



### バブル音響特性の測定

① 超音波診断装置による観察 左:レホビスト 右:シクロアミロスバブル

② バブル非線形特性の測定

送信パルス=3.33MHz 3波

ハーモニクの特長が顕著

### 高感度画像化技術:シミュレータの作成

組織伝達の非線形効果 + バブル非線形振動 ⇒ バブル振動信号=S 組織からの信号=N 検出感度(SNR)を解析的に求めた

シミュレーションによる検出方法の検討 (特許出願)

### パルス圧縮法による分解能とSNRの検討

2μm径のマイクロバブルでのシミュレーション

3MHz 4MHz

分解能 1.5mm

中心周波数 3.5MHzのチャープ信号

SNR 6dB UP

### 超音波造影剤ソナゾイドの血流モデルにおける超音波イメージ

Bモード ハーモニク(第2高調波)

- バブルと組織のハーモニクの識別感度の増強  
強い放射エコー(音圧)  
高調波の周波数の変化
- パルス圧縮法による検出感度の増強

### in vivo実験 分子捕捉能の確認

抗CD147抗体MAb12C3は、in vivoでも高い分子捕捉能を示した

未投与 2h 24h 48h 72h 96h 120h

IR Dy800CW 吸収/蛍光特性

直径 9mm 深さ 3mm

超音波による腫瘍の観察

### 今後の取り組み

#### 1. 標識マイクロ・ナバブルの開発

マイクロ・ナバブルに抗CD147抗体MAB12C3を標識した分子プローブを作製する。

DSPE-PEG + His + Peptide → DSPE-PEG + Peptide

抗体活性リン脂質 (DSPE-PEG-MAL)

抗体活性リン脂質への抗体修飾

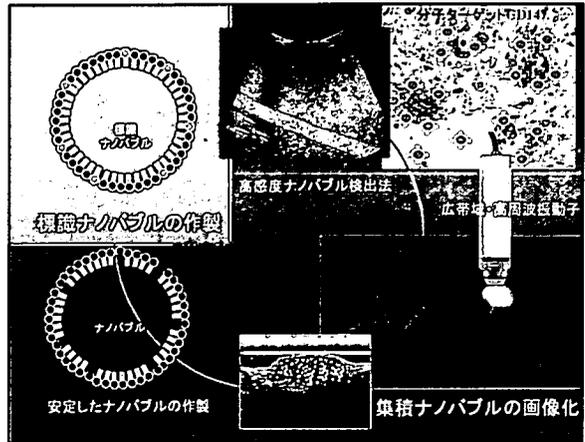
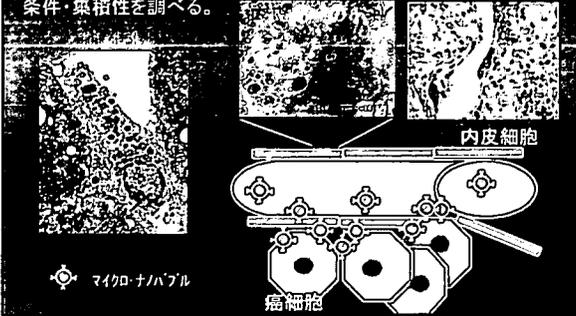
抗体を修飾したナバブルの模式図

- 新規界面活性剤 (シクロアミロスルホン界面活性剤)
- or 重合性ジ-5-ヒドロキシ界面活性剤
- 抗体活性PEGリン脂質

## 今後の取り組み

### 2. 標識マイクロ・ナノバブルの生体内動態の検討

生体内で、標識マイクロ・ナノバブルが腫瘍細胞に到達するための条件・集積性を調べる。



平成19年度 厚生労働科学研究費補助金・医療機器開発推進研究事業：ナノメディン研究  
 ナノメディン研究成果発表会  
 分子イメージング 40  
 平成20年2月27日(水) 16:50-17:00  
 (財)がん研究振興財団 国際研究交流会館

ラベル化造影剤を用いた超音波によるがんの超早期診断システムの研究開発

主任研究者: 東京慈恵会医科大学 大川 清

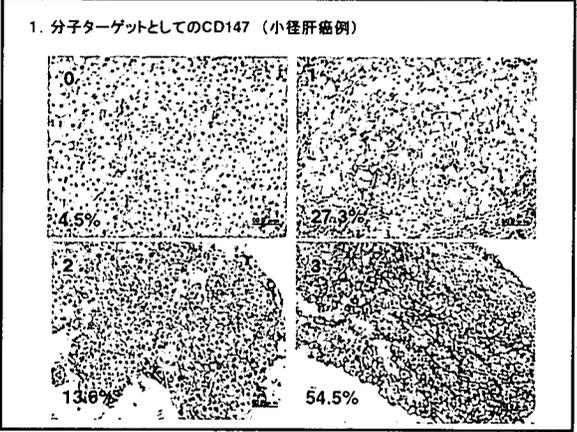
東京慈恵会医科大学  
 東京理科大学  
 ALOKA  
 Science & Humanity

(独)新エネルギー・産業技術総合開発機構・  
 分子イメージング機器研究開発プロジェクト  
 悪性腫瘍等治療支援分子イメージング機器に関する  
 先端研究  
 研究開発責任者: アロカ株式会社 伊藤 貴司

目的: 超音波分子イメージング技術の確立と超早期がん診断への応用

癌の浸潤マーカーCD147を分子ターゲットとし、造影超音波技術による微小診断システムを創出する。

- 分子ターゲットとしてのCD147  
 癌細胞表面に発現し、MMPを誘導することから癌の悪性度と相関
- 癌集積性超音波造影剤の開発  
 ①抗CD147抗体標識マイクロバブルの作製  
 ②集積性の検証
- 集積バブルを検出するための高感度画像化技術の開発  
 ①ナノバブル検出技術の開発  
 ②ナノバブル検出超音波診断装置の開発



2. 癌集積性超音波造影剤の開発  
 ①抗CD147抗体標識マイクロバブルの作製

Chemical structure of DSPE-PEG-MAL (NOF Co., Molecular weight of PEG (n) = 3400) and Maleimido group.

Reaction: DSPE-PEG-MAL + HS-(P<sub>1</sub>)<sub>2</sub> → DSPE-PEG-MAL-(P<sub>1</sub>)<sub>2</sub>-S-Keima

Antibody (Fluorescent protein) Antibody-active PEG-phospholipid

※ The maleimido group is connected with the sulfhydryl group of antibodies

It is difficult to confirm the formation of anti-CD147-labeled microbubbles directly  
 ↓  
 Fluorescent protein (Keima) was used as the model of anti-CD147

Phospholipid

Hydrophobic Hydrophilic

CH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>COOCH<sub>2</sub>  
 CH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>COOCH<sub>2</sub>  
 CH<sub>2</sub>-O-P(=O)(O<sup>-</sup>)-O-CH<sub>2</sub>-CH<sub>2</sub>-NH<sub>2</sub>  
 Dipalmitoylphosphatidylserine (DPPS) → One of the components of SONAZOID

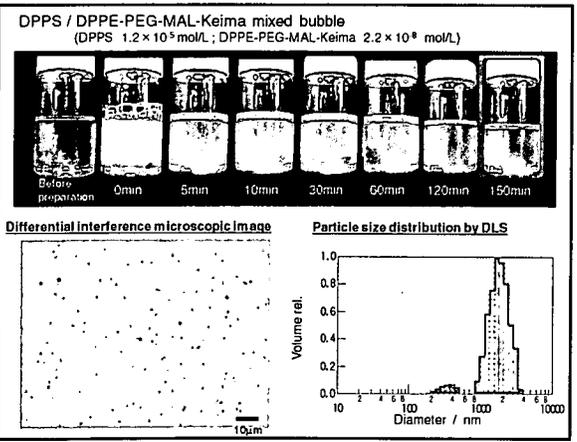
PEG-phospholipid labeled with fluorescent protein (Keima)

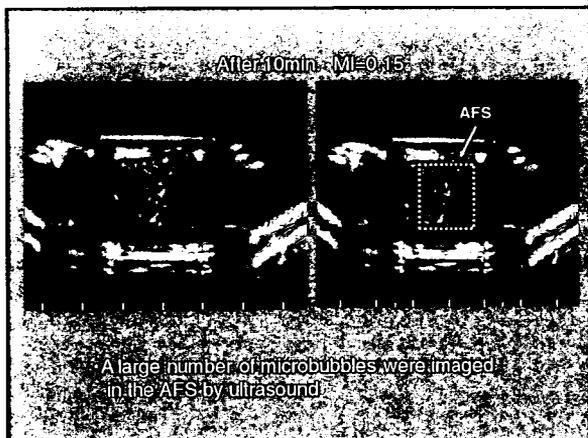
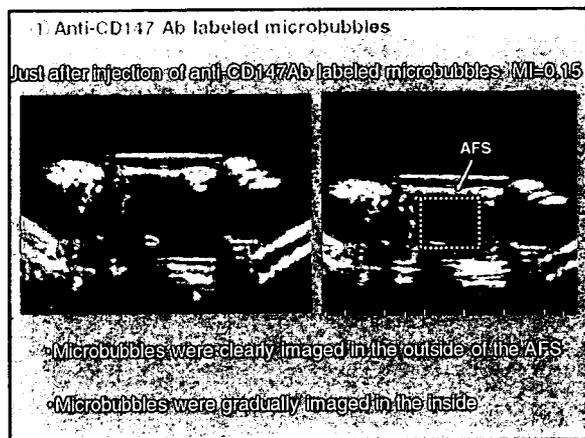
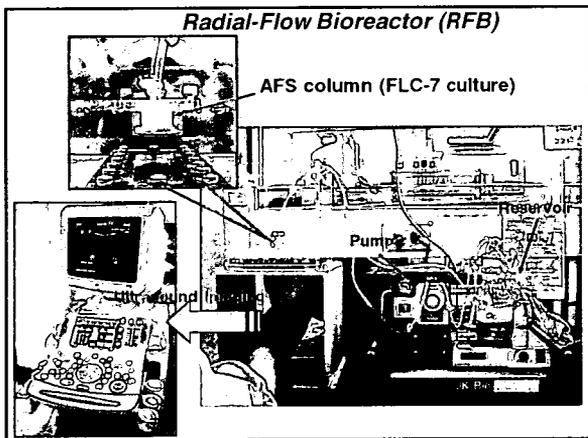
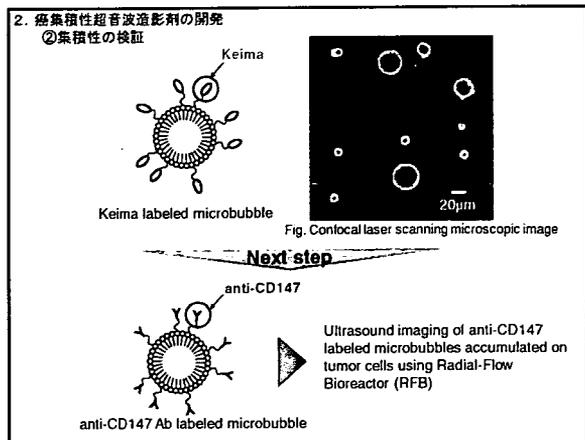
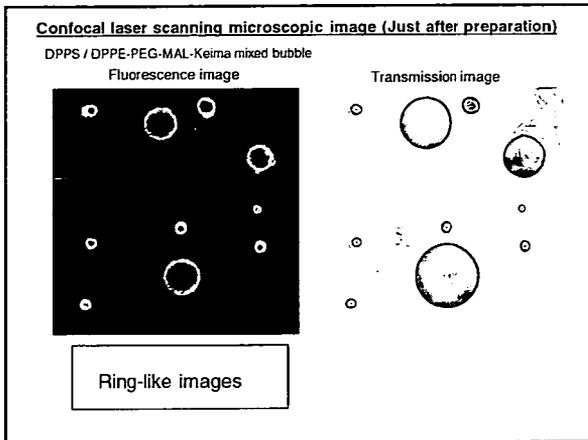
Hydrophobic Hydrophilic

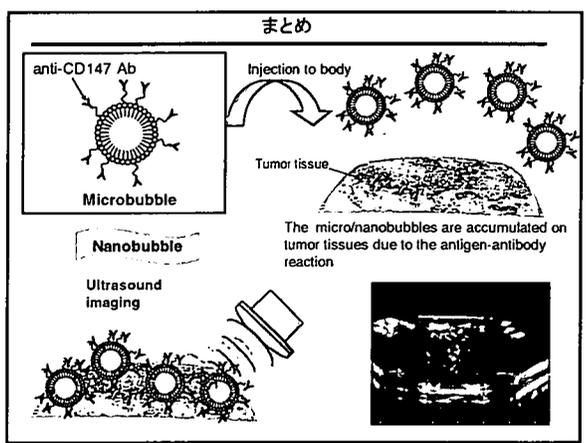
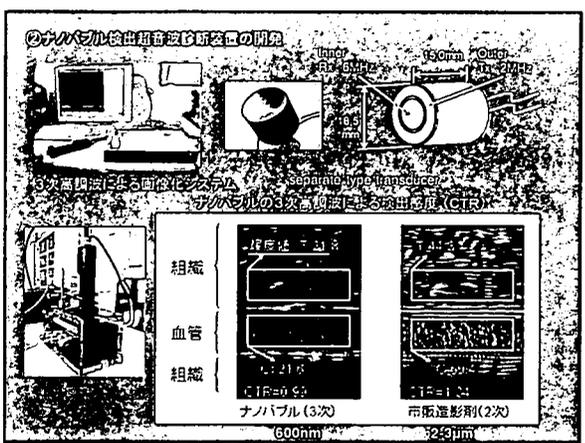
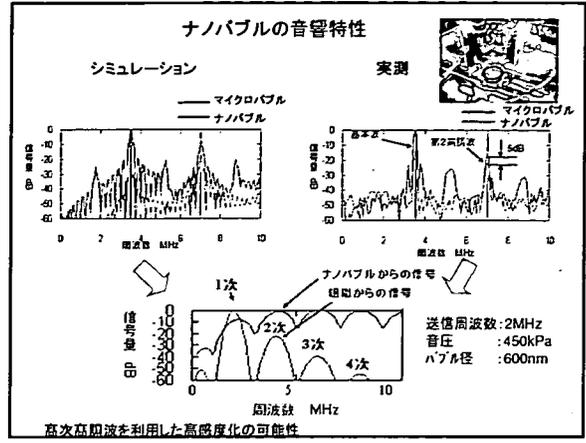
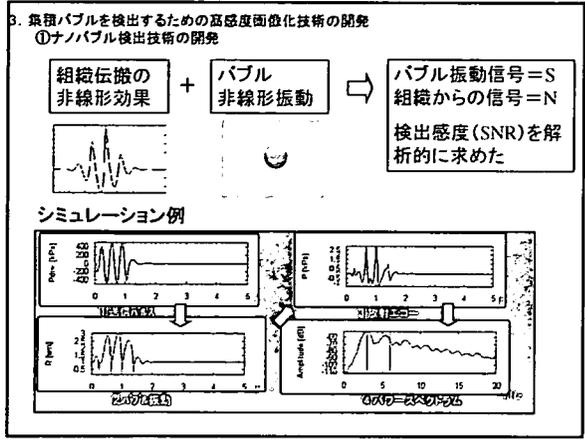
CH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>COOCH<sub>2</sub>  
 CH<sub>2</sub>(CH<sub>2</sub>)<sub>n</sub>COOCH<sub>2</sub>  
 CH<sub>2</sub>-O-P(=O)(O<sup>-</sup>)-O-(CH<sub>2</sub>)<sub>2</sub>-NHCO-(CH<sub>2</sub>CH<sub>2</sub>O)<sub>n</sub>-(CH<sub>2</sub>)<sub>2</sub>NHC(CH<sub>2</sub>)<sub>2</sub>-N  
 DSPE-PEG-MAL-Keima

Fluorescent protein Keima  
 Ex: 440 nm  
 Em: 620 nm

Preparation of mixed bubbles of DPPS and Keima-labeled PEG-DSPE





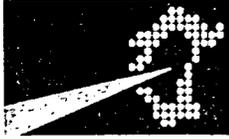


- 超音波分子イメージングの実用化に向けて—
- 今後の取り組み
1. プローブ標識集積性超音波造影剤の実用化
  2. ナノバブル作製法の確立と簡便化
  3. 3次高調波検出超音波診断装置の開発
  4. 治療への応用

---

NANO @ The University of Sydney

---



**ELECTRON MICROSCOPE UNIT\***

N A N O

People, Research Services, Programs & Training

# ***Activity Report 30 Oct – 10 Nov 2006***

A/Prof Filip Braet

Deputy Director

[filip.braet@emu.usyd.edu.au](mailto:filip.braet@emu.usyd.edu.au)

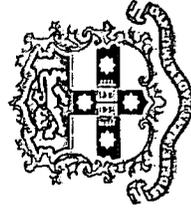
[www.emu.usyd.edu.au](http://www.emu.usyd.edu.au)

\* Incorporating the Australian Key Centre for Microscopy & Microanalysis

NANOSTRUCTURAL ANALYSIS NETWORK ORGANISATION

MAJOR NATIONAL RESEARCH FACILITY

NANO-MNRF





Monday 30 October 2006

# Micro-Nano Bubble Project I NANO

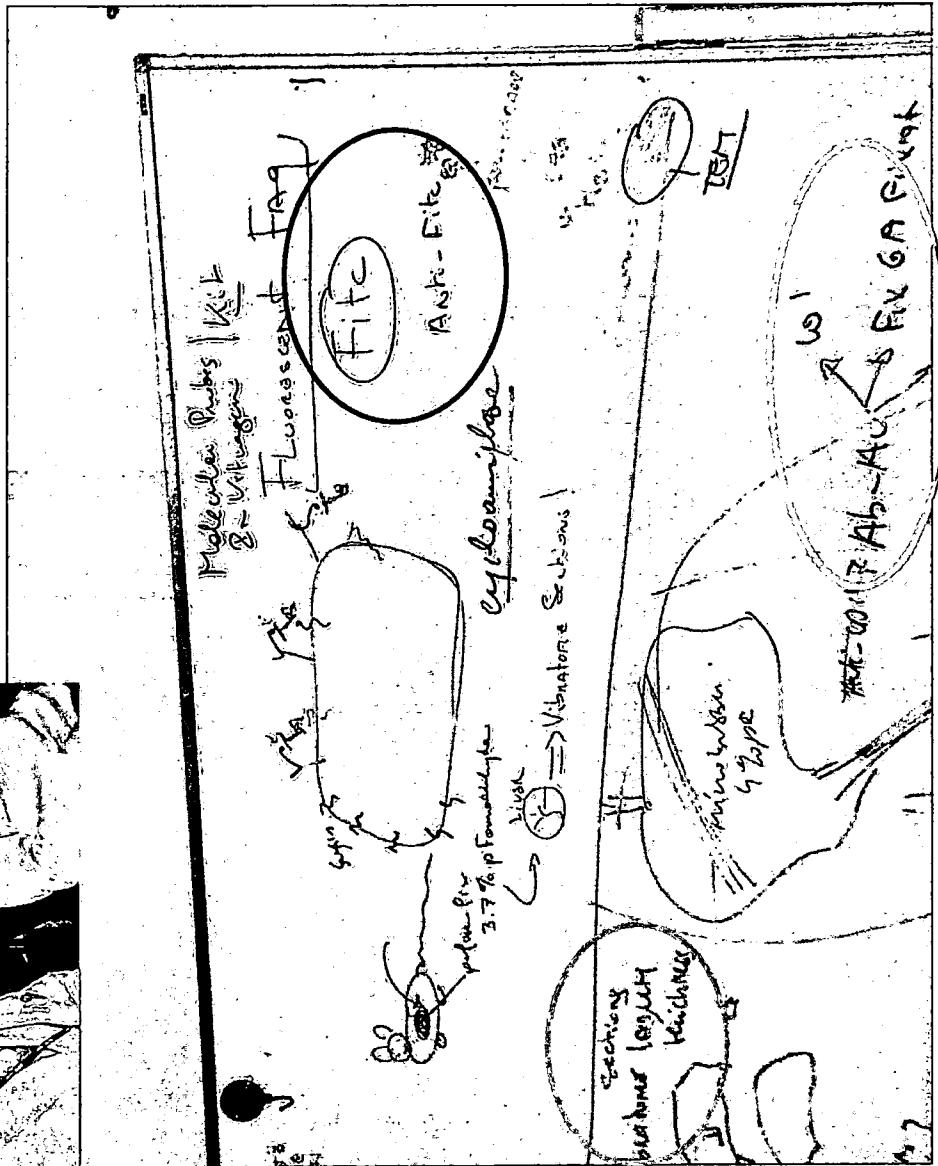


\* Information of endothelium and its pore formation ?!

\* Information of tumor vessels ?!  
RECA-1 IgG (clone HIS52 Serotec)

\* Atomic force microscopy ?! Not possible!

\* Can micro-nano bubble pass through endothelium of tumor vessels ?!









N ^ N O



- \* The **advice** given as on 31 October (increase the number of samples; RECA1 – SE1; hypothesis model)
- \* I am able to **scientifically edit** your manuscript
- \* I am able to edit the text for **English**
- \* I will check the EM immunogold labeling ( ... the part molecular part ... **immuno molecular labelling**)
- \* I will explore whether the work meets the standards for **Journal of Hepatology** & introduce it to ...





N A N O



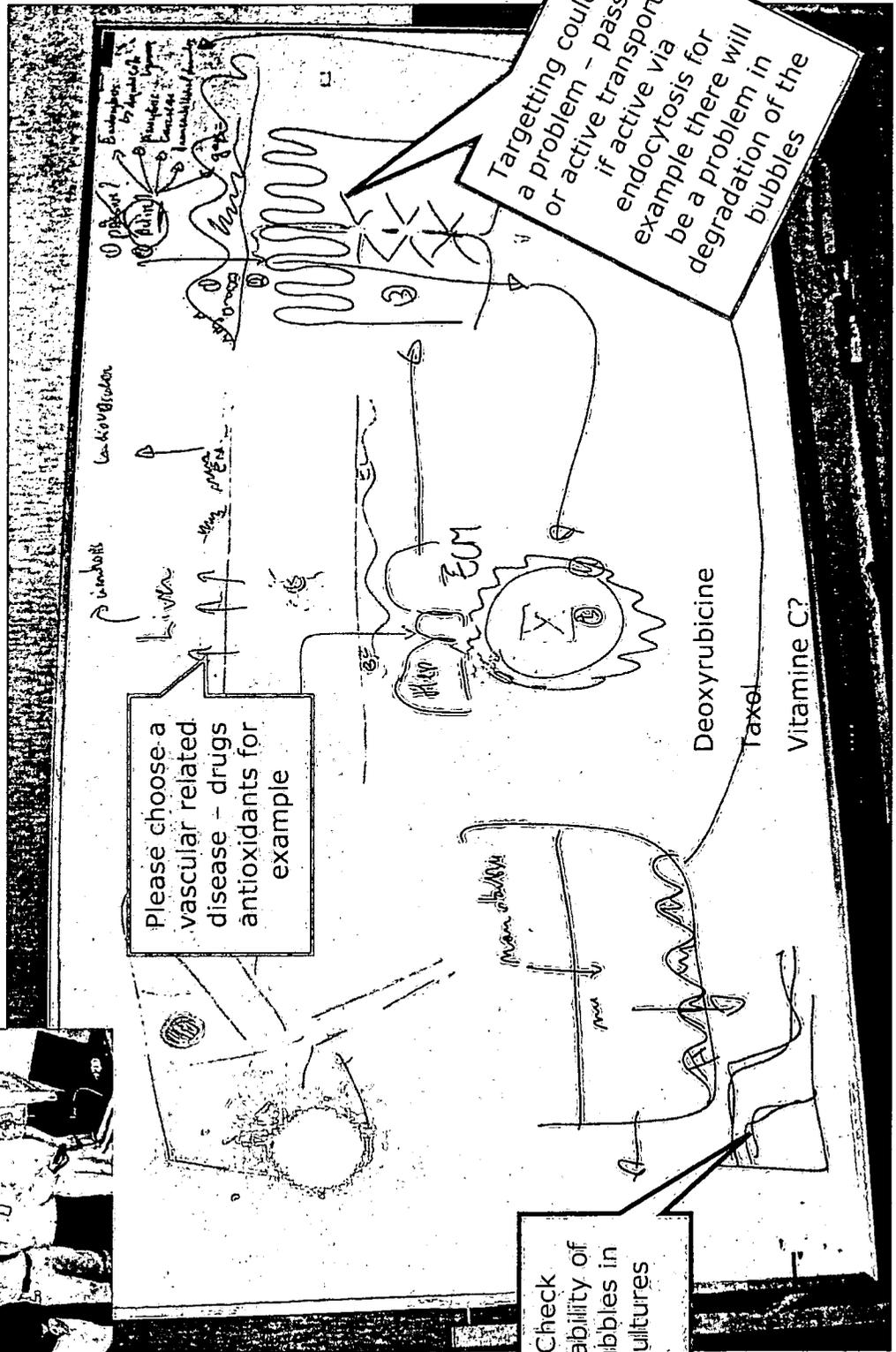
\* The **advice** given as on 31 October (perfuse rat or mice livers with the drugs or treat animals with the drugs ... perfuse-fix the livers and prepare for SEM [I am able to have a look to them])

\* I am able to **scientifically edit** your manuscript

\* I am able to edit the text for **English**

Thursday 02 November 2006

Micro-Nano Bubble Project II NANO



Please choose a vascular related disease - drugs antioxidants for example

Targetting could be a problem - passive or active transport - if active transport - endocytosis for example there will be a problem in degradation of the bubbles

Check Stability of bubbles in cultures

Deoxyribicine  
Taxel  
Vitamine C?



NANO



Oral Presentations by Filip Braet:

Braet F. Seminar: ***Probing surface and submembranous structures in living (liver) cells with the atomic force microscope.*** Niigata University, School of Physics, Niigata, Japan, 6 November 2006.

Braet F. Seminar: ***How correlative imaging techniques contributed to the study of the liver sieve.*** Jikei University Hospital, School of Medicine. Tokyo, Japan, 7 November 2006.

Braet F. Seminar: ***How correlative imaging techniques contributed to the study of colorectal liver metastasis.*** Jikei University Hospital, School of Medicine. Tokyo, Japan, 7 November 2006.

Braet F. Seminar: ***Australia's national microscopy & microanalysis research facility: An overview of biomolecular microscopy techniques.*** Tokyo University of Science. Tokyo, Japan, 9 November 2006.

---

During Visit We Wrote The  
Following Paper:



## **THE HEPATIC SINUSOIDAL ENDOTHELIAL LINING AND COLORECTAL LIVER METASTASES**

Filip Braet<sup>1</sup>, Keissuke Nagatsuma<sup>2</sup>, Masaya Saito<sup>3</sup>, Lilian Soon<sup>1</sup>,  
Eddie Wisse<sup>1</sup> and Tomokazu Matsuura<sup>4</sup>

*<sup>1</sup>Australian Key Centre for Microscopy and Microanalysis (AKCMM),  
Electron Microscopy Unit, The University of Sydney, NSW 2006,  
Australia; <sup>2</sup>Department of Pathology, The Jikei University School of  
Medicine, Tokyo, Japan; <sup>3</sup>Division of Gastroenterology and  
Hepatology, Department of Internal Medicine, The Jikei University  
School of Medicine, Tokyo, Japan; <sup>4</sup>Department of Laboratory  
Medicine, The Jikei University School of Medicine, Tokyo, Japan*

***World Journal of Gastroenterology 2007***

**THE HEPATIC SINUSOIDAL ENDOTHELIAL LINING  
AND COLORECTAL LIVER METASTASES**

Filip Braet<sup>1</sup>, Keisuke Nagatsuma<sup>2</sup>, Masaya Saito<sup>3</sup>, Lilian Soon<sup>1</sup>, Eddie Wisse<sup>1</sup>,  
and Tomokazu Matsuura<sup>4</sup>

<sup>1</sup>Australian Key Centre for Microscopy and Microanalysis (AKCMM), Electron Microscopy Unit,  
The University of Sydney, NSW 2006, Australia; <sup>2</sup>Department of Pathology, The Jikei University  
School of Medicine, Tokyo, Japan; <sup>3</sup>Division of Gastroenterology and Hepatology, Department of  
Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; <sup>4</sup>Department of  
Laboratory Medicine, The Jikei University School of Medicine, Tokyo, Japan

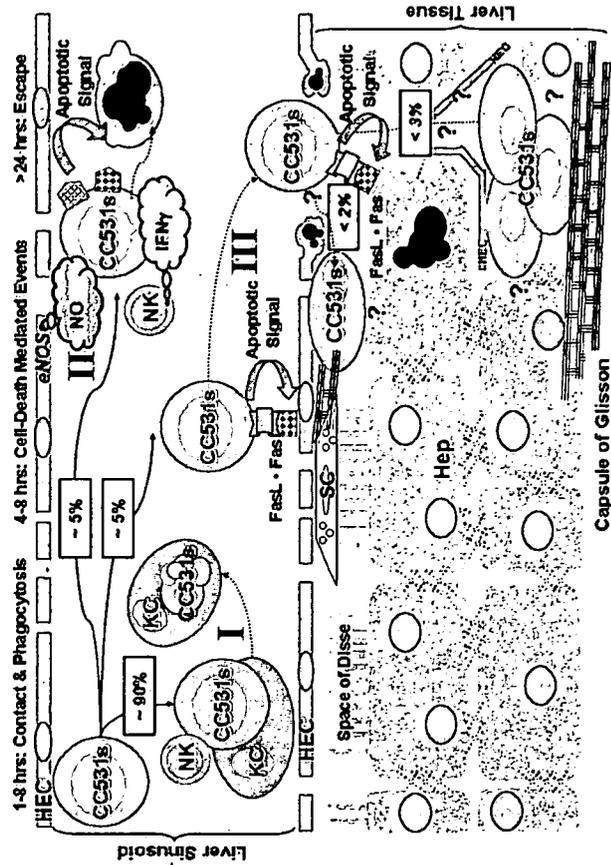
**Key words:** Apoptosis, Australia, Endothelial Cells, Hepatic Metastasis, Colorectal Cancer,  
CC531, Gaps, Interferon Gamma, Kupffer Cells, Natural Killer Cells, Nitric Oxide, Macrophages,  
Phagocytosis, Plugging, Pit Cells, Stellate Cells, X-Ray Micro-Computed Tomography.

**Correspondence to:** a/Prof Filip Braet, Australian Key Centre for Microscopy and Microanalysis,  
Electron Microscope Unit, Madsen Building F09, University of Sydney, Sydney, NSW 2006,  
Australia. Tel: + 61 2 9351 7619. Fax: + 61 2 9351 7682. E-mail: filip.braet@emu.usyd.edu.au



NANO

Will be submitted on 15  
November to World  
Journal of  
Gastroenterology



---

Grant Possibilities in Japan?



N A N O



1. Reconstructing and Modelling **Colorectal Liver Metastasis** Pathways (Filip has text proposal ready)

Or

2. **Transendothelial Transport Mechanisms** In Hepatic Endothelial Cells of the Immortomouse

Or

3. Tomo can forward me your full record (CV) to see the possibility to go for a mutual **grant under the NHMRC?**



NANO

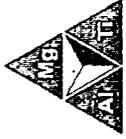
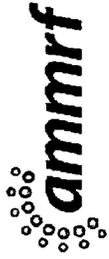
How can you help me?

- HCC tissue for X-ray Tomographic Investigation?
- My grant proposal to submit under Japanese Research Foundation?
- Possibilities to repeat visit next year around same time – in order to follow data, and prepare manuscripts?

**To do Filip for Tomo:**

*Old Immortomouse data Siegfried Hofmans*

**ELECTRON MICROSCOPE UNIT**  
People, Research Services, Programs & Training



---

Headquarters of the Australian Microscopy & Microanalysis Research Facility  
Australian Key Centre for Microscopy & Microanalysis

# ***JAAME Activity Report***

## ***11 NOV - 23 NOV 2007***



The University of Sydney

Filip Braet

