SMALL MOLECULE DISCOVERY CENTER

SMDC

High-content Analysis for Unusual Targets

Michelle Arkin

UCSF

Our Mission

Provide UCSF with small molecule discovery capabilities to

- advance understanding of cell/protein function
- identify promising new targets and leads
- advance technologies for challenging targets

Why Do Lead Discovery in Academia?

- Academic research often drives biological innovation in medicine
- Academic research can also drive technological innovation in drug discovery
- Address major unmet medical needs that are neglected by the pharmaceutical industry

SMDC Cores and Functions

Screening & Informatics

- Liquid-handling robotics
- Deck of 180K small molecules
- Genome-wide siRNA screening
- Biochemical, high-content, and fragment screens
- Chemo-informatics

Lead Discovery

- Grant-funded collaborative model
- Medicinal chemistry (8 FTEs)
- *In vitro* & *in silico* ADME-Tox
- Multiple programs in diverse TAs
  Typanosomiasis *Malaria*
  Neurodegeneration *Cancer*

Technology Development

- Fragment screening (MS, SPR)
  - *charter member of the CBC (NCI)*
  - *will focus on challenging targets*
- Targeted prodrug technology

High Content Analysis In Screening

HCA: automated microscopy

- Multi-color fluorescence and bright field
- Fixed or live cell
- Automated data collection
- Image-analysis algorithms

Benefits

- Statistical rigor
- Subcellular localization
- Multi-parameteric readouts

INCell Analyzer 1000 (GE)

50% of SMDC screening projects use HCA in drug discovery, target discovery, and developmental biology

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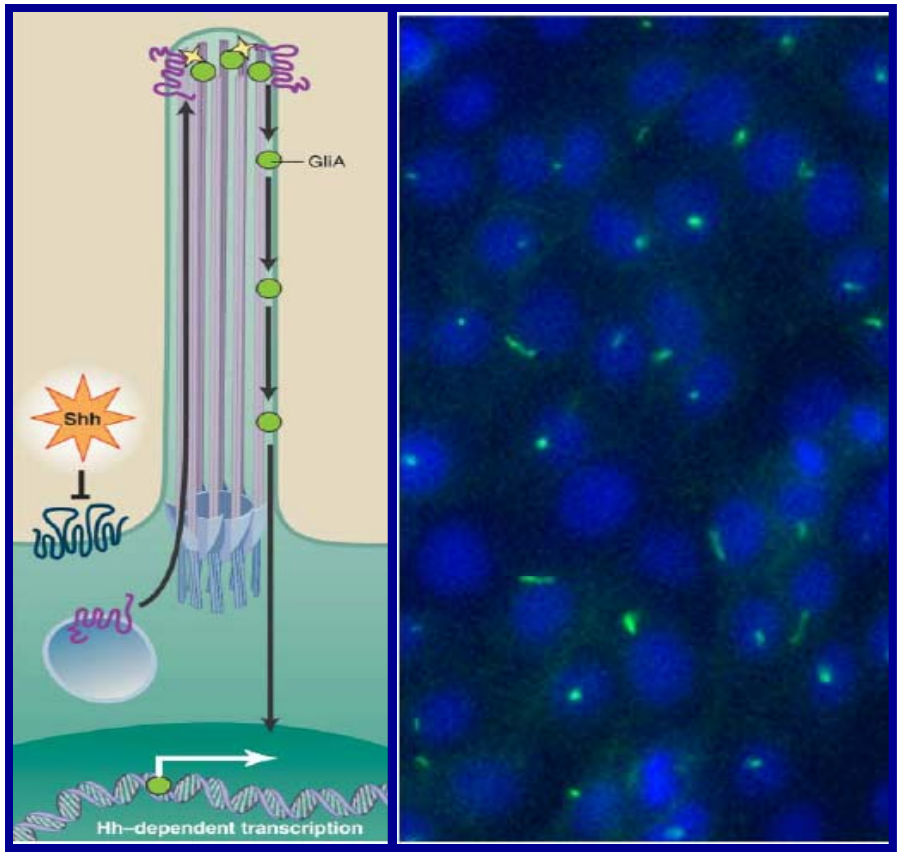
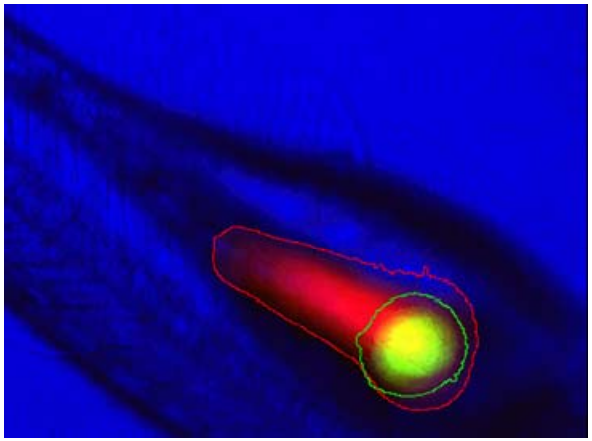
Target Discovery and Developmental Biology

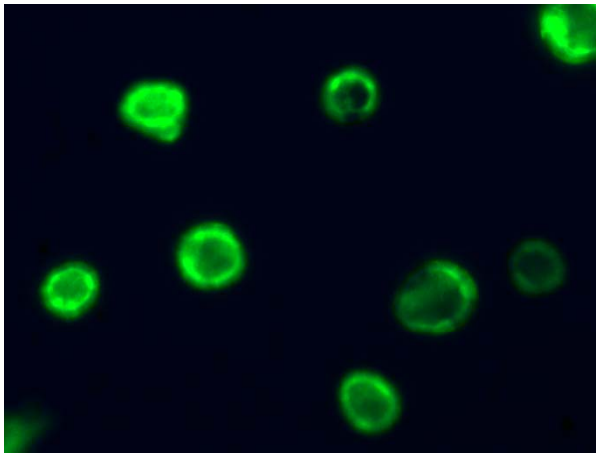
Small-molecule modulators of ciliagenesis



Pancreas regeneration



Growth of kidney-cell cysts



Victoria Wu, Jeremy Reiter; Olov Andersson, Didier Stainier; Paul Brakeman

Challenges of Tropical Parasitic Diseases

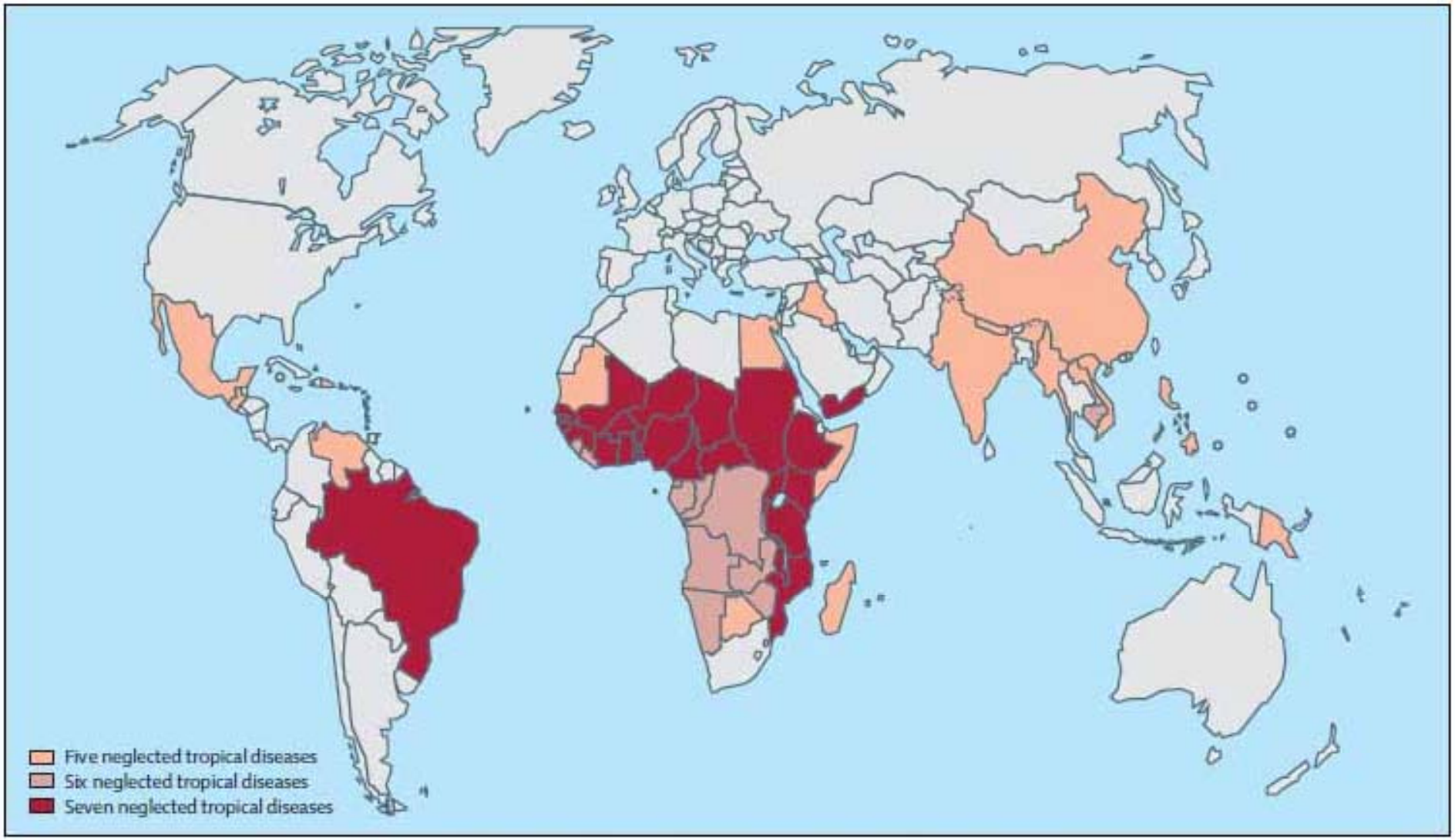


Figure 1: **Map showing geographic overlap and distribution of the seven most common neglected tropical diseases**
Reproduced with permission from Hotez.[10]

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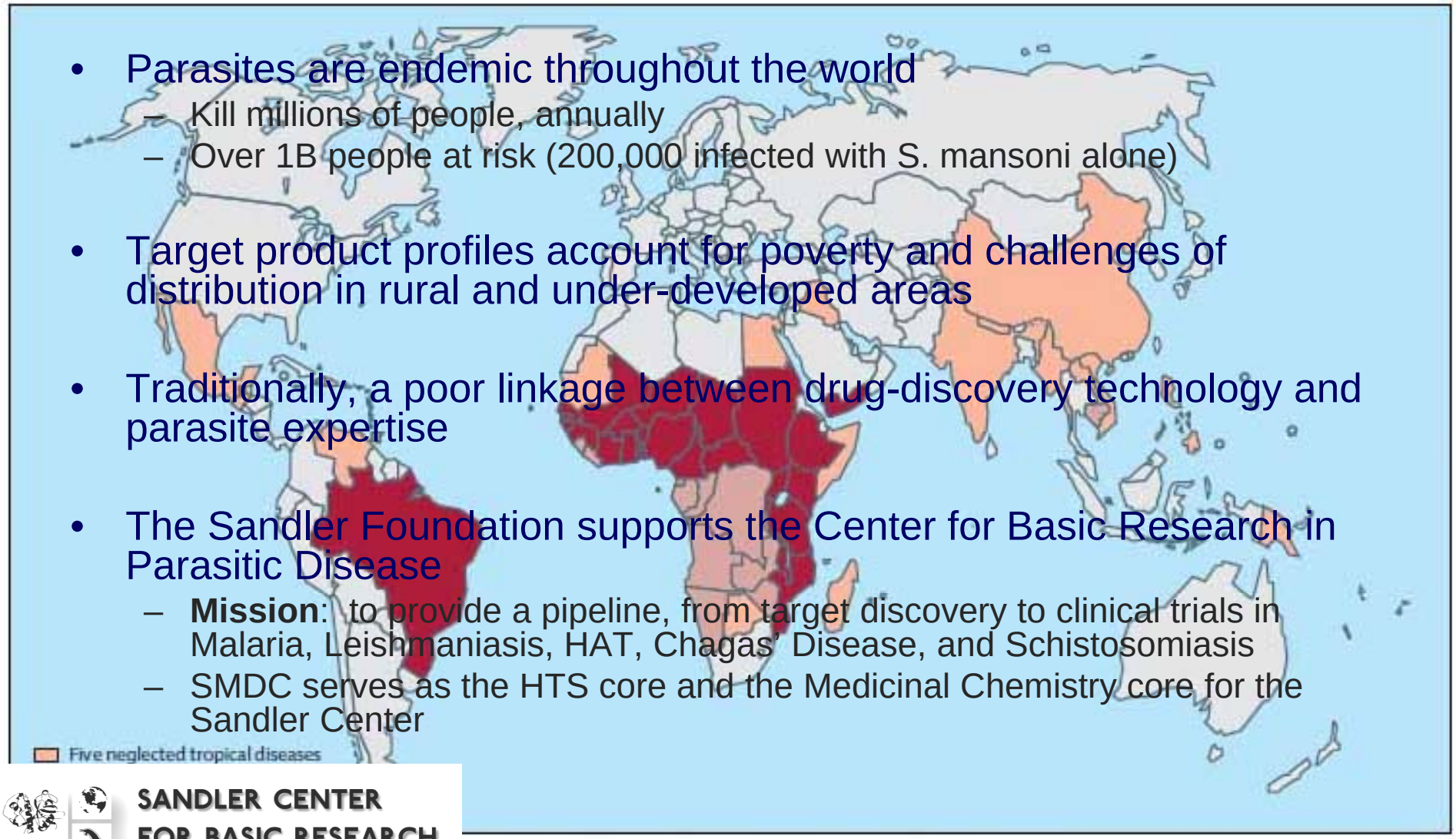
Challenges of Tropical Parasitic Diseases

- Parasites are endemic throughout the world
  - Kill millions of people, annually
  - Over 1B people at risk (200,000 infected with S. mansoni alone)
- Target product profiles account for poverty and challenges of distribution in rural and under-developed areas
- Traditionally, a poor linkage between drug-discovery technology and parasite expertise
- The Sandler Foundation supports the Center for Basic Research in Parasitic Disease
  - **Mission**: to provide a pipeline, from target discovery to clinical trials in Malaria, Leishmaniasis, HAT, Chagas' Disease, and Schistosomiasis
  - SMDC serves as the HTS core and the Medicinal Chemistry core for the Sandler Center



tribution of the seven most common neglected tropical diseases

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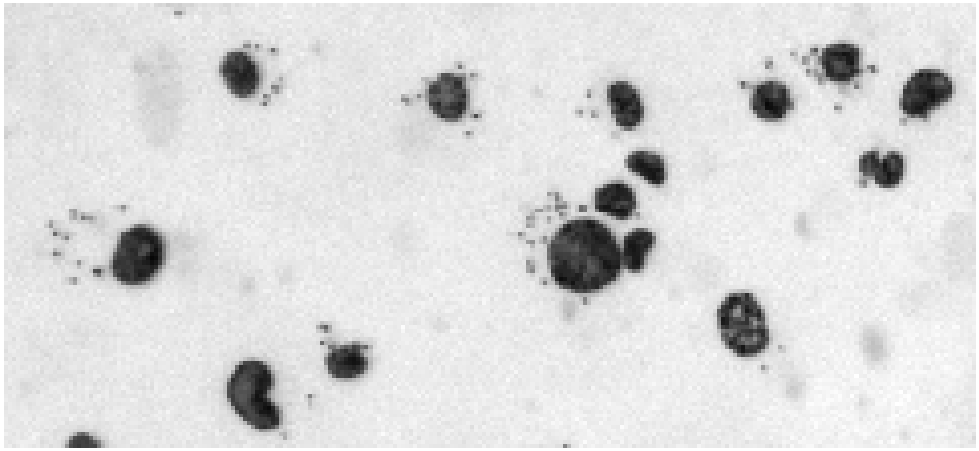
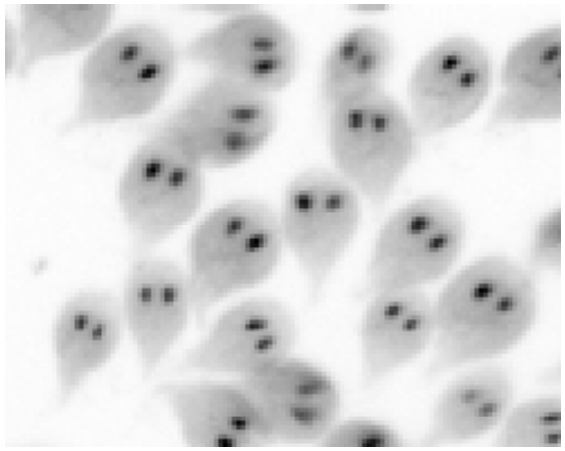
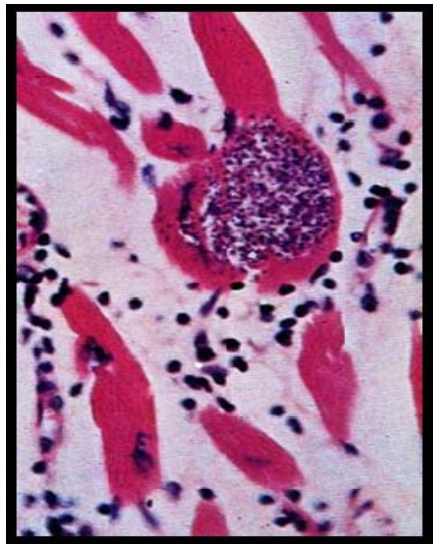
Recent Programs in Neglected Disease

- Cell-based and targeted discovery for Trypanosoma cruzi
- Cell-based assay for Leishmania
- Automated screen for Schistosoma mansoni
- Kinase-directed discovery for Trypanosoma brucei
- Targeted pro-drug therapy for P. falciparum
- HTS for Giardia, E. histolytica, Neglaria





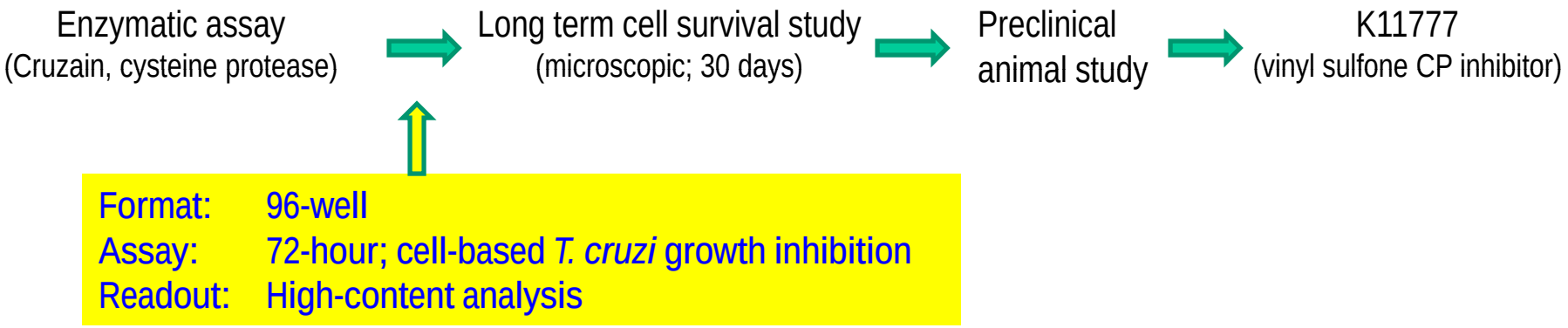
Developing HCS Assay for Amastigote stage of T. *Cruzi*



- Causative agent of Chagas Disease
  - Insect-transmitted disease affecting 18 million people in rural Latin America
  - Acute phase (blood) is not completely cleared, leading to chronic phase (heart) and sudden heart failure
- Current treatments
  - Two antiques: benznidazole and nifurtimox
  - 180-day treatment cycle
- The Sandler Center is developing K11777, a vinyl sulfone inhibitor of the cysteine protease cruzain

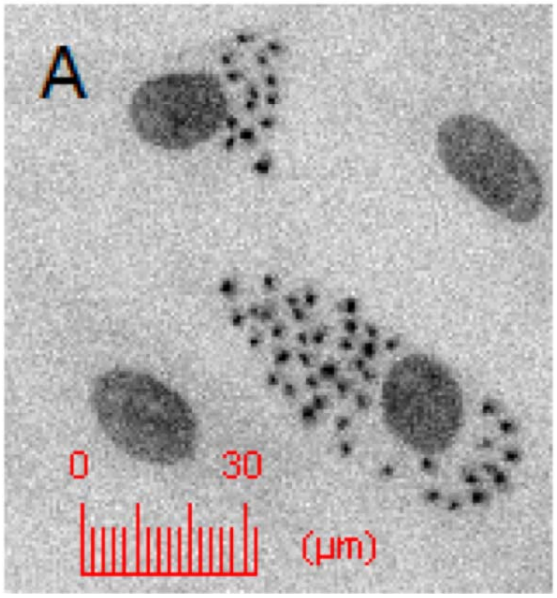
Facilitating Lead Discovery for T. cruzi

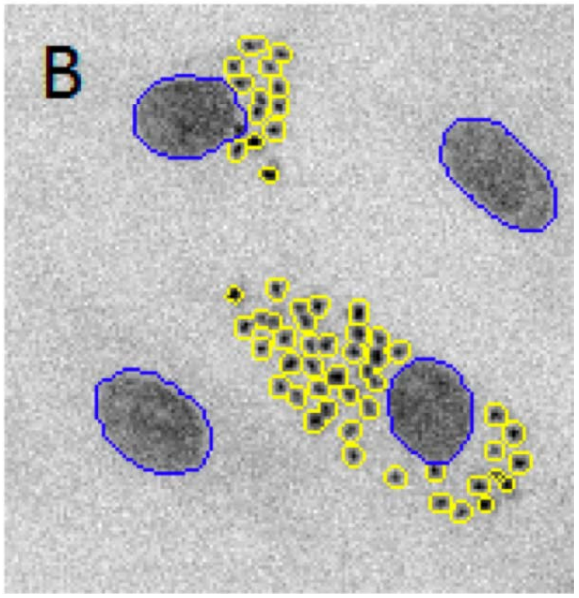
- Lead discovery assay funnel was lengthy and qualitative



Developing High-content Screen for T. cruzi

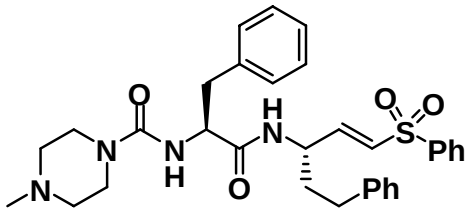
- Detect host nuclei and kinetoplast DNA with DAPI
- Simultaneous measurement of parasite inhibition and cytotoxicity
- Readily adapted to other strains and mammalian cell types





Kenny Ang, Juan Engel

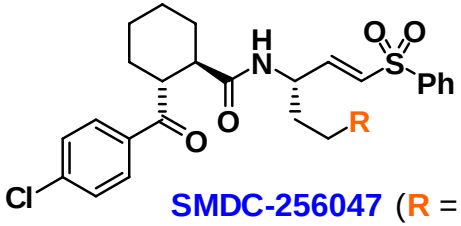
Identification of Non-peptidic Vinylsulfones



K-777

medchem

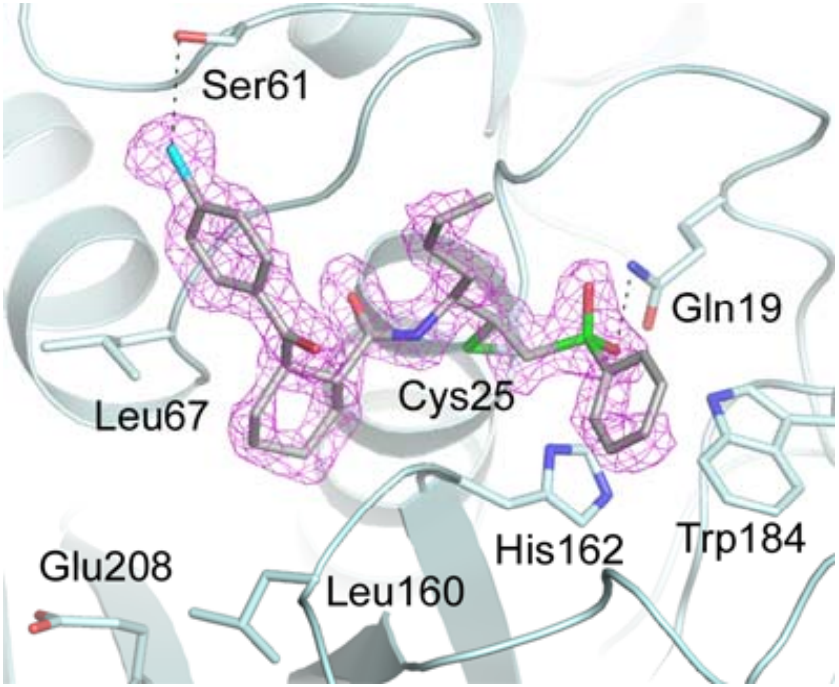
~40 non-peptidic analogs synthesized



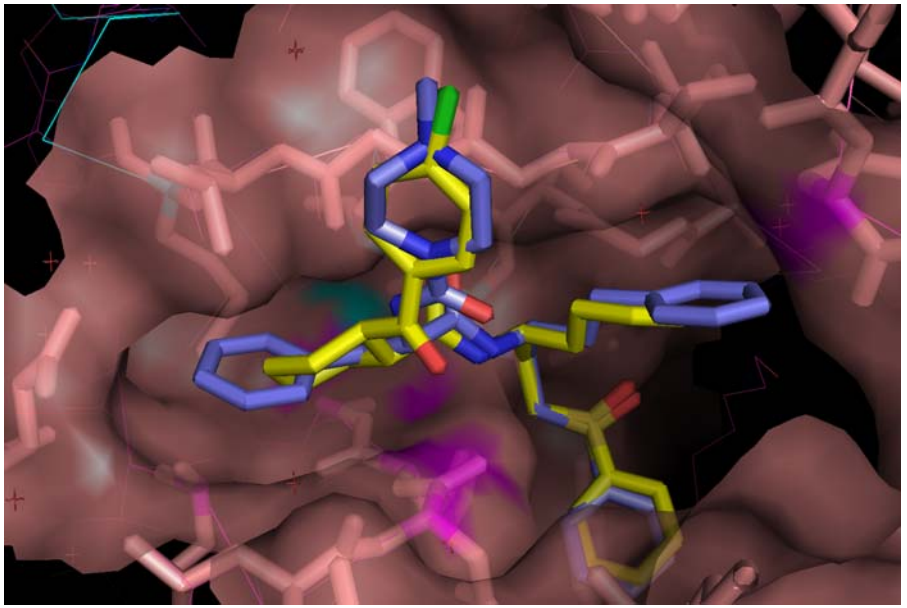
SMDC-256047 (**R** = Et)
SMDC-256002 (**R** = Ph)

Bryant, Kerr, et al *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6218-6221

SMDC-256047 bound to cruzain (1.75 Å)



Comparing 256047 (yellow) and K777 (blue)



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Correlation between HCS Screen and Survival

Compound ID	Chemotype	42-day assay	3-day assay		
		***T. cruzi*-infected J774 macrophage tested @ 10 uM (days survivial)**	*T. cruzi* IC_{50} (uM)	Cell toxicity IC_{50} (uM)	**Selectivity**
256123	vinylsulfone	**> 30**	0.4	8	**20**
256122	vinylsulfone	**> 30**	2	> 20	**> 10**
256157	vinylsulfone	**> 30**	2	20	**10**
256162	vinylsulfone	**> 30**	2	18	**9**
256037	vinylsulfone	**toxic to macrophage**	2	13	**7**
256002	vinylsulfone	**toxic to macrophage**	5	16	**3**
256064	vinylsulfone	**toxic to macrophage**	4	7	**2**
256171	oxadiazole	**19**	11	> 20	**> 1**
256189	oxadiazole	**5 (not active)**	17	> 20	**> 1**
281566	oxadiazole	**5 (not active)**	> 20	> 20	**> 1**

For Cysteine protease inhibitors, anti-proliferation correlates with trypanosidal activity

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Screening of FDA compounds identifies known and novel hits for T. cruzi

Compound	[a] Selectivity window	[b] Therapeutic use	Reported trypanosome-related effect
Furazolidone	> 125	anti-bacterial	trypanocidal
Terconazole	> 65	anti-fungal	
Nitrofurazone	> 25	anti-bacterial	trypanocidal
Amiodarone	24	arrhythmia	trypanocidal
Azelastine	16	allergy	
Nelfinavir	13	anti-viral (HIV)	anti-*Toxoplasma*
Haloperidol	12	psychosis	anti-*Toxoplasma*
Fluphenazine	8	psychosis	trypanocidal (*T.brucei*)
Dihydroergocristine	7	vascular dementia	
Raloxifene	6	osteoporosis	
Carvedilol	6	congestive heart failure	Chagas cardiomyopathy

High-content assay ready for lead optimization and screening

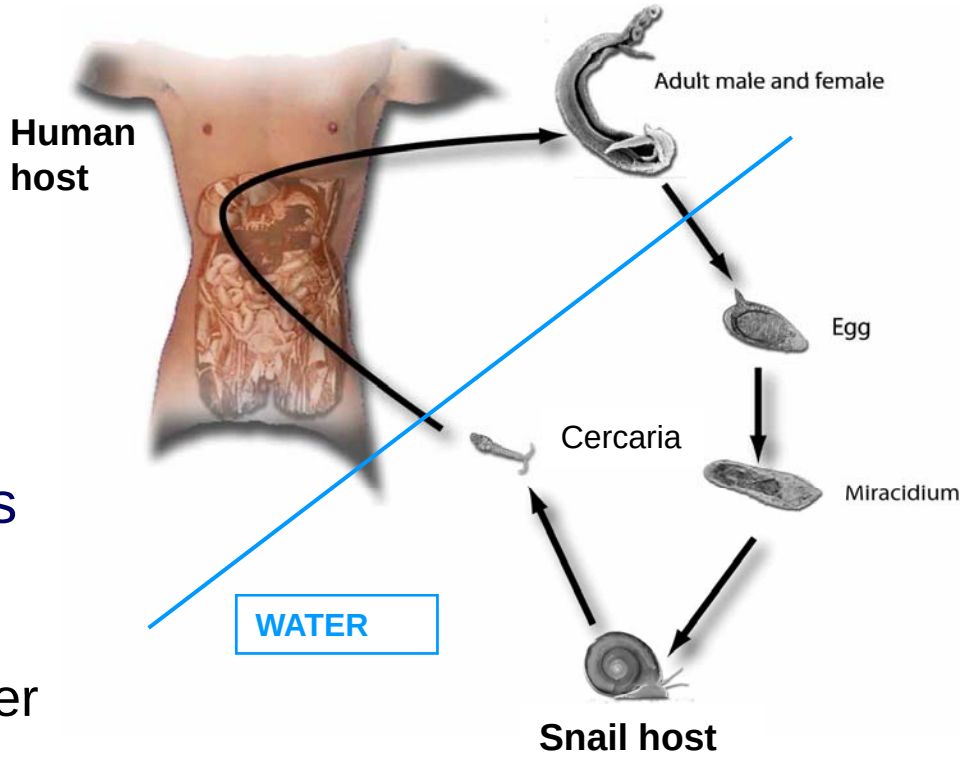
Schistosomiasis is a Major Public Health Issue

Very Prevalent

- At least 200 million currently infected, in 74 countries
- Socio-economic impact is second after malaria (among parasites)
- Praziquantel is only treatment

Assay Development Challenges

- Does not propagate in culture
- Genetically heterogeneous and possible non-sexual genetic transfer
- Multiple phenotypes expected, based on movement, death kinetics



Conor Caffrey

Current State-of-the-Art

Drug collection (eg by Microsource)

↓

Schistosomula 1º visual screen, 7d

↓

Schistosomula 2º visual screen,
24 h and 7d

↓ Prioritize fast-acting compounds
Remove known human-toxic cmpds

Adult visual screen, 7-96 hours

↓ Prioritize fast-acting compounds
Consider LD_{50}, current clinical use

Animal model of disease
PO administration, 4d
Select for efficacy/toxicity

Schistosomula *in vitro*

Adults *in vitro*

Mouse model

Selection by Visual Phenotyping

DMSO K777 PZQ

Table 1. Phenotype descriptors for schistosomula in response to known anthelmintic compounds

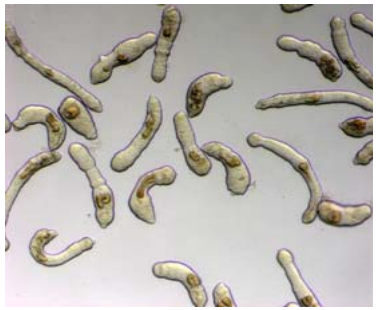
Compound*	Primary screen (7 d)	Confirmatory screen (7 d)	Confirmatory screen (24 h)
Hexachlorphene	Dead	Dead	None
Hycanthone	Dead	Dead	Dark, overactive
Niclosamide	Dead	Dead	Dead
Praziquantel	Degenerate but mobile	Degenerate but mobile	Rounded, overactive
Pyrvinium pamoate	Dead	Dead	Rounded

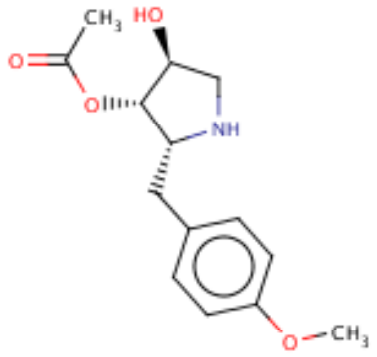
Taken from [44]. *Compounds tested at 1 μM.

Six phenotypes identified from anthelmintics and drug screen

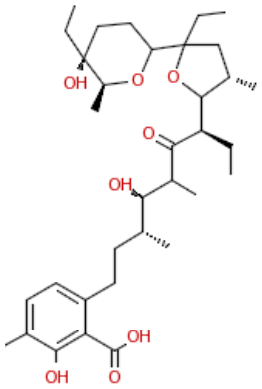
Lead Discovery for Schistosomiasis

- 96-well plates
- Visual detection of phenotypes
- Screened ~2000-compound “drugs”
- Identified hits with activity in mouse

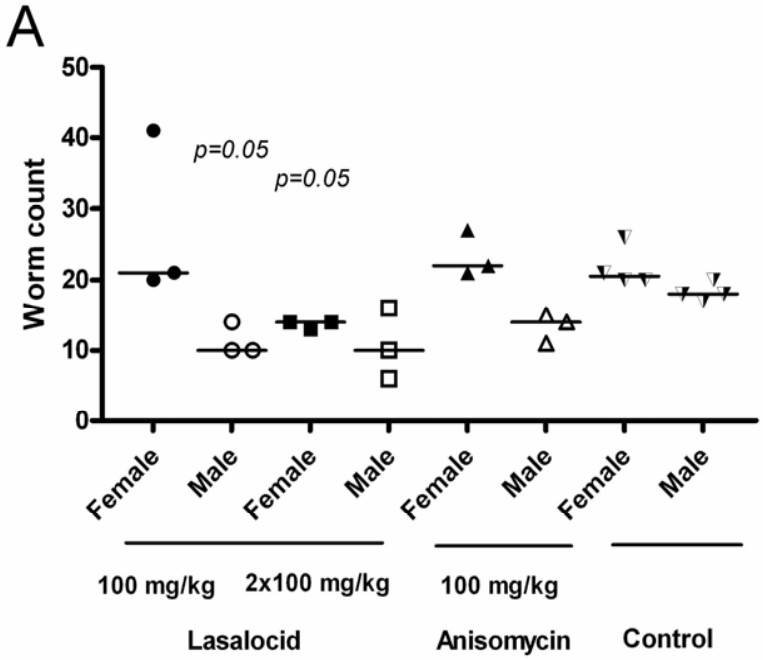




Anisomycin
(“flagecidin”)
Anti-protozoal
Anti- fungal



Lasalocid sodium
Feed additive for steers, fowl
Coccidiocide



The manual approach shows value of plate-based screening

Potential Advantages to High-Content Analysis

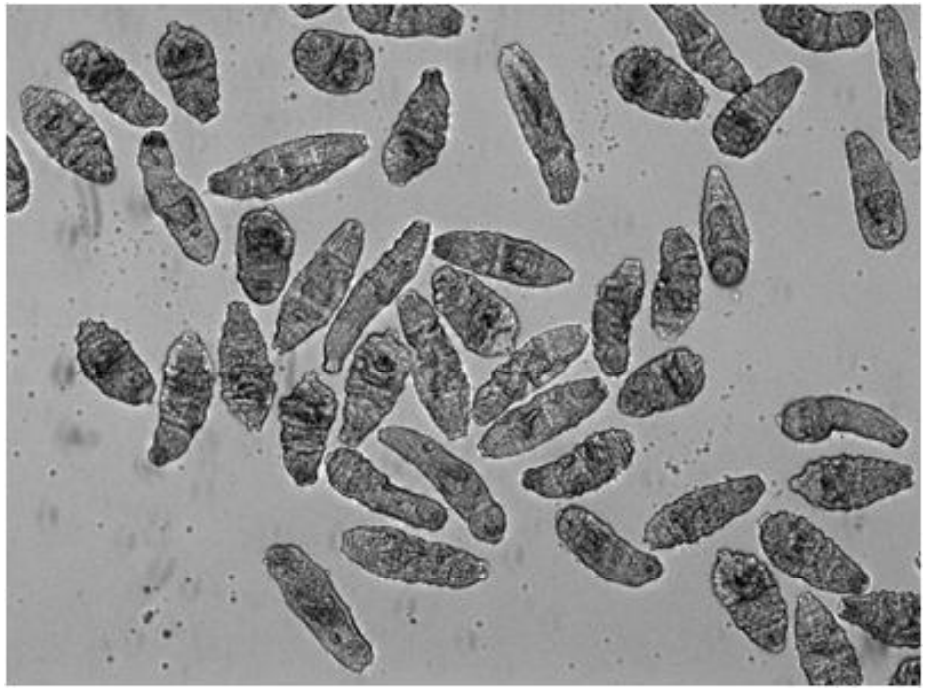
- Throughput
- Precision/quantification
- Subtlety of phenotypes
- Significant challenges in assay development and memory usage

Comparison of data-collection specifications: camera vs HCA systems

Model	IN Cell 2000	**IN Cell 1000**	AxioCam MRc
CCD Size (megapixel)	4.2	**1.2**	
Movie Frame-rate (frames/sec)	~ 10	**1.3**	15-20
Memory storage/30-sec movie	~320Mb w/o bin	**~80 Mb**	~80 Mb
Light Source	200 W Metal arc	**100 W Xe**	Tungsten
Environmental Control	Yes	**No**	No
Autofocus, automated x-y stage	Yes	**Yes**	No
Automated quantitative analysis	Yes	**Yes**	CART analysis*
3-D Deconvolution	Yes	**No**	No
Image Stitching	Yes	**No**	No

Challenges to Automation

1. Scaling up worm culture
2. Automated dispensing of worms into 96 or 384 wells
3. Collecting images
   - Plating conditions
   - Time, Z-axis stacks
4. Analyzing time-lapse images
   - Segmentation
   - Defining descriptors
   - Tracking motions



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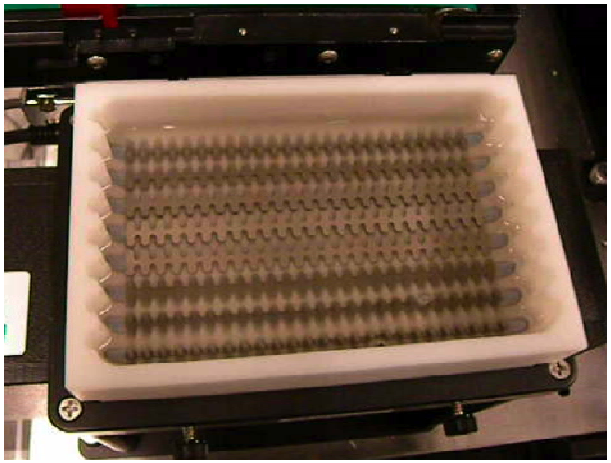
Critical Bottlenecks Identified and Addressed

1. Scale-up worm production

- Innoculate snail “farm” in lab
- Induce shed of cercariae into media
- Prepare schistosomula by filtration, removing tails
- Yield > 120,000 schisto/shed

2. Automate dispensing



- Currently 96-well format, 384 in progress
- ~30 worms/well in U-bottom or 100-200 worms/well in flat-well
- Very sensitive to liquid handling: solved through paddle stirring

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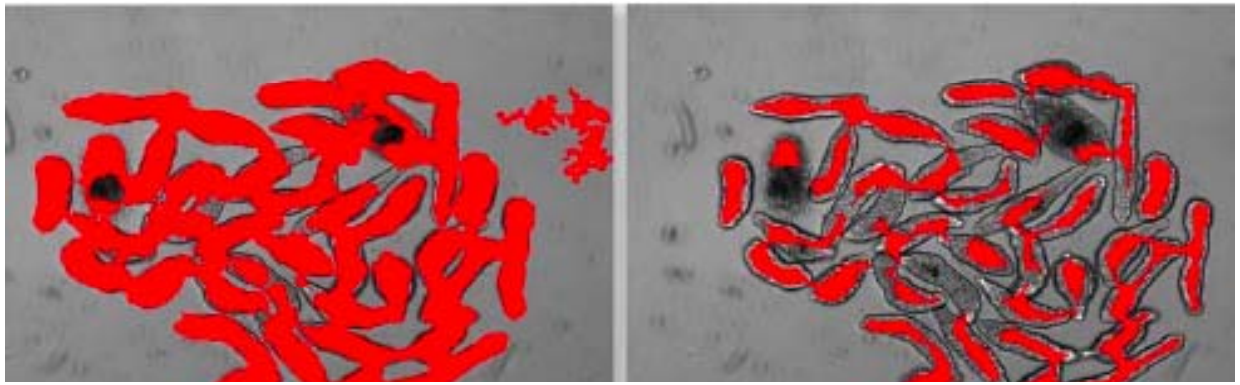
Collecting images: Bright field vs Staining

Bright field: easy to collect, hard to segment



Fluorescence: searching for the right vital dyes

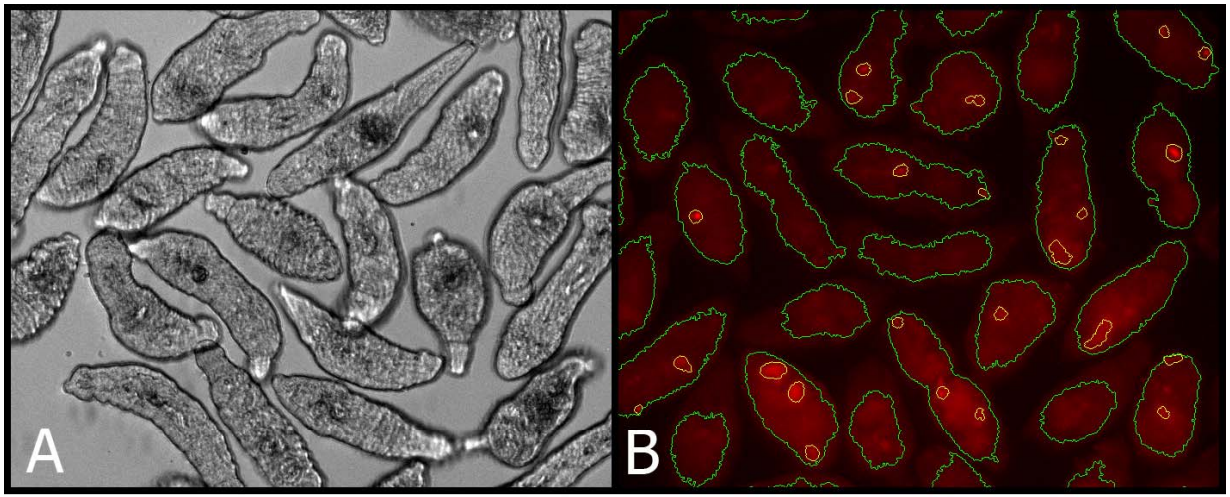


Fig Images of Schistosomula A) Bright field b) Red fluorescence and image analysis masking Green outline = body segmentation; yellow outline = small intensly stained structures

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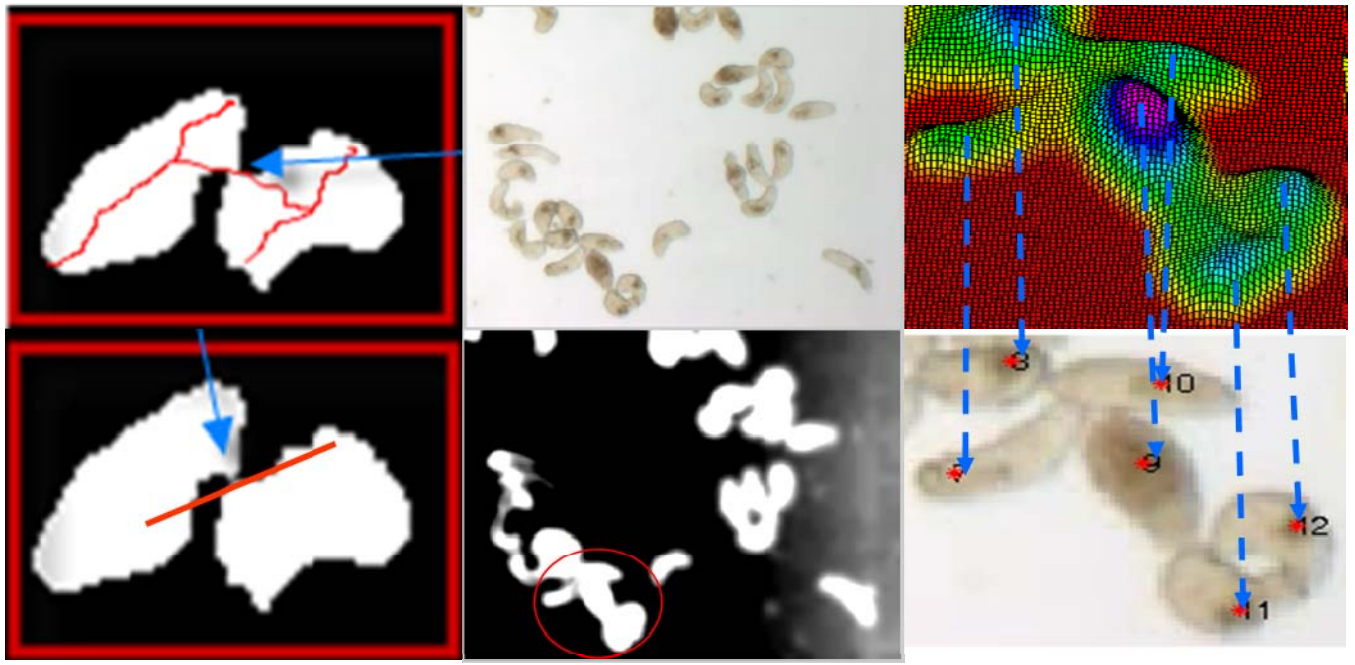
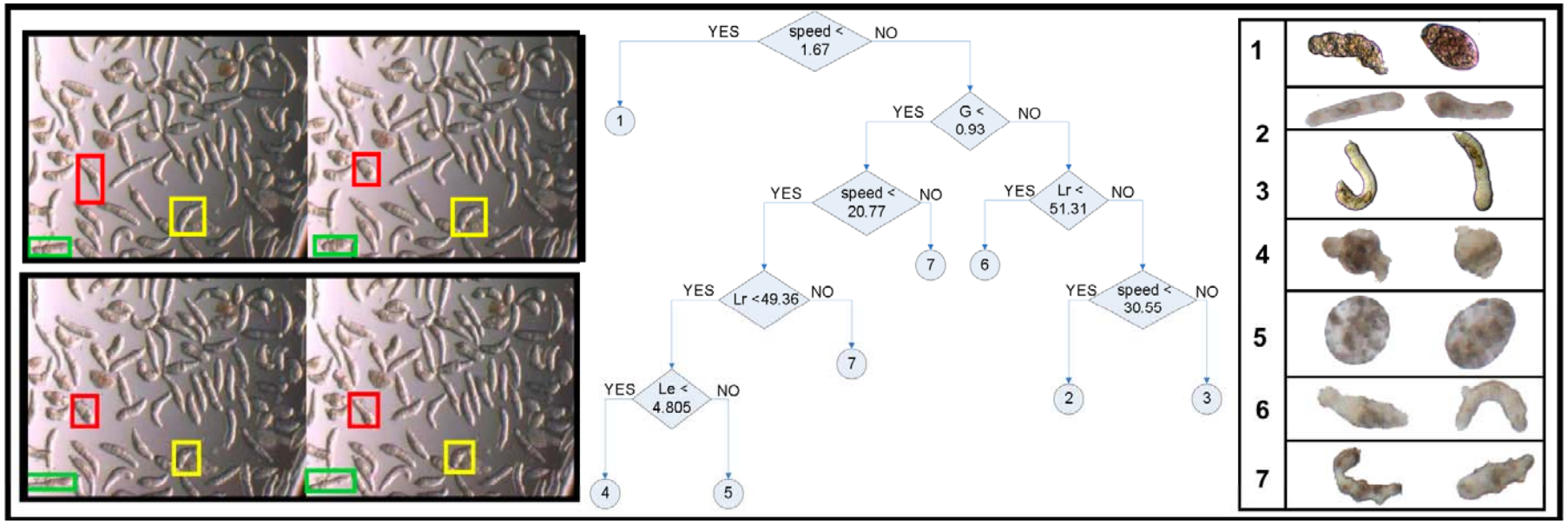
Segmentation by Seeded-Watershed Algorithm



1. Topology-driven parasite segmentation
   - identify touching concave-convex regions that are prominent enough to ensure branching of the medial axis
   - Parasites are separated by cutting these regions
2. Convolute with a multi-directional Gaussian kernel
   - Local intensity maxima correspond to the interior of each parasite

Conor Caffrey, Ido Heskia and Rahul Singh (SF State)

Algorithm-based Classification



1. Parasite segmentation
2. Track over time via mean-shift algorithm
3. Define shape and appearance-based features for each parasite in each frame
4. Seven phenotypes identified (including the 6 found from visual scoring)

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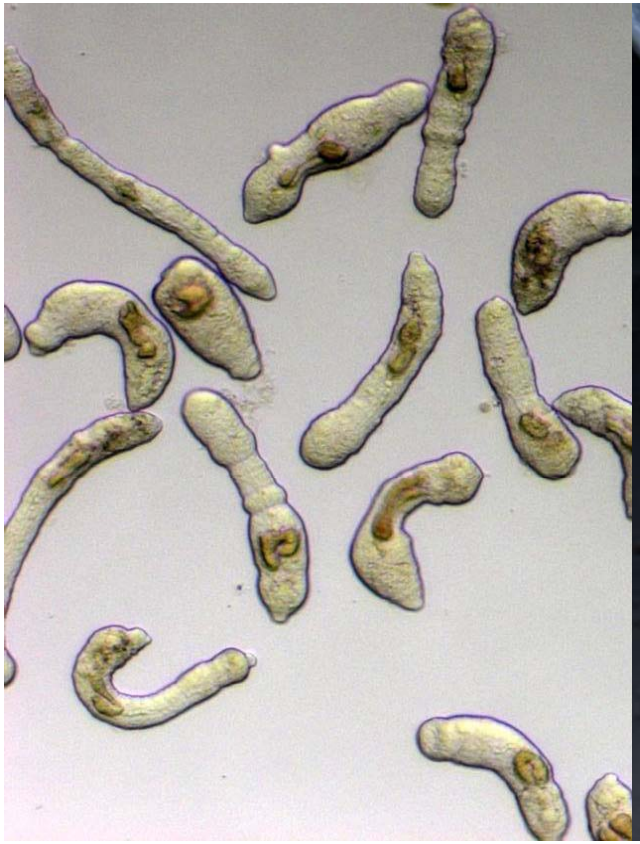
Conor Caffrey, Mixalis Pittas and Rahul Singh

And Then, There are Adult Worms



Somules: 60 x 200 um

Adults: 1 cm long, sexually dimorphic

HCA Contributes to Lead Discovery for Neglected Parasitic Diseases

- Driving biological innovation in medicine
  - High-throughput screening for lead-like molecules and siRNA
- Driving technological innovation in drug discovery
  - Replacing/supplementing low-throughput and/or low information-content assays
- Address major unmet medical needs that are neglected by the pharmaceutical industry
  - Fuel a drug-discovery engine in not-for-profit pharmaceuticals

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Our Team