---|---|---|---|---|---|
| 387 | MySample | SEP.15,2005 09:57 | 70.65 | 4.94 | 14.04 |
| 388 | MySample | SEP.15,2005 09:59 | 70.74 | 4.87 | 14.08 |
| 389 | MySample | SEP.15,2005 10:01 | 70.66 | 4.88 | 14.04 |
| 390 | MySample | SEP.15,2005 10:04 | 70.75 | 4.84 | 14.06 |
| 391 | MySample | SEP.15,2005 10:06 | 70.62 | 4.96 | 14.06 |
| 392 | MySample | SEP.15,2005 10:08 | 70.66 | 4.89 | 14.05 |
| 393 | MySample | SEP.15,2005 10:10 | 70.75 | 4.83 | 14.07 |
| 394 | MySample | SEP.15,2005 10:13 | 70.74 | 4.87 | 14.08 |
| 395 | MySample | SEP.15,2005 10:15 | 70.63 | 4.94 | 14.07 |
| 396 | MySample | SEP.15,2005 10:17 | 70.66 | 4.88 | 14.07 |
| 397 | MySample | SEP.15,2005 10:20 | 70.63 | 4.9 | 14.08 |
| 398 | MySample | SEP.15,2005 10:22 | 70.71 | 4.86 | 14.11 |
| 399 | MySample | SEP.15,2005 10:24 | 70.7 | 4.92 | 14.11 |
| 400 | MySample | SEP.15,2005 10:27 | 70.72 | 4.88 | 14.1 |
| 401 | MySample | SEP.15,2005 10:29 | 70.63 | 4.89 | 14.1 |

Record 420

Repeated measurements on a phantom sample

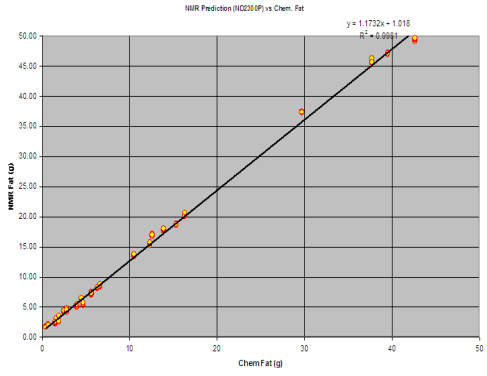


# BCA by NMR: Accuracy & Precision

Duplicate rat test results:

| Sample | Weight g | Fat % | Fluid % | Lean % |
|---|---|---|---|---|
| OM_91 | 621 | 30.2% | 4.4% | 58.6% |
| OM_91 | 621 | 30.6% | 3.8% | 58.0% |
| OM_92 | 468 | 24.3% | 3.9% | 65.9% |
| OM_92 | 468 | 24.0% | 3.7% | 65.6% |
| OM_93 | 557 | 28.3% | 3.8% | 61.2% |
| OM_93 | 557 | 28.5% | 4.1% | 61.4% |
| OM_94 | 486 | 19.1% | 3.4% | 70.5% |
| OM_94 | 486 | 19.0% | 3.6% | 70.7% |
| OM_95 | 609 | 28.5% | 4.1% | 60.8% |
| OM_95 | 609 | 28.5% | 4.1% | 60.7% |
| OM_96 | 586 | 25.9% | 3.4% | 62.8% |
| OM_96 | 586 | 25.7% | 3.4% | 63.2% |
| OM_97 | 617 | 27.5% | 4.1% | 61.4% |
| OM_97 | 617 | 27.2% | 4.4% | 61.8% |
| OM_98 | 156 | 19.7% | 4.2% | 69.9% |
| OM_98 | 156 | 20.1% | 3.8% | 69.1% |
| OM_99 | 267 | 20.8% | 3.9% | 71.0% |
| OM_99 | 267 | 20.3% | 3.9% | 71.4% |

Repeatability




NMR vs Chemical Analysis

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# BCA by NMR: Validation

Results of awake Animal

| | Fat / g | Lean / g |
|---|---|---|
| **Anesthetized Mouse:** | **4.01 (STD 0.01)** | **19.87 (STD 0.16)** |
| **Live Mouse:** | **4.14 (STD 0.11)** | **18.2 (STD 0.4)** |

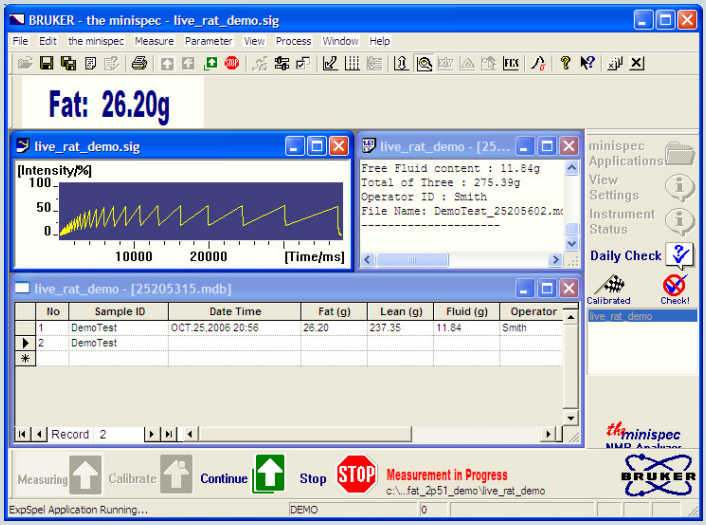
NMR vs. DEXA vs. Carcass Analysis

| | Fat(NMR) / g | Fat(DEXA) / g | Fat(Chem) / g |
|---|---|---|---|
| **Mouse I:** | **12.8 ± 0.7** | **12.6 ± 1.0** | **9.0 ± 0.6** |
| **Obese Mouse:** | **30.1 ± 0.9** | **30.2 ± 1.3** | **24.1 ± 0.9** |

DEXA: double energy X-ray absorption



# Software Interface



## Selected Customer List

***Key customers***
Sanofi-Aventis
Johnson & Johnson
Merck

***Industry customers***
Merck
Solvay
Banyu Pharmaceutical
Lilly
Boehringer-Ingelheim
Roche Pharmaceutical
Janssen Pharmaceutical
Acceleron
Metabasis
Metabolex
Develogen
Sankyo Co.

***Academic customers***
University of Graz
University of Colone
DIFE Deutsches Institut für Ernährungsforschung
Ecole Normale Superieure
CERBM-GIE
Louisiana State University
University of Florida
NIH-NIA
Penn State University
University of Michigan
Oklahoma University
Laval University
Guanjo University, Korea

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## LF90II Characteristics

| | |
|---|---|
| **NMR Frequency:** | **6.2MHz** |
| **Magnet Type:** | **Rare earth permanent magnet** |
| **Magnet Temperature:** | **Controlled to 0.01°C precision or better** |
| **NMR transmitter:** | **Broadband, 220W, linear power attenuation** |
| **NMR receiver:** | **Temperature regulated, gain range 80dB in 1dB steps** |
| **Sample size:** | **up to 850g** |
| **Parameters Analyzed:** | **Body Fat, Body Lean and Body Free Fluid** |
| **Sample Holders:** | **51mm OD for mice, 89mm OD for rats** |
| **Analysis Time:** | **ca. 2 minutes per animal** |
| **Analysis repeatability:** | **better than 0.5g for each component** |
| **Short Term Stability:** | **STDEV< 0.3 for each component within 2 hours** |
| **Long Term Stability:** | **STDEV< 0.5 for each component within 100 hours** |
| **Results data format:** | **Microsoft Access and Excel data files** |

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# LF90II Characteristics

| | |
|---|---|
| **Dimensions:** | **62 x 48 x 122 cm (D x W x H)** |
| **Weight:** | **ca. 380kg** |
| **Sitting:** | **Sealed enclosure on wheels** |
| **Power requirement:** | **115 or 220VAC, 700W** |
| **Host PC:** | **Any Pentium PC running Microsoft Windows XP** |
| **NMR software:** | **Runs with Windows XP Pro** |
| **Instrument GLP:** | |
| | **Automatic logging of system status and state of all major components** |
| | **Daily Check and Automatically logging system parameters** |
| | **Extensive diagnostics for all major components** |
| **Calibration:** | **Pre-calibrated by factory** |
| **Calibration Tools:** | **Available with the system** |
| **Calibration by User:** | **Available (optional)** |



# LF90II Characteristics

## GLP Features

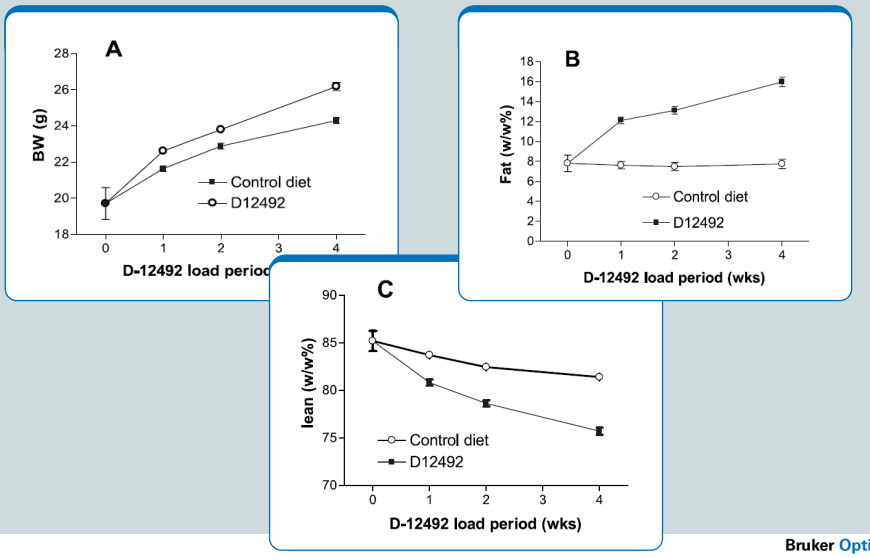
- **Self test on all units, Daily check**
- **Internal signal routing, calibration validation**
- **Automatic data logging, QC statistic mornitoring**
- **Raw data and calibration protection**
- **Remote data access**
- **Remote system diagnostic**

# BCA by TD-NMR

## Examples from publications



**Fig. 3** Changes of body weight(g), fat mass rate and lean mass rate (w/w%) in mice fed a high fat diet, D12492 (35 w/w% lard), during a 4-week period. A, body weight (g); B, fat mass rate (w/w%) to body weight; C, lean mass rate (w/w%) to body weight. Control diet, CE-2 (Clea Japan) contains 4.4 w/w% fat.



# Fat in Liver

**Table 2** Fat contents of liver in mice after overnight fasting

| Groups | liver weight (g) | NMR parameters | | fat contents in liver[1] (mg/g liver) | TG contents[2] (mg/g liver) |
|---|---|---|---|---|---|
| | | fat mass (g) | lean mass (g) | | |
| Fasting | 1.179 ± 0.044 | 0.240 ± 0.018 | 0.93 ± 0.04 | 202.9 ± 11.39 | 57.33 ± 2.13 |
| Ad lib control | 1.382 ± 0.032 | 0.208 ± 0.007 | 1.07 ± 0.03 | 151.2 ± 5.59 | 3.52 ± 0.38 |

[1]estimated using following equation: fat mass (mg) / liver wight (g), [2]enzyme assay

**Table 3** Fat contents of liver in mice orally treated with PPARγ agonist for 10 days

| Diet | Drug | n | liver weight (g) | NMR method | | | Biochemical method | |
|---|---|---|---|---|---|---|---|---|
| | | | | fat mass (g) | lean mass (g) | Fat content (mg/g liver) | TG (mg/g liver) | TC (mg/g liver) |
| HFD | vehicle (0.5%MC) | 10 | 2.472±0.061 | 0.614±0.060 | 1.716±0.061 | 243.9±13.8 | 61.68±4.87 | 5.87±0.80 |
| | PPARγ agonist, 10mpk | 10 | 1.944±0.093 | 0.331±0.023 | 1.429±0.067 | 177.7± 4.2 | 29.22±4.86 | 3.43±0.60 |
| CE-2 | vehicle (0.5%MC) | 5 | 1.941±0.058 | 0.238±0.012 | 1.416±0.065 | 122.8± 6.2 | 7.63±0.35 | 2.65±0.10 |

HFD: 35w/w% fat diet, CE-2: control diet (4.4w/w% fat diet), Biochemical method: enzyme assay using commercial kits (KYOWA determiner)

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# Tissue Analysis using other minispec instruments

**Mq-20TA**

NMR Frequency: 20MHz
Magnet type: permanent 0.47T
Magnet Tempeature: controlled
Sample range: 0.05 – 5g
Sample Holder: 18mm dia. O.D.
Sitting: On-bench or cart-mounted on wheels
Host PC: Pentium 4 or higher
ISO9001 standard certified

**Mq-60TA**

NMR Frequency: 60MHz
Magnet type: permanent 1.41T
Magnet Tempeature: controlled
Sample range: 3.0 – 150mg
Sample Holder: 7.5mm dia. O.D.
Sitting: On-bench or cart-mounted on wheels
Host PC: Pentium 4 or higher
ISO9001 standard certified

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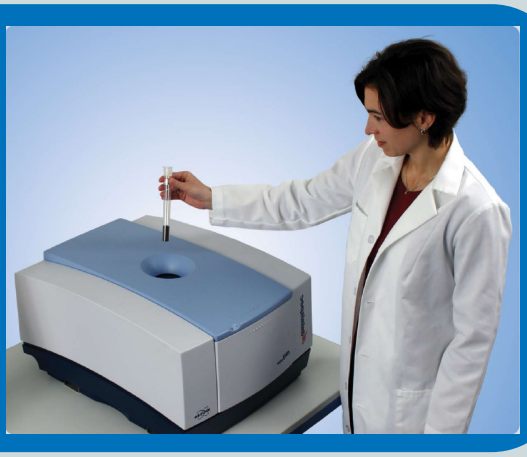
## Operation

1. Load sample into a vial, then place the vial into a test tube
2. Insert the test tube into the minispec instrument
3. Click the Measure button to start analysis.



Bruker Optics

## Summary

### TD-NMR for Body Composition Analysis

1. Animal is analyzed as **Whole**
2. **No Anesthetizing** is needed
   - Less stress for animals, and high throughput
3. **Rapid** Analysis
   - Less than 2 minutes per measurement
4. High **Reproducibility**
   - Better than DEXA and Chemical methods

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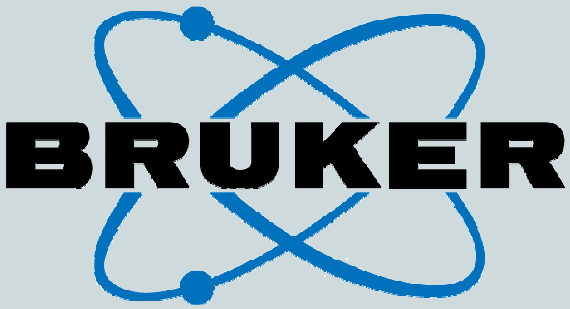


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