Through the looking glass: A review of cranial window technology for optical access to the brain

Samuel W. Cramer a, Russell E. Carter b, Justin D. Aronson b, Suhasa B. Kodandaramaiah c,d,e, Timothy J. Ebner b,*,1, Clark C. Chen a,d,1

a Department of Neurosurgery, University of Minnesota, 420 Delaware St SE, Mayo D429, MMC 96, Twin Cities, Minneapolis, MN, 55455, USA
b Department of Neuroscience, University of Minnesota, Twin Cities, Room 421, 2001 Sixth Street S.E., Minneapolis, MN, 55455 MN, USA
c Department of Mechanical Engineering, University of Minnesota, Twin Cities, MN, USA
d Department of Biomedical Engineering, University of Minnesota, Twin Cities, MN, USA
e Graduate Program in Neuroscience, University of Minnesota, Twin Cities, MN, USA

ARTICLE INFO

Keywords: Cranial window Imaging window Optical imaging In vivo imaging Neuro modulation Neurophysiology

ABSTRACT

Deciphering neurologic function is a daunting task, requiring understanding the neuronal networks and emergent properties that arise from the interactions among single neurons. Mechanistic insights into neuronal networks require tools that simultaneously assess both single neuron activity and the consequent mesoscale output. The development of cranial window technologies, in which the skull is thinned or replaced with a synthetic optical interface, has enabled monitoring neuronal activity from subcellular to mesoscale resolution in awake, behaving animals when coupled with advanced microscopy techniques. Here we review recent achievements in cranial window technologies, appraise the relative merits of each design and discuss the future research in cranial window design.

1. Introduction

Beginning in the late 18th century, exploitation of cranial defects, including the installation of glass windows for direct observation of animal and human brains, provided an early understanding of neural function (Zago et al., 2012). Since the outset it has been clear that understanding the central nervous system offers enormous challenges. For example, even “simple” mammalian brains, such as in rodents, are comprised of millions of neurons while the human brain is estimated to contain 86 billion, each of which may make thousands of connections to other neurons (Herculano-Houzel, 2009; Herculano-Houzel et al., 2007, 2006). From these massive number of interconnected cells emerge neural networks which subserve the integration of sensory inputs for perception, cognition, and transformation into motor output for interaction with the environment. Technical advancements in computational analyses, microscopy, and material science have provided neuroscientists with a large armamentarium of tools to interrogate the nervous system across scales from the entire organism down to the subcellular activity of individual neurons. Unraveling the complexities of the mammalian nervous system requires tools that afford simultaneous high spatiotemporal control and monitoring of the entire system (Weisendburger and Vaziri, 2018). Until recently, methods for investigating the nervous system such as single- or multi-cell electrophysiology have not provided such capabilities (Yang and Yuste, 2017).

The use of genetically encoded Ca2+ indicators (GECI - e.g., GCaMP6) now enables simultaneous high-resolution monitoring of the dynamics of thousands of neurons (Chen et al., 2013), while red-shifted variant Ca2+ indicators and opsins extend this imaging and neuro-modulation to deeper structures (Akerboom et al., 2013; Chuong et al., 2014). Driven by advances in sensing technology, microscopy has kept pace with two-photon (2P) imaging, becoming the instrument of choice for in vivo imaging in rodent models due to improved deep imaging over one-photon (1P) methods (Cantz and White, 1998).

Neural activity sensors and optogenetics provide the tools necessary for the optical dissection of neural circuitry (Packer et al., 2015; Rickgauer et al., 2014). The application of new technologies to dissect individual neural networks across spatial and temporal scales has provided a piecemeal view of global nervous system function. By bridging current knowledge gaps, a higher spatiotemporal resolution understanding of the nervous system will ultimately emerge. To fully

* Corresponding authors.
E-mail addresses: ebner001@umn.edu (T.J. Ebner), ccchen@umn.edu (C.C. Chen).
1 Co-senior author.

https://doi.org/10.1016/j.jneumeth.2021.109100
Received 22 November 2020; Received in revised form 7 February 2021; Accepted 9 February 2021
Available online 15 February 2021
0165-0270/© 2021 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
realize the potential of advances in optical tools microscopy, chronic optical access to large regions of the brain must be achieved.

Here we review the development of, and recent innovations in, cranial window technology. We also appraise the relative strengths and weaknesses of different optical window techniques and discuss the possible future evolution of the cranial window.

2. Approaches for chronic optical access to the brain

Chronic optical access to the brain of living mammals has been central to the current understanding of the functioning of both single neurons and neuronal networks (Forbes, 1928; Grinvald et al., 1991; Levasseur et al., 1975). In general, optical access is achieved by the creation of a transparent light path to the brain combined with hardware for head-fixation and stabilization during awake imaging sessions. There are two approaches to window construction: 1) transcranial windows created by thinning or clearing the cranium to increase and stabilize transparency of the bone; and 2) implanted windows which consists of replacing a region of the cranium with a transparent material. From these two approaches, multiple variations in optical window design have been developed including chronically implanted lenses and prisms that provide optical access to deep brain structures. Often the window technology was developed in anesthetized animals and later adapted for imaging of the brain in awake, behaving animals. First, the different methodologies for chronic optical access to the brain are described. Second, we review the advantages and disadvantages of the different approaches.

Fig. 1. Schematics of different optical window preparations and examples of large field of view (FOV) optical windows (see text for detailed descriptions of the different techniques). A. Thinned skull transcranial window is prepared by milling the bone from a small region of the cranium. Note, for awake imaging, head-posts (not illustrated) are placed with the optical chamber to immobilize the animal and stabilize the head. B. Reinforced thinned skull window consists of a thinned region of the cranium reinforced for chronic imaging by a layer of cyanoacrylate glue (yellow) and glass coverslip. The window is secured to the bone using cyanoacrylate glue and dental cement. C. Transcranial windows consist of applying a substance to the skull (often cyanoacrylate) to prevent desiccation and chronically stabilize the intrinsic transparency of the full thickness mouse cranium. D. Implanted window consists of removal of a region of the cranium and placement of a transparent material (e.g., glass, PET, or PDMS) over the dura. E. Large transcranial windows prepared by clearing a large region of the dorsal mouse cranium yield a wide FOV and are primarily used for mesoscale imaging (scale bar indicates 4 mm). F. Implanted windows that replace a large region of the dorsal mouse cranium with a transparent material (e.g., glass or PET) have recently been described which allow the simultaneous monitoring of millions of neurons (scale bar indicates 4 mm).
2.1. Transcranial windows

2.1.1. Thinned skull transcranial windows

Thinned skull transcranial windows are created by thinning a small region of the cranium to a thickness of 15–30 μm using a high-speed drill followed by the removal of remaining cancellous bone from the inner table of the skull (Fig. 1A) (Christie et al., 2001). Achieving this level of thinning is essential as skull thicknesses > 40 μm lead to severe degradation of image quality and double images (Yang et al., 2010; Zuo et al., 2005a). The thinned skull window provides excellent optical access as shown by chronic high resolution characterization of dendritic spines (Grunzender et al., 2002; Tsai et al., 2004; Yang et al., 2010; Zuo et al., 2005b,a) and dynamic microglia morphology (Nimmerjahn et al., 2005). Suturing the skull between imaging sessions and re-thinning the skull allows for two imaging sessions with an interval of up to 18 months between sessions in the same animal (Zuo et al., 2005a).

Since its conception, the thinned skull transcranial window methodology has evolved. For example, automated laser thinning of the cranium to ~24 μm over a ~3.6 mm² dorsal skull region (Jeong et al., 2013) and reinforcement of the thinned cranium with cyanoacrylate adhesive and a glass coverslip (Fig. 1B). The later advancement eliminates the need to close and re-open the scalp during repeated imaging sessions and prevents regrowth of bone, thereby allowing for repeated imaging in awake animals (Drew et al., 2010; Han et al., 2020; Marker et al., 2010; Rossi et al., 2017; Shih et al., 2012; Silasi et al., 2013; Zhang et al., 2012). In summary, thinned skull transcranial windows provide the advantage of a minimally invasive optical window preparation. The disadvantages, however, include significant training to achieve consistent thinning of the skull to ~15 μm (while avoiding over-thinning which can damage the underlying cortex) and the need for re-thinning of the skull for repeated imaging sessions.

2.1.2. Non-thinned skull transcranial windows

Non-thinned skull transcranial windows are constructed by coating the exposed cranium with transparent dental resin (prior to bone desiccation) to maintain chronic optical clarity (Fig. 1C) (Hira et al., 2009; Kuroki et al., 2018; Musall et al., 2019; Park et al., 2015; Saxena et al., 2020). Variations in construction of transcranial windows include a chronic “Clear Skull Cap” created by placing transparent dental cement over a layer of cyanoacrylate adhesive, followed by polishing and application of nail polish (Allen et al., 2017; Guo et al., 2014; Pinto et al., 2019) or a glass coverslip (Silasi et al., 2016; Vanni and Murphy, 2014; Wang et al., 2018; Xie et al., 2016). The preparations provide a stable, chronic optical window for up to 2 months (Silasi et al., 2016; Vanni et al., 2017). Transcranial windows provide optical access to nearly the entire dorsal cerebral cortex of the mouse without entry to the intracranial space (Fig. 1F). When combined with GECIs and mesoscopic imaging or optogenetics, transcranial windows allow characterization of cortical dynamics across different behaviors (Pinto et al., 2019; Saxena et al., 2020), cortical networks that subserve multi-sensory integration (Kuroki et al., 2018), and functional mapping of the motor cortex (Hira et al., 2009).

2.2. Implanted windows

2.2.1. Glass coverslip-based cranial windows

Glass coverslip-based cranial windows were utilized in the early development of in vivo 2P Ca²⁺ imaging in anesthetized animals (Fig. 1D and E;Svoboda et al., 1997). The preparation consists of removal of the bone and dura over a region of the brain which is then covered by agarose and a coverslip (Lendvai et al., 2000; Svoboda et al., 1997; Trachtenberg et al., 2002). Subsequent modifications to the glass coverslip cranial window include leaving the dura (De Paola et al., 2006; Holtmaat et al., 2012; Lee et al., 2006; Mizrahi and Katz, 2003; Portera-Cailliau et al., 2005), placing silicone on intact dura prior to coverslip placement (Dombek and Tank, 2014), and omitting the placement of agarose over the dura prior to coverslip placement (Brown et al., 2009; Choi and Wang, 2015; Chow et al., 2009; Horton et al., 2013; Keck et al., 2008; Kobat et al., 2011; O’Connor et al., 2016; Park et al., 2015; Santos and Hübener, 2014). The utility of the glass coverslip cranial window is exemplified by combining wide-field Ca²⁺ optical imaging to characterize mesoscale activity with 2P imaging of single cell neuronal activity in response to visual stimuli in awake mice (Wekselblatt et al., 2016). Other examples of experimental designs utilizing glass coverslip cranial windows include the characterization of mesoscale and single cell Ca²⁺ activity in the visual cortex and the CA1 region of the hippocampus of awake mice in a virtual reality environment (Saleem et al., 2018), and characterization of neural activity in the auditory cortex and medial geniculate body (Liu et al., 2019).

In the mouse, glass coverslip windows are placed over intact dura. Optimization of the light path, however, may require removal of the dura, as required with larger animals such as the ferret (Smith et al., 2018), non-human primate (NHP) (Grinvald et al., 1991; Stettler et al., 2006), pig (Klein et al., 2019), and rat (Kolter et al., 2019; Scott et al., 2018). Dural removal with cranial window implantation is also preferred for chronic optical imaging utilizing voltage sensitive dyes (Kuni and Takahama, 2019) or the characterization of brain tumor growth to facilitate direct access to the brain for dye uptake and tumor implantation (Orringer et al., 2010).

The glass coverslip cranial window preparation has also been adapted for chronic optical access to the cerebellar cortex. The basic approach is the same as creating an optical window over the cerebral cortex. Both craniectomy (Askoxylakis et al., 2017) and thinned skull transcranial preparations (Nishiyama et al., 2007) have been reported for the creation of ~1–6 mm diameter windows for chronic optical access to the cerebellum up to 5 months (Askoxylakis et al., 2017).

The glass coverslip cranial window and its derivatives have been widely used in chronic, awake in vivo imaging experiments in a range of model organisms (Kolter et al., 2019; Scott et al., 2018; Smith et al., 2018; Stettler et al., 2006; Wu et al., 2020) and across developmental stages, including neonatal mice (Cruz-Martin and Portera-Cailliau, 2014). Therefore, the versatility of the cranial window has led to it being the most common method for chronic optical access to the brain.

2.2.2. Molded crystalline-based cranial windows

The “Crystal Skull” (Kim et al., 2016) and similar approaches (Hatcher et al., 2020) were developed to exploit the optical clarity of glass windows while addressing the major limitation of other techniques, a restricted field of view. The Crystal Skull consists of a custom glass coverslip cut into a trapezoidal shape (~76 mm²) and press-molded to approximate the natural contour of the dorsal mouse cranium (Kim et al., 2016). Installation is performed by removing a large region of the dorsal cranium (leaving the dura intact) and the implant is secured over the cranial defect using adhesive (Kavur et al., 2020; Kim et al., 2016). The Crystal Skull exhibits excellent optical clarity, providing chronic optical access for 8 weeks (Kim et al., 2016) or 6 months in a modified application of the Crystal Skull (Sunil et al., 2020). It is estimated the Crystal Skull provides access to nearly 1 million neurons in the dorsal cerebral cortex (Kim et al., 2016).

Another approach (“Window to the Brain” - WttB) uses transparent nanocrystalline yttria-stabilized zirconia (nc-YSZ) to construct the optical window (Damestani et al., 2013; Davoodzadeh et al., 2019, 2018). The implants are custom fabricated from YSZ powder by densification into a transparent bulk ceramic slab that is then polished and cut to produce a ~4.6 mm², 300 μm thick transparent window (Damestani et al., 2013; Davoodzadeh et al., 2018). The WttB is implanted over intact dura following a craniectomy, providing optical access to the cortical surface up to 60 days (Davoodzadeh et al., 2018). Customization of the WttB across a range of dimensions allows application to almost any model organism.
2.2.3. Cranial windows and direct access to the brain

With glass coverslip based cranial windows, access for direct brain manipulation concurrent with optical imaging in small model organisms such as mice is challenging due to the size constraints. Furthermore, the requirement to avoid contamination of the brain which risks degrading optical clarity or introducing infection must be considered. Despite the challenges, the utility of a multimodal cranial window platform has driven the development of several technologies that combine optical imaging windows with direct access to the brain.

For intermittent direct access to a large region of the brain, a removable glass coverslip cranial window has been developed by modifying the basic technique described above (Holmaat et al., 2012, 2009). The window may be subsequently removed by carefully drilling the dental cement and cyanoacrylate adhesive securing the coverslip (Goldey et al., 2014). Removal of the window allows access to the brain for microinjections (e.g., viruses or tumor cell implantation), removal of bony overgrowth and thickened dura, as well as replacement of the cranial window with a microprism for imaging across multiple cortical layers. The methodology provides imaging access for up to 175 days (including up to 51 days after removal and replacement of the window) (Goldey et al., 2014).

To provide direct chronic access to the brain, a modified glass coverslip based cranial window with an integrated 1.5 mm diameter fenestration has been developed. The fenestrated implant is fabricated using a high-speed drill and the window is filled with a biocompatible silicone. The device is surgically implanted following craniectomy (leaving the dura intact) using cyanoacrylate adhesive (Roome and Kuhn, 2014). The transparent silicone plug provides direct access to the brain for microinjections and study of electrophysiology. However, after 7 weeks, and/or the repeated removal and replacement of the silicone plug, the underlying dura becomes thickened and opaque, which reduces imaging quality and impedes intracortical injections (Roome and Kuhn, 2014). A similar approach utilizes a pre-fabricated coverslip with an access port filled with silicone between experiments (Rossi et al., 2017).

Other strategies have been described to provide direct access for injection of drugs or viruses, electrode placement for electrophysiologic recordings, or optical fiber implantation in conjunction with a chronic cranial window. For example, the ‘cranula’ is a chronically implanted intracranial cannula placed in close proximity to the cranial window that provides a conduit for chronic, large volume drug administration (Zuluaga-Ramirez et al., 2015). Another approach for drug administration concurrent with optical imaging is a modified window with integrated microfluidic channels ('Lab-on-a-brain') that allows superfusion of chemicals onto the brain surface (Takehara et al., 2014).

2.2.4. Polymer-based cranial windows

In the evolution of cranial windows, polymer-based designs have emerged recently. Similar to traditional glass windows, polymer-based windows exploit the physical properties of the polymer that allow a greater flexibility in device design and function. Polymer-based cranial windows have been fabricated using polyethylene terephthalate (PET) (Ghanbari et al., 2019a), polydimethylsiloxane (PDMS, (Heo et al., 2016; Park et al., 2019)), polymethylmethacrylate (PMMA - Plexiglas, (Ghanbari et al., 2013)). Implantation of a microprism cranial window consists of a custom coverslip with the prism bonded to the lower surface is stereo.

The technique for chronic, awake Ca2+ imaging studies using microprism cranial windows was subsequently developed (Andermann et al., 2013). Implantation of a microprism cranial window consists of a craniectomy and removal of dura over the brain region of interest. The pia is then incised to approximate the dimensions of the prism and a custom coverslip with the prism bonded to the lower surface is stereotactically lowered into the brain tissue. The cranial window is bonded to the skull using cyanoacrylate followed by dental cement (Andermann et al., 2013). The microprism cranial window allows Ca2+ dynamics to be simultaneously monitored across layers 2-6 in the primary visual cortex during locomotion and in response to visual stimuli for up to 68 days (Andermann et al., 2013). In mice, implanted microprisms have been used for chronic, awake 2P imaging of neurons in the medial entorhinal cortex (Heyns et al., 2014; Low et al., 2014), somatosensory cortex (Wenzel et al., 2017), and medial prefrontal cortex (Low et al., 2014). The microprism window has also been adapted for performing 2P and wide-field epifluorescence microscopes.

2.3. Chronic optical access for imaging of deep brain structures

Traditional methods for chronic optical access to the brain utilize implanted windows to study surface structures of the cerebral or cerebellar cortex. Therefore, high resolution optical access to deep brain structures is generally limited to approximately 500–700 μm using 2P microscopy. Beyond this depth, 2P microscopy is hindered by poor signal-to-noise ratio (SNR) due to the significant photon scattering by brain tissue (Barretto and Schnitzer, 2012; Takasaki et al., 2020; Theer and Denk, 2006). Improvements in imaging technology and the introduction of new microscopy techniques such as 3-photon (3P) have pushed the boundaries of depth to 600–1000 μm using glass coverslips (Horton et al., 2013; Ouzounov et al., 2017) and transcranial glass coverslip windows (Wang et al., 2018). Other techniques involve placement of optical extenders such as prisms and gradient-index (GRIN) lenses into the brain to facilitate photoexcitation and imaging of deeper structures. Cranial windows coupled to the optical extenders are compatible with widely available optical imaging modalities such as 2P and wide-field epifluorescence microscopes.

2.3.1. Prism-based cranial windows

Using 2P microscopy, microprisms allow imaging of structures beneath the superficial cortical layers. The principle is to leverage the optical transformation provided by the prism (with a reflective coating on the hypotenuse) to translate photoexcitation from an x-y plane to an x-z plane (Fig. 2A). The first in vivo application of microprism assisted 2P imaging involved acute imaging experiments for simultaneous high resolution imaging across cortical layers (Chia and Levene, 2010, 2009a, b).

The technique for chronic, awake Ca2+ imaging studies using microprism cranial windows was subsequently developed (Andermann et al., 2013). Implantation of a microprism cranial window consists of a craniectomy and removal of dura over the brain region of interest. The pia is then incised to approximate the dimensions of the prism and a custom coverslip with the prism bonded to the lower surface is stereotactically lowered into the brain tissue. The cranial window is bonded to the skull using cyanoacrylate followed by dental cement (Andermann et al., 2013). The microprism cranial window allows Ca2+ dynamics to be simultaneously monitored across layers 2-6 in the primary visual cortex during locomotion and in response to visual stimuli for up to 68 days (Andermann et al., 2013). In mice, implanted microprisms have been used for chronic, awake 2P Ca2+ imaging of neurons in the medial entorhinal cortex (Heyns et al., 2014; Low et al., 2014), somatosensory cortex (Wenzel et al., 2017), and medial prefrontal cortex (Low et al., 2014). The microprism window has also been adapted for performing 2P and wide-field epifluorescence microscopes.
B. Lens-based windows (e.g., GRIN lens) often include a guide-tube placed within the brain (grey cylinder) which provides chronic access for lens placement and imaging across cortical layers of the parietotemporal region to evaluate chronic microvascular changes in a stroke mouse model (Beckmann et al., 2019).

Fig. 2. Window for imaging deep within the brain in the mouse (see text for detailed descriptions of the different techniques). A. Example of a prism-based window which includes removal of a region of the cranium and placement of a prism (covered with a glass coverslip) to allow simultaneous imaging across all cortical layers. B. Lens-based windows (e.g., GRIN lens) often include a guide-tube placed within the brain (grey cylinder) which provides chronic access for lens placement and imaging of deep brain regions.

Finally, simultaneous mesoscale and single cell Ca$^{2+}$ imaging using a combined cranial window microprism implant was recently described (Barson et al., 2020). Simultaneous acquisition of activity from hundreds of individual neurons and mesoscopic activity across the dorsal cerebral cortex was achieved using a wide-field epifluorescence microscope with the objective positioned perpendicular to the surface of the dorsal skull and a 2P microscope oriented tangentially to the skull surface.

2.3.2. GRIN lens-based cranial windows

GRIN lenses are cylindrical optical devices of varying diameters and lengths that are inserted into the brain to direct light to and from the target structure using glass with a negative gradient refractive index to bend and focus light. GRIN lenses are commercially available in a variety of lengths from fractions of a millimeter up to tens of centimeters, theoretically allowing their application in small mammals to NHPs. By customizing the lens’s optical parameters, a range of imaging fields may be obtained allowing investigation of subcellular structures (Attardo et al., 2015) to wide-field neuronal activity (Otis et al., 2017).

Placement of a GRIN lens for in vivo microscopy consists of a craniectomy over the target brain region. For acute experiments, the lens assembly is stereotactically inserted into the brain over the imaging target (Levene et al., 2004). Alternatively, a guide tube may be placed over the target (Barretto and Schnitzer, 2012), so that the GRIN lens may be placed during imaging sessions and then removed (Barretto et al., 2011, 2009; Bocarsly et al., 2015). The guide-tube is sealed at the insertion end and has a wider diameter flange at the superficial end to allow bonding to the skull (Attardo et al., 2015; Barretto et al., 2011, 2009). Direct insertion of the GRIN lens with a glass coverslip attached to the lens can also be used to secure the device to the cranium and seal the craniectomy (Pernici et al., 2020, 2019). Depending on the dimensions of the lens, a corticectomy may be necessary prior to lens placement to facilitate implantation of the lens or guide-tube without causing significant distortion and compression of brain tissue (Barretto et al., 2011; Jung et al., 2004; Lee et al., 2016; Moretti et al., 2016; Murray and Levene, 2012). Chronic implantation of GRIN lens assemblies have been reported up to 396 days (Barretto et al., 2011) and characterization of thalamic activity using voltage sensitive dyes (Tang et al., 2015).

In addition to prism- and GRIN lens-based cranial windows, other forms of optical extender are available and used in a fashion similar to those described above (Pilz et al., 2016; Sato et al., 2020; Velasco and Levene, 2014; Weisenburger et al., 2019).

3. Advantages and disadvantages of different approaches to chronic optical access to the brain

The choice of optical window technique ultimately depends on the experimental design. The ideal implant should minimally affect the physiologic process under investigation. Factors such as duration of optical access, requirement for direct brain access, imaging resolution, depth(s) of field(s), curvature of implant, and the impact of the window placement on cerebral physiology should be considered. The strengths, weaknesses, and limitations of the different techniques are reviewed below.

3.1. Optical properties, FOV, and longevity

In most cases, direct comparison of the properties of different cranial window techniques under identical conditions are not available. The primary comparisons reported in the literature are often made relative to the optical properties of glass coverslips themselves or glass coverslip cranial windows in conjunction with 2P microscopy.

3.1.1. Spatial resolution and imaging depth

The spatial resolution, target region, and the depth of imaging field varies by cranial window technique (Fig. 1). Thinned skull windows limit the spatial resolution and depth of tissue penetration due to the skull’s scattering effect on excitation/emission light (Heim et al., 2009). The degree of light distortion depends on the thickness and persistent inhomogeneities of residual bone remaining after cranial milling as well as on the size of the structure being examined (Heim et al., 2009). Due to these limitations, use of high-resolution 2P imaging across large cortical regions or deep (>250 μm) subcellular structures may require open skull cranial windows (Holmaa et al., 2009; Ishiki and Okabe, 2014; Nimmerjahn, 2012). The optical properties, including imaging resolution and transparency, of polymer-based windows (e.g., PET and PDMS) are nearly identical to glass coverslip (Ghanbari et al., 2019a; Heo et al., 2016). Furthermore, polymer-based windows allow high resolution imaging at depths up to ~ 600 μm below the cortical surface (Ghanbari et al., 2019a; Heo et al., 2016). Therefore, glass- and polymer-based windows have advantages in spatial resolution and imaging depth.
3.1.2. Imaging FOV

The FOV of different cranial window techniques varies considerably. Generally, cranial windows that provide large FOVs involve replacing a significant portion of the skull with a transparent implant. However, optical windows based on full thickness skull clearing, such as the Clear Skull Cap (Allen et al., 2017; Guo et al., 2014) or similar techniques (Silasi et al., 2016) also provide optical access to a large fraction of the dorsal cerebral cortex (~49–81 mm²), with the caveat that transcranial windows are suitable for mesoscale imaging applications but not for high resolution 2P microscopy (Allen et al., 2017). Except for specialized adaptations, the diameter of a typical cranial window is approximately 2–5 mm for glass coverslip windows and approximately 1–2 mm for thinned-skull preparations (Drew et al., 2010; Yang et al., 2010) (see Table 1 for comparison of FOV for different cranial window techniques).

With increased cranial window size, the likelihood of the glass coverslip applying pressure on the underlying brain tissue increases and the resultant distortion of the natural cortical anatomy may alter cerebrospinal fluid (CSF) dynamics and intracranial pressure. Therefore, a consideration for glass-based windows is the rigidity of glass, with skull curvature limiting both the surface area and location of the cranium that may be replaced. Consequently, the diameter of a typical glass coverslip cranial window is generally limited to <5 mm (Table 1). The limitations of the glass coverslip may be overcome by the fabrication of an implant that replicates the natural curvature of the animal’s skull. The “Crystal Skull” (Kim et al., 2016) and similar techniques (Hatcher et al., 2020) exploit the optical clarity of glass while addressing the major limitations of a restricted FOV and planar geometry. For example, Crystal Skulls are designed to match the curvature of the mouse skull and provide a window over the dorsal cortical surface of ~76 mm² (Kim et al., 2016), while the similarly designed See-Shells provide ~45 mm² FOV (Ghanbari et al., 2019a). While these cranial implants provide large imaging fields and their anatomically conformant designs minimize distortion of the underlying cortical anatomy, they come at the expense of increased optical aberrations compared to traditional flat cranial windows.

3.1.3. Longevity of optical windows

Differences in both device construction and experimental application underlie a wide range in the duration of quality optical access provided by cranial windows. Transcranial preparations (including reinforced thinned skull) have a useful life-time of 2–3 months (Drew et al., 2010; Silasi et al., 2016; Vanni et al., 2017). However, the duration of optical access via non-reinforced transcranial windows can be extended through repeated skull thinning for up to 18 months to >2 years (Yang et al., 2010; Zuo et al., 2005a). A limitation, however, is that chronic imaging through non-reinforced thinned skull is practically limited to ~5 imaging sessions due to repeated thinning of the cranium (Yang et al., 2010).

Windows that replace a portion of the cranium with a transparent implant provide chronic optical access for an unlimited number of imaging sessions. In the mouse, the duration of optical access provided by cranial windows varies considerably from 90 days (Davoodzadeh et al., 2018) to >300 days (Ghanbari et al., 2019a). The limiting factors are primarily thickening and opacification of the dura as well as regrowth of bone around the craniectomy perimeter (De Paola et al., 2006; Heo et al., 2016; Holtmaat et al., 2005; Trachtenberg et al., 2002). One solution is replacement of the window, providing access to the underlying bone, dura, and brain for removal of bony overgrowth, opacified dura and the microinjection of substances within the imaging field (Goldey et al., 2014).

3.2. Inflammation and alteration of intracranial physiology

The experimental design and choice of optical window should consider the impact of cranial window placement on brain physiology. Part of the consideration is the inclusion of thoughtful control conditions to account for perturbations caused by the window.

3.2.1. Transcranial windows

Surgical manipulation of the skull has the potential to influence the physiology of the underlying cerebrum. For instance, drilling of the skull generates heat that may cause subsequent changes in the underlying meninges and brain. Other changes may arise from forces exerted on the underlying tissue during surgery, including recruitment of microglia, induction of reactive astrocytes, and neuronal injury/death (Kim and Dustin, 2006; Roth et al., 2014). These effects will be reviewed in this section.

3.2.2. Cranial window and GRIN lens implantation

Surgical manipulation of the cranium and meninges can cause a range of acute and chronic changes in brain physiology. However, these effects appear study dependent. In one study, unilateral craniectomy and dural resection in a murine model is associated with decreased pulsatility of penetrating cortical arteries and impairment of global glymphatic CSF dynamics as well as astrocytic and microglial inflammatory response. These effects were associated with motor and cognitive impairment (Plog et al., 2019). Recovery of normal glymphatic CSF dynamics preceded a reduction in inflammation and recovery of motor and cognitive deficits with recovery accelerated by cranioplasty (Plog et al., 2019). Others have reported induction of reactive astrocytes, microglia recruitment, altered blood-brain barrier, and inflammatory cytokine release following surgical manipulation of the skull (Bacska et al., 2001; Drew et al., 2010; Guo et al., 2017; Holtmaat et al., 2009; Kawamura et al., 1990; Navari et al., 1978; Park et al., 2019; Shoffstall et al., 2018; Xu et al., 2007). However, such effects were not universally reported following craniectomy, suggesting potential contribution of mouse strain or surgical technique (Askoxyakis et al., 2017; Brown et al., 2018).

Table 1

Comparison of in vivo optical window properties.

| Optical window type | Imaging FOV (mm²) | Duration of access (days) | Imaging depth with 2P microscopy | Inflammation* |
|---------------------|------------------|--------------------------|---------------------------------|---------------|
| TS transcranial      | 0.2–3.1⁺⁹       | 73⁹                      | –50–250 μm²⁷⁸,¹⁶¹¹⁸           | Not observed²⁴,²⁵|
| Reinforced TS transcranial | ~9.0⁹    | 90⁹                      | –250 μm³⁴                        | Not observed⁶ |
| Transcranial         | ~49³⁷⁷          | 60³¹⁴,¹⁵,¹⁶              | –150–150 μm³⁷,²⁰              | NR            |
| Glass coverslip      | 8.0–20.0⁸,⁹,¹⁰ | 188¹⁰                    | 1600 μm³⁰                      | Not observed²⁶,²⁷,²⁸; RA + MA⁵,¹⁰,¹³,₂⁴,₂⁵ |
| Crystalline          | 4.6–76.1²,³      | 180¹                     | –700 μm¹                       | Mild RA¹      |
| Polymer              | 18.0–45.5⁵,⁵    | >300²                    | –600 μm⁵                       | Not observed ¹ |
| Prism                | 1.1–2.2⁵,⁷      | 120¹²                    | –700 μm¹¹                      | Not observed¹¹ |
| GRIN lens            | 0.02–0.03³⁵,³⁷   | 396²⁰                    | 5.0 mm²⁰                       | MA + RA²⁰,²¹ |

Abbreviations: MA (microglia activation), RA (reactive astrocytes), NR (not reported), TS (thinned skull), *See text for details. Citations: ¹(Kim et al., 2016), ²(Damestani et al., 2013), ³(Davoodzadeh et al., 2018), ⁴(Heo et al., 2016), ⁵(Ghanbari et al., 2019), ⁶(Guo et al., 2014), ⁷(Allen et al., 2017), ⁸(Drew et al., 2010), ⁹(Yang et al., 2010), ¹⁰(Holtmaat et al., 2009), ¹¹(Andermann et al., 2013), ¹²(Accanto et al., 2019), ¹³(Barretto and Schnitzer, 2012), ¹⁴(Silasi et al., 2016), ¹⁵(Vanni et al., 2017), ¹⁶(Steinzeig et al., 2017), ¹⁷(Nimmenjahr, 2012), ¹⁸(Ishikawa and Okabe, 2014), ¹⁹(Barretto et al., 2011), ²⁰(Bocarsly et al., 2015), ²¹(Lee et al., 2016), ²²(Park et al., 2019), ²³(Bacska et al., 2001), ²⁴(Xu et al., 2007), ²⁵(Guo et al., 2017), ²⁶(Nishiyama et al., 2007), ²⁷(Brown et al., 2009), ²⁸(Askoxyakis et al., 2017), ²⁹(Park et al., 2015), ³⁰(Wang et al., 2018), ³¹(Kobat et al., 2011).
et al., 2009; Ghanbari et al., 2019a; Nishiyama et al., 2007)

Despite the success of 2P, imaging of deep brain structures requires invasive procedures including either removal of overlying brain structures or insertion of optical devices into the brain (Jung et al., 2004; Levene et al., 2004; Moretti et al., 2016; Murray and Levene, 2012).

GRIN lens imaging is no exception, involving placement of the lens into the brain parenchyma and in some cases after a corticectomy. Chronic implantation of the GRIN lens causes gliosis and microglial activation for at least two weeks following implantation (Attardo et al., 2015; Barretto et al., 2011; Bocarsly et al., 2015), though the inflammatory response may subsequently subside after the initial peak (Bocarsly et al., 2015). Despite a local inflammatory response, GRIN lens implantation in the mouse prefrontal cortex did not cause significant alterations in normal brain physiology as evaluated using common behavioral assays (Lee et al., 2016). However, it is expected that the insertion location of intraparenchymal probes may impact aspects of neural function.

In contrast to GRIN lenses, microprism based 2P imaging exploits the optical properties of the prism to transform the excitation/emission (via a reflective coating on the hypotenuse) from an x-y plane to an x-z plane. Therefore, microprism based cranial windows afford a unique FOV for the imaging of deep brain structures. Translation of the imaging plane by the prism allows simultaneous monitoring of a cross-section of the cerebral cortex for investigating inter- and intralaminar cortical neuronal dynamics (Andermann et al., 2013; Chia and Levene, 2009b).

A disadvantage of GRIN lenses, and similar optical extender techniques, is that they have relatively low spatial resolution. They also tend to suffer from optical aberrations that limit imaging to the soma and preclude imaging of subcellular structures such as dendritic spines and axonal boutons (Barretto et al., 2011; Jung et al., 2004; Levene et al., 2004; Meng et al., 2019). A fixed FOV of ~130–510 μm is another limitation imposed by small diameter lenses often used for microendoscopy (Chia and Levene, 2009a; Meng et al., 2019). However, development of improved lenses and adaptive optical technology to reduce optical aberrations have begun to address limitations of microendoscopy (Barretto et al., 2011, 2009; Matz et al., 2016; Sato et al., 2020; Wang and Ji, 2013).

3.3. Effect of cranial window placement on neural activity, hemodynamics, and neovascularization

In any experimental manipulation involving a model organism, a concern is to what extent the manipulation alters normal physiology. The question is paramount in cranial window technologies. For instance, intracranial pressure or cortical inflammation may lead to altered neural activity in the absence of direct mechanical neuronal injury (Plog et al., 2019). Evoked hemodynamic and neural activity in the barrel cortex of anesthetized mice was reported to be suppressed for up to two weeks following cranial window implantation (Park et al., 2019). Suppression of evoked cortical activity paralleled the time course of neuroinflammation induced by window implantation and the altered hemodynamic and neural responses reversed with suppression of the neuroinflammatory response. The findings strongly suggest that the inflammation following cranial window placement alters cortical activity. In contrast, a voltage sensitive functional mapping study in anesthetized mice found that cortical limb representations were indistinguishable between thinned skull and open skull cranial windows (Brown et al., 2009). However, the animals underwent imaging after a recovery period of 4–5 weeks. Early perturbations of cortical physiology that occurs with optical window implantation may normalize after a sufficient recovery period.

Similarly, optical window implantation has acute effects on cortical vasculature that normalizes after 2–6 weeks. Vasodilation, defined as ~40 % increase in vessel diameter, occurred in the first 2–3 days post implant. However, these effects abated after a period of 4 weeks in larger (> 50 μm) diameter vessels (Hammer et al., 2014). Another study reported similar results with no significant change in large vessel (> 50 μm) diameter between 2 and 6 weeks post open-skull cranial window implantation (Park et al., 2019). Despite differences in vascular calibers in the early postoperative period, comparison of cerebral cortical blood flow under a cranial window to thinned skull transcranial window within the same animal found no significant difference (Li et al., 2014).

3.4. Choice of cranial window preparation and effect on cortical structure and dendritic spine turnover

A comparison of cranial windows with thinned skull transcranial preparations suggests that the former is associated with a reduction of dendritic spine density, increased microglia recruitment/activation, and enhanced reactive astrocytosis in the first two weeks immediately after the procedure (Xu et al., 2007). However, studies designed to examine the difference between cranial windows and thinned skulls ≥2 weeks after placement showed comparable densities of dendritic spines and axonal boutons (Brown et al., 2009; Chow et al., 2009; Holtmaat et al., 2006, 2005; Keck et al., 2008; Nishiyama et al., 2007; Stettler et al., 2006; Trachtenberg et al., 2002; Yang et al., 2009). Beyond the initial two weeks post-cranial window placement, spine and bouton dynamics appear stable for months (Berry and Nedivi, 2017; Holtmaat et al., 2009).

4. Future developments and applications of cranial window technology

4.1. Robotic assisted preparation and implantation of cranial windows

As technological improvements have prompted widespread adoption of wide FOV imaging, the size of cranial windows has increased from 3 to 4 mm diameter craniotomies (Holtmaat et al., 2009) to implanting coverslips across a cortical hemisphere (Sofroniew et al., 2016; Stirman et al., 2016) and recently, curved glass or polymer-based implants across the entire dorsal cerebral (Ghanbari et al., 2019a; Kauvar et al., 2020; Kim et al., 2016). Transcranial optical windows are also commonly used for in vivo imaging of mice, especially when the neuroinflammatory response to cranial window placement must be minimized (Drew et al., 2010; Shih et al., 2012; Silasi et al., 2016). Transcranial procedures in mice often require cranial milling to reduce the native thickness of 100–650 μm down to tens of micrometers. Procedures involving manipulation of the cranium require precision to avoid damaging the underlying anatomy and ensure brain quality for successful optical experimentation. Given the considerable manual dexterity required to perform procedures such as a craniectomy, bone thinning, as well as electrode or lens placement, a significant training period is required for technical mastery. Therefore, robotic assistants have been developed to aid in cranial remodeling (Coffey et al., 2013; Ghanbari et al., 2019a; Liang et al., 2019; Pak et al., 2015; Ramrath et al., 2009; Rynes et al., 2020). These tools should reduce experimental variability and facilitate adoption of cranial window studies.

4.2. Integrated multimodal cranial window platforms and diversified application of optical window technology

Advancements in material science, microelectronics, and 3D printing provide the basis for developing next generation cranial windows. For example, transparent, flexible electrodes (Qiang et al., 2018; Song et al., 2020; Thunemann et al., 2018) could be integrated into a polymer-based optical window to allow chronic simultaneous optical sensing, neuro-modulation, and electrophysiologic recordings capable of withstanding repeated penetrations of the window for access to the underlying cerebral cortex for direct manipulation of the brain (Heo et al., 2016; Park et al., 2019). This design would address a limitation of most current cranial window techniques which do not allow probe insertion into the brain to perform concurrent electrophysiologic recordings, microinjections, or neuromodulation. Another strategy for direct, chronic
manipulation of the underlying cortical physiology is provided by the “Lab-on-a-brain” window with integrated inflow and outflow ports for perfusing with drugs or different media (Takehara et al., 2014). However, the device does not provide a port for direct electrode or micro-pipette access to the brain surface.

Polymer-based cranial windows offer greater flexibility for experiments that require simultaneous optical and direct access to the brain. Soft flexible materials, such as PDMS, withstand repeated direct penetration of the window due to the self-sealing properties of the material (Heo et al., 2016). For more rigid polymers, such as PET, fenestrations may be created to access targeted brain region(s) (Ghanbari et al., 2019a).

While direct access to the brain simultaneously with chronic optical access affords greater experimental flexibility, it comes at a cost. Repeated opening of sealed fenestrations provides an opportunity for infection and inflammation, resulting in loss of optical clarity and the possibility of animal drop out due to compromised health with reduced success rate in longitudinal studies.

Ideally, the next generation cranial window would have a modular design consisting of a rigid frame bonded to the transparent window and be fabricated out of entirely MRI compatible materials for easy modification of the window for applications across a wide array of experimental conditions and anatomical regions. Finally, part of the flexible multimodal cranial window design would accommodate reversible coupling to a mini-microscope to perform optical imaging in freely behaving mice (Ghosh et al., 2011; Skocek et al., 2018; Zong et al., 2017).

As genetic tools, optical sensing, and neuromodulation become more widespread in neuroscience and the technological advances afford the ability to monitor larger tissue volumes across different model organisms, lessons learned in the mouse may be applied to the development of cranial windows for larger model organisms such as rats, ferrets, and NHP species. Chronic mesoscale and 2P Ca2+ imaging has been performed in NHPs (Arieli and Grinvald, 2002; Chen et al., 2002; Ju et al., 2010; Li et al., 2017). However, with the expanding FOV provided by advances in imaging technology, lessons learned designing conformational cranial windows for the mouse may be applied to the custom fabrication of large optical windows specifically designed to replace targeted regions of the skull.

For example, a large cranial window implant fabricated with a polyetheretherketone (PEEK) plastic chamber with transparent artificial dura (Gong et al., 2020) and glass coverslip has been developed for use in NHPs (Macknik et al., 2019). The chamber consists of a 2 cm diameter window with a silicone insert to distribute fluctuations in intracranial pressure across the surface of the optical window, preventing cortical injury as well as dural and subdural hemorrhage by inhibiting migration of inflammatory cells. Using the large field provided by the cranial window implant, > 60,000 cells have been recorded using 2P imaging (Macknik et al., 2019).

5. Conclusion

There is compelling beauty in visualizing the physiology that underlies our neurologic function. The nexus of genetic engineering, microscopy techniques, and cranial window technology has yielded a view of this fascination. Despite the quantum leaps in our understanding of cerebral physiology provided by these technologies over the course of more than 200 years, their true potential has yet to be realized. Continued development of these techniques and their application to queries pertinent to human health should afford new horizons in therapeutic and diagnostic development.

CRediT authorship contribution statement

Samuel W. Cramer: Conceptualization, Writing - original draft, Writing - review & editing. Russell E. Carter: Writing - review & editing. Justin D. Aronson: Writing - review & editing. Suhasa B. Kodandaramiah: Supervision, Funding acquisition, Writing - review & editing. Timothy J. Ebner: Supervision, Funding acquisition, Writing - review & editing. Clark C. Chen: Supervision, Writing - review & editing.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

Acknowledgements

We thank Alexander Cramer at the University of Minnesota Imaging Center for assistance in generating graphics. Research reported in this publication was supported by the University of Minnesota MnDRIVE (Minnesota’s Discovery, Research and Innovation Economy) initiative. We also acknowledge the partial support of 1R01NS111028-01 (SBK and TJE), 1P30DA048742-01 (SBK and TJE), 1R24NS116105-01A1 (SBK), 1RF1NS113287-01 (SBK) and R61/R33 NS115089-01 (TJE), Minnesota Spinal Cord Injury and Traumatic Brain Injury Research Grant Program (TJE, SBK, CWC, and CCC).

References

Akerboom, J., Calderon, N.C., Tian, L., Wahng, S., Prigge, M., Tolli, J., Gordus, A., Orger, M.B., Severi, K.E., Macklin, J.J., Patel, D., Pulver, S.R., Wardill, T.J., Fischer, E., Schiller, C., Chen, T.W., Sarkisyan, K.S., Marvin, J.S., Bargmann, C.I., Kim, D.S., Kögl, S., Lagno, L., Hegemann, P., Gottschalk, A., Schreiter, E.R., Looger, J.L., 2013. Genetically encoded calcium indicators for multi-color neural activity imaging and combination with optogenetics. Front. Mol. Neurosci. 6. https://doi.org/10.3389/fnmol.2013.00002.

Allen, W.E., Kauvar, I.V., Chen, M.Z., Richman, E.B., Yang, S.J., Chan, K., Gradinaru, V., Deverman, B.E., Lao, J., Deisseroth, K., 2017. Global representations of goal-directed behavior in distinct cell types of mouse neocortex. Neuron 94 (891–907). https://doi.org/10.1016/j.neuron.2017.04.017.

Andermont, M.M., Gilroy, N.A., Goldey, G.J., Sachdev, R.S.N., Wölfel, M., McCormick, D.A., Reid, R.C., Levene, M.J., 2013. Chronic cellular imaging of entire cortical columns in awake mice using microprobes. Neuron 80, 900–913. https://doi.org/10.1016/j.neuron.2013.07.052.

Arieli, A., Grinvald, A., 2002. Optical imaging combined with targeted electrical recordings, microstimulation, or tracer injections. J. Neurosci. Methods 116, 15–28. https://doi.org/10.1016/S0165-0270(02)00022-5.

Asokylakis, V., Badeaux, M., Robarge, S., Batista, A., Kirkpatrick, N., Snuderl, M., Amooghar, Z., Serra, G., Ferraro, G.B., Chatterjee, S., Xu, L., Fukumura, D., Duda, D.G., Jain, R.K., 2017. A cerebellar window for intravital imaging of normal and disease states in mice. Nat. Protoc. 12, 2251–2262. https://doi.org/10.1038/nprot.2017.101.

Attardo, A., Fitzgerald, J.E., Schnitzer, M.J., 2015. Impermanence of dendritic spines in adult live CA1 hippocampus. Nature 523, 592–596. https://doi.org/10.1038/nature14467.

Backes, B.J., Kajdasz, S.T., Christie, R.H., Carter, C., Games, D., Seubert, P., Schenk, D., Hyman, B.T., 2001. Imaging of amyloid β deposits in brains of living mice permits direct observation of clearance of plaques with immunotherapy. Nat. Med. 7, 369–372. https://doi.org/10.1038/nm5525.

Barretto, R.P.J., Schnitzer, M.J., 2012. In vivo optical microendoscopy for imaging cells lying deep within tissue. Cold Spring Harb. Protoc. 7, 1029–1034. https://doi.org/10.1101/pdb.top71464.

Barretto, R.P.J., Messerschmidt, B., Schnitzer, M.J., 2009. In vivo fluorescence microscopy with high-resolution microlenses. Nat. Methods 6, 511–512. https://doi.org/10.1038/nmeth.1339.

Barretto, R.P.J., Ko, T.H., Jung, J.C., Wang, J.T., Capps, G., Waters, A.C., Ziv, Y., Attardo, A., Recht, L., Schnitzer, M.J., 2011. Time-lapse imaging of disease progression in deep brain areas using fluorescence microendoscopy. Nat. Med. 17, 223–229. https://doi.org/10.1038/nm.2292.

Beckmann, L., Zhang, X., Nadkarni, N.A., Cai, Z., Batra, A., Sullivan, D.P., Muller, W.A., Sun, C., Kuruvan, R., Zhang, H.F., 2019. Longitudinal deep-brain imaging in mouse using visible-light optical coherence tomography through chronic microprobes cranial window. Biomed. Opt. Express 10, 5235. https://doi.org/10.1117/11.2005235.

Berry, K.P., Nedivi, E., 2017. Spine dynamics: are they all the same? Neuron 96, 43–55. https://doi.org/10.1016/j.neuron.2017.08.028.

Boccardi, M.E., Jiang, W., Wang, C., Dudaam, J.T., Ji, N., Aponte, Y., 2015. Minimally invasive microendoscopy system for in vivo functional imaging of deep nuclei in the mouse brain. Biomed. Opt. Express 6, 4546. https://doi.org/10.1364/boe.6.004546.

Brown, C.E., Aminotirejat, K., Erb, H., Winsdip, L.R., Murphy, T.H., 2009. In vivo voltage-sensitive dye imaging in adult mice reveals that somatosensory maps lost to stroke are replaced over weeks by new structural and functional circuits with prolonged modes of activation within both the peri-infarct zone and distant sites. J. Neurosci. 29, 1719–1734. https://doi.org/10.1523/JNEUROSCI.4249-08.2009.
