New Imaging Contrast Agents and Imaging Protocols in Contrast-Enhanced Background-Free Ultrasound and Photoacoustic Imaging

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Microbubble contrast agent imaging and considerations
- Gas core
  - C₃F₈ (Octafluoropropane)
  - C₄F₁₀ (Decafluorobutane)
  - C₅F₁₂ (Perfluoropentane)
  - C₆F₁₄ (Perfluorohexane)
- Shell (typically lipid)
- Mechanical index
  - What is the peak negative acoustic pressure resulting in cavitation for a microbubble at resonance?
  - MI = \( \frac{p}{\sqrt{f_0}} \)
  - FDA limit: 1.9 for non-contrast
  - 0.8 for Definity

Molecular ultrasound imaging and extravascular delivery
- Microbubbles
  - Commonly used clinically
  - Too large to reach extravascular targets
- Solid nanoparticles
  - Too small to respond to acoustic field

Ideal contrast agent for contrast-enhanced molecular ultrasound
- Small (sub-micrometer) to penetrate endothelium and escape vascular compartments
- Large acoustic cross-section to respond to ultrasound field

Adapted from Kaneko OF and Willmann JK. Quant Imaging Med Surg 2012
Ideal contrast agent for contrast-enhanced molecular ultrasound

- Small (sub-micrometer) to penetrate endothelium and escape vascular compartments
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**Molecular ultrasound imaging and extravascular delivery**

US or laser-triggered perfluorocarbon nanodroplets (PFCnD)

**Boiling point of encapsulated nanodroplets**

<table>
<thead>
<tr>
<th>Boiling points</th>
<th>Bulk gas</th>
<th>200 nm droplet</th>
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</thead>
<tbody>
<tr>
<td>C₃F₈</td>
<td>-36.7 °C</td>
<td>37 °C</td>
</tr>
<tr>
<td>C₄F₁₀</td>
<td>-1.7 °C</td>
<td>79 °C</td>
</tr>
<tr>
<td>C₅F₁₂</td>
<td>29 °C</td>
<td>119 °C</td>
</tr>
<tr>
<td>C₆F₁₄</td>
<td>57 °C</td>
<td>150 °C</td>
</tr>
</tbody>
</table>

Core gas in nanoscale droplets experiences several atmospheres of pressure → increased boiling point

**Imaging encapsulated nanodroplets**

Two approaches for imaging:
1. Single vaporization
2. Temporary vaporization followed by re-condensation
1. Imaging localized ultrasound-activation of phase change nanodroplets

Approaches:
- Pulse inversion imaging of resulting microbubbles
- Differential imaging before/after activation

Goal: Image spatial distribution of nanoscale agent
Requires activation beam and imaging before/after activation

Both can work well for broad activation of high concentration agent, little motion

Challenges:
- Sensitivity to low concentration in regions of localized activation (molecular imaging, delivery)
- Physiological motion

No motion, C₃F₈ core, 10⁶/ml, room temperature

Pre-activation B-mode image

Pulse inversion
Differential

No motion, C₄F₁₀ core, 10⁶/ml, 37° C
Localized ultrasound activation and imaging

Challenges:
• Sensitivity to low concentration in regions of localized activation (molecular imaging, delivery)
• Physiological motion

No motion, C₄F₁₀ core, 10⁶/ml, 37°C
Pre-activation B-mode image

No motion 1 mm/s

High rate physiological motion

C₄F₁₀ core, 10⁶/ml, 37°C
Pulse inversion, 20 mm/s

Differential, 20 mm/s

A new imaging approach

What if we image at high frame rate and activate between imaging pulses?

Ultrafast inter-frame activation imaging sequence

Acquisition rate: 2000 Hz
In vivo imaging

Proof of concept: Activate by steering focused beam to desired location in 6 x 6 mm grid

Imaging activation in the presence of physiological motion

Conventional techniques

Imaging in the heart

Rat liver

Activation beam sweeping through at 2000 frames/sec

Post-activation image

Ultrafast frame rate: 10000 frames/sec

Proof of concept: Activate by steering focused beam to desired location in 6 x 6 mm grid

Imaging in the presence of physiological motion

Conventional techniques

Pulse inversion

Differential

Inter-frame

Visualizing locations of activation

Imaging in the heart

Rat heart
2. Laser-activated phase change contrast agents

Perfluorohexane (PFH) NanoDroplets

Core: PFC+Dye

Shell: Lipid

Laser-activated PFH (boiling point: 57°C) nanoDroplets produce time-varying US (PA) contrast

Liquid nanodroplet

Gas microbubble

Anechoic

Hyperechoic

Laser pulse

Recondensing

Linear Ultrasound Intensity (a.u.)

0 dB - 25 dB

Ultrasound Imaging

1 mm

20 µm

Optical Microscopy

0

0.75

1.5

Linear Ultrasound

Intensity (a.u.)

Liquid nanodroplet

Gas microbubble

Anechoic

Hyperechoic

Laser pulse

Recondensing

PA signal
Laser-activated PFPnDs produce time-varying contrast in US imaging.

Tumor Metastases in Sentinel Lymph Node

- Mouse model of oral cancer
- Metastatic foci are formed in sentinel lymph node(s) within 2-3 weeks

Contrast Enhanced, Laser-Assisted Ultrasound Imaging

- Imaging protocol
  - Injection of perfluorocarbon (PFC) nanodroplets
  - 30 min wait
  - Ultrasound imaging of laser-activated PFCnDs
Clinical ultrasound-guided photoacoustic imaging

Dual contrast imaging agent (PFC nanodroplets) + MDA-MB-231 xenograft

1064 nm, 20 MHz

Sentinel Lymph Node (SLN) Imaging

Contrast Enhanced Ultrasound + Photoacoustic Imaging of Sentinel Lymph Node

MDA-MB-231 xenograft:
1064 nm, 20 MHz

US/PA

Molecularely targeted PFCnDs

Untargeted

Targeted

SK BR 3

3B5 (anti-HER2) PFCnDs
Summary

• Nanoscale PFC contrast agents enable extravascular imaging
• Ultrasound or laser activation can provide high contrast images with high frame rate ultrasound imaging techniques
• Potential for lymphatic imaging and non-invasive image-guided therapy

Thank you

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