ENGINEERING M13 BACTERIOPHAGE NIR-II PLATFORMS FOR TUMOR IMAGING APPLICATIONS

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GIRLS WHO BUILD
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Finding Tumors

Cancer cells

Normal cells
Our Tools

NIR-II imaging agent

Custom-built NIR-II imager

Imaging Applications

SWNT

M13 virus
Probe: M13 Bacteriophage

Figure. Structure of the M13 bacteriophage

~ 880 nm

~ 6.5 nm

Figure. AFM Image of M13 bacteriophage

Figure. Schematic of phage replication cycle

Courtesy of ViralZone. Used with permission.
Probe: Second Window Near-Infrared NanoTube

**Figure.** Schematic of SWNT fluorescence through brain tissue

Our wavelengths of interest

\[ \lambda_{\text{ex}} = 808 \text{ nm} \]
\[ \lambda_{\text{em}} = 1350 \text{ nm} \]

Courtesy of Nature Photonics. Used with permission.
Figure. M13 bacteriophage designs. i). Tumor targeting at p3 end, fluorescent imaging agent at p9, and doxorubicin/drug release sites at p8. ii). Tumor targeting at p3 end and single walled carbon nanotube (SWNT—a deep tissue, near infrared imaging agent) binding at p8.

• Biocompatibility
• Extended blood circulation time, accumulation in diseased tissue
• Successful targeting to sites of biomedical interest
Real Time Intraoperative NIR-II Imaging System

Ovarian Cancer Model
~225,000 women diagnosed annually
~140,000 death toll

- Early diagnosis is key, before metastatic stage
- Thorough therapy, small tumor detection prolong survival
Gen-1 Application: Image-Guided Surgical Planning

SWNT NIR-II image guidance (pre-surgical planning): helps in better excision of sub-mm tumors

Randomize tumor cohort

Image-guided surgery

Measure tumor nodules

Unguided surgery

Gen-2 Application: Real Time Intraoperative NIR-II Imaging System

Massachusetts General Hospital surgeon, Dr. Na, demonstrates the NIR-II imaging system, where camera detects probes attached to tumors, and displays their "glow" on a monitor as he performs surgery.

Figure. Serial cytoreduction of ovarian tumor at sub-millimeter resolution (scale—1cm)

i) Pre-injection
ii) Re-surgery
iii) Post unguided surgery
iv) Post SWNT-M13 guided surgery

Courtesy of National Academy of Sciences, U. S. A. Used with permission.
Project 1. Small \{Inho\} Phage

Construction of smaller phage to improve on the blood trafficking of our M13 probe systems while retaining its multi-functionality which allows us to simultaneously target, detect, and deliver various agents to cancer masses.

Project 2. Glioma Phage

Cloning for peptide display on the tail p3 capsid protein of M13 to allow for passage across the blood-brain barrier and targeting to glioma cells.
M13 Small {Inho} Phage

Figure. Atomic force microscopy inho1960, 475, 285 images

A) Inho1960

B) Inho475

C) Inho285

D) BASE SIZE ~100nm

BASE SIZE ~50nm

BASE SIZE ~280nm

880nm

280nm
M13 Small {Inho} Phage

Figure. Atomic force microscopy inho1960, 475, 285 images

A) Inho1960  
BASE SIZE ~100nm

B) Inho475  
BASE SIZE ~50nm

C) Inho285

D) Inho285
BASE SIZE ~280nm

Inho475

Inho285
Glioma Targeted Phage Project

Functionalization of M13 phage to cross the blood-brain barrier

- Expand phage probes for usage with our NIR-II deep imager (10cm depths)
- Utilize the internalization of phage and localization near the nucleus to consider gene therapies (siRNA)

**Figure. M13 phage shuttles localizes to the brain tumor mass.**
Phage shuttles (carrying red dye) have selectively gathered at the brain tumor site (expressing green dye).

  - GFP – Tumor
  - Cy3 – Phage

**Figure. Phage Internalization.** Internal cellular localization of glioma targeting phage (red) in relation to Golgin-97 (green) and the nucleus (blue) in human glioma cell line U87MG.

  - 6hrs post incubation
  - 24hrs post incubation
In Summary

Two new platforms for early detection and treatment of hard-to-reach tumors

1. **Inho-phage** retains the multi-functional structure of M13 bacteriophage and will allow us to explore the benefits of new smaller geometries in trafficking to and extravasation into tumors as well as various other materials applications.

2. **Glioma targeted phage** can induce passage across the blood-brain barrier, target brain tumor cells, and internalize to the golgi region of cancerous cells.

Demonstrated the medical impact of tumor de-bulking surgery guided by NIR-II imaging and targeted M13-CNT probes
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Commonly Used Imaging Modalities

<table>
<thead>
<tr>
<th>Technique</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>MRI</td>
<td>Good Resolution; Good Penetration Depth</td>
<td>Costly; Long Imaging Time</td>
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<tr>
<td>CT</td>
<td>Penetration Depth</td>
<td>X-ray radiation; Only Used for Bone and Lungs</td>
</tr>
<tr>
<td>PET</td>
<td>Good Penetration Depth</td>
<td>Radioactive Material; Long Imaging Time; Expensive</td>
</tr>
<tr>
<td>Ultrasound</td>
<td>Good Resolution; Cost Effective</td>
<td>Poor Penetration (cm)</td>
</tr>
<tr>
<td>Near-Infrared (650-950 nm)</td>
<td>Low Tissue Absorption; Safe Light Source; multiplex imaging</td>
<td>Autofluorescence; scattering</td>
</tr>
<tr>
<td>Second Window Near-Infrared (950-1650 nm)</td>
<td>Long Wavelength; Penetration Depth (10 cm); Non-Radioactive; Inexpensive; Quick Imaging Time (seconds-min)</td>
<td>Requires Sensitive Equipment for Detection</td>
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Adapted from Weissleder and Pittet, Nature 452 (2008)