My word

Is our retina really upside down?

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One of the first things we find in textbooks when introducing the vertebrate retina is that it is 'upside down', 'inverted' or 'back to front'. Looking at how the intricate network of photoreceptors, interneurons and finally projection neurons is arranged in the human eye — all alongside a multitude of glia and blood vessels — it is easy to see how we might come to this conclusion (Figure 1). To reach the light-sensitive engines of our eyes, the outer segments of the rods and cones, light must travel through the entire depth of the retina[1]. On this journey, some of the light is scattered and absorbed within the non-photosensitive parts of the retina, deteriorating image quality. To make things worse, on the other side of the retina, the axons of the retinal ganglion cells must somehow exit the eye to reach the brain. The only way for them to do so is to penetrate through the retina, which results in the blind spot, a region of the eye where photoreceptors must give way to the optic nerve head. From an engineer's perspective, these problems could be trivially averted if the retina were the other way round, with photoreceptors facing towards the centre of the eye. Accordingly, the human retina appears to be upside down. However, here we argue that things are perhaps not quite so black and white. Ranging from evolutionary history via neuronal economy to behaviour, there are in fact plenty of reasons why an inverted retinal design might be considered advantageous.

We share our seemingly awkward retinal arrangement with all other vertebrates[2,3], but there are animals that appear to have got it right. Cephalopods[4] (octopus, cuttlefish and squid) have well developed eyes, superficially almost identical to vertebrate eyes. However, a close look at their retina reveals that the photoreceptor cells point the opposite way to those of vertebrates[5,6] (Figure 2). In the cephalopod retina, photoreceptors are arranged with

their light sensitive segment aiming towards the light, and their axons emerging from the back of the eye. This arrangement is called an everted retina, in contrast to the inverted retina of vertebrate eyes.

Among invertebrates there are many more examples of eyes with everted retinas, but also a few more cases of inverted ones[7]. The reasons for one or the other type of retinal orientation goes a long way back to photoreceptor arrangements in the simple light-sensitive spots that evolved before real eyes[7]. The specific reason for our own retinal orientation is the way the nervous system was internalised by invagination of the dorsal epidermis into a neural tube in our pre-chordate ancestors. The epithelial orientation in the neural tube is truly inside out. The vertebrate eye cup develops from a frontal (brain) part of the neural tube, where the receptive parts of any sensory cells naturally project inwards into the lumen of the neural tube (the original outside)[8]. In contrast, the eyes of octopus and other cephalopods develop from cups formed in the skin, and the original epithelial outside keeps its orientation[9].

Accordingly, the early phases of development, which evolved before real eyes, make the retina inverted in some animal groups and everted in others. The question here is whether cephalopods ended up with a lucky draw and the poor vertebrates got stuck with a backwards retinal design. Arguments for a basically flawed orientation of the vertebrate retina are built around an eye that we encounter in a grossly enlarged state compared to its humbler origins. We are now more than 500 million years down the road from where vertebrate vision started in the early Cambrian[10,11]. At this time, highly resolved spatial vision in any animal had only just gotten going. In fact, together with the emergence of increasingly complex locomotor abilities and brains to match up the information, the appearance of spatially resolved visual systems in the Cambrian has been widely credited as one of the pillars supporting the evolution of behaviourally complex animal life on the planet.

Almost inevitably in the evolutionary history of sensory structures, small precedes large. The ancient

forerunners of vertebrate eyes, predating the Cambrian, started out as collections of screened photoreceptors that eventually clustered into eye-cup-shaped arrangements (Figure 3A). Then, gradually, came primitive lenses. In these early eyes there was no vitreous space separating the lens and the retina. This is still the case in ragworms and many other invertebrates. Such an arrangement yields a strongly under-focused image, but an image, nonetheless (Figure 3B). Good enough to guide orientation in relation to major structures in the environment, but inadequate for seeing other animals and interacting with them[11].

Next, building an eye that is approximately in focus requires distancing the light sensitive parts of the photoreceptors from the lens (Figure 3C). This requirement is already initiated in eyes with an inverse orientation of the photoreceptor cells and would have provided a selective advantage for this arrangement: If outer segments face towards the back of the eye, their distance to the centre of the eye is maximised. In this respect, an 'inverted' photoreceptor placement is therefore the better solution in these smallest of eyes.

Beyond basic optics, any extra space that emerged by gradually distancing the photoreceptors from the lens would have been prime neuronal real estate. For the measly price of slightly deteriorating image quality in a probably still moderately under focused eye, budding inverted retinas would have been able to introduce increasingly powerful local circuits immediately downstream of their photoreceptors. It seems plausible that this packing of extra neurons into the eye would have been able to occur largely decoupled from the need to further increase eye size, or indeed of brain size.

In the lineage that would ultimately give rise to all vertebrates, cone-like photoreceptors connected directly to ganglion cells that projected to the rest of the brain[10]. The photoreceptors kept the original orientation, with their light sensitive segments facing the back of the eye. These compact photoreceptor cells remain present in all extant vertebrate eyes where they translate incoming photon flux into synaptic
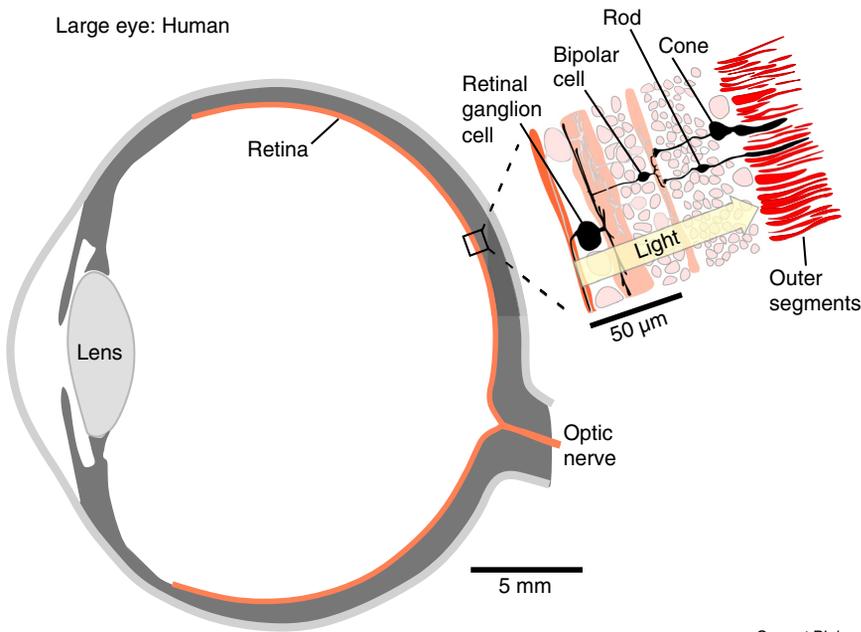
Figure 1. Longitudinal section of the human eye and retina.
The light sensitive outer segments of rod- and cone-photoreceptors (dark red) face away from the light. (Retina schematic modified from Baden *et al.*[2].)

release in a graded manner[10]. Early on, the ganglion cells diversified and gave rise also to local interneurons that have been critical in driving computational finesse of vertebrate eyes[12]. Bipolar cells emerged, slotting in between the cones and the ganglion cells to provide a second synaptic layer right in the sensory periphery. Further finesse came through the addition of horizontal and amacrine cells[10]. The result is a structurally highly stereotyped sheet of neuronal tissue present in all extant vertebrates, of typically at least 100 μm thickness, and sometimes substantially more[3].

Such full-blown retinas are even found in the smallest of extant vertebrate eyes. For example, the 150 μm radius eyes of larval zebrafish pack a whopping 100 μm worth of retina[13], taking up >90% of the total eye volume (Figure 4). At this life stage, about half of this animals' central neurons are in the eyes, rather than the brain[14]! Similar eye sizes also exist in some adult vertebrates, for example in the ~11 mm body length *Danionella cerebrum*[15], or the ~8 mm stout infantfish (*Schindleria brevipinguis*)[16].

To fully understand the merits of the inverted design, we need to consider how visual information is best processed. The highly correlated structure of natural light means that the vast majority of light patterns sampled by eyes are redundant[17]. Using retinal processing, vertebrate eyes manage to discard much of this redundancy, which greatly reduces the amount of information that needs to be transmitted to the brain. This saves colossal amounts of energy and keeps the thickness of the optic nerve in check, which in turn aids eye movements. An intuitive example might come from considering a clear blue sky. Encoding the presence of such a spatially, temporally, and spectrally

uniform scene using a population of visual neurons that are constantly active would be incredibly wasteful. A much more efficient representation is one that accentuates change. In this case, neurons 'looking' at this sky could be silent most of the time, but spring into action if anything were to change[18,19] — for example, upon the sudden appearance of a bird's silhouette. This is where the vertebrate retina truly excels. The extensive local circuitry within the eye — enabled by two thick and densely interconnected synaptic layers, achieves an amazingly efficient, parallel representation of the visual scene[1,12,20]. By the time the signal gets to the ganglion cells that form the optic nerve, spikes are mostly driven by the presence of the unexpected. If there is nothing new to report, the nerve remains essentially silent.

Returning to our central narrative, the intraocular space of vertebrate eyes is an ideal location for such early processing, hinting that the vertebrate retina is in fact cleverly oriented the right way! This is particularly true for the smallest of aquatic vertebrates where retinal neurons fill the intraocular space — the part which in larger species is taken up by the vitreous. Taking larval zebrafish as the best studied example, the somata and axons of their ganglion cells are squished up right against the lens, while on the other end the outer segment of photoreceptors sits neatly inserted into the pigment epithelium that lines the eyeball. Clearly, in these smallest of perfectly functional
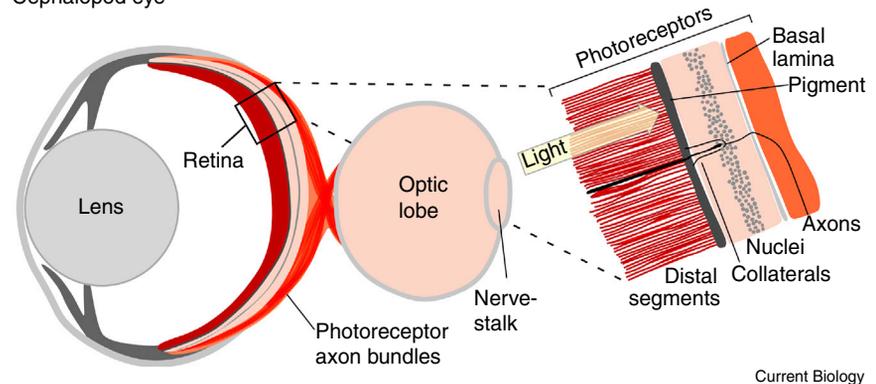

Figure 2. Longitudinal section of an octopus eye, optic lobe, and retina.
The light sensitive outer segments of the photoreceptors (dark red) face towards the light. (Eye cross-section redrawn based on Young[5]; retina schematic redrawn from Anadón[6].)

A First spatial vision: No focus

B Low-quality spatial vision: Under-focused

C High-quality spatial vision: Focused

+Lens

Enlarge

"usable space!"

"primitive" photoreceptor with outer segment and screening pigment
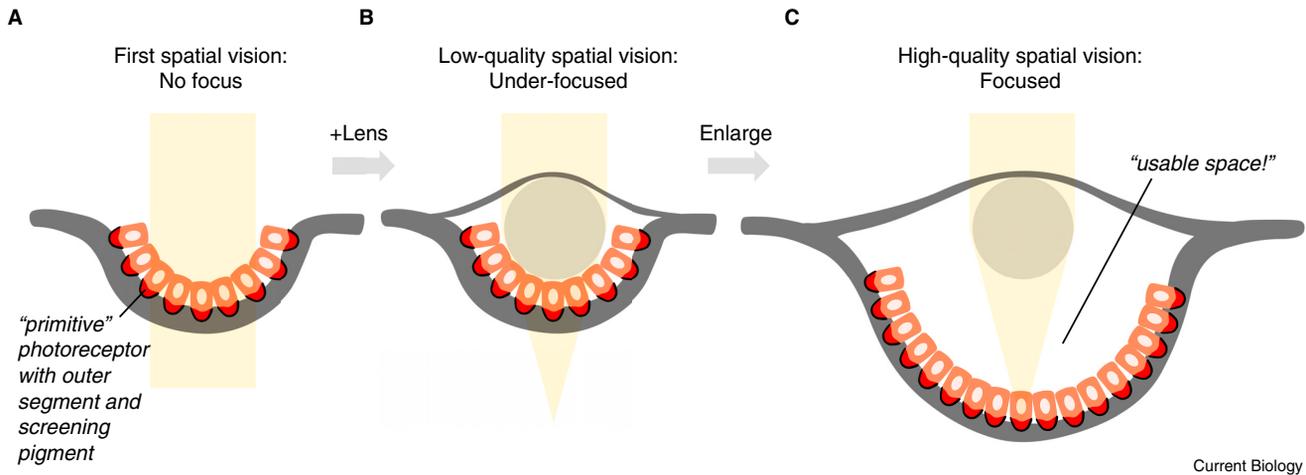
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Figure 3. Evolution of an eye with an 'inverted' retina.
(A) A patch of pigment-screened 'primitive' photoreceptors is beginning to invaginate to form an eye cup allowing for the first rudiments of spatial vision. Light-sensitive parts of the photoreceptors are facing away from the light. (B) The addition of a spherical lens immediately above the eyecup enables low-resolution spatial vision; however, the eye remains severely under-focused. (C) Gradual enlargement of the eye distances the lens from the photoreceptors, improving image resolution. Eventually, the eye will be in focus. At this point, the eye offers much "empty" space between the lens and the photoreceptors. This space could be usefully filled by the addition of neurons that locally pre-process the image picked up by the photoreceptors.

vertebrate eyes, the inverted retina has allowed efficient use of every cubic micron of intraocular real estate.

In contrast, now it is suddenly the cephalopod retina that appears to have an awkward orientation. Developmental constraints mean that any early processing must be housed proximal to the light sensitive layer, while the space between the lens and the light sensitive part of the photoreceptors is left empty and unused even in the smallest of cephalopod eyes. Instead, the neural circuitry for early visual processing must be placed outside the optical part of the eye, thus making the eyeball larger — or the optical part of the eye smaller. For tiny eyes, the everted design wastes extremely valuable space inside the eye whereas the inverted retinal design is a blessing. With this reasoning, cephalopods have an unfortunate retinal orientation and, contrary to the general notion, it is the vertebrate retina that is the right way up.

Does this mean that the general textbook account now ought to be revised? Perhaps not quite! When eyes grow larger, the impact of retinal orientation diminishes. Vertebrate eyes can afford a vitreous body that occupies much of the space inside the eye, and cephalopod eyeballs are only marginally larger from the external layers of nerve plexus and

axon bundles. Both the inverted and the everted principles of retinal design have their advantages and their challenges, or shall we say 'opportunities'. The good points with the vertebrate way of making an eye is that it provides ample space close to the photoreceptors for early visual processing, and in very small animals, the visual system can be made extremely compact. Good points with the cephalopod everted retina are that there is no blind spot in the visual field, and the space in front of the photoreceptors is free of optically compromising neural tissue.

But perhaps the most illuminating and surprising comparisons come from the ways design challenges have been met. Looking out through a layer of neural tissue may seem to be a serious drawback for vertebrate vision. Yet, vertebrates include birds of prey with the most acute vision of any animal[21], and even in general, vertebrate visual acuity is typically limited by the physics of light, and not by retinal imperfections[7]. Likewise, photoreceptor cell bodies, which in vertebrate eyes are also in the way of the retinal image, do not seem to strongly limit visual acuity. Instead, in several lineages, which include species of fish, reptiles and birds, these cell bodies contain oil droplets that improve colour vision[22] and/or

clumps of mitochondria that not only provide energy but also help focus the light onto the photoreceptor outer segments[23].

The other often-noted downside of the 'inverted' retina — the blind spot — is actually not all that bad either. First, compared to the size of the whole retina it tends to be vanishingly small. For example, in humans it takes up less than 1% of the retinal surface[24]. Second, if like in our own eyes the blind spot falls into a region of binocular overlap, the brain can fill in much of the missing information using the signal from the other eye. Moreover, as required eye and/or body movements can ensure suitable sampling of visual scenes despite this nuisance. After all, when is the last time you have felt inconvenienced by your own blind spots?

So, in general, the apparent challenges with an inverted retina seem to have been practically abolished by persistent evolutionary tweaking. In addition, opportunities that come with the inverted retina have been efficiently seized. In terms of performance, vertebrate eyes come close to perfect. Can we say the same about cephalopod eyes? This is not easy to answer because with cephalopod vision, most things are yet unknown[25], and this lack of knowledge may easily be interpreted
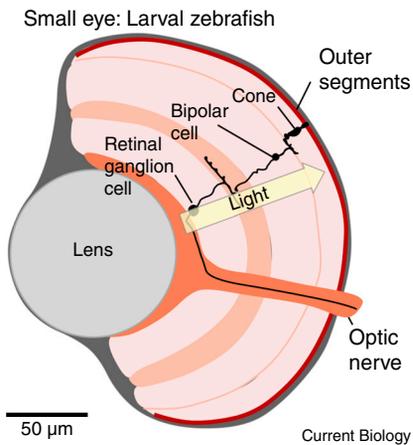
Figure 4. Longitudinal section of a larval zebrafish eye and retina.
As in humans (Figure 1), the light sensitive outer segments of cone-photoreceptors (dark red) face away from the light. However, unlike in humans, in the tiny larval zebrafish eye, the entirety of the space between the lens and the photoreceptors is taken up by the neural retina. (Schematic modified from Baden *et al.*[2].)

in favour of the extremely well studied vertebrates. For anyone who has observed visually guided behaviour in squid or cuttlefish, performance appears in no way inferior to that of fish. A challenge that comes with the everted retina is to find suitable space for early neural processing. The solution seems to have been to make an absolute minimum of early processing in the retina: photoreceptor axons project straight to the optic lobes[26], which lie directly behind the eyes. No other neurons have their cell bodies inside this retina. The only known intra-retinal processes are photoreceptor collaterals, presumably for local processing alongside some putative contributions from glia, and dopaminergic efferent neurons that are thought to play a role in controlling adaptational state[4]. Early reports noted the presence of both 'On' and 'Off' as well as 'fast' and 'slow' responses on the axon bundles connecting the retina to the optic lobe[27,28]; however, their neural substrate has remained unknown for now more than 50 years.

Presumably, cephalopods keep retinal processing to the bare essentials and the photoreceptor cells have to single-handedly code local contrasts through collaterals and convert graded receptor potentials to spike trains. The arrangement implies that each photoreceptor sends an axon to the optic lobe, and there can be no convergence and/or parallelisation onto a smaller number of ganglion cells as in vertebrates. Especially for peripheral vision in larger vertebrate eyes, this convergence is massive and reduces the number of axons in the optic nerve to a tiny fraction of the number of retinal photoreceptors. In cephalopods, any such convergence will have to happen in the optic lobe, which, perhaps for this reason, is attached directly to the back of the eye through a massive chiasm of axon bundles. There is no consolidated optic nerve that facilitates free rotation of the eye. The huge optic lobes are in turn connected to the central brain through a thick neural stalk. How these animals achieve their sophisticated eye movements remains anyone's guess!

In the end, vertebrates and cephalopods embarked on very different routes for acquiring eyes and vision. Retinal photoreceptors became oriented in opposite directions, leading to different advantages and different challenges. Features that may appear awkward were ironed out with the efficiency and ingenuity of evolution. The good use of space in small eyes may have contributed to make early vertebrates more efficient than they would have been with an everted retina. But in general, it is not possible to say that either retinal orientation is superior to the other. It is the notions of right way or wrong way that fails. Our retina is not upside down, unless perhaps when we stand on our head.

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