3D technologies to accelerate HTAN atlas building efforts

On behalf of the NCI HTAN CMIT: Shannon Hughes, Philipp Oberdoerffer
Recommendation I: Generation of Human Tumor Atlases

Create dynamic 3D maps of human tumor evolution to document the genetic lesions and cellular interactions of each tumor as it evolves from precancerous lesion to advanced cancer.

Overall Goal of this concept:

Rapid implementation of promising new technologies for time-efficient, three-dimensional (3D) molecular characterization of intact human tumor tissue for dynamic 3D tumor atlas construction.
The NCI Human Tumor Atlas Network

- Construct dynamic 3D atlases of human cancers
- **Integrate** molecular, cellular, and tumor tissue composition and architecture, including the microenvironment and immune milieu
- Focus on **high-risk** cancers; including those responsive / non-responsive to immunotherapy; pediatric cancers
- Represent a **diverse patient population**, including minority and underserved patients
- Describe **transitions during cancer**: pre-malignant lesions to malignancy, locally invasive to metastatic cancer, & the development of therapeutic resistance
- Enable **predictive modeling** to refine therapeutic choices for patients.

https://humantumoratlas.org/
Cyclic IF reveals staged immunoediting in early cancer (melanoma \textit{in situ})

**Ongoing HTAN efforts illustrate the importance of spatial tumor context**

Multiplex imaging allows mapping of up to 100 proteins / 1000 transcripts to assess tumor heterogeneity

Progression of distinct immune editing states across a ~ 2 mm tumor section.

\textit{Left:} immune cells have cleared or are active against the tumor

\textit{Right:} melanoma with horizontal growth,

\rightarrow Drivers and mechanisms of progression?

\textit{Kindly provided by Peter Sorger, Harvard HTAN Center}
Ongoing HTAN efforts illustrate the importance of spatial tumor context

Diffuse Immune Infiltrate (DII) Colorectal Cancer

Clinical Annotations

Multiplex imaging

Prognostic Value

Tumor function is driven by complex cell-subset interactions

→ Current HTAN efforts are largely focused on 2D analyses of ~ 5 µm tissue slices

Limitations of 2D spatio-molecular mapping

- Marked heterogeneity within single biopsies can result in image selection bias and failure to detect rare cell types or key physiological landmarks.

- Limited preservation of spatial relationships, particularly irregular structures (vasculature, microenvironment).

- 3D views require sequential tissue sectioning, which is time-consuming and destructive to tumor tissue.

→ Impediment to the Blue Ribbon Panel recommendation to create dynamic 3D tumor maps.
Emerging examples for 3D characterization of intact tissue

<table>
<thead>
<tr>
<th>3D Approach</th>
<th>Assay</th>
<th>Validated in…</th>
<th>Tissue Depth</th>
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</thead>
<tbody>
<tr>
<td>Light Sheet Microscopy</td>
<td>IF</td>
<td>Human tumor tissue</td>
<td>~ 3 mm</td>
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<tr>
<td>Transparent tissue tomography (T3)</td>
<td>IF</td>
<td>Core needle biopsy</td>
<td>0.8 mm</td>
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<td>StarMAP</td>
<td>RNA FISH</td>
<td>Mouse brain</td>
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<td>DNA microscopy</td>
<td>Custom RNA-Seq</td>
<td>Tissue culture</td>
<td>N/A</td>
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<tr>
<td>Paired-cell sequencing</td>
<td>scRNA-Seq</td>
<td>Mouse liver</td>
<td>N/A</td>
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</tbody>
</table>

→ Suitable 3D technologies were not available or mature enough at time of HTAN awards

Synergy

2D assays: Discovery of tumor-specific cell types and suitable markers

3D assays: Map cell types identified by 2D in the context of intact tumor microenvironment
Proposed funding mechanism – UH2

- UH2 Cooperative Agreement to integrate with existing HTAN U2C and U24 grants.
  - 3 - 4 UH2 Grants
  - $250,000 / year - informed by HTA pilot project
  - Duration: 2 years
  - Total costs for all years: ~ $3.3 M total cost for 4 awards
- All PIs with relevant expertise are encouraged to apply. Non-HTAN grantees are expected to be part of HTAN and encouraged to use HTAN-PROCURED biospecimen.
- Preliminary data demonstrating the “shovel-readiness” of the technology in an HTAN-relevant tumor will be required.
- HTAN-focused program that leverages and complements other NIH and NCI imaging efforts.
Integration with existing HTAN Research Network

- Leverage **shared HTAN tumor sources** via trans-network efforts (currently colon, breast)
- Encourage identification of **collaborators** within HTAN-funded research centers
- Agree to data use and sharing policies
- Deposit data, protocols and SOPs with the HTAN Data Coordinating Center
- Participate in relevant HTAN Working Groups and biannual Face-to-Face meetings
Portfolio analysis of active 3D imaging awards across NIH

- Mostly low-res clinical imaging
- Core support
- BRAIN initiative

Limited support of non-destructive high-resolution imaging in the cancer space.

- Light Sheet Microscopy (LSM) ~250
- 16 LSM in tumors
- 159

- Transparent Tissue Tomography (1)
- DNA microscopy (1)
- STARMAP (1)
Thank you / Questions?