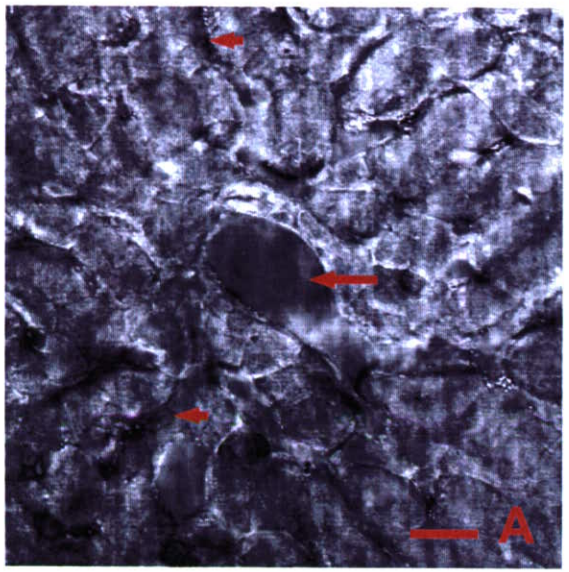
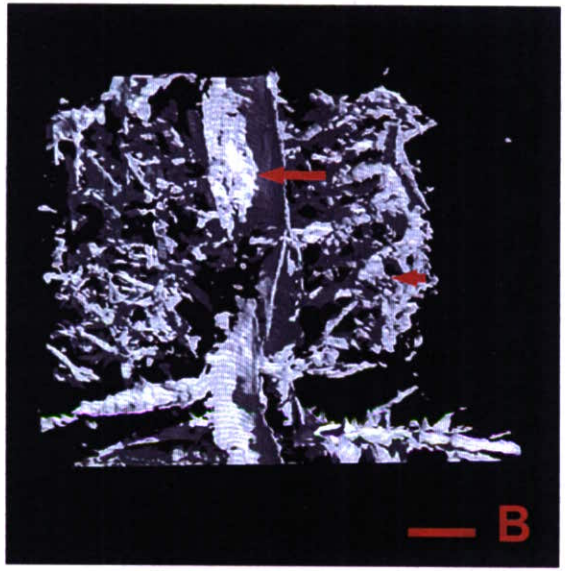
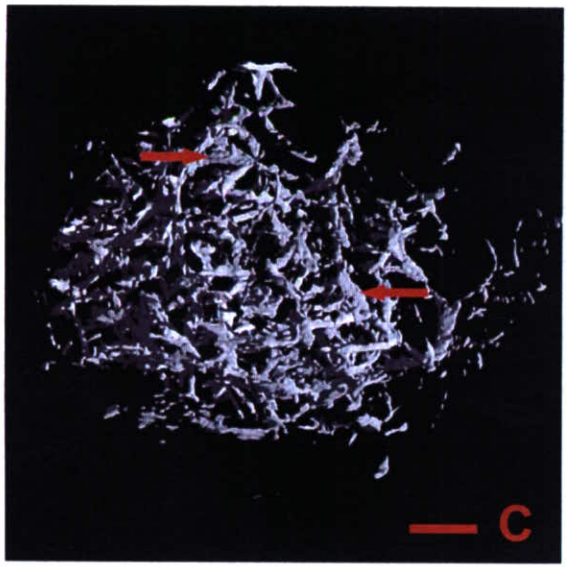
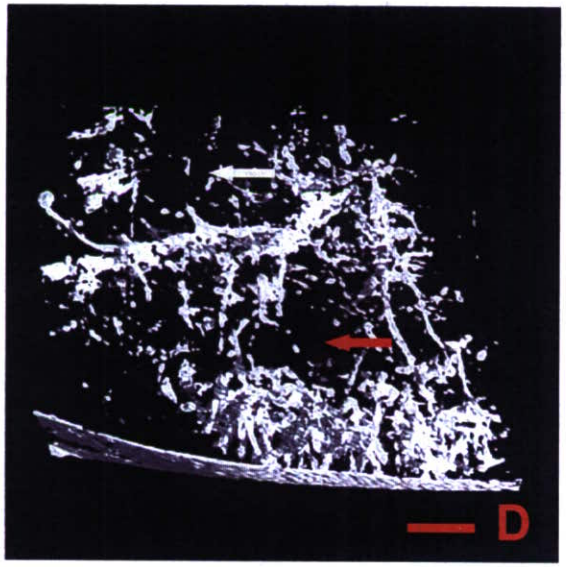
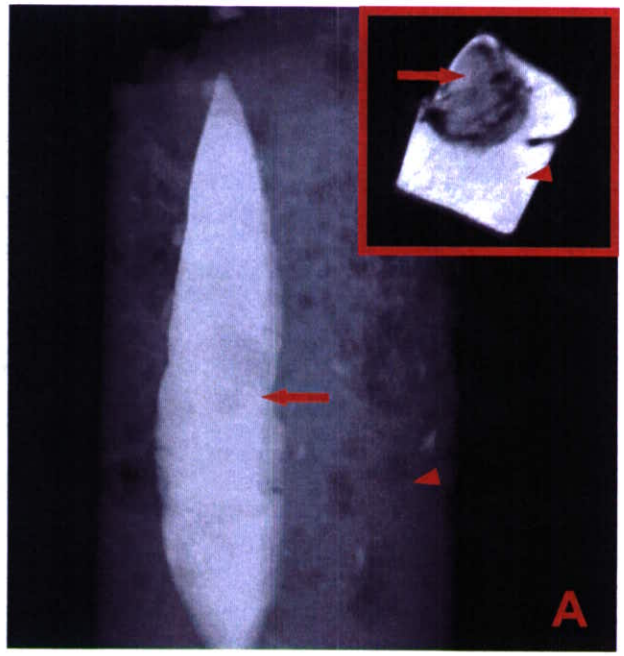
2006 X-Ray Micro CT Research On Liver Tissue

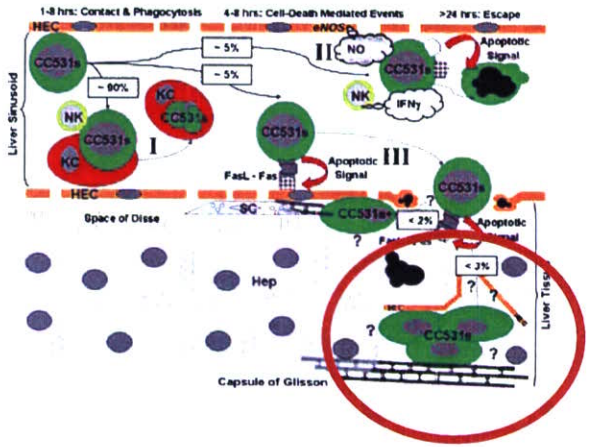






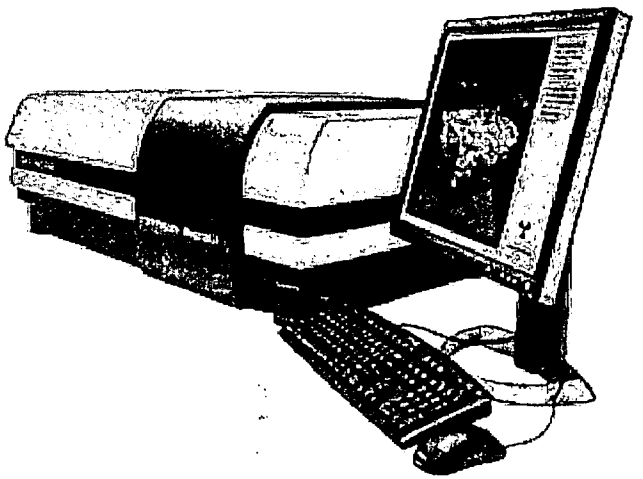


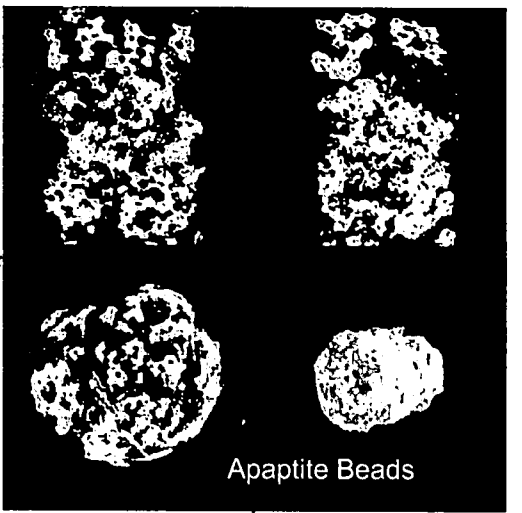


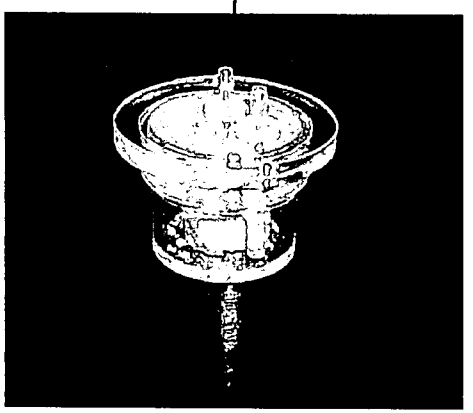


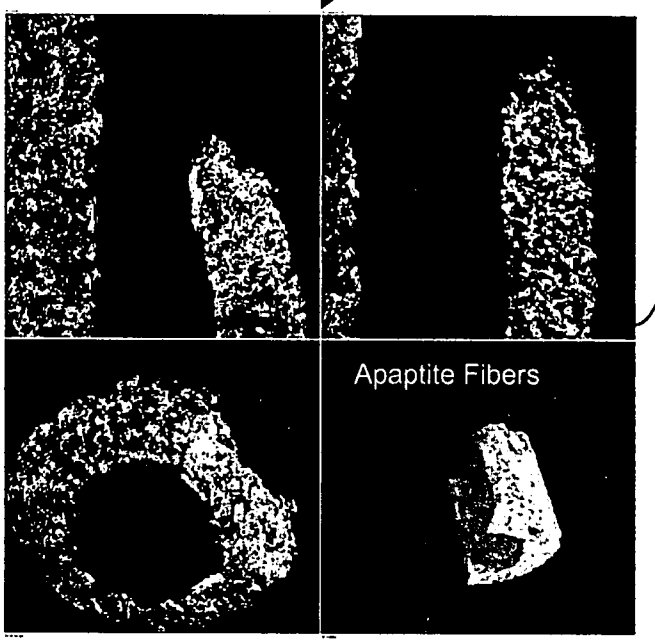
Braet F, Nagatsuma K, Saito M, Soon L, Wisse E, Matsuura T. The hepatic sinusoidal endothelial lining and colorectal liver cancer metastases [Editorial] [Editorial Commentary pg. 824]. World J Gastroentero 2007:13;821-825.

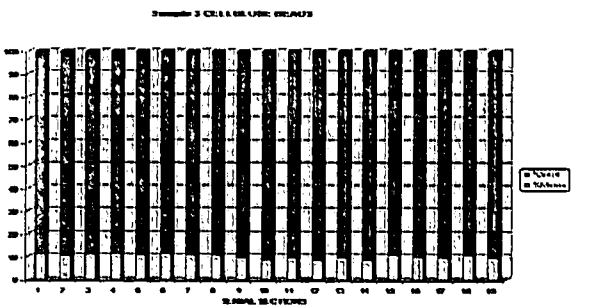
2007 X-Ray Micro CT Research On Liver Bioreactor Tissue

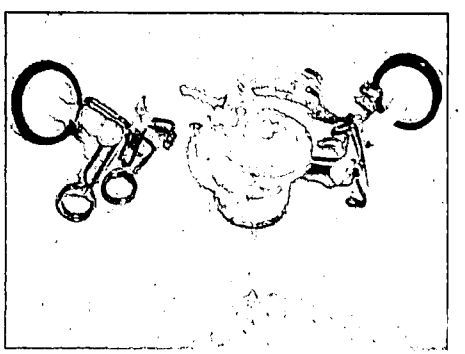




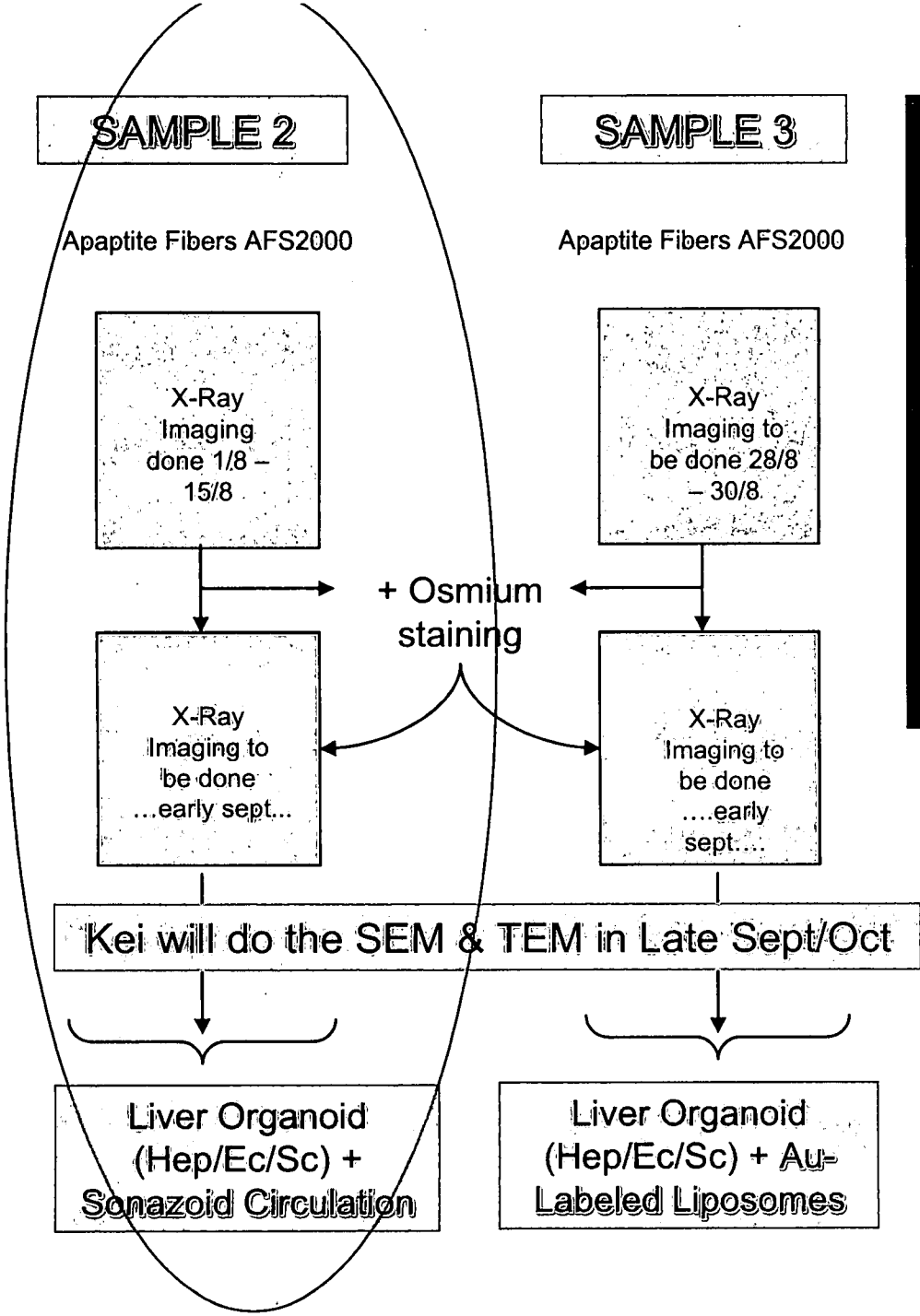


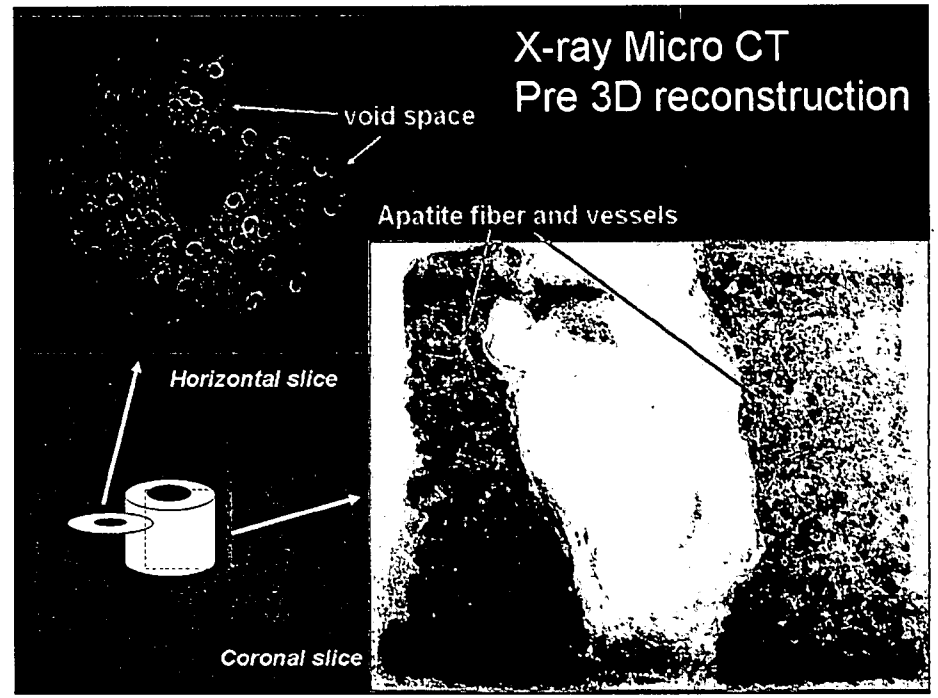




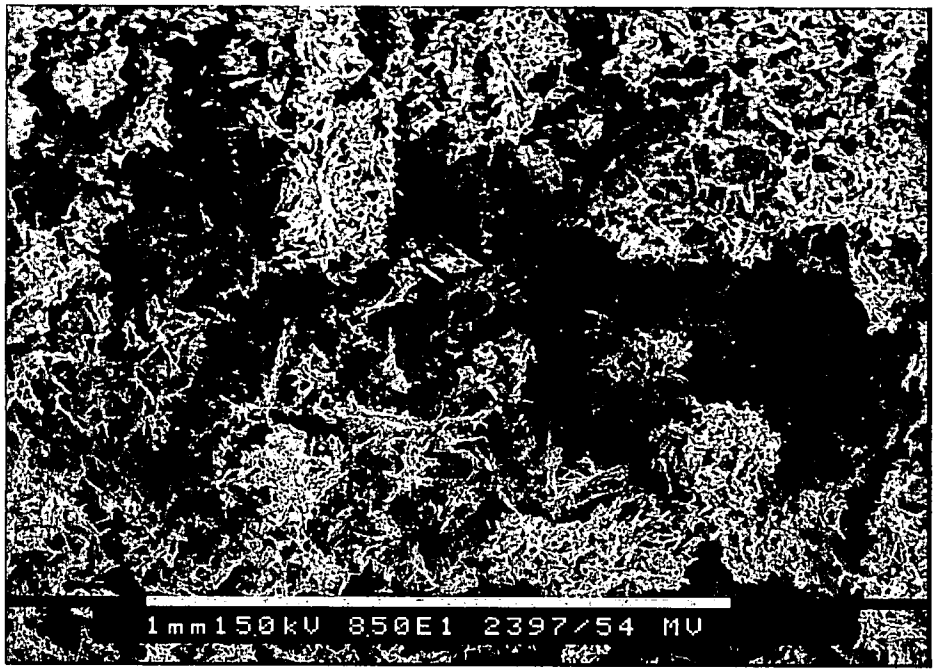


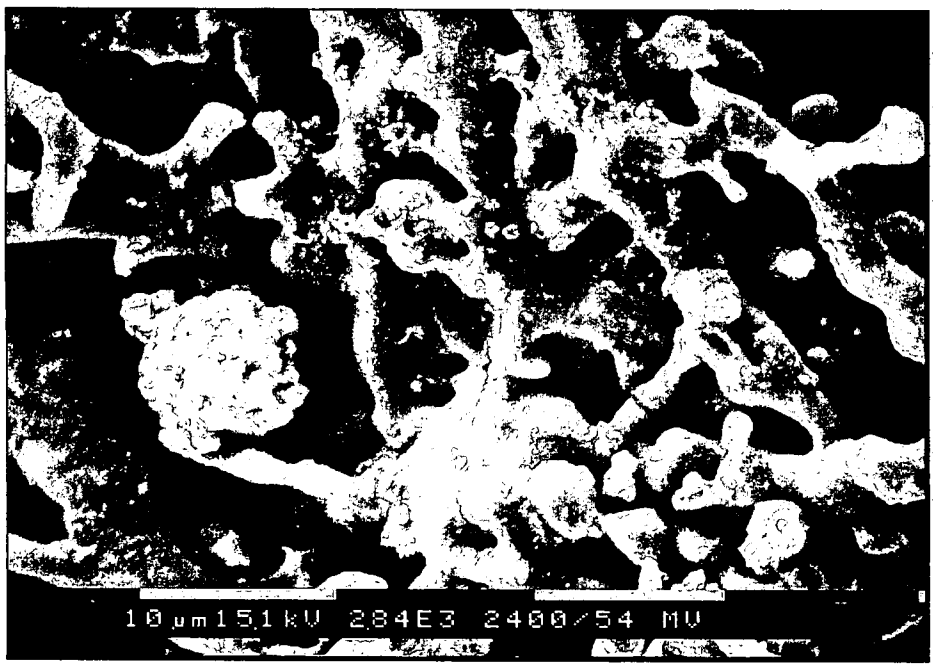
Doctor Nagatsuma reports:

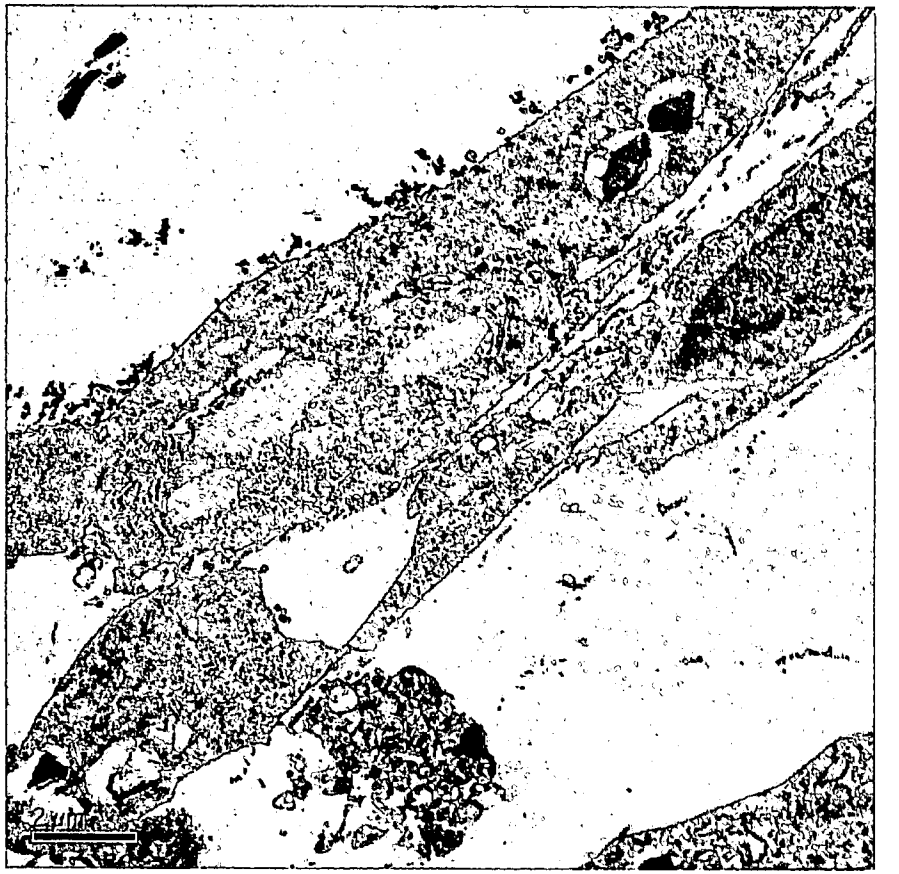




To Be Computer Modelled in Sydney November – December 2007







Apaptite Fibers AFS2000

Liver Organoid
(Hep/Ec/Sc) +
Sonazoid Circulation

SAMPLE 2

Apaptite Fibers AFS2000

X-Ray Imaging done 1/8 – 15/8

X-Ray Imaging to be done ….early sept...

SAMPLE 3

Apaptite Fibers AFS2000

X-Ray Imaging to be done 28/8 – 30/8

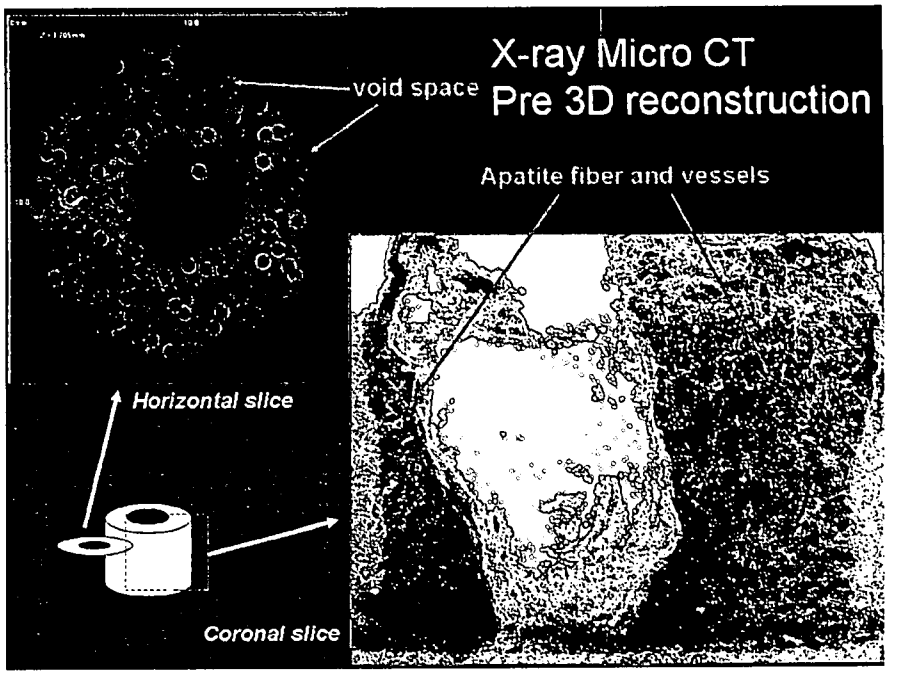
+ Osmium staining

X-Ray Imaging to be done ….early sept….

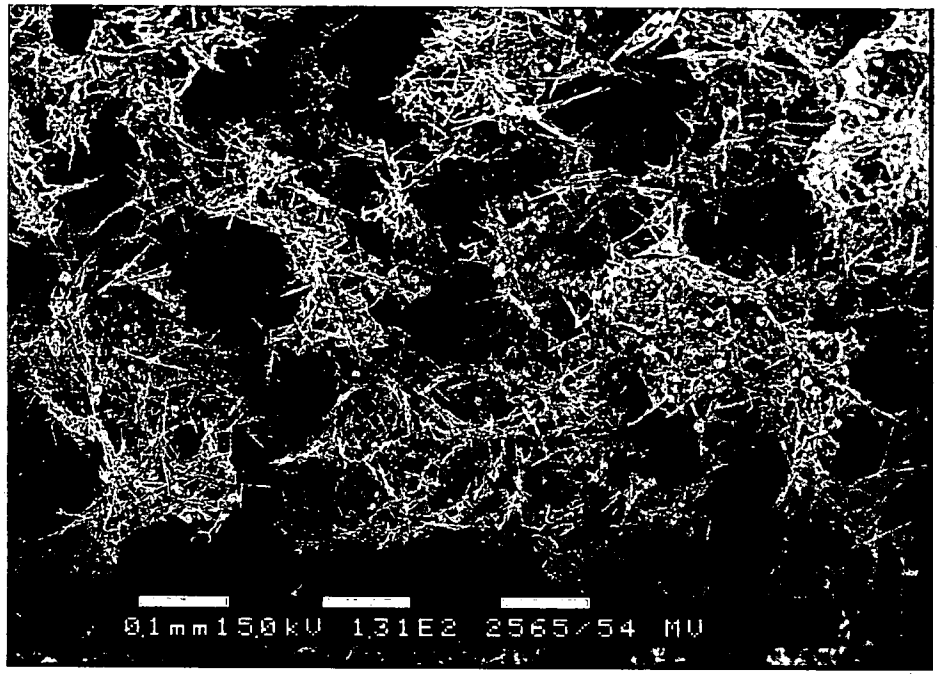
Kei will do the SEM & TEM in Late Sept/Oct

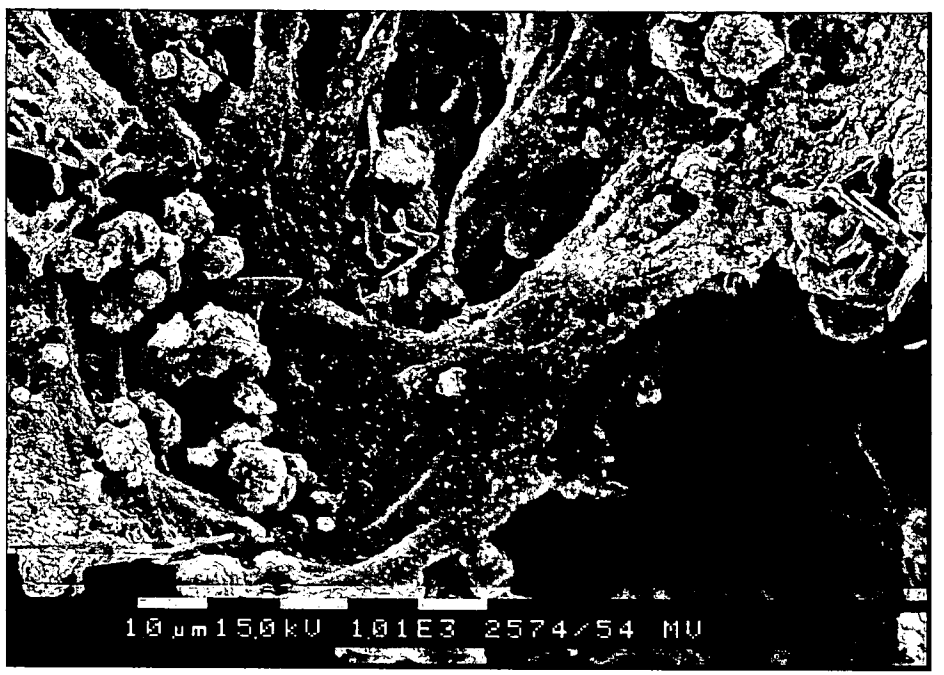
Liver Organoid (Hep/Ec/Sc) + Sonazoid Circulation

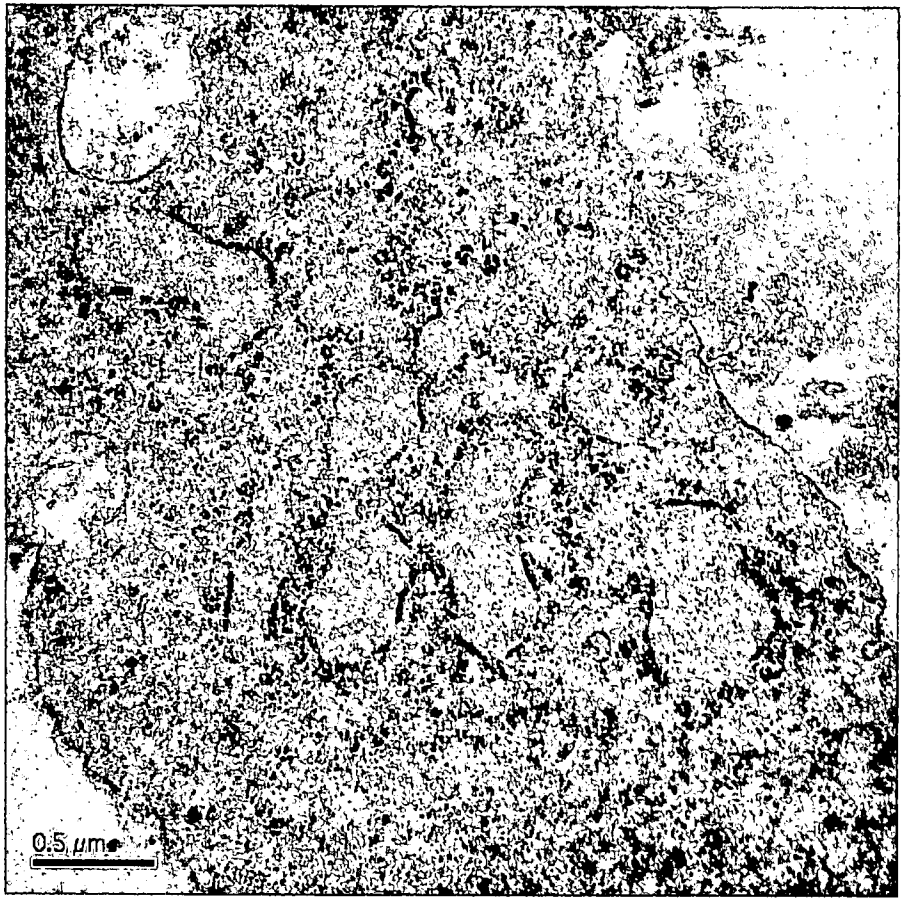
Liver Organoid (Hep/Ec/Sc) + Au-Labeled Liposomes



To Be Computer Modelled in Sydney November – December 2007

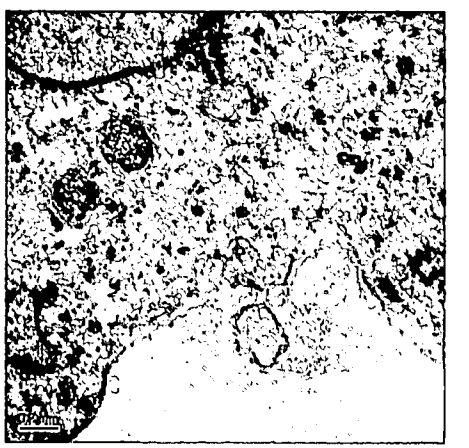






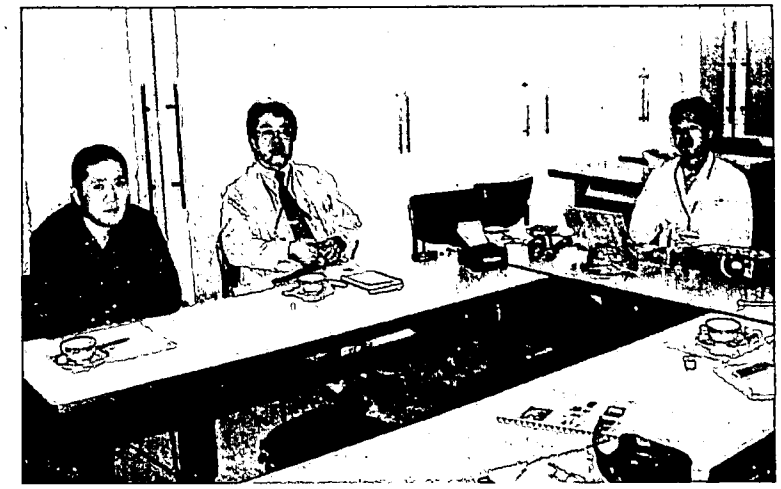
Apaptite Fibers AFS2000

Liver Organoid (Hep/Ec/Sc) + Au-Labeled Liposomes

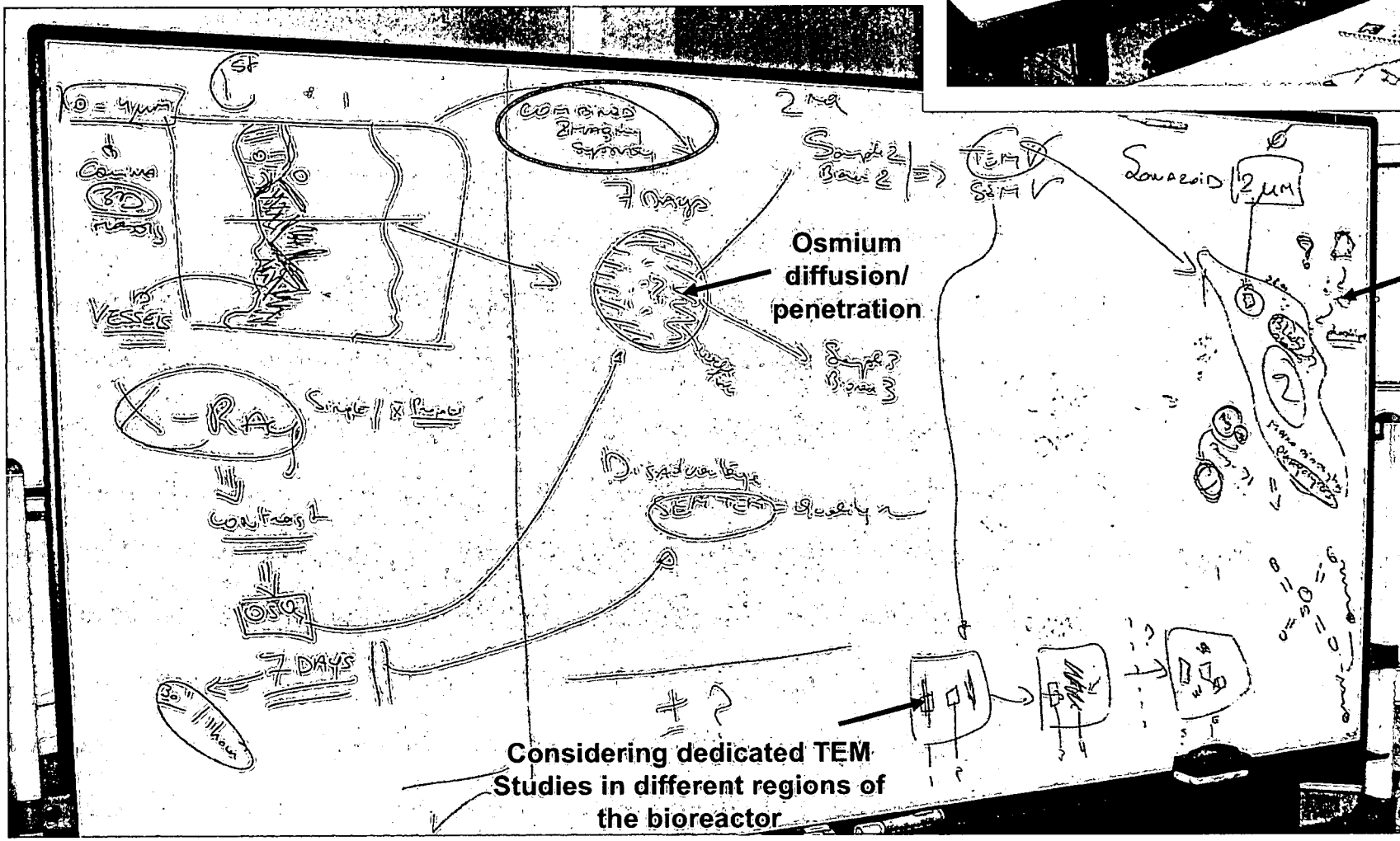


Monday 12 November 2007

Micro-Nano Bubble Research Project Meeting – Jikei University Results

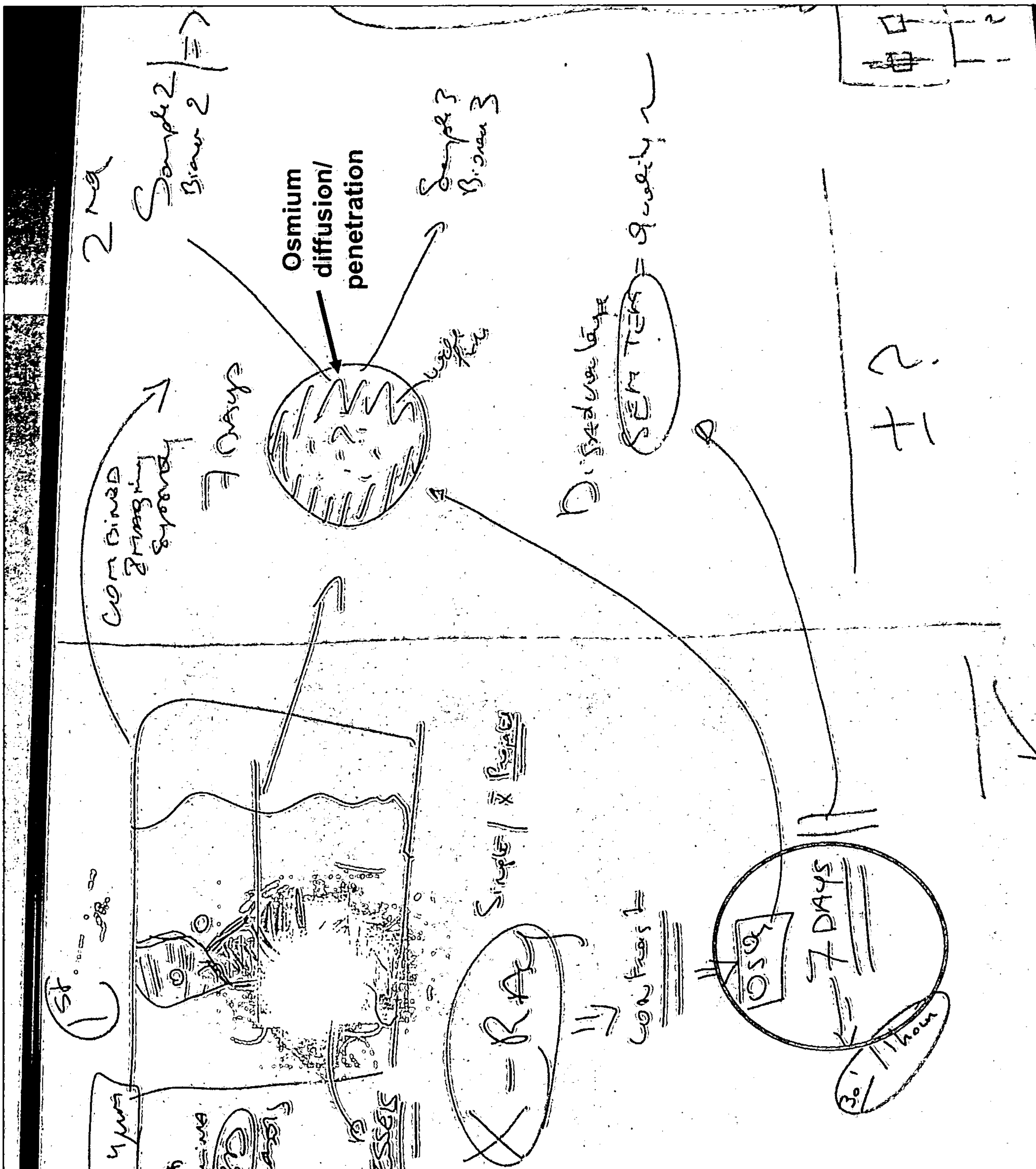


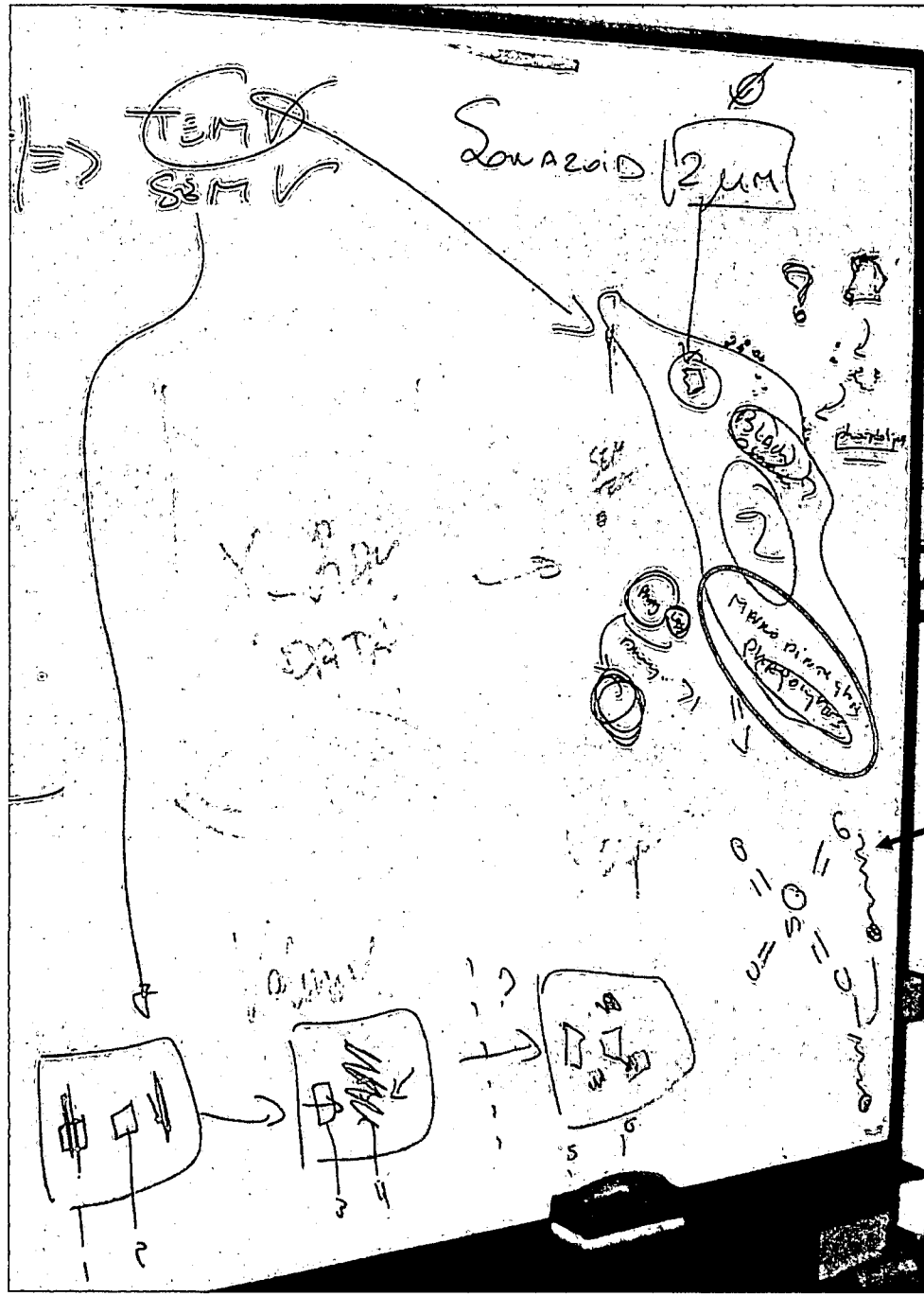
Doctor Braet reports:



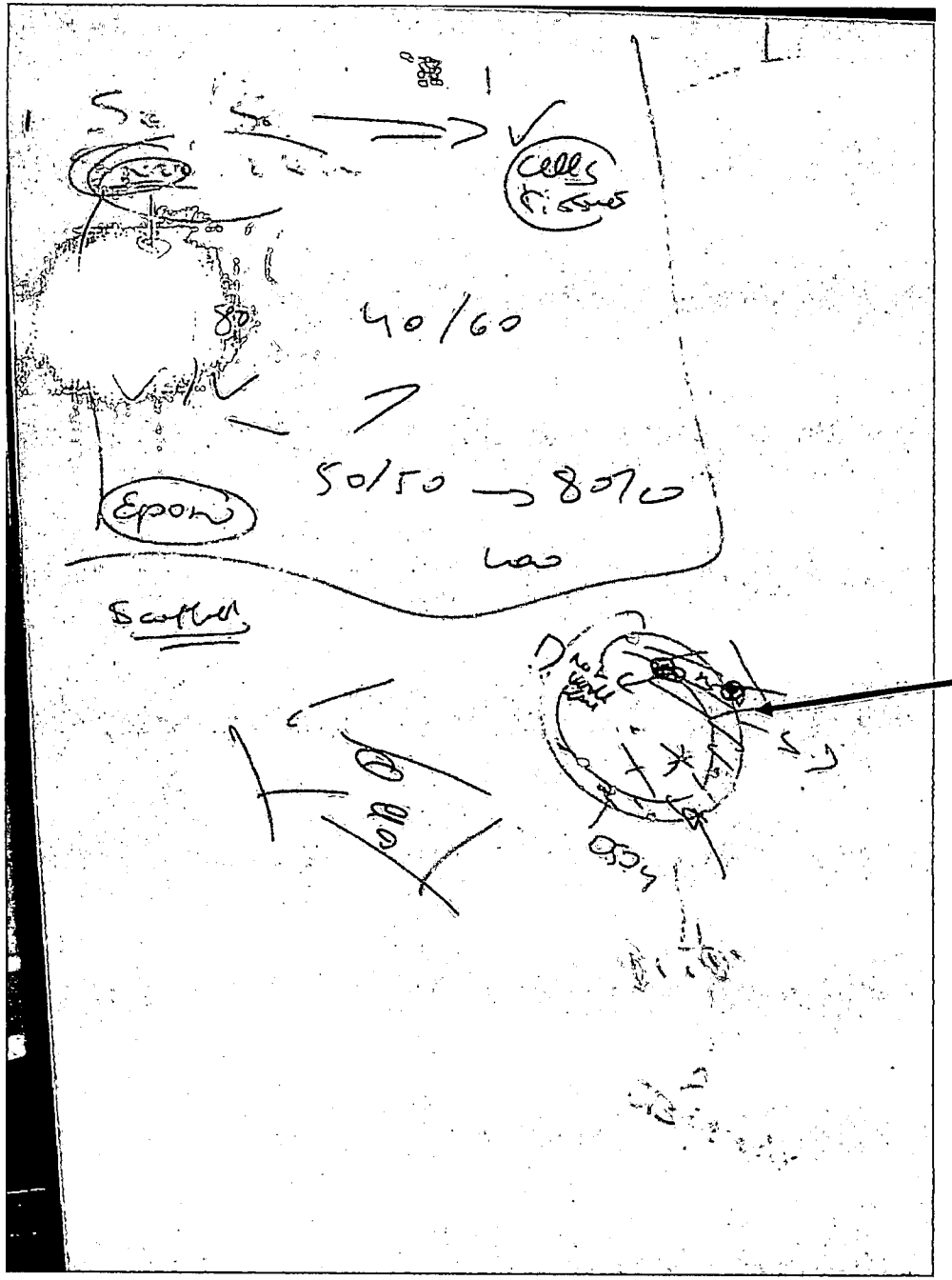
Uptake Mechanims of Sonazoid

X-Ray micro CT Advantage of Large Volume & Later the data can be used to be compared with Ultrasound data


Osmium
diffusion/
penetration
7 DAYS
X-RAY
VESSELS
COMBINED
SEM TEM



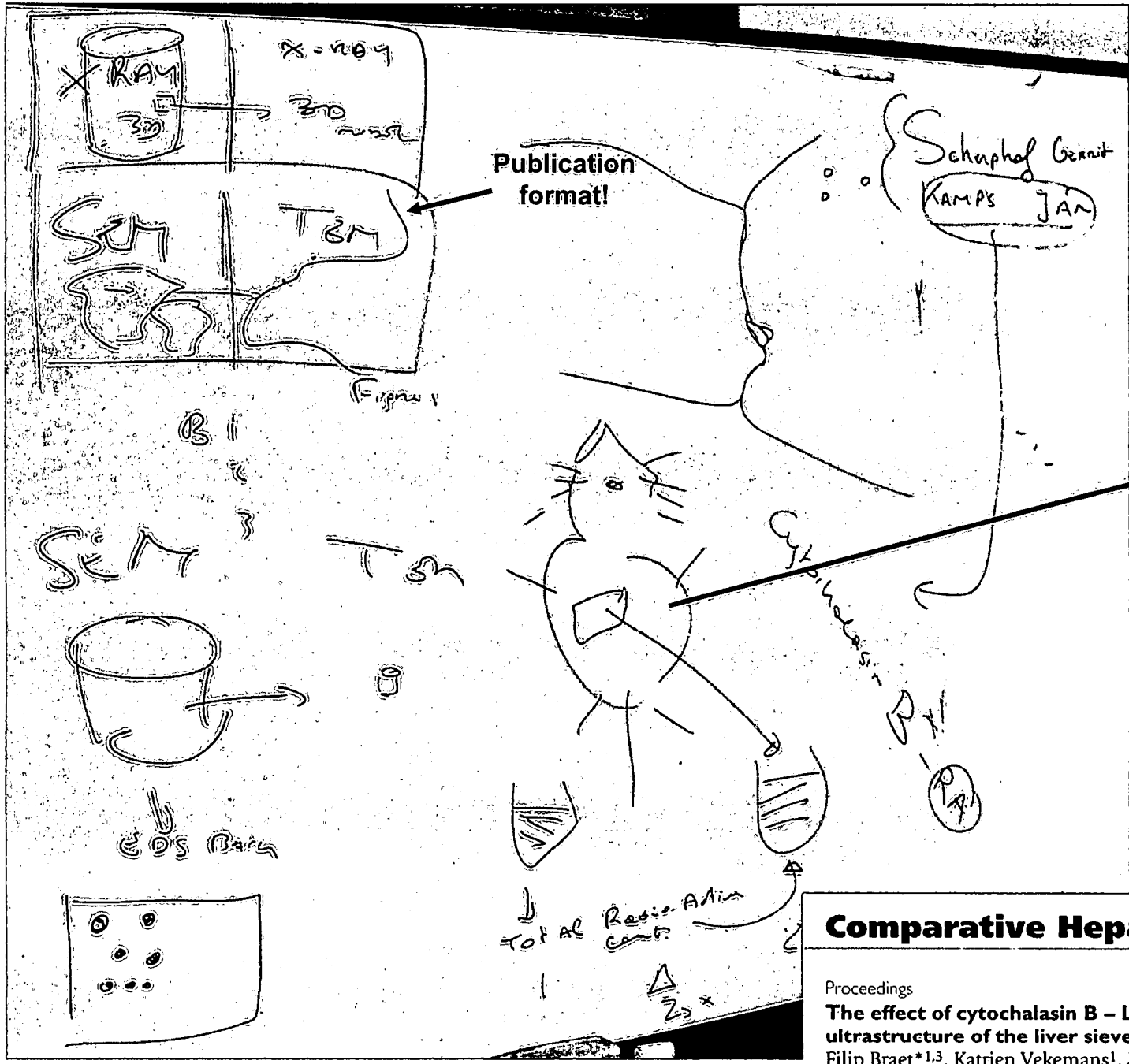
The long osmium impregnation staining may explain why the sonazoid is so dark stained: phospholipid – osmium complex



Advice for future TEM studies:

1. <u>Sampling</u>, exactely record place here tissue samples is taken from – now we took samples in a double-blind ad random manner

2. <u>Embedding</u>, start with 20/80 Epon/EtoH, than 40/60, 60/40, 80/20, 100/100, 100/100. Will result in better infiltration



Comparative Hepatology

BioMed Central

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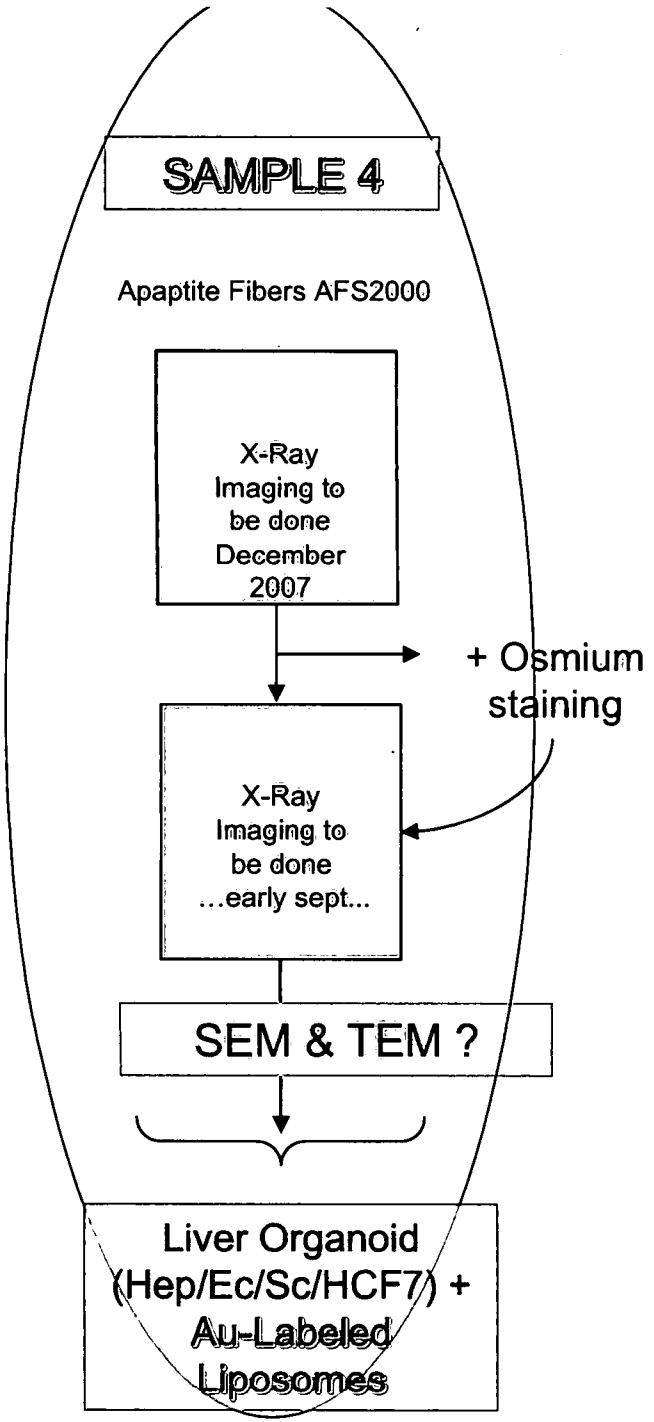
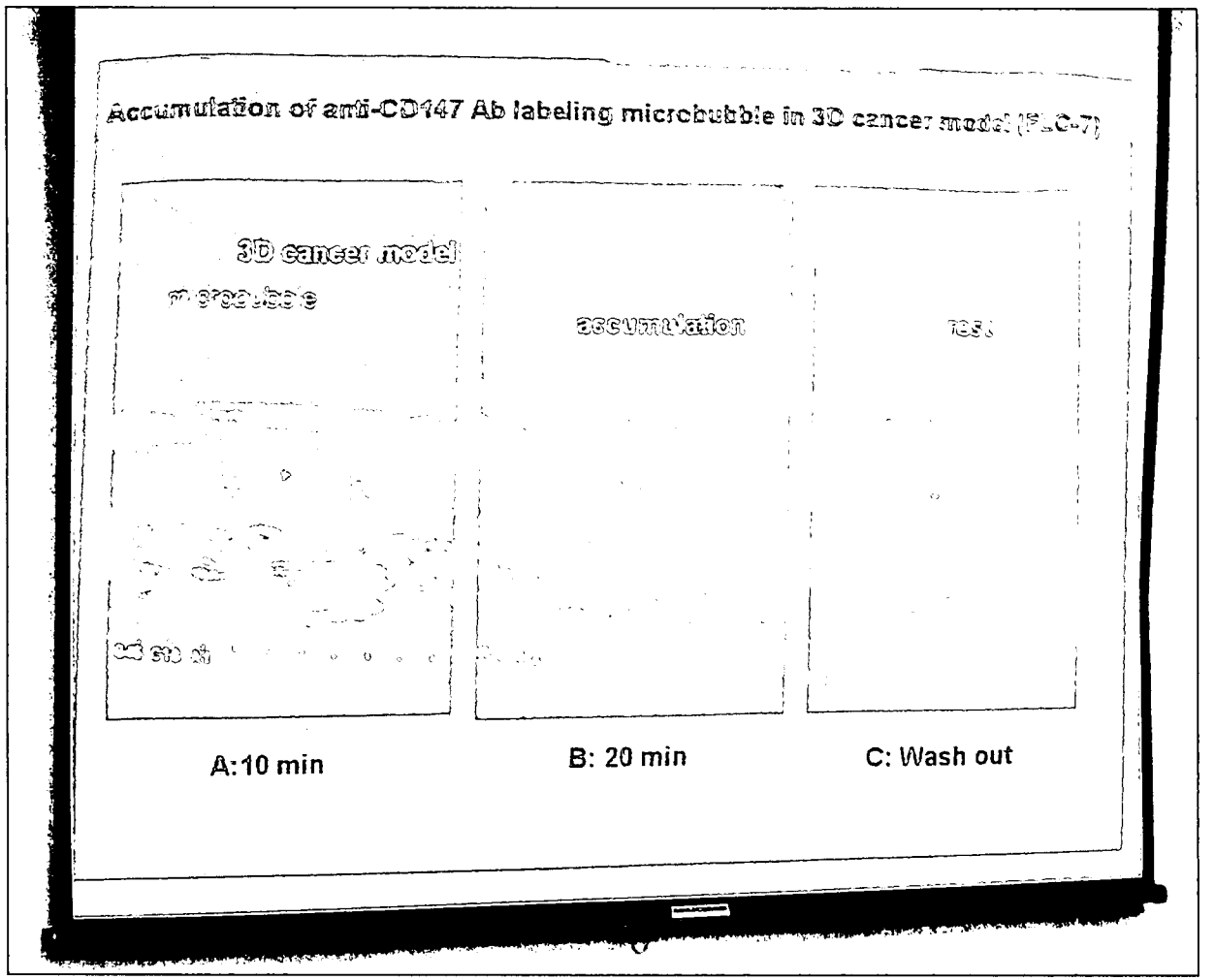
The effect of cytochalasin B – Loaded liposomes on the ultrastructure of the liver sieve

Filip Braet*[1,3], Katrien Vekemans[1], Henriette Morselt[2], Ronald De Zanger[1], Eddie Wisse[1], Gerrit Scherphof[2] and Jan Kamps[2]

Doctor Matsuura reports about the 24 October 2007 experiment:





Tuesday 13 November 2007

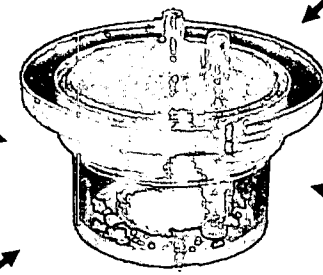
Micro-Nano Bubble Research Project Meeting – Research Partner Presentations at Jikei Hospital

Doctor Braet advice:

1. With KEIMA labelled bubbles - *in vitro* co-culture experiments can be considered to study uptake

2. Ultrasound data can be elaborated with total imaging of fluorescent labelled bubbles in the bioreactor – i.e., comparative kodak imaging (similar to total animal imaging studies)

3. The technology of making fluorescent labelled bubbles is now available – so we can consider FRET studies; this model would help us to study uptake mechanism by detecting another fluorescent peak etc …

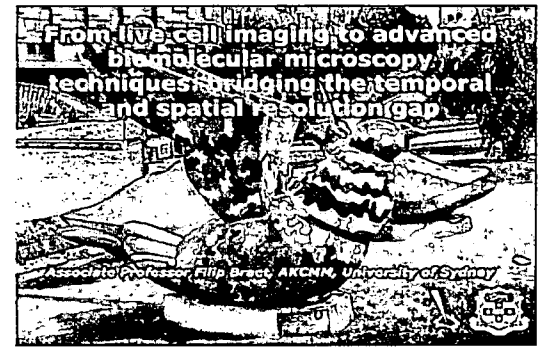


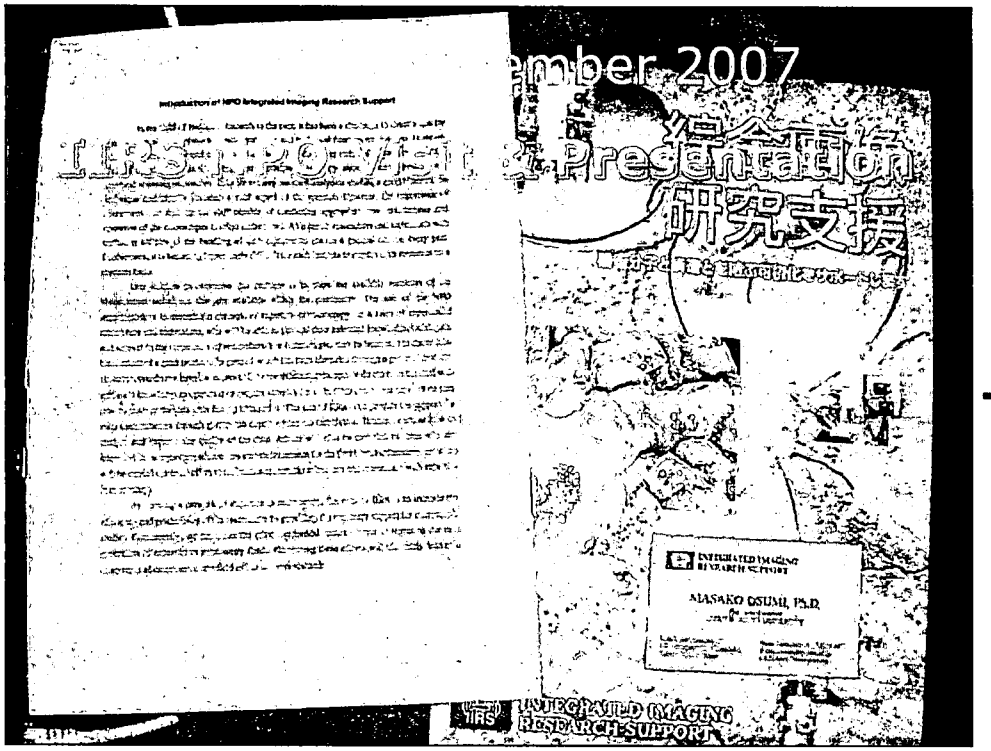
5. We have now KEIMA labelled microbubbles – it would be interesting to have a CYP transfected HCF7 cell line – As such we can study if indeed the green lablled bubbles reach the blue labelled cancer cells for example

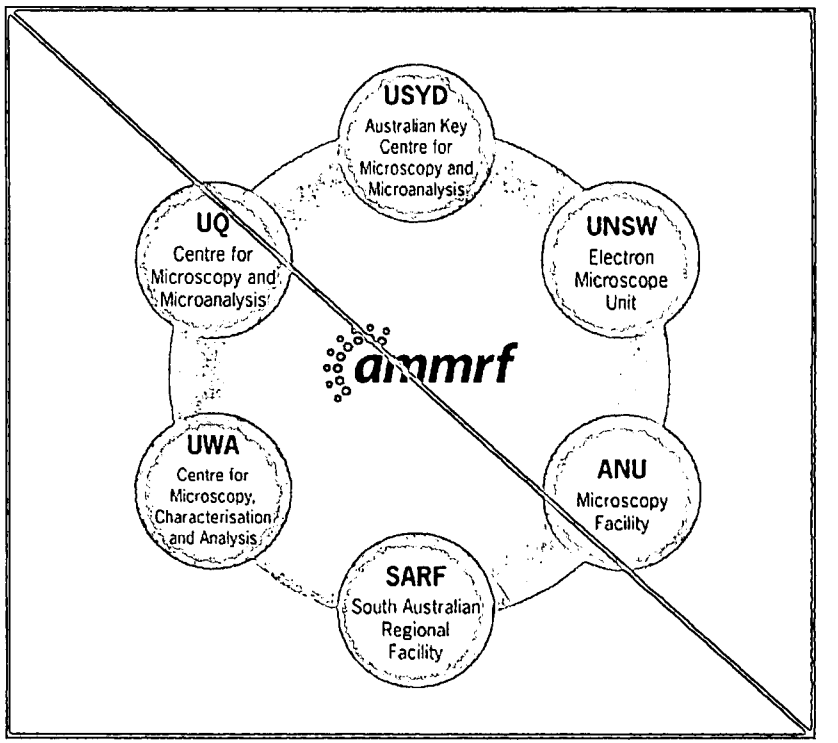
4. The group of Scherphof and Kamps did already a lot of work in making liposomes – PEG liposomes were modified with HAS for example which significantely changed the uptake of particles and as such different liver cell types could be targettted

The day was concluded with a seminar at Jikei Hospital:

Braet F. Seminar: From live cell imaging to advanced molecular microscopy techniques: bridging the temporal and spatial resolution gap. Jikei University Hospital, School of Medicine. Tokyo, Japan, 13 November 2007







The interactive business meeting was based on a seminar at Japan Women's University:

Braet F. Seminar: Australian microscopy & microanalysis research facility (AMMRF): A joint venture between Australian university-based microscopy and microanalysis centres. NPO Integrated Imaging Research Support, Japan Women's University. Tokyo, Japan, 15 November 2007

IIRS Members Present:

Professor Masako Osumi (IIRS) Japan Women's University); Dr Yoshiaki Hataba (Head of Research Center IIRS); Professor Yoshinobu Mineyuki (University of Hyogo / TBC Visitor to Sydney); Professor Shohei Yamashina (Kitasato University); Professor Hideyo Yamaguchi / Teikyo University); Doctor Tomoyuki Matsuura (Jikei University)

Main Outcome:

Visit IIRS/NPO delegation to Sydney nodes USyd & UNSW ~ in the week of December 10, 2007

IIRS committee member proposal International (F. Braet) & National (T. Matsuura) scientific & technical committee

Thursday 15 November 2007

Business Dinner with Biott ABLE® Group (Dr Shutaro Ishikawa) & TORII PHARMACEUTICAL Co. (Dr Tadashi Mizuima)

In this evening meeting I had the opportunity to meet with Dr Matsuura's collaborators:

(i) Mr Ishikawa explained me about the technology of the Bioreactors & products available; and,

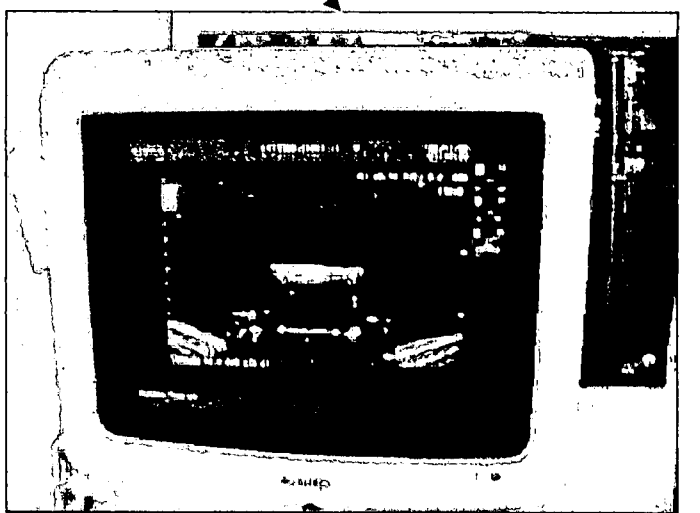
(ii) Mr Mizuima explained me about Nafamostat Mesilate, a protease inhibitor launched in Japan under the name Futhan.

Tuesday 20 November 2007

Microbubble Experiment Dr Matsuura's Laboratory

Follow-up experiments after last week's meeting on Tuesday 13 November 2007

Step1:sample preparation Liver bioreactor with anti-CD147 Ab labeling microbubbles



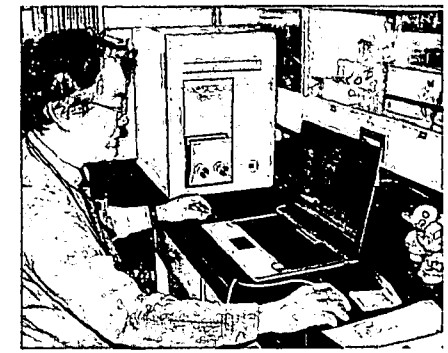
Step 2: Imaging with US

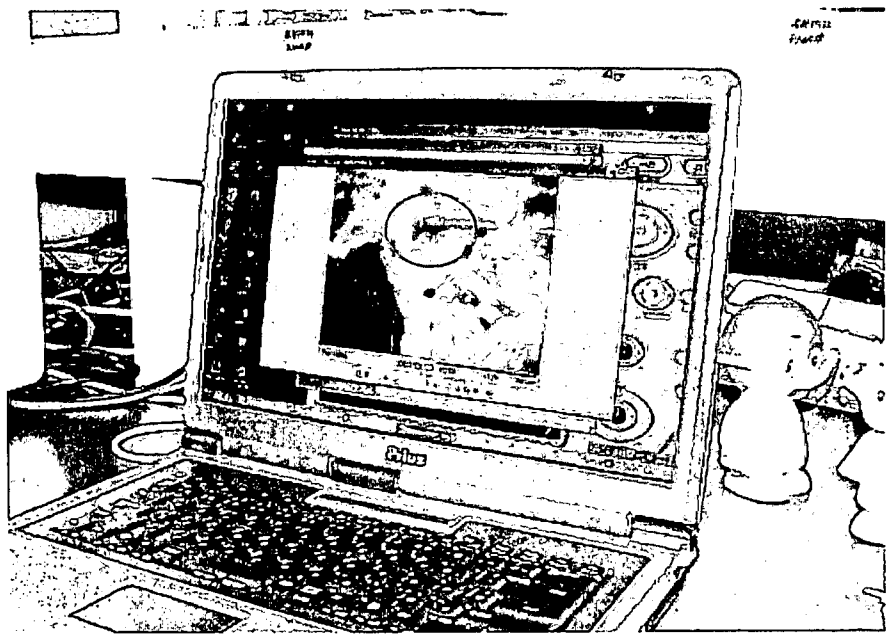
Step 3: The interpreation with Dr.Matsuura's team

Tuesday 20 November 2007

Hitachi eSEM Table-top Experiment @ Dr Matsuura's Laboratory

Hitachi eSEM Miniscope TM-1000 (Aus$ 50K): The instrument has superb / excellent imaging capabilities under environment conditions. Wet liver tissue was investigated at 10,000× that was first fixed with glutaraldehyde, rinsed for 10' in PBS and made conductive with a Hitachi Platinum solution.





Advice given herein:

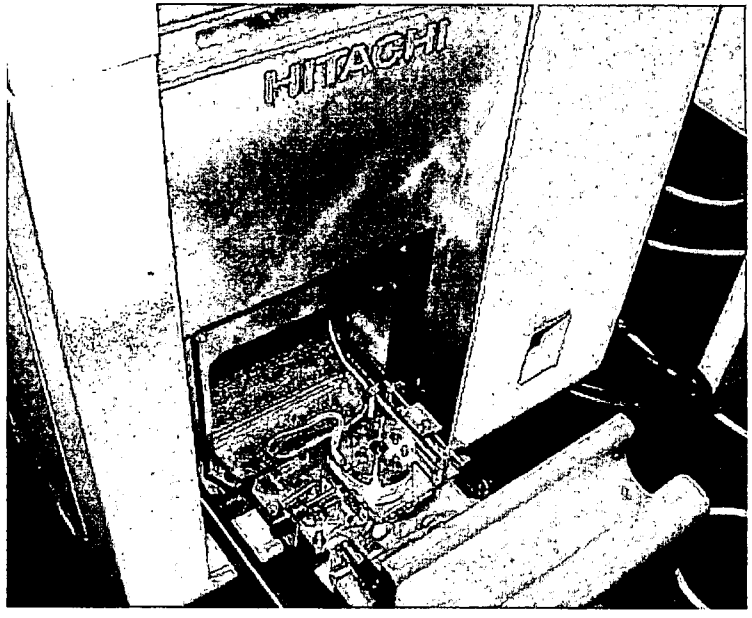
- Filip will sent the SEM samples obtained in Sydney by Dr Nagatsuma to Dr Matsuura for comparative analysis between classical SEM & eSEM

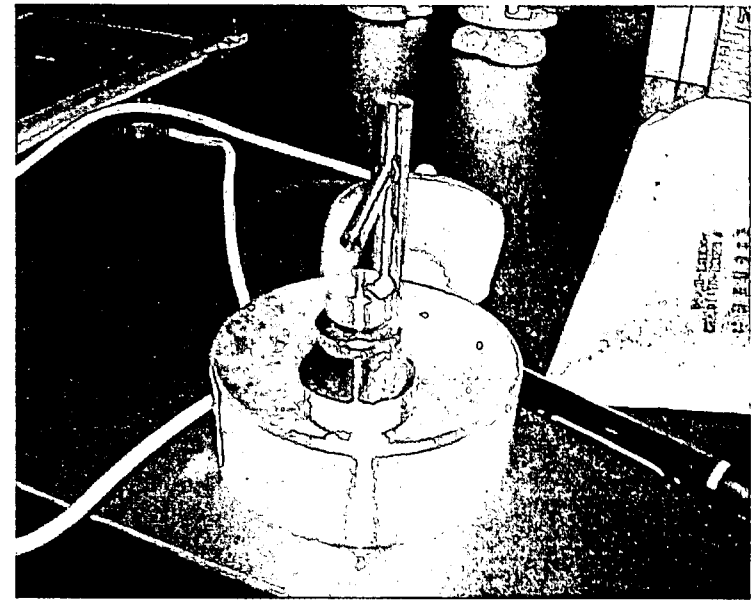
- Compare PBS washing with ammonium acetate buffer washing = reducing phosphate crystal formation / check if this results in improved image quality?!

- Another point to consider is to treat it with 2% osmium – even osmium is not needed for eSEM imaging ... it can however enhance the contrast of the images as osmium is a metal which will give rise to an enhanced electron scattering

- The eSEM images are (1) the first SEM images recorded under wet conditions in the liver endothelial field: THIS is NEW information and as such is an interesting observation and wortwhile to publish in one or another format; and (2), this eSEM images also <u>undoubetly show</u> fenestrae, sieve plates, coated pits in the cell line.

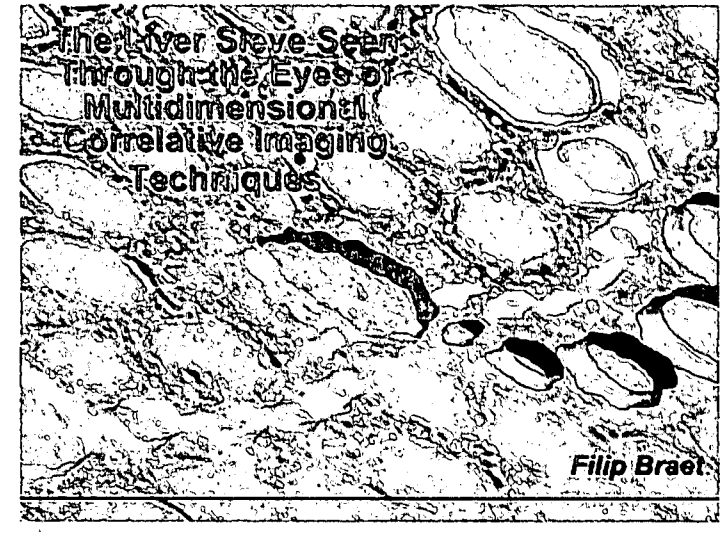
=> Point 1 and point 2 can be a communication in LIVER INTERNATIONAL in 2008





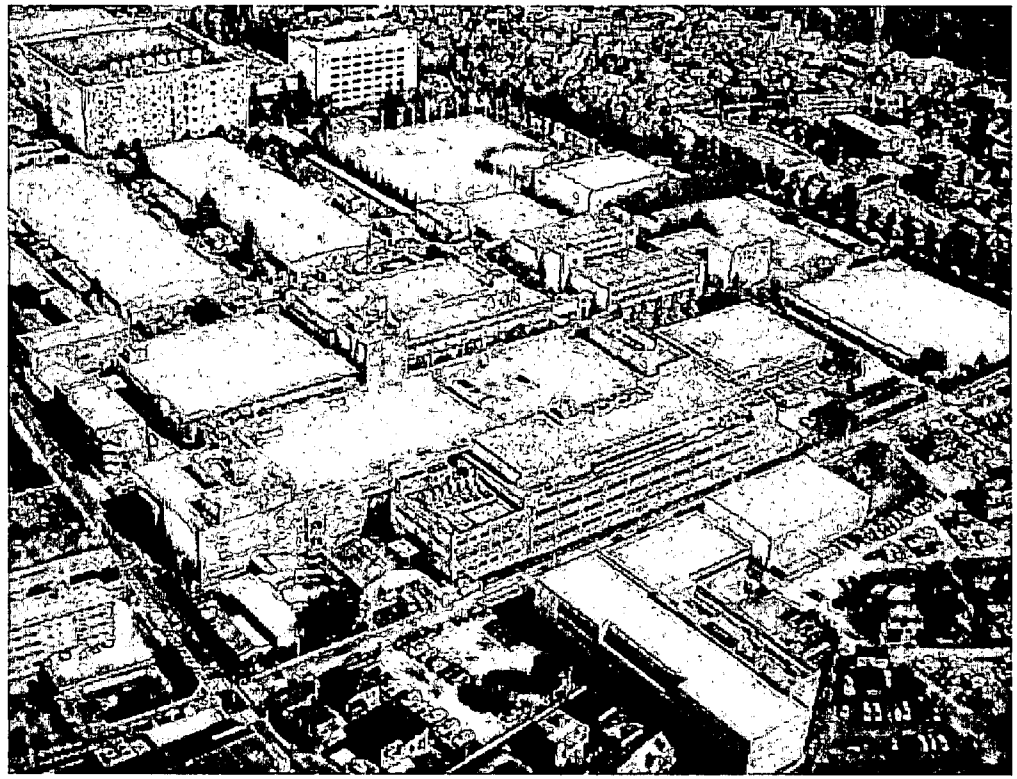
The day (20 November) was concluded with a seminar at Sanno Hospital:

Braet F. 1st Seminar IUHW International Seminar at Sanno Hospital: The liver sieve seen through the eyes of multidimensional correlative imaging techniques. International University of Health and Welfare. Minato-ku Tokyo, Japan, 20 November 2007



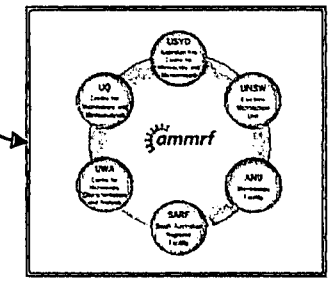
Wednesday 21 November 2007

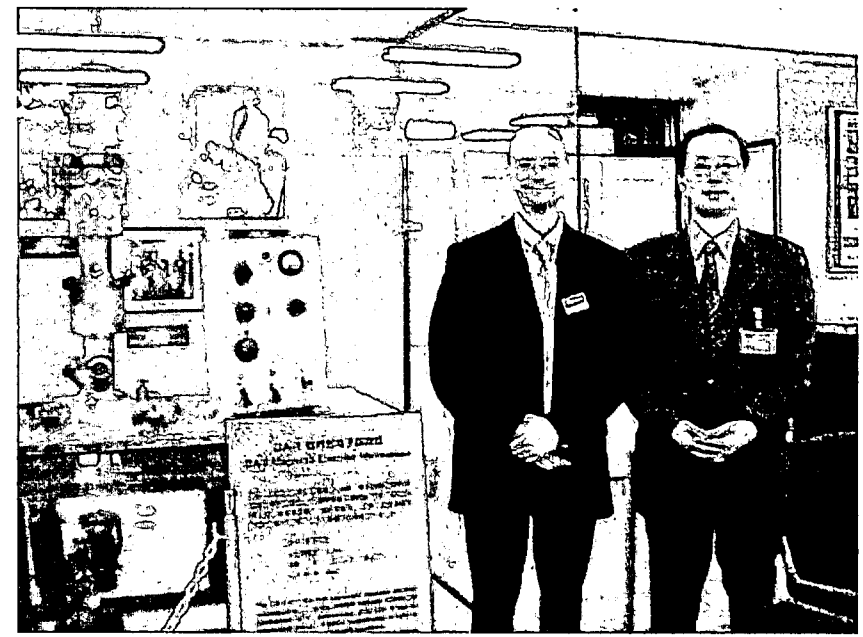
Visit Mr Yoshida Jeol- Discussing TEM procurement, AMMRF & NPO



Met with cryo 2100 specialists, TEM holders specialists, Tomography specialists, TMP technology

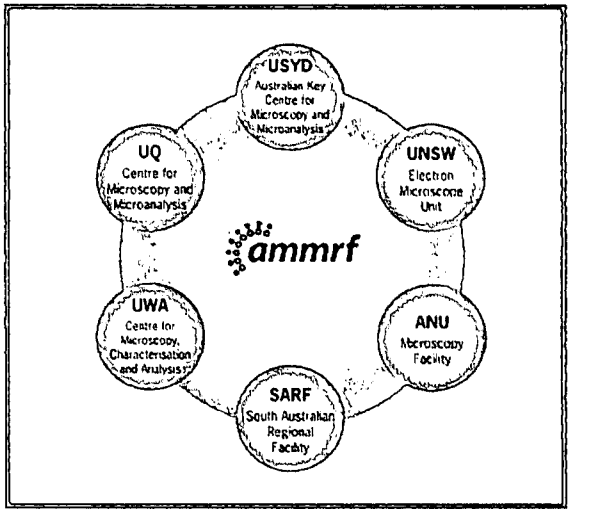
Presentation: AMMRF: A joint venture between Australian university-based microscopy and microanalysis centres

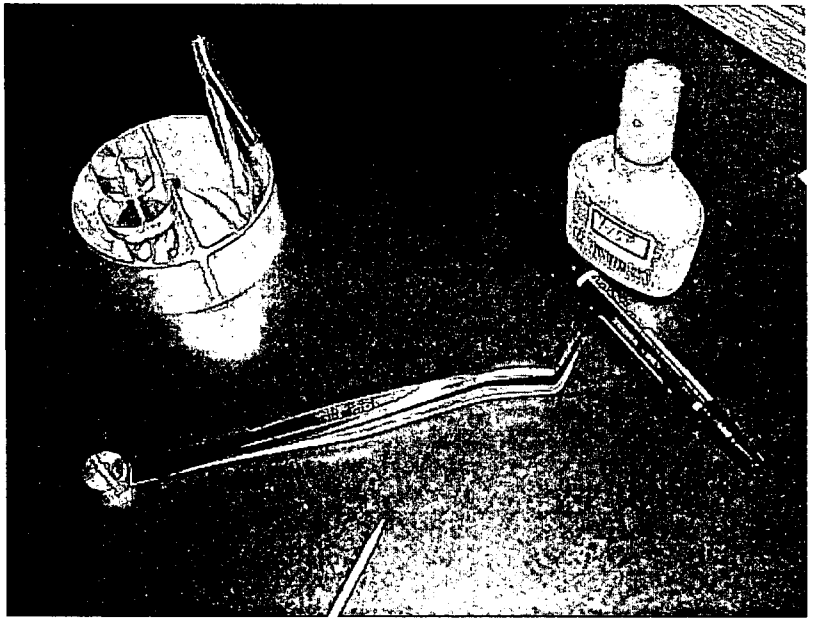




Thursday 22 November 2007
Visit Hitachi (Katsuta)- Discussing SEM Microscope TM-1000
Research Project Dr.Matsuura, Hitachi in Australia, AMMRF & NPO,
And demonstration model in Sydney

Presentation: AMMRF: A joint venture between Australian university-based microscopy and microanalysis centres





Duscussion ample prep for low vacuume observation