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Necessity of Bio-imaging Hybrid Approaches Accelerating Drug Discovery Process (Mini-Review)

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ABSTRACT

Imaging technologies have made a significant improvement in the past few decades and their application made a great impact on accelerating the process of drug discovery and development. The ability to non-invasively image an animal model or co-cultured live cells and validate potential drug target, biomarkers of drug efficacy and assess a pharmacological drug interaction significantly contributes to the process of translating molecules into medicines. This paper summarizes current trends in bio-imaging technologies and their application on the process of drug discovery. In particular, High Content Screening (HCS) and Virtual Screening (VS) are reviewed, and their respective examples are discussed to gain insight into state-of-the-art bio-imaging methodologies used for extracting knowledge and its application to drug discovery. This paper argues the need to reduce the gap between experimental (e.g. HCS based assays) and theoretical (e.g. VS based assays) assays. Although HCS and VS are leading drug discovery choices for the pharmaceutical industry and such investigations have been carried out in their respective campaign, the potential effects of these approaches together to facilitate the process of drug discovery has rarely been reported. Further, the prevalent research trends on developing hybrid approaches such as VS complementing HCS implies substantial enhancement to the goal of reliable drug candidate identification.

Keywords

Bio-imaging, drug discovery, high-content screening, virtual screening

1. INTRODUCTION

It is critical for the pharmaceutical industry to refine streamline processes which early identify the candidate molecule for further clinical development. Early pivotal stages of drug-screening and preclinical testing, accelerate speed and quality of decision-making, improve efficiency, provide accurate predictivity for clinical experimentation and can save a lot of money. Imaging techniques (ITs) used in drug discovery platforms uniquely provide quantitative information, accurately assessing every part of the process. The focus of this review is to present the imaging techniques used in drug discovery stage which have made a significant step beyond prevailing methods of digital imaging. The advantages of High Content Screening (HCS) and Virtual Screening (VS), in their individual campaigns, as well as approaches developed from their combination are creating new knowledge from a massive number of throughputs without extensive human interaction fundamentally changed the concept of drug discovery.
The paper organization is as follows: In the next section 2, a brief description of drug discovery and development multilayer process is presented. Section 3 is a review, describing the multiplicity of digital imaging applied through all stages. A detailed representation of the wide scope and complexity of HCS and VS technologies in drug discovery process with examples, is made in section 4. This leads to a discussion on the attributes of HCS and VS for drug discovery, which is presented in section 5 and finally section 6 presents with concluding remarks and furture trend in drug discovery.

2. OVERVIEW

Generally, for approval of a new drug from beginning takes more than 10 years, making the drug development process a lengthy, high-risk, and costly endeavor. Furthermore, in 2013, among more than 5000 medicines in development, less than 1% of those were approved by the Food and Drug Administration (FDA) [3]. The selection of promising drug candidates is critical in the early phase of successful drug development. Advances in imaging techniques both from hardware and software perspectives, are making their contributions at different stages of the drug discovery and development process. A typical drug discovery and development process consists of five stages (shown in Figure 1). 1. Target selection, 2. Drug discovery, 3. Drug development, 4. Drug approval and 5. Clinical use. In the following section, a brief review of imaging techniques for drug discovery is described.

3. REVIEW OF THE IMAGING TECHNIQUES IN DRUG DISCOVERY

Imaging sciences have grown exponentially during the past three decades, and many techniques, such as magnetic resonance imaging, nuclear tomographic imaging, and

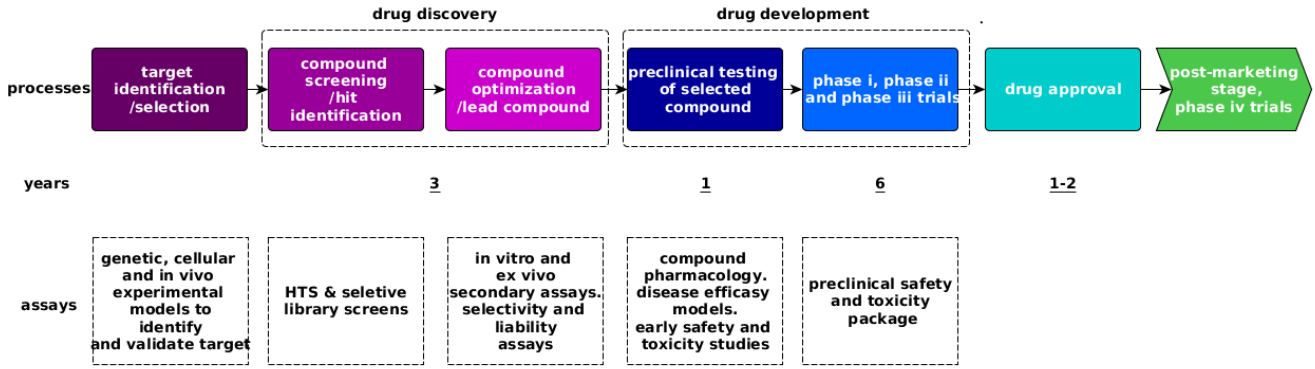


Fig. 1: Top Row: An illustration of stages involved in Drug Discovery and Development process. Middle Row: Number of years (approx.) needed for different stages of the drug discovery and development lifecycle. Bottom Row: Types of assays involved at different stages.

X-ray computed tomography have become indispensable in clinical use. Following sections present a brief review of some of the imaging techniques for different stages of drug discovery.

3.1 Target identification

Advantages of imaging technology advances can be explored to identify new targets [41, 50] at the earliest stage of drug discovery and development process. For example, Drevets et. Al. in [12] could find lower metabolic activity and decreased cortical volume in patients with bipolar and unipolar depression by imaging the medial prefrontal cortex (mPFC). They measured the brain activity from rate of glucose metabolism and positron emission tomographic (PET) images of cerebral blood flow. An extended study of this work can be found [13].

3.2 Drug discovery

A brief review of the imaging techniques used in sub-phases namely compound screening and compound optimization for drug discovery are presented.

3.2.1 Compound screening. The compound screening phase aims to find the 'hit' molecule. For this purpose, a plethora of compound screening assays are developed such as cell-based assay [13], virtual screening [6, 35, 28] etc.
Fluorescent-imaging Plate Reader (FLIPR)

The fluorescent-imaging plate reader (FLIPR) utilizes the charge-coupled device, imaging of the whole plate and captures the fluorescent readouts. [43]. FLIPR enables functional screening of the largest membrane proteins in the human genome, G protein-coupled receptors (GPCRs). FLIPR enables functional screening of the largest membrane proteins in the human genome, G protein-coupled receptors (GPCRs). FLIPR is sensitive, homogenous, amenable to automation but cannot be used for inverse agonist screens, and suffers from fluorescence quenching [46].
High-through Put Screening (HTS)/High-content screening (HCS)
High content screening is an automated imaging approach which consists of both the acquisition and analysis of digital images in a multi-well microtiter plate with and without other substrates. High content screening differs from high-throughput screening in regards to its capability, to simultaneously monitor multiple phenotypes. On the other hand, HTS measures a signal averaged over all cells within a microplate well. Hence, HCS provides deeper insights into biological processes [29]. With technological advancements in imaging, fast automated microscopes capable of auto-focusing and sample positioning acquire high-resolution images. Integrated software platforms coupled with the automated microscope are running the analysis by extracting quantitative measurements at the pixel level from acquired digital images in an unbiased manner. This multiparametric quantitative data is a result of the algorithmic extraction of number, size, texture, fluorescent distribution, fluorescent intensity changes per pixel, the spatial distribution of objects, statistical analysis, application of deep learning methods to detect unusual cell morphologies and network access to databases via commercial or open source components [39]. Besides the technological advancements, development in HCS assays highly depends on physiologically relevant models which include – primary cells, engineered cell lines, 3D-cell cultures and whole organisms. The pharmaceutical industry has been implementing HCS technology in all stages of contemporary drug discovery, and it is considered as a mainstream technology [51]. The process results are optimized, by increasing target confidence, decreasing the time taken for screening drug libraries, reducing the number of animals in experiments, better exclusion criteria, reproducible endpoints and a better understanding of preclinical pharmacology. *Virtual screening*
Virtual Screening (VS) approaches provide the possibilities to process molecules that are physically non-existent in an investigators collection and can readily acquire through purchase or synthesis. [4]. Thus, making this type of compound screening time and cost-effective as compared to alternative compound screening techniques. Based on the target or existing ligands information, VS can be divided into two approaches; structure-based

VS (SBVS) and ligand-based VS (LBVS). SBVS aims to understand the molecular basis of a disease by using the known three-dimensional (3D) structure of a biological target in the process. The available protein (3D)structure of interest and a synthesized compound library of small molecules is investigated by docking into the active site of the biochemical target using computer algorithms and scoring [48, 17, 22]. LBVS explores biological data to identify known active or inactive compounds form biological data to retrieve other potentially active molecular scaffolds based on similarity measures such as common descriptor values. A combination of these two approaches has also been proposed previously [42].
Recently, SBVS has been proven to be more effective than the other traditional ways of drug discovery [30]. In SBVS, a 3D structure of the target for processing is obtained from imaging techniques such as X-ray, NMR or neutron scattering spectroscopy, besides homology modeling, or from Molecular Dynamics (MD) simulations. However, SBVS approaches are prone to shortcomings such as tools developed for specific cases, struggle with very potent leads, unable to perform in congeneric series etc., making its use in drug discovery debatable.

3.2.2 Compound optimization. High attrition rate of compounds entering the clinical phase implies that academic-industry partnerships could really add value preclinically and this eventually could help bring more effective drugs to patients. For lead optimization, techniques such as hit evaluation [7], (Bio)isosteric replacements [18] and hit fragmentation [44] could be used. However, these techniques are beyond the scope of this review and will not be discussed further.

3.3 Drug development

Non-invasive molecular imaging is making a great contribution towards chronic investigational animal models, to image the same animal at different stages of disease progression, and extract data for the consistency of drug effect and safety profile over a long period of treatment.
For use of imaging, to be considered reliable in drug development, it needs to be robust, quantitative and easy to implement across multiple centers which poses a major challenge. For data acquisition and analysis including applications for small animals, a variety of imaging modalities are available such as Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT), optical imaging, Magnetic Resonance Imaging/Spectroscopy (MRI/MRS), ultrasound and computed tomography (CT).
Using PET by [5] and SPECT by [27], promising results for drug development have been reported with a premise of the translation of preclinical studies into clinical applications. Different imaging modalities give complimentary rather than competitive results, and the choice of appropriate imaging technique primarily depends on the specific question to be addressed. It can be concluded that a combination of different imaging modalities holds great promise.

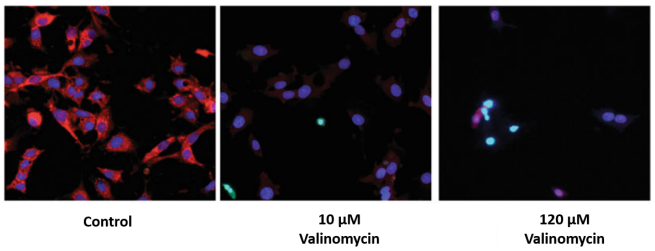
4. HCS AND VS ASSAYS IN DRUG DISCOVERY

This section discusses the impact of imaging techniques on drug discovery with the aid of recently reported examples; an HCS assay example reported and VS assay example from [24].

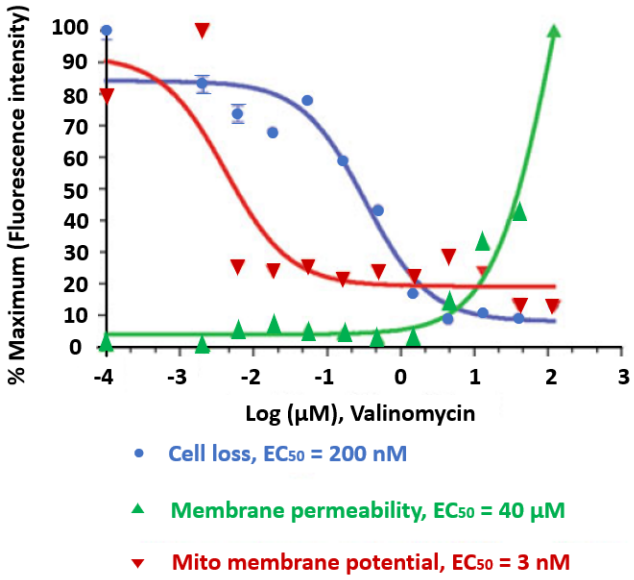
4.1 HCS Assay

High content screening is effectively used for multiparametric measurement of early cytotoxicity testing and cell health assessment, during the drug discovery process. Upon treatment, drugs are mainly metabolized by the liver or in other words, enzymatically converted into less active or inactive compounds. Water-soluble drug metabolites are then easily excreted by the kidney [40]. Some of the routinely screened biomarkers in drug-induced liver injury and toxicity are mitochondrial dysfunctions, plasma membrane permeability, oxidative stress, accumulation of lipids in lysosomes and defects in lipid metabolism. In an HCS assay, depolarization of mitochondrial potential in living cells is possible by using MitoTrackers organic dyes [32, 38, 21]. These fluorescent dyes, stain mitochondria in live cells and their accumulation are dependent on membrane potential and measure membrane dysfunction. This physiological parameter is in correlation with cells' capacity to produce ATP (adenosine triphosphate) and deal with oxidative stress.

A multiparametric HCS assay demonstrating mitochondrial health and cytotoxicity is shown in (Figure 2a). In the experiment, human liver cancer cell line Hep G2, displaying robust morphological and functional differentiation is chosen as a suitable model for 'in vitro' studies [36]. Cells are stained with three different dyes, to detect in detail the processes that occur after being treated with 10μM and 120μM dose of Valinomycin for 24h. Valinomycin is an ionophore which can destroy the electrochemical gradients of membranes and lead to cell death. The Image-iT® DEAD Green^{TM} fluorescent dye is permeant for cells which plasma membranes are compromised and does not affect healthy cells. MitoHealth stain in red and is a reagent that accumulates in active mitochondria thereby the signal decreases when mitochondrial membrane depolarises. The third signal in blue is from Hoechst nuclear stain which gives signal from the nuclei of cells that has not lost their integrity and intensity decline, measuring the quantity of cell loss.
Control cells not treated with Valinomycin are shown on the left side of panel A in (Figure 2a) with intact plasma membranes and strong visualisation of their active mitochondria in red and functional nuclei in blue. The administration of 10 μM Valinomycin, rapidly changes the fluorogenic excitation with almost complete loss of active mitochondria, a weak signal of compromised plasma membranes and small number of lost cells. When 120 μM Valinomycin are administered the level of cytotoxicity increases and the excitation from mitochondria, and nuclei significantly decrease while excitation from compromised plasma membrane increases. After data analysis, according to fluorescent intensity changes per pixel, the half-maximal dose response (EC50), can be calculated with a great accuracy for each biomarker. The HCS in vitro fluorescence-based method is routinely used for early cytotoxicity testing in



(a) Representative images showing the fluorescent excitation from cells in the control well (left), cells treated with 10 μM Valinomycin (middle) and cells treated with 120 μM Valinomycin (right).



(b) Dose-response curves, showing the change in the intensity of fluorescent excitation according to the concentration of Valinomycin. Imaging and analysis of the experiment was done on a Thermo Scientific Cellomics® ArrayScan® VTI [34].

Fig. 2: A multiparametric HCS assay demonstrating mitochondrial health and cytotoxicity.

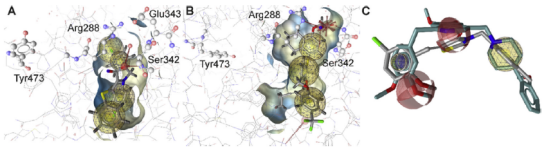
order to predict which chemical entities should proceed in drug development stage.

4.2 VS assay

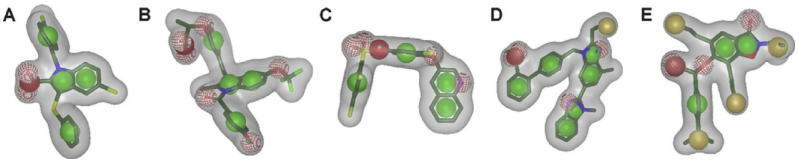
To elaborate the virtual screening process for drug discovery, authors of [25, 24] evaluated common virtual screening tool, which is used to identify novel bioactive molecules for cyclooxygenases-1 and -2 as representatives of classical enzymes [25] and to identify novel peroxisome proliferator-activated receptor (PPARγ) ligands [24]. PPARγ belongs to nuclear receptor class, and is a valuable drug target. These upon activation, form heterodimers with the retinoid X receptor (RXR) that regulates the expression of genes involved in adipogenesis, lipid homeostasis, and glucose metabolism [8]. Research on these receptors focuses on the discovery of novel partial agonists since full activation of the nuclear receptor adds to unwanted side effects. An example of such case is evaluated by [24] which by employing pharmacophore, shape-based virtual screening and docking, independently and in parallel to identify novel PPARγ ligands. These models are briefly explained to elaborate its effectiveness in the drug discovery. For all three models, virtual screening of the commercial Maybridge database[1] was used.

For pharmacophore modeling, 10 models from five Protein Data Bank (PDB) entries (2Q5S, 3H0A, 2Q5P, 3FUR, and 3QT0), and one additional ligand-based model generated with LigandScout 3.1 [24], were used. Three optimized

[1]www.maybridge.com



(a) A. based on the crystal structure of PPARγ in complex with the partial agonist nTZDpa (PDB entry 2Q5S). B. based on the crystal structure of tetrahydronaphthalene derivative 1 in complex with PPARγ (PDB-entry 3H0A) C. ligand-based model generated with the known partial agonists GQ-16 2 and PA-082 3.



(b) The models were generated with (A) nTZDpa (PDB-entry 2Q5S), (B) MRL24 (PDB-entry 2Q5P), (C) INT131 (PDB-entry 3FUR), (D) telmisartan (PDB-entry 3VN2), and (E) one low-energy conformation of the known partial agonist isoxazolone derivative 4.

Fig. 3: a) Pharmacophore models for PPARγ partial agonists. b) Shape-based models for PPARγ partial agonists [24].

models were selected as shown in Figure 3a(A-C), based on their ability to find the majority of compounds in the "partial agonist" dataset. In the prospective screening of the commercial Maybridge database (52,000 entries), 9231 unique compounds mapped at least one of the models and virtual hits were ranked by their relative geometric pharmacophore fit score [24].

Secondly, employing shape-based modelling, 50 models using vROCS 3.0.0 tool [2] were generated and a selection of best performing models were chosen. The co-crystallized ligands of PPARγ -compound complexes were selected for model generation, as they describe the biologically relevant conformations. It also contains a model based on, one low-energy conformation of the known partial agonist, isoxazolone derivative 4, which has identified most of the compounds in the "partial agonist" dataset. The final shape-based models are depicted in Figure 3b (a-e). Color features were added to refine the shape models: green sphere, ring feature; red sphere, anion; blue sphere, cation; yellow sphere, hydrophobic; red mesh, hydrogen bond acceptors (HBA). For all virtual hits, the relative ComboScore calculated from shape and color features, was subsequently used to rank all mapping compounds. Table 1 also known as prediction matrix, contains list of selected compounds and their relative ComboScores.

Further, docking process, that contributes to predicting both the strength and type of signal produced, was generated with GOLD v5.2 [23, 1] tool, using the eight crystal structures 2Q5S, 2Q5P, 2Q6S, 2YFE, 3FUR, 3V9Y, 4A4V, and 4A4W [24]. As a result 809 unique compounds were docked into the binding site of PPARγ with a GoldScore of $\geq$124.0. Virtual hits were ranked by their GoldScore (shown in Table 1). Top 10 compounds from virtual hit ranking list for each of the three models are further investigated. Overall hit list obtained from the three models contains 29 unique compounds as shown in Table 1. All these compounds are subject to further investigation with the external bioactivity profiling tools such as SEA [26], PASS [16] and PharmMapper [31] (shown in Table 1). Lastly, biological testing could confirm the binding of nine out of the 29 selected test compounds.

5. DISCUSSION

HTS/HCS and VS are conceptually different (experimental vs theoretical) but widely used for lead compound discovery. On the one hand, both strategies have their advantages and limitations when employed individually(summary presented in Table 2). On the other hand, a combined approach can impact favorably on lead compound discovery due to synergies between VS and HTS technology. This section briefly discusses the potential impact of VS-HTS/HCS approaches. One of the major challenges for HTS/HCS is the need for a large and diverse source of compounds. This particularly affects smaller pharmaceutical companies. VS methods such as presented by an example in section 4.2, showed the ability for compounds selection. Such utility of VS techniques provides augmentation of in-house compound databases. Furthermore, the availability of quality compound libraries can benefit HTS/HCS before the screening. This can be achieved by compound filtering, to enrich libraries with molecules that have preferred properties. Therefore, it is vital that the compound filtering 4.2) achieved by VS methods, is applied as a 'front-end' technique, before screening such as reported in [10, 49]. HTS/HCS assays are prone to errors, both random errors,

Table 1. : Prediction matrix for overall hit list. Values obtained in the top-ten hit lists of the respective methods are highlighted in bold.

	Name	LigandScout[a]	ROCS[b]	GOLD[c]	SEA[d]	PASS[e]	PharmMapper[f]	Activity[m]
Top-ranked pharmacophore modelling hits	**Compound 5**	**0.97[h]**	**-**	**-**	**-**	**-**	**-**	**-**
	Compound 6[g]	**0.97[h]**	**1.215[j]**	**-**	**-**	**-**	**-**	**-**
	Compound 7	**0.97[h]**	**-**	**-**	**-**	**-**	**-**	**-**
	Compound 8	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**-**
	Compound 9	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**+**
	Compound 10	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**+**
	Compound 11	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**-**
	Compound 12	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**+**
	Compound 13	**0.96[h]**	**-**	**-**	**-**	**-**	**-**	**-**
	Compound 14	**0.95[i]**	**-**	**-**	**-**	**-**	**-**	**-**
Top-ranked shape-based modelling hits	**Compound 15**	**-**	**1.265[k]**	**-**	**-**	**-**	**0.607**	**+**
	Compound 16	**-**	**1.254[j]**	**-**	**-**	**-**	**-**	**-**
	Compound 17	**-**	**1.251[j]**	**-**	**-**	**-**	**-**	**-**
	Compound 18	**-**	**1.233[l]**	**-**	**-**	**-**	**-**	**+**
	Compound 19	**0.93[h]**	**1.217[l]**	**-**	**-**	**-**	**-**	**-**
	Compound 20	**-**	**1.198[j]**	**-**	**-**	**-**	**-**	**-**
	Compound 21	**-**	**1.196[l]**	**-**	**-**	**-**	**-**	**-**
	Compound 22	**0.93[h]**	**1.192[j]**	**127.019**	**-**	**-**	**-**	**-**
	Compound 23	**-**	**1.189[k]**	**-**	**-**	**-**	**-**	**-**
	Compound 24	**-**	**-**	**146.089**	**9.93eˆ-4**	**-**	**-**	**+**
Top-ranked docking hits	**Compound 25**	**-**	**-**	**144.178**	**-**	**-**	**0.634**	**-**
	Compound 26	**-**	**-**	**141.653**	**-**	**-**	**-**	**+**
	Compound 27	**-**	**-**	**141.154**	**-**	**-**	**-**	**-**
	Compound 28	**-**	**1.011[l]**	**140.461**	**-**	**-**	**-**	**-**
	Compound 29	**-**	**-**	**139.719**	**-**	**-**	**-**	**+**
	Compound 30	**0.93[h]**	**-**	**139.554**	**-**	**-**	**-**	**-**
	Compound 31	**-**	**-**	**138.331**	**-**	**-**	**-**	**-**
	Compound 32	**-**	**-**	**37.578**	**-**	**-**	**-**	**+**
	Compound 33	**-**	**-**	**136.966**	**-**	**-**	**-**	**-**

[a] Only highest relative pharmacophore fit score is listed for every compound, high values are desirable.
[b] Only highest relative ComboScore is listed for every compound, high values are desirable.
[c] Only highest GoldScore is listed for every compound, high values are desirable.
[d] Only lowest E-value below the activity cut-off is listed for every compound, low values are desirable.
[e] Only Pa values above activity cut-off are listed, high values are desirable.
[f] Highest relative pharmacophore fit score retrieved with a model with at least 6 features, high values are desirable.
[g] Consensus hit ranked in the top-ten of both the pharmacophore- and shape-based modeling hit list.
[h] Identified with model pm-2q5s.
[i] Identified with model pm-3h0a.
[j] Identified with model shape-2q5s.
[k] Identified with model shape-3vn2.
[l] Identified with model shape-3fur.
[m] + active in the biological testing, - inactive in the biological testing.

such as noise, and systematic errors that are associated with consistent or over-underestimated activity across the screening collection [11, 14]. This has also been reflected by examining the examples discussed in section 4.1. To mitigate this limitation, VS can play key role in extraction of knowledge from HTS experiments and the data derivation that is required for predictive models of activity for database mining. Finally, the compound reusability factor is a major HTS/HCS issue. Nevertheless, in section 4.2, VS techniques allowed to analyze the known crystal structure. Such VS techniques allow the creation of compound subsets. These compound subsets are biased towards the target

Table 2. : Summary of relavant VS and HTS factors

	Type	Effectiveness	Integration and automation	Cost	Drug target knowledge
HTS/HCS	**Experimental, testing as may compounds as possible**	**depends on compounds screened**	**HTS/HCS benefits from automation. However, integrating ever increasing advances in computational technologies pose a continuous challenge**	**HTS/HCS are still costly because of the large amount of resources required in relation to the number of active compounds discovered**	**no prior target information is required**
VS	**theoratical, uses prior biological information to identify active compounds.**	**depends on quality and completeness of input training sets for model generation and validation**	**VS benefits both from automation, and integration with state-of-the-art computational approaches contributing to overall task of compound screening and optimization for drug discovery**	**cost effective - after a large number of possible new ligands are found, only then these active compounds are purchased and tested.**	**detailed knowledge of the target is required**

Table 3. : Successful applications of vHTS.

Target	Main contribution	Method	Reference
DNMT	Nanaomycin as selective DNMT3b inhibitor	Structure-based	[37]
Chk-1 kinase	Thirty-six inhibitors with IC50values between 68 nM and 110 μM	Ligand-centric, pharmacophore-based and structure-based	[33]
mGlu4 receptor	Six agonists from a library of 720 000 compounds	Structure-based	[47]
Neurokinin-1 receptor	One compound with IC50 = 0.25 μM	Pharmacophore-based and structure-based	[15]
Fructose 1,6-bisphophatase	Three compounds from ZINC6 database with IC50 values between 1.1 and 32 μM	Structure-based	[20]
Serine/Threonine and tyrosine kinases	Substituted 2-arylbenzothiazoles EC50 = 60 nM.	Structure-based	[45]

class for which structure-activity relationships (SARs) exist across the different lead chemotypes or active sites [19]. These compound subsets, also known as focused screening (illustrated in the appendix), can provide sufficient hits in the drug discovery phase, without screening the whole molecular inventory.
In light of these complimentary benefits offered by VS and HCS in the individual campaigns, further research developing hybrid approaches could mitigate their limitations and increase the efficiency of the drug discovery process. Some of the successful approaches based on the combination of VS and HCS are summarized in Table 3 below.

6. CONCLUSION AND FUTURE REMARKS

In this paper, we briefly present a review of the two leading imaging techniques i.e HCS and VS, used for drug discovery to identify potential compound candidates. From disucssion it can be deduced that the ever-increasing amount of available compound activity and biomedical data is leading to the emergence of new hybrid approaches from the HCS and VS. To mine, efficiently large-scale chemistry data for such approaches make it a plausible solution for drug discovery. Deep learning techniques provide the flexibility to create neural network architectures custom-build for specific problems. Deep learning techniques are more complex and large at scale. Some of the applications for deep learning include compound property and activity prediction, predicting reactions and retrosynthetic analysis, predict ligand-protein interactions, Benchmark datasets within chemoinformatics, biological imaging analysis etc. In the context of drug discovery, deep learning has been found to be a suitable solution for tasks with structured input descriptors such as bioactivity prediction [9].

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