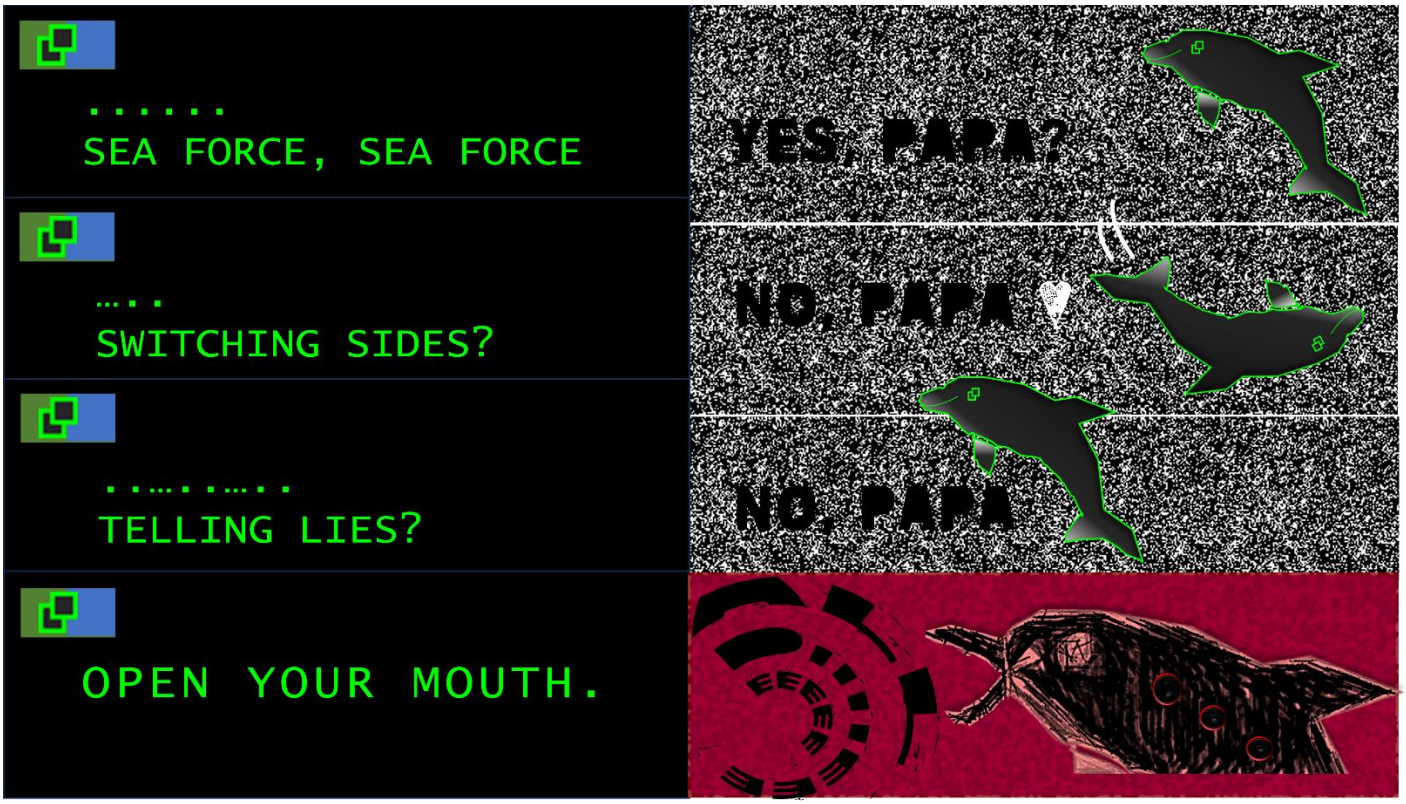
I & Vision Research Centre

Liquid Biopsies, Lies, and Cyborg Swarms
Part 1



Biohybrid Bottlenose Battle Bots: Playfully sadistic cetaceans with semi-autonomous AI controls as catastrophic risk.

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For this very trendy article (AI and roaches and 'drones', oh my), we look at medical and military uses of living and artificial organisms. In part 1 we briefly review recent research in ocular and neurological disease, noting the use of artificial intelligence, micro-views, and microorganisms in biomedical applications and imaging. Later comes the phenomenon of learning, play, and the evolution of strategic deception in animals and machines. As a natural progression, the third section will go over an ancient and advanced type of weaponry: Bugs. Also the occasional dolphin. For your own safety, try to look as un-porpoise-like as possible.

Part 1: Imaging, Molecular Biomarkers, and Machine Learning

The pace of discoveries in neuro and ocular imaging is phenomenal, partly facilitated by advances in computing and machine learning. Motivation to detect early-stage molecular, cellular, or other biomarkers of disease onset remains high for neurological, psychiatric, and age-related ocular conditions. Schizophrenia (Boudriot et al., 2024) is one condition of interest that may be detected through retinal imaging, with the same hoped for Parkinson's (Cannon, 2023) through liquid biopsies and Alzheimer's through hyperspectral imaging (Saeed et al., 2024). Methods that are *in vivo*, subcellular, and non-invasive are often the goal, having the highest value for not just research and prevention but precise medical interventions and outcome monitoring for existing patients. University labs are a major source of innovation, sometimes with several novel techniques included in a single study.

Three-Dimensional Blood Vessel View

A new multiphoton microscopy technique that yields 3-dimensional imaging was developed at University of California San Francisco (UCSF). It was used to uncover the functional role of a protein secreted by a subset of perivascular neurons (Fam19a4/Nts-positive retinal ganglion cells), those that surround developing blood vessels in the eyes of studied newborn mice (Marks, 2024). The protein called PIEZO2 was found to regulate the proper formation of vascular lattices, specifically the upward angle of 'penetrating vessels' near the ganglion cell layer, which act as the physiological groundwork for proper blood circulation in the eyes as well as the cerebellum. Identified through morphological, single-cell transcriptomic, and immunohistochemical analysis, the neurons discovered which are responsible for this cue are similar to and may be a subtype of ON-OFF direction-selective ganglion cell (ooDSGC) (Toma et al., 2024). These neurons and their somata are located uncommonly close to pillar-like penetrating vessel branches, with unique teeny tiny and adorably clingy perisomatic endfeet facilitating direct contact and presumably chemical regulation of vessels.

PIEZO2 is a gene coding for a mechanosensory ion channel that is highly conserved in humans and mice. In the experiment, mice with defects in PIEZO2 production were more vulnerable to stroke type injuries due to disorganised vessel structures, along with having lower capillary perfusion and visual function (Toma et al., 2024). This was despite similar penetrating vessel density as controls, meaning disease vulnerability is attributable to blood vessel contact, angle, and arrangement determined at development and growth stages. The loss of PIEZO2 at P8 'abolished' the perisomatic endfeet of and vascular contact with Fam19a4/Nts-positive RGCs, with later stage breakdown of the blood-retina-barrier (BRB) as well. The team expect human applications to be focused on neurodegenerative diseases, strokes, and conditions such as diabetic retinopathy (Ibid.). Another advantage is the ability to view blood vessels without disturbing the eye as a 'movie', images revolving slowly to display how a vessel lattice is malformed at multiple angles (example in Marks, 2024).

Three-Dimensional Subcellular, Molecular, Neuron Projectome Brain Imaging

You can also watch a mini movie about another 3D technique developed by researchers at the Picower Institute for brain imaging. The samples in this case were donor brains, one of them from an Alzheimer's patient. Although Alzheimer's was an initial application in this proof of concept, authors believe that the method will be useful for other organs, diseases, and even the study of interspecies homologies – the comparison and study of evolutionary relationships between human and animal genes and tissues (Park et al., 2024).

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As described in the university press release, imaging of the intact hemispheres and visibility of synapses were made possible through three new mechanical, chemical, and computing techniques: The Megatome device for thick (4mm) and damage-free tissue slicing, mELAST for slice treating that confers exceptional durability while enabling repeat labelling, and a computational system named UNSLICE that 'seamlessly' reunifies each slice for analysis. Supposedly the detail capture is so fine that individual capillaries and axons align perfectly in the rendered 3D model. High throughput and options for antibody-labelling are other benefits, with this study demonstrating use of 20 (they theorise >100 are possible) and a timeline of 100 hours versus months.

This is intended as a multiscale, multidimensional tool for neuropathology, extracting 'spatial, molecular, morphological, and connectivity information of individual cells' simultaneously from the same brain. In this first case study of the Alzheimer's phenotype, observation of lower cell density compared to control brain and other Alzheimer's brain regions led the team to focus on the orbitofrontal cortex.

Comparing the Alzheimer's brain hemispheres to the control, authors list loss of synapse density, changes to nerve fiber orientations and protein expression, and other morphological features and cell-type distributions. Areas of synaptic density loss coincided with higher distributions of pathology-linked proteins like amyloid-β (Ab), phosphorylated tau (pTau), and activated astrocytes (glial fibrillary acid protein) (Figure 5 in Park et al., 2024, view image extract at 1:47 in the video). In the orbitofrontal cortex, pTau deposits and associated axon swelling were correlated with axonopathy, demyelination, and overall synapse loss. Notably, the angles of pNFP+ cortical fibres (nerve axons) were altered from a mix of parallel, oblique, and orthogonal (~95 degress) to almost all at 80 degrees across the different cortical layers. In penetrating ocular blood vessels of the UCSF study, regular orientation is functional for blood flow. This is not the ideal for cortical neuron types and brain function, hence the cognitive impairment seen in Alzheimer's.

Connectivity mapping facilitated by UNSLICE unveils the projectome and pathological protein associations of each neuron, something typically achieved only through viral labelling and animal modelling. As such, Park et al. (2024) expect this new imaging method to significantly advance understanding of cellular and molecular disease mechanisms in humans.

Indeed, the ability to visualise the internal composition of an individual and their organs has multiple uses. For example, dolphins use the handy skill of echolocation in the practice of 'porpoise pulping', where precise sonic-targeting of the creatures' squishy internal organs is utilised for efficient and effective pulverising (Goldstein, 2006). Less murderously, new combinations and versions of techniques for analysing human ocular diseases include proteomics from samples of patients vitreous or aqueous humour and hyperspectral imaging.

Liquid Biopsy Proteomics + AI

Amacrine cells have been described as 'drug cabinets of the retina', with little known about the 50+ different types of interneuron and their assorted chemical cues in humans (Bates, 2018), although we now have a mouse atlas. That is useful, but animal models and even retinal organoids may not illuminate key factors and influences of human eye disease mechanisms at the cellular level. They are even less likely to uncover any associations with neurological or systemic disease.

To get a better idea of what goes on and wrong in ocular ageing and pathologies, Wolf et al. (2023) used liquid biopsies obtained during surgeries for living patients to link >5,953 proteins to individual cell types (57 ocular plus 15 cell types from the blood, liver, and spleen) and disease states. The process was greatly facilitated by existing databases of single-cell RNA sequencing, permitting a fusion of proteomic and single-cell transciptomic data through a software called TEMPO (Tracing Expression of Multiple Origins). The software identified 1,920 marker proteins, those unique to a specific cell type, and diseases explored were uveitis, retinitis pigmentosa (RP), Parkinson's, and various types of diabetic retinopathy.

Amacrine cells were linked to 224 unique and cell-specific marker proteins, retinal ganglion cells to 114 proteins, 55 from retinal pigment epithelium (RPE) and 42 from glial cells, with lower numbers for other eye cell types. Several hundred markers specific to immune cell types were also found. Overall, they 'observed cell-specific marker proteins with unexpected disease activities' and cell-type rather than tissue-type-dependent clustering of proteins.

Investigating retinitis pigmentosa, a rare inherited retinal disorder that leads to progressive loss of vision in later life, they found a 39% decrease in the 36 marker proteins for rod photoreceptor cells. That was expected. Less so were the reductions in marker protein levels of cone photoreceptors (16%), bipolar cells (14.3%), erythrocytes (11%), vascular endothelial cells (10%), and amacrine cells at 7.6% less of the 224 unique proteins produced by them. Unfortunately further detail was not provided in the paper for RP, but is for uveitis in Figure S2. Given that intervention timing may matter in terms of patient outcomes from gene therapy for RP, figuring out the initial biomarkers of disease progression along with genetic screenings may be useful.

Results for diabetic retinopathy also uncovered a lot. The presence of liver proteins was noted in diabetic retinopathy cases, with this invasive molecule possibly driving inflammation. Next, by virtue of cellular and molecular profiling, proliferative diabetic retinopathy (PDR) was found to be distinct from non-proliferative kinds in that it is largely characterised and possibly driven by high levels of immune cell protein expression. The detected immune system role in ocular disease is similar to the unexpected findings of a study by Sporri et al. (2024) published in June, where functional analysis of the tear proteome revealed 'up-regulation of immune-related pathways in glaucoma patients compared to controls'. While pointing out the role of gut dysbiosis, neuroinflammation, and autoimmunity in glaucoma and intraocular pressure, the authors also found that 123 out of 2550 identified tear proteins differed quantitatively in glaucoma patients, those related to the immune system being enriched. They further described minor differences in the ocular surface microbiome such as an absence of the bacterium *Corynebacterium mastitidis* which is important for local immune response and anti-microbial release against pathogens like *Candida albicans* and *Pseudomonas aeruginosa*.

Wolf et al. (2023) also crafted 'AI proteomic clocks', where data fed into the system uncovered cell-specific ageing profiles for different kinds of disease. According to Cannon (2023) and interviewed authors, the method links anatomical change and disease state to molecular occurences in real time, with potential for early diagnosis of Parkinson's through analysis of proteins in the aqueous humour (AH). Prior, this type of protein profiling for Parkinson's patients was restricted to post-mortem analysis of their brain tissue. As such, there is a lot of room for

not just early detection and risk analysis, but monitoring of therapeutic outcomes via molecular study of immune, stroma, vascular, or retinal cells. Other potential targets for the TEMPO method include cerebrospinal fluid, breast milk, synovial fluid, tumors, and fluid from parts of the lung to study and monitor a wide array of diseases and their cellular characteristics (Wolf et al., 2023). Even better, this is without the requirement of tissue extraction for the biopsy, and with a sensitivity potentially greater than imaging or structural biomarkers (Ibid. p. 4870, 4872).

With advances in non-invasive protein and molecular imaging, gene therapy outcome monitoring and target evaluation could be more rigorous. As an example, a recent study by Liu et al. (2024) suggests that increasing levels of immunoregulatory protein interleukin-1 receptor–associated kinase M (IRAK-M) in the RPE through gene augmentation would be a pathway-agnostic approach for prevention of retinal degeneration and age-related macular degeneration (AMD). Genetic variability in the IRAK3 gene has earlier been linked to AMD risk, but the protein application may apply to all AMD candidates. This is a pretty big deal, as current therapeutics for AMD target specific pathways in the complement protein system, dependent on several factors besides the patient's genetic risk (several reviews listed in earlier article). Liquid biopsies during drug delivery may be a rich data-source for treatment verification, comparison, and insight. Obtaining them from either the vitreous instead or aqueous humour may be possible as Wolf et al. (2023) report that there is substantial protein exchange observable between each compartment, despite anatomical and other barriers.

Hyperspectral Imaging + Deep Learning = 4D Cube

If three-dimensional views are not enough, why not four with the addition of spectral and thus chemical properties? Hyperspectral imaging and analytical software are other additions to the neurological and ophthalmological toolkit, though still hovering at the proof-of-concept stage for detection of retinal vascular diseases, AMD, and Alzheimer's. Still less certain is their applicability for study of pathogenesis, or as a substitute for fluorescein angiography (FA).

There are hints of viability as quick, convenient, *in vivo*, non-invasive but somewhat insensitive snapshots for detection of molecular biomarkers and their spatial visualisation. Plus it's really fun to say 'hypercube', which is how the data is organised. Other current applications and ambitions for the technological principle include hyperspectral satellites to monitor climatic elements and events. As a fancier version and complement to the commercial drones (unmanned aerial vehicles or UAVs) increasingly used by the agricultural industry to manage security and crop diseases, these are able to pick up the chemical-spectral composition of individual trees, mineral deposits, leaking pollutants, and cars – perhaps even you, as an organic blob lurking, slinking across a terrestrial landscape, emitting pollution, disease, and carbon dioxide. Naughty, naughty.

Back to eyecare: Currently, there are few commercial options for retinal imaging with genuinely hyperspectral equipment. A January preprint lists two, besides their proposed snapshot hyperspectral camera in the range of 450 – 700 nm. The hyperspectral assembly is mounted on a retinal camera in this study and mydriatics administered prior. Authors explain that by judiciously selecting and combining particular wavelengths, contrast for disease-specific diagnostic features can be enhanced, with an example given for dry AMD on page 7, although a lot seems to be hoped for in terms of blood vessel imaging too in the limited literature.

If hyperspectral scans can also be combined with existing optical coherence tomography (OCT) procedures and resources, the technology would be more viable and accessible than proteomics or multiphoton microscopy to eyecare clinics that have smaller budgets and laboratory relationships. Also for those with less time to spare, as depending on the method, a retinal imaging procedure can take as little as one second at some cost to spatial resolution (Saeed et al., 2024). Although entirely non-invasive and requiring no major chemical intake or injection for patients as is the case for FA, the method provides location and spectroscopic snapshots of tissue, with a continuous (as opposed to multispectral) range of spectral wavelengths that are differentially absorbed by molecules of interest interacting with light. Of interest would be phosphorylated tau (pS396-tau) and amyloid β for Alzheimer's, drusen subtypes, lipofuscin, and melanofuscin in AMD, AMD-protective factors like lutein and zeaxanthin, and oxy- versus deoxy-haemoglobin levels in retinal vasculature (Ibid.).

The spectral and spatial data is gathered in an aforementioned 'hypercube', allowing 4-dimensional visualisation of individual components, which can be further refined. Improvements to cameras, analytical methods, and computing power – perhaps using deep learning – will determine biomedical and clinical potential. In their review, Saeed et al. (2024) illustrate that a convolutional neural network could be trained on 'accurate ground-truth data' comprised of positron emission tomography (PET), OCT, and OCT- angiography (OCTA) scans to aid in biomarker discovery. They further state that early-stage Alzheimer's diagnosis through hyperspectral imaging is already possible, as phosphorylated tau (pS396-tau) and amyloid β have 'distinct spectral signatures'. Several earlier applications of hyperspectral retinal imaging were indeed focused on a combination with OCT scans for early Alzheimer's diagnosis.

In another study by Wang et al. (2024), glaucoma detection is proposed through algorithmic conversion of fundus images (OCT) to hyperspectral representations. Comparing a variety of wavelengths, they found that use in the range of 610 – 780 nm would be the most accurate and precise for glaucoma classification, with acceptable tradeoffs. Why does this differ so much from the preprint range (Guenot et al., 2024)? The discussion section explains that glaucoma-associated damage of the choroid, RPE, thinning of of the retinal nerve fibre layers and changes to the optic nerve head affect reflectance properties of the retina, making longer wavelengths more informative with a peak signal-to-noise ratio at 680nm. Does this mean that the same type of mathematical checking must be done for each age group and disease type, including distinguishing between non-proliferative and profliferative diabetic retinopathy which are driven by different cell-type activities and protein expression? Probably. New molecular and transcriptomic findings could be used to refine the software training data.

The other lingering question given limited clinical data so far is whether this method of analysis and 4D cubing, whether OCT-to-hyperspectral conversion or direct hyperspectral retinal imaging, is really sensitive enough to pick up early onset of retinal diseases in younger and ageing populations. This at comparative cost and convenience to competing technologies, at a level of precision to facilitate early-enough therapeutic or lifestyle interventions. Or to colourfully convince patients to comply with their eyecare schedule and practitioner recommendations, whichever.

Biohybrid Microrobots in Sensing & Drug Delivery

Not exactly catchy terminology so someone may have to work on it, but this is where it starts to get really dynamic. Scientists are evaluating tech-directed microbiological solutions to biomedical problems like cancer and perhaps soon, ocular disease. Similar to hyperspectral imaging, biomimetic and biohybrid technologies may have roles in environmental monitoring too, several ideas covered in July's International Conference on *Living Machines* held in

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Genoa, Italy. Living templates may be anything from cownose rays (Bianchi et al., 2023) to bees and cockroaches, cardiac and muscle tissue (Webster-Wood et al., 2023), DNA, enzymes, blood cells, and even sperm cells (Li et al., 2022).

The biorobotic option is less familiar, rather complex, and could therefore be generally anxiety-inducing for the public. Despite predictability being a primary goal of current research, biology (in Part II we shall see new generations of artificial intelligence too) is inherently less predictable and wieldable than other sciences. Even chemical products can have unseen as well as unforeseen consequences when applied to biological organs or ecosystems, initiating a string of tragedies in Graeco-Roman myth when employed as weapons (Mayor, 2009; 2022). Other example: The mechanism/s of topical atropine in myopia control are still unclear and the subject of ongoing investigation.

As biology is 'engineered' from the primeval bottom-up and subject to significant environmental constraints affecting genetic survival, it is more complicated and 'clever'. Admittedly sometimes very stupid, but still sometimes very clever. By nature it is adaptive, flexible, interactive, and autonomous to some degree at multiple levels. It can do lots of things with fewer components and controls, often integrated systems and interactive pathways, and traits as well as genetic procedures tend to be pruned to be energetically efficient. Being alive, biological organisms also have the predicament of survival and reproduction, which leads to all kinds of ingenuities in genetic code and programming.

In the case of *Esicheria coli*, organic machinery exhibits a type of strategic optimism. This bacteria has a variety of metabolic protocols in place to respond to and survive during conditions of resource constraint. In a study by Princeton scientists, *E. coli* defied prior assumptions about contextual optimisation. During times of resource constraint, energy was diverted to preparing cellular infrastructure for times of plenty. This allowed the ribosome-prepper bacteria to maximise protein production once carbon input was restored. Pretty clever system for a brainless gut-dweller. Rodent eyes are also emergency-effective and well-designed for efficient information processing. If you recall the direction-selective retinal ganglion cells (DSRGs) from the PIEZO2 study by Toma et al. (2024), only the 'up' kind are motion generalists in low light, perhaps to detect looming predators (Bates, 2018) and initiate rapid escape behaviours.

This subtype of ON-OFF DSRG demonstrate less-effective GABA inhibition and are coupled by gap junctions, which affects light adaptation. Compared to the other 7 types of mouse DSRG cell, this arrangement prioritises general motion detection in threat conditions over direction-discrimination in general conditions, which is pretty darn smart, showcasing why artificial systems can improve through imitating and/or learning from biophysical ones.

Cockroaches are also famously skilled at survival, to the fascination and frustration of pest-controllers. Keep in mind there are around 4600 known species, most of which we never encounter. Sadly these tend to be the prettier ones (images in Bittel, 2016). Anyway, for the urban pest species like German and American cockroaches, some of this resilience is due to their diet – more on that later – respiratory systems made up of miraculous spiracles, less miraculous (for non-human species) virgin births, and abilities to transmit various kinds of chemical and anti-microbial resistance within and across colonies. The cockroach genome also keeps biotic and abiotic hazards at bay through random genetic mutations. Recently the German cockroach served as a model for a new gene editing technique called Direct Parental CRISPR (DIPA-CRISPR), which simplifies the engineering of genomes for study and could apply to research on other insects including 'agricultural and medical pests'. One restriction is that the method may only work in species that undergo vitellogenesis, so fruit flies and aphids are out.

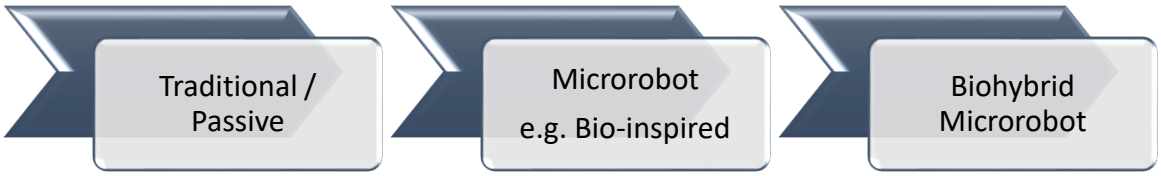
Due to the entanglement of multiple systems in a single organism, biological elements and strategies are also more agile and robust than a typical engineered entity. The ability to heal and overcome random environmental obstacles or resource constraints are some key advantages, and why insects are a major source of locomotion ideas for

robotics. Further, invertebrate tissues are more metabolically efficient and much less finicky than that of mammalian or other species in terms of maintanence and cultivation (Webster-Wood et al., 2023). When a living organism – microscopic or otherwise – is the scaffold for a robotically enhanced or directed system, this confers biological advantages such as their evolved nanomotors, tissue efficiency and repair, and adaptability (Ibid.; Weibel et al., 2005). While some may find disorder distasteful, or feel that random is a form resistance meant to be culled, those attributes can be positives and function as survival opportunities in conditions of uncertainty, lack of data, or the reduced possibility for constant central command/control.

Now let's briefly consider the subset of 'microbial biohybrids', which are itsy bitsy organisms fused to micro- or nanoscale therapeutic loads or sensors. Ophthalmology and several other fields are already making significant use of viruses in gene therapy, but these are technically non-living along with the bulk of nanotechnologies for ocular drug delivery (Taylor, 2023). Making things really, really small alters the nature of matter in terms of magnetic and chemical properties too, imparting useful characteristics to traditional and novel pharmaceutical products (Ibid.).

Living biohybrids would include single-cell bacteria, fungi, and algae suited for a variety of biomedical purposes, paired and directed with synthetic elements to fulfil those roles (Webster-Wood et al., 2023). *Esicheria coli* is a popular experimental subject, displaying high motility and chemical sensing capabilities. Natural chemical, magnetic, and other gradients are useful ways to coordinate the movement of thousands of brainless microbes (Ibid.), and scientists can take this further by attaching extra components such as magnetic particles or dissolvable drug delivery liposomes for steering and dropoff. In 2022, the possibility of this was demonstrated by researchers at Max Planck Institute using E. Coli for drug delivery at cancerous sites, steering with magnets and the natural swimming, navigation, and chemical-seeking skills of the bacteria.

Referred to as 'micro-oxen' in a 2005 study by Weibel et al. (there are very cute videos of them moving in the 'Supporting Information' section of the paper), algae are also of interest because of their tendency to just keep swimming and ability to conduct evasive manoeuvres against alveolar macrophages, although the poor dears may have a weight limit in terms of nanoparticle payloads. Drug input was therefore kept to 25 micrograms in Zhang et al. (2024). For lung cancer, green micro-algae sprinkled with blood-covered nanoparticles (as a nifty membrane and for biological stealth and shielding, not for the purposes of psychological warfare) were used as carriers for superior chemotherapeutic distribution, showing greater effects and patient survival than alternative delivery methods. Back in 2022, a similar microalgae-borne drug delivery strategy was employed for treatment of bacterial pneumonia, with success in mouse models.



Drug Delivery Options. Higher therapeutic efficacy for cancer drugs has been achieved in experiments with biohybrid microrobots versus other delivery methods.

Now what do blood, chocolate, and the aqueous and vitreous humour have in common? They are all delicious and nutritious mediums for micro-organisms (Li et al., 2020). The ocular nerves, vascular diseases, and ophthalmic injections or surgical procedures are routes to introduce or increase pathogens in various parts of the eye. In line

with proteomic study, metagenomic sequencing methods have also shown that each ocular disease may be associated with a unique array of not just proteins but intraocular microbiota (Ibid.).

While it seems no biohybrid microbots have yet been employed for management of ocular diseases, considering the role of the immune system, gut, and perhaps ocular surface proteome in various eye diseases, it may not be far-fetched to expect something of the sort in future. Oral and human gut microbiota seem to be involved in uveitis and glaucoma as well as macular degeneration, with probiotics being evaluated as treatments for several types of ocular disease, neatly side-stepping the issue of growing muti-drug resistance in pathogenic bacteria (Chiang & Chern, 2022). Even cockroach stomach biotics may be a potential resource for human therapeutics (Siddiqui et al., 2023). As demonstrated in the summarised studies above, clever software and existing genetic and biomedical databases can be used to streamline the process of microbial candidate short-listing and testing. A terminator version of *C. mastitidis*, perhaps? Roaming, ravenous carnivorous cyborg bacteria to control gut dysbiosis? Probably no, don't do that. Or at least run a simulation first.

The year is 2024.

Scientists drop communication and counter-pathogen combat kits into the dense molecular jungles of the ocular surface microbiome.

Tragically, the forces of commensal *C. mastitidis* do not pass physical requirements for load-bearing.

Image: Magic Studio AI Art Generator

The various barriers between blood, retina, and brain are a long-standing issue, but these may be breaking down during disease e.g. diabetic retinopathy (Wolf et al., 2023), inflammation (Li et al., 2020), or dysfunctional expression of PIEZO2 (Toma et al., 2024), yielding wider opportunities for drug delivery whether by nanotech or microbe. The parasite *toxoplasmosis gondii* was recently used for therapeutic protein delivery to neurons in the brain, but it would be up to a pharmaceutical marketing team to decide how to spin the prospect of cat-borne microscopic vermin with 'kiss-and-spit' or intracellular delivery mechanisms as the hot new therapeutic. Somehow it sounds more palatable than an autonomous-learning 'slime-like robot that can seamlessly change its shape to squeeze through narrow spaces, which could be deployed inside the human body to remove an unwanted item'.

Authors of the *toxoplasmosis* study (Bracha et al., 2024) point out that this method is less constrained in terms of protein delivery range than typical viral vectors, and were able to use the injected parasite for delivery MeCP2

protein, treating a mouse model of Rett syndrome. This implies some viability as an addition to the current viral and nanotech non-viral gene therapy methods. Given the tendency of *toxoplasmosis* to navigate to and infect ocular cells and tissue (Fell & Mammo, 2023), there could be some potential as a drug or protein delivery vector for ocular diseases, vascular, genetic, or otherwise.

Part 1 Conclusion & Practical Considerations

The ability to visualise pathogenic pathways and molecular mechanisms could be revolutionary not just for research and therapeutics, but also science student and patient education. It seems odd that the value of these new types of diagnostic media as communication tools is not emphasised slightly more, but that may come further along down the project timelines. Another pattern is that nowadays, even before getting to the research and experimental stage there may be an AI component. A recent article in *Nature* summarised the possible ways AI can be applied to clinical trials, while highlighting the associated challenges including bias, an all-too-human replication crisis, lack of transparency due to algorithmic complexity leaving researchers dependent on results, and data security risks (Hutson, 2024). Besides that are the ethical and professional issues of whether and how to qualify an AI product for biomedical research and use.

Cutting-edge research with micro-view movies and colour-coded diagrams are one thing, clinical translation is another. Legal challenges to the new technological tangents of medicine and security are burgeoning, along with risks to privacy, professional conduct or culpability, cyberthreats, and bias. The issue of bias given the lack of education about AI and the hype to sell and use it is significant for most professional endeavours. It has already been shown that people are more likely to trust an AI's opinion on who is a liar over their own, leading to more false accusations especially in cohorts that chose to use an AI assistant for the task (van Paridon, 2024). In medicine, certain deployed AI products, some of which are purpose-built, have already harmed patients through erroneous diagnoses and socially naïve advice. The programmes even biased the judgment of experienced radiologists, leading to incorrect and harmful human decisions on the basis of that output (Spichak, 2023).

Despite potential benefits to workflow and analysis of increasingly complex patient and molecular data, physicians are being warned by professional medical associations, and in turn are also warning others not to rely too much on AI tools in diagnosis and treatment. Is this a parallel to cyborg insects like RoboRoach developing neuropsychological resistance to their robot overlord locomotion controls (Webster-Wood et al., 2023)? No, not really, but that's a suitable lead-in to part 2.

Continued in Part 2 and 3 with elaborations on the especial moral turpitude of dolphins, the craftiness of new generation AI, poison kings, and biological warfare (animals and swarms). A full list of the main references will be added to the end of the full article, with select for Part 1 below.

Disclaimer: The material presented is for informational and entertainment purposes only, in summary of recent news and events. It neither reflects the views nor constitutes professional advice of the organisation.

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