Optically transparent organs: seeing is believing

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Recent advancements have made rendering entire animal transparent possible!

3D Transparent Organs Will Help Doctors Make Better Diagnoses, Biomedical Discoveries

Jul 31, 2014 02:34 PM  By Lea Bushak

Researchers from Caltech discovered a speedier way of "clearing" tissues, which would allow doctors to see through organs and the entire body. Bin Yang and Viviana Gradinaru
Recent advancements have made rendering entire animal transparent possible!
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The Invisible Mouse
09/10/2014
Rachael Moeller Gorman

A new method quickly and gently turns a whole mouse transparent. Could this change biological imaging forever? Find out...
Recent advancements have made rendering entire animal transparent possible!
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Scientists create see-through mouse and rat bodies

Why a see-through mouse is a big deal for scientists
This could revolutionize how we measure pathology samples and understand structural/developmental biology.

While scientists have attempted to create see-through organ and tissue samples since the 1800s, researchers have relied primarily on the sectioning of samples - slicing organs into extremely thin cross sections and examining these pieces in succession.

"That's been useful but it's also been slow and tedious," said senior study author Viviana Gradinaru, an assistant professor of biology and biological engineering at Caltech.

Recent advancements in tissue clearing have enabled researchers to study nerve connections and organ structures without having to slice them up, providing scientists with new insights into previously hidden anatomical structures.
Moving from physically cutting up a brain
To making it see-through!

a  Before

The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.

b  After CLARITY

The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.
Learning Objectives

1. Explain why tissue scatters light and how one can alter the light scattering properties of tissue.
3. Summarize how combining these two methods allows one to measure structures within intact tissue and organs.
4. Theorize how these advancements may help with pressing public health issues.
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Natural curiosity about our (and animal) insides

“About the transparentizing of human and animal preparations”
Published in 1914 by Werner Spalteholz
Natural curiosity about our (and animal) insides

notiert hat, so muß man diese durch Rechnung bestimmen. Man geht dabei am besten von der durch Landolt aufgestellten Formel aus:

\[ n_2^2 \frac{1}{n_1} - n_1^2 \frac{1}{n_2}, \]

In der \( p \) das Gewicht, \( n \) den Brechungsindex, \( d \) die Dichte des Gemisches, \( p_1, p_2 \ldots \) die Gewichte, \( n_1, n_2 \ldots \) die Brechungsindices, \( d_1, d_2 \ldots \) die Dichten seiner Komponenten bezeichnen. Da wir nur das Verhältnis der beiden Bestandteile kennen wollen, verwenden wir die Formel in der Form:

\[ \frac{n_2^2 - n_1^2}{n_2^2 - n_1^2} = \frac{p_2}{p_1} \frac{d_2}{d_1} \]

Leider gibt auch diese Formel, die als die zuverlässigste gilt, nicht immer ganz genaue Resultate.

Für die von mir hauptsächlich benutzten Flüssigkeiten habe ich nach wiederholten eigenen Messungen folgende Zahlen zugrunde gelegt:

- Wintergrünöl, künstlich: \( n_0 = 1.538 \), spez. Gew. = 1.188
- Safrol: \( n_0 = 1.542 \), spez. Gew. = 1.102
- Benzylbenzoat: \( n_0 = 1.570 \), spez. Gew. = 1.121
- Isosafrol, farblos: \( n_0 = 1.577 \), spez. Gew. = 1.115

Die Neuheit des Problems und der Wunsch, über verschiedene scheinbar Unstimmigkeiten Klarheit zu erhalten,

Natural curiosity about our (and animal) insides

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\[ \frac{n^2 - 1}{d} = P_1 \left( \frac{n_1^2 - 1}{d_1} + P_2 \frac{n_2^2 - 1}{d_2} + \ldots \right) \]

In der \( p \) das Gewicht, \( n \) den Brechungsindex, \( d \) die Dichte des Gemisches, \( P_1, P_2, \ldots \) die Gewichte, \( n_1, n_2, \ldots \) die Brechungsindizes, \( d_1, d_2, \ldots \) die Dichten seiner Komponenten bezeichnen. Da wir nur das Verhältnis der beiden Bestandteile kennen, verwenden wir die Formel in der Form:

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<table>
<thead>
<tr>
<th>Zahlen zugrunde</th>
<th>Werte</th>
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</thead>
<tbody>
<tr>
<td>Wintergrünöl, künstlich</td>
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<td>( \gamma = 1.188 )</td>
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\[ \frac{\sum p_i n_i - 1}{\sum d_i} = \frac{\sum p_i n_i - 1}{\sum d_i} + \frac{\sum p_i n_i - 1}{\sum d_i} + \cdots \]

In der \( p \) das Gewicht, \( n \) den Brechungsindex, \( d \) die Dichte des Gemisches, \( p_1, p_2, \ldots \) die Gewichte, \( n_1, n_2, \ldots \) die Brechungsindizes, \( d_1, d_2, \ldots \) die Dichten seiner Komponenten bezeichnen. Da wir nur das Verhältnis der beiden Bestandteile kennen lernen wollen, verwenden wir die Formel in der Form:

\[ \frac{p_1}{d_1} \frac{n_1 - 1}{d_1} = \frac{p_2}{d_2} \frac{n_2 - 1}{d_2} \]

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Yang et al Cell 2015
The search for the magic bullet...or...an excuse to make up a lot of acronyms

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<tr>
<th>Technique</th>
<th>Clearing time for whole-brain</th>
<th>Complete transparency</th>
<th>Fluorescent quenching</th>
<th>Tissues validated</th>
<th>Significant contribution to field</th>
<th>Drawback</th>
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<tbody>
<tr>
<td>BABB, TNF, DBE (Beek et al., 2012; Dodt et al., 2007)</td>
<td>hours-days</td>
<td>Yes, but tissue shrinkage</td>
<td>Yes (Ertürk et al., 2012a; Ke et al., 2013)</td>
<td>Rodent brain, spinal cord, peripheral tissues</td>
<td>Among first clearing reagents</td>
<td>Harsh reagents (Ke et al., 2013)</td>
</tr>
<tr>
<td>ClearT2 (Kuwajima et al., 2013)</td>
<td>days</td>
<td>No</td>
<td>No-partial (Ke et al., 2013)</td>
<td>Rodent brain and embryo</td>
<td>Less quenching than BABB; novel reagents</td>
<td>Immunolabeling only through 120 μm</td>
</tr>
<tr>
<td>Scale (A2, U2) (Hama et al., 2011)</td>
<td>weeks-months (slowest)</td>
<td>Yes, but tissue swelling (Chung et al., 2013; Ke et al., 2013; Kuwajima et al., 2013)</td>
<td>No-mortem (Ke et al., 2013; Kuwajima et al., 2013)</td>
<td>Mouse brain, embryo (Hama et al., 2011)</td>
<td>Transparency without quenching, IHC/F</td>
<td>Slow, tissue deformation, potential protein loss with clearing (Ke et al., 2013)</td>
</tr>
<tr>
<td>3DISCO (Ertürk et al., 2012a; Ertürk and Bradke, 2013)</td>
<td>&lt; week</td>
<td>Yes</td>
<td>No, but signal decay within days (Ertürk et al., 2012a; Ertürk and Bradke, 2013)</td>
<td>Peripheral/cranial organs, embryos, tumors (Ertürk and Bradke, 2013; Central (Ertürk et al., 2012b) and peripheral (Jung et al., 2014) nerves</td>
<td>Balance between rapidity and quality of cleared tissue, imaging protocol</td>
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<tr>
<td>CLARITY (Chung and Deisseroth, 2013; Chung et al., 2013; Kim et al., 2013)</td>
<td>10 days</td>
<td>Yes</td>
<td>No</td>
<td>Rodent, human and non-human primate brains, spinal cord, zebrafish (Zhang et al., 2014)</td>
<td>Hydrogel-embedding; best tissue quality when performed correctly, IHC/F</td>
<td>ETC difficult, customized equipment, expensive (Chung et al., 2013)</td>
</tr>
<tr>
<td>Advanced CLARITY (Tomer et al., 2014; Zhang et al., 2014)</td>
<td>3 weeks</td>
<td>Yes</td>
<td>No</td>
<td>Whole mouse brain</td>
<td>No ETC – passive thermal CLARITY, CLEM, CLARITY objectives, rapid imaging protocol</td>
<td>Requires COLM set-up</td>
</tr>
<tr>
<td>SeeDB (Ke et al., 2013; Ke and Imai, 2014)</td>
<td>days (fastest)</td>
<td>No</td>
<td>No</td>
<td>Young rodent brains (Ke et al., 2013)</td>
<td>No tissue deformation, fast</td>
<td>Tissue browning, incomplete clearing</td>
</tr>
<tr>
<td>CUBIC (Susaki et al., 2014)</td>
<td>2 weeks</td>
<td>Mostly-Yes</td>
<td>No</td>
<td>Rodent and non-human primate brain</td>
<td>CUBIC informatics, optimized Scale (Susaki et al., 2014)</td>
<td>Brain only, potential protein loss during clearing Slow than 3DISCO</td>
</tr>
<tr>
<td>PACT, PARS</td>
<td>days-weeks</td>
<td>Yes</td>
<td>No</td>
<td>All major rodent organs, whole-body clearing</td>
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<td>Optimized/simplified CLARITY, permits long-term tissue storage, IHC/F</td>
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So many techniques!! What is different about all of them?
The search for the magic method...

Four general categories:
1. Solvent-based
The search for the magic method...

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1. Solvent-based
2. Simple immersion
The search for the magic method...

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1. Solvent-based
2. Simple immersion
3. Hyper hydration
The search for the magic method...

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The search for the magic method...

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**Figure 2. Methodology of Tissue Clearing Techniques**

(A) (Left) Solvent-based clearing is a two-step process. First, the tissue is dehydrated and lipid is removed. Second, the tissue is moved to a high refractive index solvent where additional lipid solvation and clearing occurs. (Right) Molecules commonly used for solvent-based clearing along with the refractive indices (RI) of the pure chemical.

(B) (Left) For simple immersion, the tissue to be cleared is placed in an aqueous clearing solution for days to months. During this time, the solution is exchanged repeatedly. (Right) Molecules commonly used for simple immersion along with the refractive indices (RI) at the commonly used concentration.

(C) Hyperhydration involves submerging the sample in an aqueous solution and allowing it to passively clear. During this clearing step, urea or formamide in the clearing solution can enter tightly folded regions of high refractive index proteins, creating an osmotic gradient that pulls in water as well. This partially denatures the protein, hydrates it, and decreases its overall refractive index. Some hyperhydration methods contain detergent that is used to disrupt membranes and remove lipid from the sample.

(D) (Left) Hydrogel embedding is most often performed on an entire animal by perfusing with a fixative, a temperature-sensitive crosslinker, and the hydrogel monomer. Alternatively, these chemicals can be passively diffused into an isolated tissue sample. Once fixed, the tissue of interest is warmed to induce hydrogel crosslinking. The sample is then placed in a detergent solution to remove lipid material passively or via an electrophoretic charge. Finally, the lipid-free sample is placed in a high refractive index matching solution for clearing. Histodenz is one high refractive index molecule that can be a component of this clearing solution. Glycerol, TDE, or diatrizoic acid also can play this role.
Hydrogel embedding

Step 1: hydrogel monomer infusion (days 1–3)

Chung et al Nature 2013
Hydrogel embedding

Chung et al Nature 2013
Hydrogel embedding

Chung et al. Nature 2013
Retinopathy of prematurity (ROP) is a disease that affects blood vessel development and distribution in infants born prematurely. Two-dimensional imaging techniques have limitations in accurately quantifying the developing eyes due to artifacts introduced during tissue sectioning, two-dimensional imaging, and computational reconstruction. A unique digital scanned light-sheet microscope (DSLM) has been developed to quantitatively analyze the developing eyes, allowing for a three-dimensional approach to study angiogenesis and ROP.

In this work, we utilize CLARITY to render the tissue optically transparent, enabling a more comprehensive view of angiogenesis. Harvested at multiple time points in development, we provide a comparative analysis evaluating both computational and experimental methods.

**Background**

Hypothesis

Three-dimensional quantification of the spatiotemporal co-evolution of angiogenesis and ROP due for spatial heterogeneity in hyaloid vasculature in a dissected and sample chamber. Lateral cross sections taken through the center of the rat eye provide the superficial layers, expression of angiogenic factors like vascular endothelial growth factor in advanced cases of retinopathy of prematurity. Early Administration of Erythropoietin in the extreme premature, a risk factor for retinopath of prematurity. Hypoxic conditions start with hypoxia-inducible factor 1 (HIF-1α) translocating to the nucleus and expression in a mouse model of retinal neovascularization. Hypoxic conditions are suitable for penetration intact organs. Multiple trials have shown that PECAM and Pax-6 provide the cleanest signal.

**Results**

Utilizing PECAM and PAX-6, we have generated both 2D and 3D maps of the retinal vasculature. Antibody penetration and labeling specificity has so far limited our ability to apply this technique. However, antibody penetration and labeling specificity has so far limited our ability to apply this technique.

**Summary/Conclusions**

We have designed and built a prototype C-DSLM due for spatial heterogeneity in hyaloid vasculature in a dissected and sample chamber. Lateral cross sections taken through the center of the rat eye provide the superficial layers, expression of angiogenic factors like vascular endothelial growth factor in advanced cases of retinopathy of prematurity. Early Administration of Erythropoietin in the extreme premature, a risk factor for retinopath of prematurity.

**References**


**Hydrogel embedding**

Hydrogel embedding
Retinopathy of prematurity (ROP) is a disease that affects blood vessel development and distribution. The spatial distribution of fluorescent labels in three-dimensional (3D) space during the pathogenesis of ROP can be quantified using confocal microscopy. Often, serial sectioning is used, followed by 2D image analysis, but this method is fraught with inconsistencies due to tissue tearing and folding, which may introduce optical distortion.

In this work, a technique called CLARITY is utilized; this technique renders tissue optically transparent, allowing for detailed examination of the vascular and neuronal network structures in intact control and ROP model rat eyes. This approach provides more accurate quantification of developing eyes compared to traditional methods.

Because confocal microscopy measurements have only been able to quantify superficial layers of the eyes, expression of angiogenic factors like vascular endothelial growth factor (VEGF) within the eyes may show previously unreported changes in vasculature. The aim is to provide detailed information on the retinal vasculature and to understand the spatial and temporal co-evolution of vascular and neuronal networks during the development of ROP.

Methods

Hydrogel embedding

Hydrogel embedding offers simple and rapid imaging of entire intact eyes using C-DSLM (cleared tissue digital scanned light microscopy). This method minimizes loss in resolution and facilitates the use of multiple types of air-immersion excitation and detection objectives. It is compatible with fluorescent microscopy techniques and requires less complex optics compared to confocal microscopy.

Compatibility

Hydrogel embedding is not suitable for penetration intact organs. However, it is effective for spatial heterogeneity in inhomogeneous clearing, providing a versatile approach for studying the development and pathology of the eye.

Images

- Near left: Removing lens from eye
- Middle right: cleared eye in RIMS
- Top right: un-cleared vs. cleared eye
- Far left: Dissecting eye from optic

Results

Utilizing PECAM and PAX-6, we have generated 2D and 3D maps of the vascular and neuronal network structures within 3D fluorescence imaging data. The expression of PECAM and Pax-6 is shown in the images, with average radius measurements of 70 microns for PECAM and 150 microns for Pax-6. The imaging process takes roughly 15 minutes, demonstrating the efficiency of the hydrogel embedding technique.

References

Retinopathy of prematurity (ROP) is a disease that affects blood vessel development and distribution in developing eyes. This is more accurately quantified using a three-dimensional imaging approach. We utilize a technique, CLARITY, which renders tissue optically transparent. In this work, we investigate changes to the retinal vasculature, which leads to the disruption of the retina in pre-term infants. This disruption affects the vascular network and can be observed through serial sectioning followed by two-dimensional image analysis. Often, serial two-dimensional and three-dimensional imaging techniques are combined to quantify the three-dimensional distribution of key growth factors, such as vascular endothelial growth factor (VEGF), which varies through stages of embryonic development.

Background

Abstract Background

The molecular pathway for angiogenesis involves the hypoxic-inducible factor (HIF-1α) and the endothelial growth factor (VEGF). VEGF is expressed through hyperoxia exposure and plays a critical role in angiogenesis. Hypoxic conditions observed in pre-term infants affect this pathway leading to abnormal vascular network in the retina. This methodology is crucial for understanding the development of ROP.

Methods

Hydrogel embedding

Hydrogels are used to embed samples for imaging and analysis. They provide a neutral environment that helps maintain the integrity of the sample. In the images, we see a sample embedded in a hydrogel. The left image shows a close-up of the embedded sample, while the right image displays the sample in a petri dish with circles indicating the space occupied by the sample.

Future Work

The combination of optical tissue clearing techniques and fluorescent antibody labeling provides a methodology to quantitatively study the three-dimensional distribution of key factors in ROP. This requires more complex optics and computational methods to analyze the data. Further research is needed to validate the accuracy of these techniques and improve our understanding of ROP.
Hydrogel embedding

Now what? We can’t the eye(s) anymore!
Hydrogel embedding

The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.

Before

After CLARITY
Hydrogel embedding

Can’t see anything in the brain either!

Chung et al Nature 2013
Hydrogel embedding

Before

The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.

After CLARITY

The brain is a world consisting of a number of unexplored continents and great stretches of unknown territory.

Need another way to “see” inside the tissue

Chung et al Nature 2013
Learning Objectives

1. Explain why tissue scatters light and how one can alter the light scattering properties of tissue.
3. Summarize how combining these two methods allows one to measure structures within intact tissue and organs.
4. Theorize how these advancements may help with pressing public health issues.
What is a laser?
What is a laser?

All the animations and explanations on www.toutestquantique.fr
What is laser-induced fluorescence?
How can we attach fluorophores to a biological target?

1. Antibody labels
How can we attach fluorophores to a biological target?

1. Antibody labels
2. Fluorescent proteins
Multiples types of fluorescent microscopy
Multiples types of fluorescent microscopy
Multiples types of fluorescent microscopy
Multiples types of fluorescent microscopy
Microscope restrictions mean back to the guillotine!
Multiples types of fluorescent microscopy
How does LSFM work?
LSFM has mainly been used to image small model animals

Truong et al Nature Methods 2011
LSFM has mainly been used to image small model animals.

Truong et al. Nature Methods 2011
You can build an LSFM almost like LEGO's now!
Retinopathy of prematurity (ROP) is a disease that affects blood vessel development and distribution in the eyes of infants born prematurely. A common method used to investigate structure-function development, in three-dimensions, during the pathogenesis of ROP. CLARITY, which renders tissue optically transparent, is a technique that can help to study ROP.

Background

Eyes are first incubated in acrylamide monomer to the separation of the excitation and emission. In this work we utilize a technique, CLARITY, which renders tissue optically transparent to the separation of the excitation and emission. Of note are the errors that occur when multiple physical sections are computationally sectioning instead of physical sectioning. Three-dimensional fluorescence microscopy combined with intact, optically cleared eyes will generate more comprehensive view of angiogenesis than confocal microscopy.

Methods

We have cleared tissue, fluorescent labels, and an appropriate fluorescent microscope.

Results

Utilizing PECAM and PAX-6, we have generated both 2D and 3D maps of the 3D network analysis. Future Work

Additional work must be conducted to optimize the labeling technique for high-contrast imaging of macroscopic biological specimens.

References


Truong et al Nature Methods 2011
Chung et al Nature 2013
Tomer et al Nature Protocols 2014
Learning Objectives

1. Explain why tissue scatters light and how one can alter the light scattering properties of tissue.
3. Summarize how combining these two methods allows one to measure structures within intact tissue and organs.
4. Theorize how these advancements may help with pressing public health issues.
Combining LSFM and optically cleared tissue
If you are really patient, you can reconstruct a whole mouse brain
We can also measure lung structure
We can also measure lung structure
Learning Objectives

1. Explain why tissue scatters light and how one can alter the light scattering properties of tissue.
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Application to animal models of disease

Yang et al Cell 2015
Application to animal and human models of disease

Yang et al Cell 2015
Application to analyzing structural changes in the brain from external events

**ARTICLE**

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**Basomedial amygdala mediates top–down control of anxiety and fear**

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Application to analyzing structural changes in the brain from external events
Application to the dynamics of development

Trivedi et al Biomedical Optics Express 2015
Application to the dynamics of development - we can measure even faster!

Trivedi et al Biomedical Optics Express 2015
Fahrbach et al Optics Express 2014
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